



**The effect of Fleckvieh crossbreeding in dairy cattle on the
conjugated linoleic acid content of milk produced in intensive and
pasture-fed systems**

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DECLARATION

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ABSTRACT

Conjugated linoleic acid (CLA), a fatty acid (FA) present in the meat and milk of ruminant animals, is considered a functional nutrient for humans. The interest in CLA is attributed to its many health benefits, such as having anti-carcinogenic, anti-atherogenic, anti-diabetic and anti-adipogenic effects. Dairy products are a rich natural source of CLA, and preliminary research indicates that the levels in milk can vary based on cattle breed and feeding system.

The Fleckvieh is a popular dual-purpose breed recently introduced to the Western Cape. It is used in crossbreeding programs with Holstein and Jersey herds to increase fertility and beef production. Holstein and Jersey cattle are the two most common South African dairy breeds, but little information is available on the effect of crossbreeding on FA content and CLA levels in the milk. The primary objective of this study was to compare milk FA composition and CLA content of these two dairy breeds and their respective Fleckvieh crosses. A second objective was to investigate the effect of lactation stage on CLA levels. Jersey (J) and Fleckvieh x Jersey (F×J) cows were kept in a pasture-based system (PBS) for the duration of the study. Holstein (H) and Fleckvieh x Holstein (F×H) cows were housed in a feedlot system and fed a total mixed ration (TMR) for the duration of the study. The FA and CLA content of feeds from these two feeding systems were also analysed and compared. All cows used in the study were housed at the Elsenburg Research Farm of the Western Cape Department of Agriculture.

Milk samples were collected every 35 days from the cows in each feeding group, starting 10 days after calving. Samples were kept in a freezer at -20 °C until laboratory analyses. The total number of milk samples collected was 1044. The milk FA composition and CLA in particular were determined by gas chromatography. Differences between groups were statistically analysed by two-way repeated measures of analysis of variance (ANOVA).

Results show that milk CLA levels are higher in Fleckvieh-crossbred cows when compared to purebred cows. The higher CLA content of milk from Fleckvieh crossbred cows demonstrates the feasibility of using Fleckvieh bulls in South African crossbreeding programs, and are also an indication that genetic selection for increased CLA content is possible. The analysis of lactation stage showed that milk CLA is higher during late lactation. A comparison of feed samples from the two feeding systems revealed that pasture feeding resulted in higher omega-3 FA in milk.

Key words: Conjugated linoleic acid, milk fatty acids profile, diet, breed, health benefits, Fleckvieh, Holstein, Jersey, diet, pasture based system, total mixed ration system,

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“My grace is sufficient for you”- Corinthians 12: 9

DEDICATION

I dedicate this thesis to my parents, Pumza and Mzuvukile Sasanti, for their unconditional love, support and guidance. They taught me to believe in myself, to work hard to reach my goals and to search for wisdom and truth in the word of God.

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GLOSSARY OF ABBREVIATIONS

ALA	Alpha-linolenic acid
ANPI:	Animal Production Institute
CLA	Conjugated linoleic acid
CSOCM	Cotton seed oil cake meal
FA's	Fatty acids
FAME	Fatty acid methyl esters
FxH	Holstein cow crossed with Fleckvieh sire
FxJ	Jersey cow crossed with Fleckvieh sire
GC	Gas chromatograph
H ₂ O	Water
HIP	Hexane:isopropanol
HPLC	High performance liquid chromatography
LA	Linoleic acid
MUFA's	Mono-unsaturated fatty acids
MUN	Milk Urea Nitrogen
N	Nitrogen
PROMECC	Program on Mycotoxins and Experimental Carcinogenesis
PUFA's	Polyunsaturated fatty acids
SFA's	Saturated fatty acids
SCC	Somatic cell count
TAG	Triacylglyceride
TFA	Total fatty acids
TMR	Total mixed ration
PROC MIXED	Mixed model procedure

ACRONYM/ABBREVIATION	DEFINITION/EXPLANATION
ALA	An omega-3 fatty acid found in plant foods, including grasses, canola, soybeans oil, and flaxseeds. It is a precursor of EPA and DHA essential in the human diet.
Cis	Chemical configuration in which the two pieces of carbon chain on either side of a double bond are on the same side of the molecule.
Concentration	Cattle feed containing a mixture of high-energy grains and protein-rich feed sources such as cottonseed oilcake, soya oilcake or fishmeal. Concentrates are higher in energy and protein and lower in fiber content than forages.
CLA	Collective term for about 20 conjugated isomers of linoleic acid (LA) found primarily in dairy products but also in beef.
FA	A chemical unit of fats composed of chains of 4-30 carbon atoms with hydrogen atoms attached.
Grass-fed	A term generally used to describe a dairy or beef production system in which close to 100 percent of a cow's diet over the course of its lifetime consist of forage.
Isomer	A compound that has the same molecular formula as one or more other compounds but in a different arrangement of atoms.
LA	Linoleic acid is the most common poly-unsaturated fatty acid in plant and animal tissues. This essential nutrient is found in large quantities in many plant seeds and oils such corn, soy, sunflower and peanut. Moderate intake levels of LA reduce the risk of coronary heart disease.
Lipids	Also known as fats in the body. These substances store energy, transport fat-soluble vitamins, regulate biological functions and serve as a building block of membranes.
Omega-3 FA	Unsaturated fatty acid of a kind occurring chiefly in fish oils, with double bonds between the carbon atoms that are third and second from the end of the hydrocarbon chain. These fatty acids have generated interest among

	nutritionists and consumers because of its potentially beneficial health effects.
Osteoporosis	A condition where the bones become thin and weak, breaking easily. It frequently goes undiagnosed until a fracture occurs. The spine, wrist and hips are particularly vulnerable to fracture.
Rotational grazing	Cows are moved to fresh pastures once or twice a day so their grazing does not damage any pasture beyond its ability to regenerate. This can also be called management-intensive grazing.
Ruminants	Ruminants are able to utilize fiber in plant-based feeds by microbial fermentation in a specialized stomach called the rumen. The Rumen is the forestomach, one of four, the others being the Reticulum, Omasum and the Abomasum.
Silage	Animal feed in the form of plant material, which has fermented while in storage.
TMR	A total mixed ration is a complete feed mixture given to dairy or beef cattle. It typically contains forages, such as lucerne hay or silage, and concentrates consisting of grains, high-protein feeds, minerals and vitamins.
Trans	A chemical configuration in which the two pieces of the carbon chain are on opposite sides of the double bond.
PROC MIXED	The SAS [®] mixed linear model procedure, allowing modelling the means of data (as in the standard linear model) and their variances and co-variances as well.

STYLE

The language and style used in the thesis are in accordance with the requirements of the CPUT article thesis format. Each chapter is presented as an individual entity and some repetition between chapters has, therefore, been unavoidable.

Contribution to the Thesis

Conference presentations (oral or poster)

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2. Muller, C.J.C., Sasanti, B., Abel, S. and Schmulian, A. 2013. The milk fatty acid composition and conjugated linoleic acid content of Jersey and Fleckvieh x Jersey cow milk in a pasture-based feeding system. *Fleckvieh World* 2013:20-21.
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CHAPTER 1

GENERAL INTRODUCTION

Conjugated linoleic acid (CLA) is a fatty acid (FA) found predominantly in products such as milk and meat of ruminant origin (De la Fuente *et al.*, 2006; Flower *et al.*, 2007). During the past decade, CLA has received a considerable amount of attention due to the identification of a link with beneficial effects in humans and animals. A number of studies based on cell cultures, animal models and some human studies found CLA to be a potent anti-carcinogen. Additional health benefits include anti-atherogenic, immune modulating, anti-diabetic and cholesterol lowering properties (McGuire and McGuire, 1999; Cruz-Hernandez *et al.*, 2007).

A large component of the global population consumes milk on a regular basis. Some studies have suggested that dairy products contribute to heart disease, as a number of dairy products are high in fat and cholesterol. Other studies suggest that there is a possible link between dairy consumption and cancer (Peng *et al.*, 2006; Haug *et al.*, 2007). However, milk and dairy products also contain beneficial components, such as CLA, omega-3 FA's, short- and medium- chain FA's, vitamins, minerals and bioactive compounds. These have been linked to positive health effects and confirm the beneficial aspect of milk as part of the Western diet (Muller and Delahoy, 1988; Clancy, 2006).

The type of feeding system used by dairy farmers appears to have a large effect on FA content and profile of cows' milk. Over the past decade, researchers have shown that the meat and milk from pasture-fed cattle contain higher FA levels (Khanal and Olson, 2004; Kreuzer and Leiber, 2005). Clancy (2006) compared the fat levels in beef and dairy products from cows in two feeding systems, total mixed ration (TMR) and pasture. The cows on pasture showed higher levels of polyunsaturated fatty acids (PUFA's) in meat compared to cows in a TMR feeding system. Collomb *et al.* (2002) found that meat and milk from cattle raised on pasture contained less total fat than cows on a TMR feeding system. Lastly, Liebenberg (2007) indicate a positive relationship between the intake of feed containing high levels of PUFA's, such as linoleic and linoleic acid, and increased CLA content of milk.

Song and Kennelly (2003) suggested that breed influences the CLA content of milk. Research done in Canada has shown that Fleckvieh x Holsteins cows (F x H) have higher levels of CLA in their milk than Holsteins on similar diets and management programs (Lock and Bauman, 2004). Grega *et al.* (2005) found significant differences in the CLA content of milk from three Polish cattle breeds, the Polish Red, Simmental and Black and White. Similar experiments have not been conducted under local South African conditions.

The Fleckvieh, a Simmental-derived breed from Bavaria in Germany, is a dual-purpose breed, known for its beef and milk production. The advantage of using the Fleckvieh sires in a crossbreeding program with specialized dairy breeds, is that high quality weaner calves are produced for the feedlot, without sacrificing the milk yield of the cows (Patrick *et al.*, 2000).

Recent studies conducted in the Western Cape, South Africa show a performance advantage in Fleckvieh crosses over purebred dairy breeds, with regards to beef and veal production in steers, and reproduction in heifers (Muller and Botha, 2012; Muller *et al.*, 2012; Metaxas *et al.*, 2013). If the same advantage exists concerning CLA content of these Fleckvieh crosses, then genetic selection or crossbreeding with Fleckvieh may become a profitable industry-wide practice.

The research results discussed above give a preliminary indication that breed as well as dietary conditions have a significant influence on the CLA content of milk. The aim of the study is to determine if significant differences in milk CLA content occur in South African breeds, under specific South African farming and feeding conditions.

1.1 Research Objectives

The main objective of this study is to compare the milk and feed FA composition, particularly CLA content of cows maintained in two different feeding systems. The effects of crossbreeding and lactation stage on each group will also be determined for:

1. Jersey and Fleckvieh x Jersey cows in a pasture-based feeding system.
2. Holstein and Fleckvieh x Holstein cows in a TMR feeding system.

References

- Collomb, M., Butikofera, U., Siebera, R., Jeangros, B. and Bosset, J.O. 2002. Correlation between fatty acids in cows' milk fat produced in the Lowlands, Mountains and Highlands of Switzerland and botanical composition of the fodder. *Int. Dairy J.* 12: 661-666.
- Clancy, K. 2006. Greener Pastures: How grass-fed beef and milk contribute to healthy eating. Cambridge, Massachusetts: Union of Concerned Scientists Publications.
- Cruz-Hernandez, C., Kramer, J.K., Kennelly, J.J., Glim, D.R., Sorensen, B.M., Okine, E.K., Goonewardene, L. A. and Weselate, R.J. 2007. Evaluating the conjugated linoleic acid and *trans* 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. *Amer. Dairy Sci. Assoc.* 90:3786-3801.
- De la Fuente, M.A., Luna, P. and Juarez, M. 2006. Chromatographic techniques to determine conjugated acid isomers. *Trends Anal. Chem.* 25(9):917-926.

- Flower, G., Ibrahim, S.A. and AbuGhazaleh, A.A. 2007. Milk fatty acid composition of grazing dairy cows when supplemented with linseed oil. *J. Dairy Sci.* 91:722-730.
- Grega, T., Sady, M., Najgebauer, D., Domagala, J., Pustkowiak, H. and Faber, B. 2005. Factors affecting the level of conjugated linoleic acid (CLA) in milk from different cow's breeds. *Biotechnol. Anim. Husb.* 21:241-244.
- Haug, A., Hostmark, A.T. and Harstad, O.M. 2007. Bovine milk in human nutrition: A Review. *Lipids Health Dis.* 6:115-122.
- Khanal, R.C. and Olson, K.C. 2004. Factors affecting conjugated linoleic acid content in milk, meat and eggs: A review. *Pak. J. Nutr.* 3:82-89.
- Kreuzer, M. and Leiber, F. 2005. Milk from grass-fed cows higher in Vitamin E. *Lipids* 40:191-202.
- Lock, A.L. and Bauman, D.E. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids* 39:1197-1206.
- Liebenberg, L. 2007. Effect of nutrition on conjugated linoleic acid content of milk. MSc thesis, University of Stellenbosch, South Africa.
- McGuire, M.A. and McGuire, M.K. 1999. Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects on human health. *Proc. Amer. Soc. Anim. Sci.* 1-8.
- Metaxas, L., Muller, C.J.C., Dzama, K. and Botha, J.A. 2013. The beef production of Holstein and Fleckvieh x Holstein calves as veal or steers. *46th SASAS Congress, Bloemfontein, 24-26 June 2013.*
- Muller, C.J.C. and Botha, J.A. 2012. The growth rate of heifers as affected by crossbreeding using Fleckvieh sires. *45th SASAS Congress, East London, 9-12 July 2012.*
- Muller, C.J.C., Botha, J.A. and Potgieter, J.P. 2012. The reproduction performance of purebred Holsteins and Jerseys in comparison to Fleckvieh crossbreds. *45th SASAS Congress, East London, 9-12 July 2012.*
- Muller, L.D. and Delahoy, J.E. 1988. Conjugated linoleic acid (CLA) implications for animal production and human health.
<http://www.extension.psu.edu/animals/dairy/nutrition/nutrition-and-feeding/nutrition-and-health> [15 February 2013].
- Patrick, R., O'Quinn, J.L., Robert, D. and Michael, D.T. 2000. Conjugated linoleic acid. *Anim. Health Res. Rev.* 1:35-46.
- Peng, Y., West, G.D. and Wang, S. 2006. Consumer attitudes and acceptance of CLA Enriched dairy products. *Can. J. Agr. Econ.* 54:663-684.
- Song, M.N. and Kennelly, J.J. 2003. Biosynthesis of Conjugated Linoleic Acid and its incorporation into ruminant's products. *Asian-Aust. J. Anim. Sci.* 16(2):306-314.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Milk is a nutrient-rich food item that provides a significant portion of different dietary components essential to the diet of humans. More than 6 billion people worldwide consume milk and milk products, and it is a key element of the Western diet. Global per capita milk consumption ranges between 150Kg/capita/year in high consumption countries such as the US, and 30Kg/capita/year in medium consumption countries such as Southern Africa and Latin America (FAO, 2012).

Milk fit for human consumption is obtained from just about every domestic species of livestock utilized by man, including camels, yak, horses and even reindeer. Although there is a large variation in the composition of milk between species, it is an especially good source of calcium, magnesium, selenium, riboflavin, vitamin B and pantothenic acid. On the other hand, it is a poor source of iron and folate (FAO, 2013). Milk contributes a significant amount of lipids, lactose and proteins, to the human diet (Nantapo and Muchenje, 2013).

Milk has recently been studied for numerous additional health benefits due to presence of bioactive components such as oligosaccharides, conjugated linoleic acid, nutraceuticals and enzymes (Shahzad *et al.*, 2010). Several studies have also focused on the risks of consuming milk fat more specifically for its perceived role in increasing the risk for coronary heart disease (CHD). However, no convincing evidence exists to support this perception, and several studies have found a lack of association between milk consumption and CHD (Haug *et al.*, 2007).

There appears to be a mismatch between the evidence from long-term studies and common perceptions of harm from consuming dairy products. However, meta-analysis suggests a reduction in the incidence of vascular disease and diabetes in subjects with high dairy consumption (Elwood *et al.*, 2010). In a large population based cohort study of 1782 men surveyed 12 years apart, a high intake of dairy fat was significantly associated with lower risk of central obesity and low dairy fat intake was associated with higher risk (Holmberg and Thelin, 2013).

A comprehensive review of 16 studies showed that high-fat dairy intake was inversely associated with measures of adiposity in 11 of the studies. The evidence indicates that high-fat dairy foods does not contribute to obesity or cardio-metabolic risk, and suggests that high-

fat dairy consumption within typical dietary patterns is inversely associated with obesity risk (Kratz *et al.*, 2013). High levels of dairy consumption may also be associated with reduced blood pressure and risk of stroke (Massey, 2001). Milk fat contains a number of potential anti-carcinogenic components including conjugated linoleic acid, sphingomyelin, butyric acid and ether lipids (Parodi, 1997).

2.2 Composition of milk fat

On average, milk contains 33 g total lipid per litre (Jensen and Newberg, 1995). Triglycerides, also called triacylglycerols, account for about 95 % of the lipid fraction, and formed when three fatty acids (FA's) of different lengths combine with a glycerol molecule. The FA's differ in length (4-24 C-atoms) and degree of saturation (Jensen, 2002). The other milk lipids are diacylglycerol (about 2% of the lipid fraction), cholesterol (less than 0.5 %), phospholipids (about 1%), and free fatty acids (FFA) accounting for less than 0.5% of total milk lipids (Haug *et al.*, 2007).

Milk contains three main types of FA's. Saturated fatty acids (SFA's) occur when every carbon atom in the FA chain is linked by single bonds and only attached to hydrogen atoms. Unsaturated fatty acids contain one (Mono-unsaturated) or more double bonds (Poly-unsaturated) between two carbons on the chain. High levels of saturated fat in the diet have long been associated with a risk of CHD (Clancy, 2006). Mono-unsaturated fatty acids (MUFA's) and poly-unsaturated fatty acids (PUFA's) are considered "good fats" as they play an important role in lowering cholesterol levels and reducing the risk of heart diseases (Qureshi *et al.*, 2012).

Essential FA's (EFA's) are PUFA's which cannot be synthesized by the animal and therefore, have to be provided through the diet (Stark *et al.*, 2008). According to Simopoulos (2005) there are two so-called parent family essential FA's: Alpha-linolenic acid (ALA) which is an omega-3 FA and linoleic acid (LA) which is an omega-6 fatty acid. The family types of these FA's are determined by the final double bond being either at the omega-3 or omega-6 position counting from the omega or methyl terminal end of the carbon chain (Clancy, 2006). They have widely been studied because they are both essential FA's and are beneficial to human health (Clancy, 2006). Omega-3 FAs have the potential to prevent diseases such as cardiovascular and neurological disorders, and cancer (Sprecher, 2000; Simopoulos, 2005).

Conjugated linoleic acids (CLA) are a family of at least 28 isomers of LA found mostly in the meat and dairy products derived from ruminants. They can be either *cis*- or *trans*-fats and the double bonds of CLA's are conjugated and separated by a single bond between them (Banni,

2002). In dairy products *cis*-9, *trans*-11 18:2 CLA (*c9,t11* CLA) is the most significant isomer and accounts for about 75-90% of the total CLA in milk (Bauman *et al.*, 1999).

2.3 Health benefits of CLA

This FA is considered to be a functional food for humans, and it is associated with biological and physiological health benefits, such as anti-atherogenic, anti-diabetic, anti-carcinogenic and anti-adipogenic effects (Khanal and Dhiman, 2004; Bauman *et al.*, 2004; Dhiman *et al.*, 2005; Grega *et al.*, 2005; Cruz-Hernandez, 2007; Mitchell and McLeod, 2008; Yassir *et al.*, 2010; Mierlita *et al.*, 2011).

Conjugated linoleic acid also assists in the modulation of food allergic reaction, the reduction in the growth of melanoma, leukaemia, mesothelioma, and glioblastoma (Khanal and Dhiman, 2004; De la Fuente *et al.*, 2006; Tanaka *et al.*, 2011). Prostrate, ovarian and breast cancer are also modulated by it (Kelly *et al.*, 1998; Bauman *et al.*, 1999; Pariza *et al.*, 1999; Lawless *et al.*, 1999; McDonald, 2000; Khanal and Dhiman, 2004; Yassir *et al.*, 2010; Yassir *et al.*, 2012). The mechanisms whereby this takes place are at present unknown, but some theories are that CLA reduces cell proliferation and alters various components of the cell cycle inducing apoptosis (Pariza *et al.*, 1987; Belury, 2002).

Despite the immense expenditure performed on cancer research during the past three decades and outstanding progress made in this field, the death rate for cancer patients with invasive and metastatic carcinoma of the colon, breast, lung, pancreas, prostate and bladder has not decreased very much (Bauman *et al.*, 2003).

Further suggested benefits of CLA are its ability in reducing body fat, delaying onset of Type II diabetes, slowing the development of atherosclerosis, improving the mineralization of bone, and modulating the immune system (Bauman *et al.*, 2003; McDonald, 2000). A National Academy of Science report showed that CLA is the only FA that has been shown to suppress carcinogenesis in experimental animals (Chin *et al.*, 1992). The anti-cancer effect found with consumption of CLA products is the most extensively investigated of all the identified health benefits.

It is clear that prevention rather than therapy should be an important strategy for fighting cancer and the use of therapeutic food such as CLA-enriched milk can be part of a cancer prevention approach (Chin *et al.*, 1992).

2.4 Synthesis of CLA

Several studies have shown that ruminant tissues contain more CLA than non-ruminant tissues (Chin *et al.*, 1992; Gavino *et al.*, 1999; Kelsey *et al.*, 2003; Khanal and Dhiman, 2004; Dhiman *et al.*, 2005; Mitchell and Mcleod, 2008). Milk and dairy products are the richest source of CLA and derived naturally from ruminants (Bauman *et al.*, 1999; Kelsey *et al.*, 2003; Bauman *et al.*, 2004; Khanal, 2004; Clancy, 2006; Bauman and Lock, 2010; Mierlita *et al.*, 2011).

Ruminants produce CLA naturally from dietary LA and *trans*-11 18:1 FA (Bauman *et al.*, 2004). The *cis*-9, *trans*-11 (rumenic acid) isomer is the principal form of CLA (Chin *et al.*, 1992). Bacteria in the rumen are the key to the formation of CLA, which explains why CLA production is unique and found almost exclusively in food products (milk and meat) produced from ruminant animals (McDonalds, 2000). Synthesis of CLA occurs naturally in two ways, either in the rumen during ruminal biohydrogenation of FA's or in the tissue (Kelly *et al.*, 1998; Lawless *et al.*, 1999; Khanal and Dhiman, 2004; Bauman and Lock, 2010; Mierlita *et al.*, 2011).

Conjugated linoleic acid is a product of biohydrogenation and a portion is taken up by the body and incorporated into milk fat and body fat. The main CLA isomer, *c9,t11*, is synthesised by the ruminant animal itself (so-called *de novo* synthesis) while other intermediates are absorbed in the mammary glands (Kepler *et al.*, 1966; Bauman *et al.*, 1999; Larsen and Toubro, 2003; Collomb *et al.*, 2006).

Conjugated linoleic acid is first produced in the rumen from dietary LA. Additionally, dietary LA and ALA are converted to *trans*-11 18:1 (vaccenic acid, VA), which is transported to the mammary gland, where it is enzymatically converted to CLA by $\Delta 9$ desaturase enzyme activity. Conjugated linoleic acid is then transported into the milk from the udder (Bauman *et al.*, 1999; McDonald, 2000; Song and Kennelly, 2003; Bauman *et al.*, 2004; Khanal, 2004).

2.5 Factors influencing milk CLA content

Recent studies have shown that cattle breed and diet are important determinants of the CLA content of milk (Song and Kennelly, 2003; Griinari *et al.*, 2000; Mierlita *et al.*, 2011). Conjugated linoleic acid content in milk fat is affected by a number of other factors, such as forage to concentrate ratio, level of intake and the intake of PUFA's, especially plant oils that are high in LA (Kelly *et al.*, 1998). Other factors such as the stage of lactation and seasonal variation of feed also play a role in influencing the CLA milk content of dairy cows (Lawless *et al.*, 1999). Milk produced from cows fed low grain rations or pastures, contained higher levels of CLA (Schroeder *et al.*, 2003; Berry, 2009).

Testing individual cows, Elgersma *et al.* (2006) showed that, even if the patterns in reaction to the diet changes were similar, the concentration of CLA differed between cows. This indicates a genetic effect. Variation may also be due to factors that are associated with rumen fermentation, because CLA originates from the incomplete biohydrogenation of unsaturated FA's in the rumen (Kelly *et al.*, 1998). Other specific factors that may cause this variation have not been investigated extensively; although, it remains clear that diet and breed are of major importance (Kelly *et al.*, 1998; Kelsey *et al.*, 2003; Mitchell and Mcleod, 2008).

Research conducted in Canada and North America, on different dairy breeds, have shown that Fleckvieh crossbred cows, i.e. Fleckvieh x Holstein (FxH) and Fleckvieh x Jersey (FxJ), have higher concentrations of CLA in their milk than pure-bred Holstein and Jersey cows on similar diets and management programs (Berry, 2009). Worldwide, farmers have considered crossbreeding as an alternative goal to increase efficiency of milk production and to improve milk composition, as well as fertility, health and survival of dairy breeds (Caraviello, 2004). Traditionally, dairy farmers have continued breeding with pure breeds; however, the interest in crossbreeding dairy cattle is increasing because of a demand from commercial dairy farmers.

Jersey cows are a small breed of dairy cattle and make up a significant portion of dairy cattle in South Africa. Originally bred in the Channel Islands of Jersey, the breed is popular for its milk which is higher in fat than other breeds (Samkova *et al.*, 2012). The breed is also popular for its lower maintenance costs, because of its lower bodyweight, as well as its genial temperament. Holsteins on the other hand are known for their outstanding volume of milk production and are the most predominant dairy breed in South Africa (Grega *et al.*, 2005), although their milk is less creamy than milk from the Jersey (White *et al.*, 2001; Samkova *et al.*, 2012).

The Fleckvieh breed (a Simmental-derived breed) from Bavaria in Germany, is a dual-purpose breed with good milk and beef production characteristics (Grogan *et al.*, 2005). It is suited to crossbreeding with dairy cows as well as beef breeds, resulting in increased performance due to heterosis. Recent studies have shown that crossing Jersey and Holstein with Fleckvieh increases the beef production of these two dairy breeds, while not affecting the milk yields of crossbred females negatively (Muller and Botha, 2008). According to Muller *et al.* (2006) FxH cows not only have higher lifetime production and reproductive performance in comparison to Holstein cows, but may also produce milk with higher CLA

levels than other dairy breeds. In one study, Fleckvieh dairy cows produced 14% more omega-3 and -6 FA's from pastures than Holsteins and Jerseys (Lock and Bauman, 2003). CLA and omega-3 content of milk increase when dairy cows are grazing pastures (Dewhurst *et al.*, 2006; De Wit and de Vries, 2008). The reasons for this increase are not fully understood and require further investigation. However, pasture contains high levels of PUFA, mainly LA, which may act as substrate to increase precursors of CLA and *trans*-11 18:1 (Kelly *et al.*, 1998; Whiting, 1999; Rego *et al.*, 2004). Dairy cows on pastures produce milk with a FA profile with higher proportions of CLA, VA and LA than non-grazing cows (Zachut *et al.*, 2010).

Dietary factors can greatly affect the composition of milk of dairy cows, especially the fat and protein components (Santos, 2002). Feeding adequate LA, as a dietary constituent, provides ruminal substrate for optimal growth of bacteria producing LA isomerase, and maximal CLA output (Parodi, 1999; Liebenberg, 2007). Diet seems to have the largest effect on CLA content of milk with results indicating that cows on green pastures may produce up to 500% higher CLA content in milk in comparison to total mixed rations (Kelly *et al.*, 1998; Dhiman *et al.*, 1999; Kay *et al.*, 2004; De Wit and de Vries, 2008; Mitchell and Mcleod, 2008).

Three dietary sources provide substrates for increased CLA production, i.e. increased dietary supplements of LA in the diet, increasing dietary oil content (Parodi, 1999; Dhiman *et al.*, 1999) and increased forage to concentrate ratio resulting in a variation of the rumen population of bacteria.

Cows on lush spring pasture will have a milk fat content of CLA that is 2- to 3-times greater than corn-based TMR diet (Mitchell and Mcleod, 2008). There are marked seasonal variations in the CLA content, with values during the summer often several times higher than during the winter (Parodi, 1999; McDonald, 2000; Grega *et al.*, 2005).

Lactation stage has been reported to be one of the important factor influencing milk production and concentration of milk FA's. Qureshi *et al.* (2011) reported that during late lactation stage milk yield decreases, but as lactation progresses, the proportion of most *de novo* FA's increases. This leads to higher CLA levels at the end of lactation stage with dairy cows on pastures than with cows on TMR (Kelly *et al.*, 1998; Dhiman *et al.*, 1999).

2.6 Recent South African studies on CLA in milk

While a number of studies have been done in Europe, little information is available in South Africa. A study at Stellenbosch University suggested that diet affects milk CLA levels and dairy cows grazing pasture have high levels on CLA and omega-3 than dairy cows on TMR

feeding system (Liebenberg, 2007). A study at the University of Free State noted a great difference in milk FA's between different dairy breeds kept in the same feeding and living conditions (Myburgh *et al.*, 2012).

2.7 Consumer perceptions

In Western societies, the consumption of milk has decreased during the last decades. This trend may partly be explained by the claimed negative health effects that have been attributed to milk and milk products (Samkova *et al.*, 2012). This criticism has arisen especially because milk fat contains a high portion of saturated FA's assumed to contribute to heart diseases, weight gain and obesity (Haug *et al.*, 2007).

Medical research has recently found the positive correlations between milk consumption and the reduction of risks such as coronary heart diseases, diabetes, obesity, atherosclerosis, strokes, as well as boosting the immune system (Bauman *et al.*, 1999; Bauman and Lock, 2006; Peng *et al.*, 2006). Positive health effects may be promoted by the content of oleic acid, CLA, omega-3 FA's, short- and medium chain FA's, vitamins, minerals and bioactive compounds (Haug *et al.*, 2007). The dairy industry has been adjusting its product to adapt to changing consumer preferences. Functional dairy products have recently been the drivers of growth in the dairy industry. Consumers are focusing more on health benefits and nutritional content of dairy products (Peng *et al.*, 2006).

Peng *et al.* (2006) reported that the dairy industry is working actively with scientists to help determine consumer acceptance of these products. CLA received a "no objection" letter from the US Food and Drug Administration (FDA) on its Generally Recognized as Safe (GRAS) status for the functional milk products that have already been launched in the market, such as yoghurt, milk, butter and cheese (US Food and Drug Administration, 2003). This has paved the way for mass marketing of CLA in the US.

There is overwhelming evidence that CLA is beneficial to health and milk can be considered a good source. Suggested health benefits include anti-atherogenic, immune modulating, anti-diabetic and cholesterol lowering effects. The overarching objective of this study is to compare the milk and feed FA composition, particularly CLA content, for cows housed in pasture and TMR feeding systems in the Western Cape.

References

- Banni, S. 2002. Conjugated linoleic acid metabolism. *Current Op. Lipidol.* 13 (3): 261–266
- Bauman, D.E., Baumgard, L.H., Corl, B.A. and Griinari, J.M. 1999. Biosynthesis of conjugated linoleic acid in ruminants. *Proc. Amer. Soc. Anim. Sci.* 1999:1-15
- Bauman, D.E., Perfield, J.W. and Lock A.L. 2004. Effect of trans fatty acids on milk fat and their impact on human health.
https://ansci.cornell.edu/bauman/cla/conference_proceedings/articles/2004_Bauman_Perfield_and_Lock_SWNC.pdf [20 July 2012].
- Bauman, D.E., Perfield, J.W., de Veth, M.J. and Lock A.L. 2003. New perspectives on lipid digestion and metabolism in ruminants.
https://ansci.cornell.edu/bauman/cla/conference_proceedings/articles/2003_cnc_bauman_et_al.pdf [25 July 2012].
- Bauman, D.E. and Lock A.L. 2010. Milk fatty acid composition: challenges and opportunities related to human health. *26th W. Buiatrics Congr.* Santiago, 14-18 November 2010.
- Bauman, D.E and Lock, A.L. 2006. Animal products and human health: Perceptions opportunities and challenges.
https://ansci.cornell.edu/bauman/cla/conference_proceedings/articles/2006_CNC_Bauman_&_Lock.pdf [20 July 2012].
- Belury, M.A. 2002. Inhibition of carcinogenesis by conjugated linoleic acid: Potential mechanism of action. *J. Nutr.* 132(10):2995-2998.
- Belury, M.A., Mahon, A. and Banni, S. 2003. The conjugated linoleic acid (CLA) isomer, t10c12- LA, is inversely associated with changes in body weight and serum leptin in subjects with type 2 diabetes mellitus. *J. Nutr.* 133(1): 257-260.
- Berry, R. 2009. Milk from grass using Fleckvieh crossbreds: A study on milk components, conjugated linoleic acid (CLA) and productivity. *Fleckvieh World 2009:* 12-17
- Caraviello, D.Z. 2004. Crossbreeding Dairy Cattle. <http://www.babcock.wisc.edu/node/508> [20 February 2012].
- Chin, S.F., Liu, W., Storkson, J.M., Ha, Y.L. and Pariza, M.W. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Compos. Anal.* 5:185-197.
- Clancy, K. 2006. Greener Pastures: How grass-fed beef and milk contribute to healthy eating. Cambridge, Massachusetts: *Union of Concerned Scientists Publications.*
- Collomb, M., Schmid, A., Sieber, R. and Wechsler, D. 2006. Conjugated linoleic acid in milk fat variation and physiological effects. *Int. Dairy J.* 16:1347-1361.
- Collomb, M., Bisig, W., Butikofer, U., Sieber, R., Bregy, M. and Etter, L. 2008. Fatty acid composition of mountain milk from Switzerland: comparison of organic and integrated farming system. *Int. Dairy J.* 18:976-982.

- Cruz-Hernandez, C., Kramer, J.K., Kennelly, J.J., Glim, D.R., Sorensen, B.M., Okine, E.K., Goonewardene, L. A. and Weselate, R.J. 2007. Evaluating the conjugated linoleic acid and *trans* 18:1 isomers in milk /fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. *Amer. Dairy Sci. Assoc.* 90:3786-3801.
- Dewhurst, R.J., Shingfield, K.J., Lee, M. R. F. and Scollan, N. D. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim. Feed Sci. Tech.* 131:168-20.
- De la Fuente, M.A., Luna, P. and Juarez, M. 2006. Chromatographic techniques to determine conjugated acid isomers. *Trends Anal. Chem.* 25:917-926.
- De Wit, J. and de Vries, A. 2008. Feed composition and strategies to improve polyunsaturated fatty acid levels in organic cow milk. *16th IFOAM Organic World Congress*. Modena, 16-20 June 2008.
- Dhiman, T.R., Anand, G.R., Satter, L.D. and Pariza, M.W. 1999. Conjugated linoleic acid content of milk from cows fed different diets. *J. Dairy Sci.* 82:2146-2156.
- Dhiman, T.R., Nam, S.H. and Ure, A.L. 2005. Factors affecting conjugated linoleic acid content in milk and meat. *Crit. Rev. Sci. Nutr.* 45:463-482.
- Elgersma, A., Tamminga, S. and Ellen, G. 2006. Modifying milk composition through forage. *Anim. Feed Sci. Tech.* 131:207-225.
- Elwood, P.C., Pickering, J.E., Givens, D.I., Gallacher, J.E. 2010. The Consumption of Milk and Dairy Foods and the Incidence of Vascular Disease and Diabetes: An Overview of the Evidence. *Lipids* 45(10): 925-939.
- FAO 2013. Milk and dairy products in human nutrition. <http://www.fao.org/docrep/018/i3396e/i3396e.pdf> [5 January 2014].
- FAO 2012. Dairy production and products. <http://www.fao.org/agriculture/dairy-gateway/milk-and-milk-products/en/#.VLLNd8mrHSo> [5 January 2014].
- Gavino, V.C., Gavin, G., Leblanc, M.J. and Tuchweber, B. 1999. An Isomeric mixture of conjugated linoleic acids but not pure *cis*-9, *trans*-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters. *J. Nutr.* 130:27-29.
- Gregga, T., Sady, M., Najgebauer, D., Domagala, J., Pustkowiak, H. and Faber, B. 2005. Factors affecting the level of conjugated linoleic acid (CLA) in milk from different cow's breeds. *Biotechnol. Anim. Husb.* 21:241-244.
- Griinari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V.V. and Bauman, D.E. 2000. Conjugated linoleic acid is synthesized endogenously in lactation dairy cows by Δ^9 -desaturase^{1,2}. *J. Nutr.* 130:2285-2291.
- Grogan, A., Wichham, B., Olori, V. and Cromie, A. 2005. Strategies of European Fleckvieh cattle breeding to optimize dual-purpose breeding particularly for beef production. *Stocarstvo.* 59(5):339-353.
- Haug, A., Hostmark, A.T. and Harstad, O.M. 2007. Bovine milk in human nutrition: A

- Review. *Lipids Health Dis.* 6:25.
- Holmberg, S. and Thelin, A. 2013. High dairy fat intake related to less central obesity: A male cohort study with 12 years' follow-up. *Scand. J. Prim. Health. Care.* 31(2): 89–94.
- Jensen, R.G. 2002. The composition of bovine milk lipids. January 1995 to December 2000: A review. *J. Dairy Sci.* 85(2):295-350.
- Jensen, R.G. and Newburg, D.S. 1995. Bovine milk lipids. In Jensen, R., G. (ed). *Handbook of milk composition.* San Diego: Academic Press: 543-575.
- Kay, J.K., Mackle, T.R., Auld, M.J., Thomson, N.A. and Bauman, D.E. 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pastures. *J. Dairy Sci.* 87(2):369-378.
- Kelly, M.L., Kolver, E.S., Bauman, D.E., Van Amburgh, M.E. and Muller, L.D. 1998. Effect of intake of pasture on concentration of Conjugated Linoleic Acid in milk of lactating dairy cows. *J. Dairy Sci.* 81(6):1630-1636.
- Kelsey, J.A., Botha, B.A., Collier, R.J. and Bauman, D.E. 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *J. Dairy Sci.* 86(8):2588-2597.
- Kepler, C.R., Hirons, P.K., McNeill, J.J. and Tove, S.B. 1966. Intermediates and products of biohydrogenation of linoleic acid by *Butyrivibrio Fibrisolvens*. *J. Biol. Chem.* 241:1350-1354.
- Khanal, R.C. 2004. Potential health benefits of conjugated linoleic acid (CLA): A review. *Asian-Aust. J. Anim.* 17:1315-1328.
- Khanal, R.C. and Dhiman, T.R. 2004. Biosynthesis of Conjugated Linoleic Acid (CLA): A review. *Pak. J. Nutr.* 3:72-81.
- Khanal, R.C. and Olson, K.C. 2004. Factors affecting conjugated linoleic acid content in milk, meat and eggs: A review. *Pak. J. Nutr.* 3:82-89.
- Kratz, M.L., Baars, T. and Guyenet, S. 2013. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. *Eur. J. Nutr.* 52(1):1-24.
- Kreuzer, M. and Leiber, F. 2005. Milk from Grass-Fed Cows Higher in Vitamin E. *Lipids.* 40:191-202.
- Larsen, T. M. and Toubro, S. 2003. Efficacy and safety for dietary supplements containing conjugated linoleic acid (CLA) for the treatment of obesity-evidence from animal and human studies. *J. Lipid Res.* 44:2234-2241.
- Lawless, F., Stanton, C., Escop, P.L., Devery, R., Dillon, P. and Murphy, J.J. 1999 Influence of breed on bovine milk *Cis*-9, *trans*-11-conjugated linoleic acid content. *Livest. Prod. Sci.* 62: 43-49.
- Liebenberg, L. 2007. Effect of nutrition on conjugated linoleic acid content of milk. MSc thesis, University of Stellenbosch, South Africa.

- Lock, A.L. and Bauman, D.E. 2003. *Dairy products and milk fatty acids as functional food components*.
https://cncps.cornell.edu/bauman/cla/conference_proceedings/articles/2003_cnc_lock_bauman.pdf [20 July 2003].
- Massey, L.K. 2001. Dairy food consumption, blood pressure and stroke. *J Nutr.* 131(7):1875-1878.
- McDonald, H.B. 2000. Conjugated Linoleic Acid and Disease Prevention: A review of Current Knowledge. *J. Amer. Col. Nutr.* 19:111-118.
- Mitchell, L. and Mcleod, S. 2008. Conjugated linoleic acid and atherosclerosis: studies in animal models. *Biochem. Cell Biol.* 86(4):293-301.
- Mierlita, D., Daraban, S.T. and Lup F. 2011. Effects of breed on milk fatty acid profile in dairy ewes, with particular reference to *cis*-9, *trans*-11 conjugated linoleic acid. *S. Afr. J. Anim. Sci.* 41:224-231.
- Muller, C.J.C., Botha, J.A., Meshelle, G., Van Pittius., Grupp, T., Swart, M.J. and Neser, F.W.G. 2006. Preliminary results on the growth performance of Holstein and Fleckvieh x Holstein bull and heifer calves. *J. Elsenburg.* 3:1-12.
- Muller, C. J. C. & Botha, J. A. 2008. Preliminary results on crossbreeding Jerseys with Fleckvieh sires. *J. Elsenburg.* 1: 2-3.
- Myburgh, J., Osthoff, G., Hugo, A., De Wit, M., Nel, K. and Fourie, D. 2012. Comparison of the milk composition of free-ranging indigenous African cattle breeds. *S. Afr. J. Anim. Sci.* 42:1-14.
- Nantapo, C.T.W. and Muchenje, V. 2013. Winter and spring variation in daily milk yield and mineral composition of Jersey, Friesian cows and their crosses under a pasture based dairy system. *S. Afr. J. Anim. Sci.* 43:17-2.
- Pariza, M.W., Park, Y. and Cook, M.E. 1987. Mechanism of action of Conjugated linoleic acid: Evidence and speculation. *Exp. Biol. Med.* 223(1): 8-13
- Pariza, M.W., Park, Y. and Cook, M.E. 1999. Conjugated linoleic acid and the controls of cancer and obesity. *Toxicol. Sci.* 52(2):107-110
- Parodi, P.W. 1997. Cow's milk fat components as potential anticarcinogenic agents: *Crit. Rev. J. Nutr.* 127:1055-1060.
- Parodi, P.W. 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *J. Dairy Sci.* 82(6): 1339-1349.
- Peng, Y., West, G.D. and Wang, S. 2006. Consumer attitudes and acceptance of CLA enriched dairy products. *Can. J. Agr. Econ.* 54:663-684.
- Qureshi, M.S., Jan, S., Mushtaq, A., Rahman, I.U., Jan, M. and Ikramullah. 2012. Effect of age on milk fatty acids in dairy buffaloes. *J. Anim. Plant Sci.* 22(2):108-112.
- Rego, O.A., Portugaal, P.V., Sousa, M.B., Rosa, H.J.D., Vouzela, C.M, Borba, A.E.S. and Bessa, R.J.B. 2004. Effect of diet on the fatty acid pattern of milk from dairy cows.

- Anim. Res.* 53:213-220.
- Samkova, E., Spicka, J., Pesek, M., Pelikanova, T. and Hanus, O. 2012. Animal factors affecting fatty acid composition of cow milk fat: A review. *S.Afr. J. Anim. Sci.* 42:83-99.
- Santos, J.E.P. 2002. Feeding for milk composition. *Proc. 6th Int. Congr. Bov. Med.* 2002: 163-172.
- Schroeder, G.F., Delahoy, J.E., Vidaurreta, I., Bargo, F., Gagliostro, G.A. and Muller, L.D. 2003. Milk fatty acid composition of cows fed a total mixed ration or pasture plus concentrates replacing corn with fat. *J. Dairy Sci.* 86(10):3237-3248.
- Shahzad, M.A., Mukhtar, N and Sarwar, M. 2010. Nutrient modelling to fabricate dairy milk constituents: Let milk serve more than a food item.
<http://waset.org/publications/11841/nutrient-modelling-to-fabricate-dairy-milk-constituents-let-milk-serve-more-than-a-fooditem> [25 July 2012].
- Simopoulos, A.P. 2005. The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and other chronic diseases: A review.
http://www.cmcgc.com/media/handouts/320121/M10_Artemis_Simopoulos.pdf
[15 January 2012]
- Song, M.N. and Kennelly, J.J. 2003. Biosynthesis of Conjugated Linoleic Acid and its incorporation into ruminant's products. *Asian-Aust. J. Anim. Sci.* 16(2):306-314.
- Sprecher, H., 2000. Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochim. Biophys. Acta.* 1486:219-231.
- Stark, A.H., Crawford, M.A. and Reifen, R. 2008. Update on α -linolenic acid. *Nutr. Rev.* 66: 326-332.
- Tanaka, T., Hosokawa, M., Yasui, Y., Ishigamori, R. and Miyashita, K. 2011. Cancer chemopreventive ability of Conjugated Linoleic Acids. *Int. J. Mol. Sci.* 12:7495-7509.
- US Food and Drug Administration. 2003. Question and answers about trans fat nutrition labeling. <http://www.cfsan.fda.gov/~dms/gatrans2.html>. [12 January 2012]
- White, S.L., Bertrand, J.A., Wade, M.R., Washburn, S.P., Green, J.T Jr. and Jenkins T.C. 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. *J. Dairy Sci.* 84:2295-2301.
- Whiting, C.M. 1999. Thesis. Transfer of alpha-linolenic acid from forage diets to milk in Holstein cows. *The University of Guelph, Canada.*
- Yassir, M.A., Arifah, A.D., Yaakub, H., Zuraini, A. and Zakaria, Z. A. 2010. Comparison of Conjugated Linoleic Acid and other fatty acid content of milk: Fat of Mafriwal and Jersey cows. *J. Anim. Vet. Adv.* 9:1318-1323.
- Yassir, M.A., Arifah, A.K., Yaakub, H., Zuraini, A., Zakaria, Z.A. and Somchit, M.N. 2012. Effect of breed on *cis*-9, *trans*-11 and *trans*-10, *cis*-12 Conjugated Linoleic Acids concentrations in milk fat of dairy cattle and the relationship of these CLA

with other unsaturated C18 fatty acids. *Pertanika J. Trop. Agric. Sci.* 35:165-172.

Zachut, M., Arieli, A., Lehrer, H., Livshitz, L., Yakoby, S. and Moallem, U. 2010. Effects of increased supplementation of n-3 fatty acids to transition dairy cows on performance and fatty profile in plasma, adipose tissue, and milk fat. *J. Dairy Sci.* 93:5877-5889.

CHAPTER 3

MILK FATTY ACID COMPOSITION AND CLA CONTENT OF JERSEY AND FLECKVIEH X JERSEY COWS IN A PASTURE-BASED FEEDING SYSTEM

3.1 Abstract

The aim of the study was to analyze the effect of crossbreeding on the milk FA content and composition of 38 Jersey (J) and 43 Fleckvieh × Jersey (FxJ) cows in a pasture-based feeding system. The study was conducted at the Elsenburg Research Farm of the Western Cape Department of Agriculture.

All cows were fed the same diet consisting of kikuyu-ryegrass pasture in a rotational grazing system supplemented with a standard concentrate mixture of seven kilograms of pellets per cow per day. Milk samples were collected at 35-day intervals and this was repeated seven times during the lactation period of each cow. The variables that were measured during each milk test were the milk fat percentage, CLA isomers, total SFA's, total PUFA's, total MUFA's, essential FA's content as well as the ratio of LA to ALA and Omega 3 FA's to Omega 6 FA's. Concentration levels of FA were determined by gas liquid chromatography and 36 different FA's were detected in the milk.

Statistical analysis was carried out using two-way repeated measures of analysis of variance (ANOVA) and a mixed model procedure. The model included the treatment effect (breed), time effect of observation (test) and interaction effect of treatment and time as fixed effects, whereas animal influence within treatments was specified as a random effect. The measured variables obtained at each milk test (i.e. 1, 2, 3, 4, 5, 7, 8) during the trial were considered as repeated observations of a particular block.

FxJ cows produced milk with higher levels of *c9,t11* CLA isomer than J cows (0.584 ± 0.023 vs. 0.678 ± 0.018 g FA/100g fat). FxJ cows also produced significantly ($P < 0.05$) more total CLA content than J cows (0.630 ± 0.024 and 0.728 ± 0.018 g FA/100g fat). The CLA content of the milk increased from early to late lactation for both breeds. CLA content ranged from 0.565 ± 0.040 to 0.847 ± 0.033 g FA/100 g fat in FxJ cows over the lactation period. It was concluded that FxJ crossbred cows could be more productive on average than purebred Jersey cows with respect to CLA content most probably owing to heterosis.

3.2 Introduction

For many years milk fat has been regarded as unhealthy due to research finding negative effects on chronic diseases like heart diseases and other forms of cancer caused by

saturated fatty acids (SFA's) to humans (Salter, 2005). For this reason, fat-free or low-fat milk (0 or 2% fat respectively) has become a popular choice among many consumers. Contrary to these negative claims, recent research has shown milk FA's, particularly CLA to have beneficial effects on the health status of people such as having anti-carcinogenic, anti-diabetic and anti-inflammatory benefits (Gavino *et al.*, 1999; Khanal and Dhiman, 2004).

Several studies have contributed in providing insight about the nutritional value and characteristics of milk, indicating that it can be considered a multifunctional food product due to its known beneficial health components. Milk provides proteins, lipids, vitamins and minerals (Steinmetz *et al.*, 1994; Atti *et al.*, 2006; Collomb *et al.*, 2008; Descalzo *et al.*, 2012).

The Jersey breed is popular among pasture-based dairy farmers, especially in the Southern Cape area of South Africa (Muller and Botha, 2008). There has been an increasing interest in the crossbreeding of dairy cows in South Africa resulting in Jersey cows being crossed with the dual-purpose Fleckvieh breed (Muller and Botha, 2008). Fleckvieh animals are internationally recognized for important qualities (Grogan *et al.*, 2005) and crossbreeding improving the lifetime production (i.e. beef and milk yield) and reproduction (i.e. fertility, calving) performance (Bauman *et al.*, 1999; Muller *et al.*, 2006; Bauman *et al.*, 2004). In several studies on CLA, it has been recorded that Fleckvieh cows produce milk with higher CLA levels than any other breeds. Fleckvieh dairy cows also produce 14% more omega-3 and omega-6 FA on grazing pastures than Jersey cows (Lock and Bauman, 2004).

Lock and Bauman (2004) have shown that the milk fat from Jersey crossbred cows have higher CLA levels in comparison to purebred Jersey cows on similar diets and management programs. Breed appears to have an effect on total milk CLA content, while CLA concentration varies widely among herds (Kelly *et al.*, 1998; Patrick *et al.*, 2000). This variation may be associated with factors that are linked to rumen fermentation because CLA originates from the incomplete biohydrogenation of unsaturated FA's in the rumen (Kelly *et al.*, 1998).

Other factors that affect the milk CLA level besides breed are diet and lactation stage (Lawless *et al.*, 1999). Samkova *et al.* (2012) reported lactation stage as one of the important factors influencing milk production and concentration of milk and its FA's. Qureshi *et al.* (2011) stated that the CLA content in milk increases as the lactation stage progresses, i.e. from early to late lactation. The effect of diet on CLA includes forage to concentrate ratio, level of intake and the intake of PUFA's, especially plant oils that are high in LA (Kelly *et al.*, 1998; Collomb *et al.*, 2008).

Mitchell and McLeod (2008) reported that the amount of CLA in cow's milk is determined mostly by their diet. Healthy FA's increase when cows are on pasture or when diets are supplemented with extracted soy beans and cottonseed (Collomb *et al.*, 2006). The CLA found in dairy products is a direct reflection of the diet of animals. Evidence suggests CLA increases linearly when breeds are pasture fed and decreases when pasture intake declines (Kelly, 2001; Smith *et al.*, 1993).

Compared to cows fed on a TMR-based system, cows on cultivated pastures produce milk with a higher proportion of PUFA's such as omega-3, omega-6 and CLA (Atti *et al.*, 2006; Dewhurst *et al.*, 2006). Pastures in the Western Cape consist predominantly of kikuyu (*Pennisetum clandestinum*) with varying levels of perennial ryegrass (*Lolium perenne*) or clover (*Trifolium*) species (Botha *et al.*, 2008). Kikuyu is utilised by many farmers in pasture-based feeding systems (Quinlan *et al.*, 1975), and it is one of the most important summer pastures in South Africa (Miles *et al.*, 1995). Kikuyu grass is considered a highly productive grass during summer and autumn and is capable of sustaining a high stocking rate (Bell *et al.*, 2011; Kaizer, *et al.*, 1993). In winter the growth is relatively low (Botha *et al.*, 2006 and Botha *et al.*, 2008), which means it contributes very little towards dairy cow nutrition. Pasture production can be increased by over-sowing winter-spring growing species such as annual or perennial ryegrass and white clover with kikuyu to increase productivity (Kaizer *et al.*, 1993).

The aim of the study was therefore:

1. to compare the essential FA's content of Jersey (J) and Fleckvieh x Jersey (F×J) cows in a pasture-based feeding system over the lactation period, and
2. to provide a reference for the average CLA content of the milk of Jersey (J) and Fleckvieh x Jersey (F×J) cows under similar pasture feeding systems in the Western Cape Province of South Africa.

3.3 Methods and Materials

3.3.1 Site description

The experimental location of the study was the Elsenburg Research Farm of the Western Cape Department of Agriculture. Elsenburg is situated approximately 50km east of Cape Town at an altitude of 177m, longitude 18° 50' and latitude 33° 51' and is situated in the winter rainfall region of South Africa. The area is characterized by a Mediterranean climate with cool wet winters and long dry summers. The long-term average annual rainfall is 650mm.

3.3.2 Experimental animals

Dairy cows used in the study form part of a long-term project by the Western Cape Department of Agriculture to investigate the production characteristics of Fleckvieh crossbred dairy cows and steers. Fleckvieh bull semen from Bavarian Fleckvieh Genetics (BFG) had previously been used to breed crossbred cows from purebred Holstein and Jersey breeds at the Elsenburg experimental farm. The herd consisted of 280 Holstein (H), 350 Jersey (J), 180 Fleckvieh x Holstein (F×H) and 210 Fleckvieh x Jersey (F×J) cows at the initiation of the study. The experimental herd was divided into groups based on breed and cows were randomly and independently selected from each group to form part of the current study. A total of 38 pure Jersey (J) cows and 43 Fleckvieh x Jersey (F×J) experimental cows were selected for this study. Data was collected as these cows calved over a period from November 2011 to December 2013.

3.3.3 Feed Management

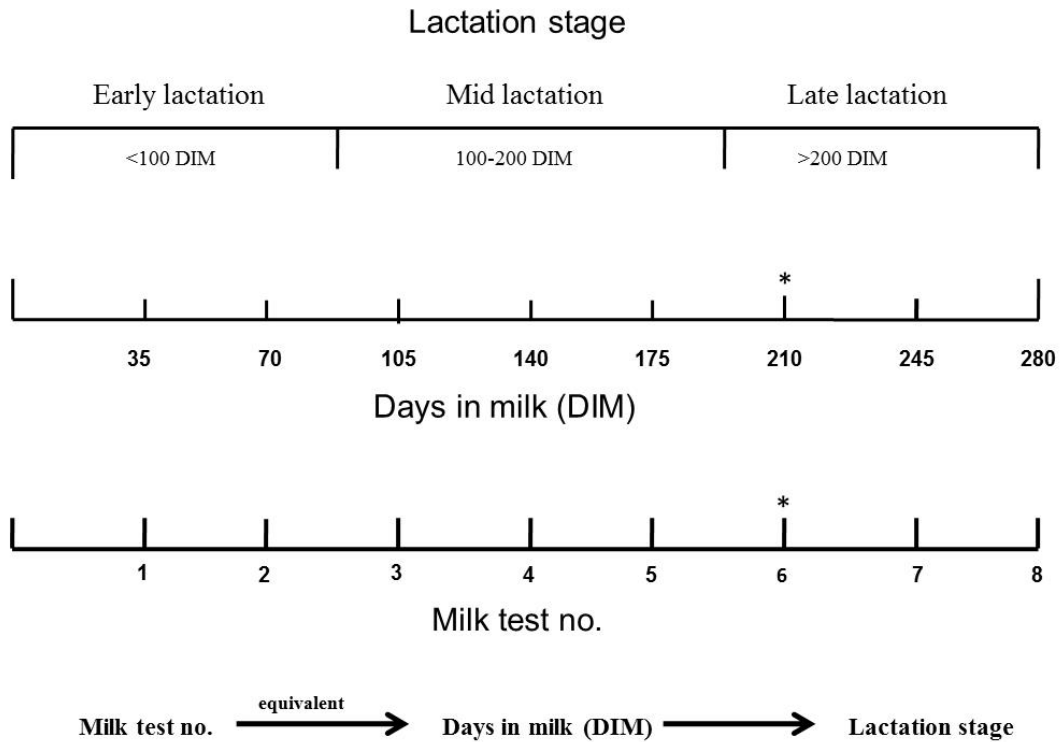
During the study, cows were rotated on pastures and supplemented with a pelleted concentrate mixture twice a day after milking (in the morning and in the afternoon). Each cow received 7 kg of pellets per day (3.5 kg in the morning and 3.5 kg in the afternoon) of a commercial concentrate meal in a post-parlor feeding facility. Each cow consumed approximately 10 kg dry matter (DM) of pasture per day. Pasture composition on the farm was similar in all paddocks utilized for the study, and consisted of commercial kikuyu and perennial ryegrass mixture. This pasture mix represents a popular combination planted by dairy farmers in the Western Cape containing sufficient nutrients to sustain milk production (Fulkerson, 2007; Mears, 1970). Extra lucerne and oat-hay was provided as additional roughage during winter when pasture availability was low.

3.3.4 Sample Collection

Samples of milk, pasture and feed were collected for FA analysis. Milk samples were collected at the evening and the next mornings' milking and pooled for each cow. A total of seven milk FA tests were completed per cow over the course of lactation. The 100 mL milk samples were collected every 35 days. Samples were immediately cooled to 7 °C and then frozen at -20 °C until laboratory analysis by gas liquid chromatography. A total of n=265 (J) and n=302 (F×J) were used to compare breeds for FA content.

3.3.5 Data collection

Milk recording was done according to the standard milk recording procedures. The FA tests were denoted 1,2,3,4,5,7,8, while test 6 (210 DIM) was not included in the study. The rationale of repeating the tests was to determine the effect of lactation stage on milk FA composition and content.



* No samples were collected at milk test 6 (210 DIM)

Figure 3.1. Time frame of milk sampling over the lactation stage

Samples were collected during seven milk-recording events per lactation, viz the first to fifth and the seventh and eighth milk recording tests. The milk sampling procedure is represented in Figure 3.1. Tests corresponded with the three stages of lactation: Early (first 100DIM), mid (100-200DIM) and late (200-280DIM) lactation. Standard milk recording procedures were followed, i.e. the milk yield of cows was recorded at the evening and following morning's milking session and total yield weighed accordingly. Only milk fat data was recorded in the study. Milk tests 1-2 correspond within the early-lactation stage, milk tests 3-5 correspond with mid-lactation and milk tests 7-8 correspond with late-lactation.

Pasture sampling was based on the methods used by Atti *et al.* (2006) and Mel'uchova *et al.* (2008). A pasture sample consisted of herbage collected from five area sites representing the grazing area of the cows. A 50 cm×50 cm quadrat of pasture was cut at six cm above the ground to obtain the herbage at each site. Pasture samples were collected monthly over the two-year period in order to determine seasonal variations to pasture quality.

Pelleted concentrate feed was sampled according to the method followed by Schroeder *et al.* (2005). Monthly 500g samples were collected and analyzed once per season or every three months.

3.3.6 Milk fat analysis

The milk FA composition and CLA content of milk samples was analyzed by gas chromatography (GC) at the Program on Mycotoxins and Experimental Carcinogenesis (PROMEC Unit) of the Medical Research Council at Tygerberg, Western Cape, South Africa.

Solvents and chemicals were obtained from Sigma-Aldrich: hexane, iso-propanol, chloroform, methanol, diethyl ether, butylated hydroxytoluene (BHT), anhydrous sodium sulphate, (Na_2SO_4), sodium methoxide (0.5M), oxalic acid, methyl acetate ($\text{CH}_3\text{CO}_2\text{CH}_3$). Triacylglyceride (TAG) 17:0 was obtained from Avanti Polar Lipids (USA). The FA standard mixture (4:0-24:1) for chromatogram peak identification was purchased from Nu-Chek Prep (USA).

The following solvents and solutions were used for milk FA extraction and methylation: HIP solvent (Hexane: Isopropanol; 3/2, vol/vol) with 0.01% BHT as antioxidant, internal standard $\text{C}_{17:0}$ TAG (300 mg TAG/2 mL chloroform), saturated anhydrous sodium sulphate solution (1 g anhydrous salt in 15 mL distilled H_2O), oxalic acid in diethyl ether (1g oxalic acid in 30 mL diethyl ether) and transmethylation reagent (methanol/18 M sulphuric acid; 95:5, v/v) for grass FA methylation and 0.5M sodium methoxide for milk FA methylation. All solvents were HPLC grade or double distilled.

All glassware were soaked for a minimum two hours (or overnight) in a 2-5% phospholipid-free soap (Contrad, Merck) and rinsed in warm tap water (5x), distilled water (5x) and oven dried at 100 °C for 24 hours. Prior to use, all glassware was rinsed with HPLC-grade methanol and dried in a fume hood.

Milk samples were analyzed for fat percentage with the aid of a Milk-O Scan 133B at the ANPI dairy lab of the Milk Recording Testing Scheme of the Agricultural Research Council at Elsenburg. For fatty acid extraction, separation of milk fat was based on the method described by Chouinard *et al.* (1999). Milk (~500 mL) stored at -20 °C was thawed at 36-38 °C and homogenized using a polytron. An aliquot of milk (~40 mL) was measured into a 50 mL Falcon tube and centrifuged at 4000 g for 30 minutes at 8° C. The top fat layer (fat cake) was removed with a sterile/clean plastic spatula and placed in a Falcon tube (20 mL) then flushed with N_2 gas and stored at -20 °C for further extraction.

The lipid from the isolated fat cake was extracted based on the method of Hara and Radin (1978). A 100 mg fat cake was removed and placed into a Teflon-capped glass tube and HIP solvent (5.5 mL) was added together with the internal standard 17:0 TAG (100 µl/15 mg TAG). The tube was flushed with N₂ gas, closed, vortexed for 2 minutes and the mixture was filtered, under vacuum, through a sinterglass funnel lined with glass microfibre filter paper and rinsed with 3 x 0.6 mL HIP solvent. Saturated sodium sulphate solution (3.5 mL) was added and the sample vortexed for 1 minute and centrifuged at 500 rpm for 12 minutes at 8 °C (Sorvall model centrifuge). The top hexane-rich layer of the biphasic solution, containing lipid extract, was removed with a glass Pasteur pipette into a Teflon-capped glass tube, flushed with N₂ gas and stored at -20 °C

Transmethylation of FA's in the extracted lipids was done according to Christie (1982) and Christie (1993) with modifications by Chouinard *et al.* (1999). An aliquot (2 mL) of the hexane lipid extract was removed and evaporated, until dry, under N₂ gas, in pre-weighed Teflon-capped glass tubes, and the dried lipid weight recorded. One mL of hexane and 40 µL methyl acetate were added and the mixture vortexed. Methylation reagent (40 µl, 0.5M sodium methoxide) was added, samples vortexed, flushed with N₂ gas and tubes tightly closed with a Teflon cap. Samples were allowed to react for 15 minutes at 50 °C, and thereafter cooled to room temperature. The methylation reaction was terminated by adding 60 µL of oxalic acid/diethyl ether solution and vortexed after which the samples were centrifuged at 1000 rpm for 12 minutes. A 1 µL aliquot, containing FA methyl esters (FAMES), was removed for GC analyses.

3.3.7 Pasture analysis

Feed samples were stored at -20 °C at the PROMEC Unit until further analysis. A portion of the pellets was removed from each sample collection bag and finely ground. One gram of feed was measured into a Petri dish covered with a filter paper and dried in a microwave oven until no further change in weight was recorded. Samples were cooled in a glass desiccator in between heating intervals. Thereafter, the dried samples were ground fine in liquid nitrogen for lipid extraction.

To prevent plant lipase activation and hydrolyzation of phospholipid glycolipids, pasture lipids were extracted with isopropanol based on a modified method by Nichols (1963) and Hara and Radin (1978). An accurately weighed sample (50 mg) of finely ground sample was transferred into a Teflon-capped glass tube. Six mL of hexane (12 volumes) and 4 mL (8 volumes) of isopropanol were added after which the mixture was vortexed for one minute and centrifuged for 8 minutes at 500 rpm using a Sorvall RC-3B centrifuge. The lipid extract was transferred to a new tube and the sample re-extracted with 3 mL HIP (7:2 hexane: iso-

propanol with 0.01% BHT). Two mL saturated sodium sulphate solution were added to the extract, vortexed and centrifuged for 12 minutes at 1000 rpm using a Sorvall RC-3B centrifuge. The top sodium sulphate phase was discarded and the bottom solvent lipid phase was flushed with N₂ and stored at 4 °C.

Sample lipid extracts were evaporated under N₂ gas until dry, re-dissolved in 1 mL methanol/18 M sulphuric acid (95:5, v/v) and transmethylated for 2 hours at 70 °C as described by Tichelaar *et al.* (1989) and Smuts *et al.* (1994). Thereafter, samples were cooled to room temperature and 2 mL hexane was added together with 1 mL distilled H₂O and vortexed. After phase separation, the top hexane layer containing the FAMEs was removed for analysis on a Varian 3300 Gas Chromatograph. Lipid extraction and FA transmethylation of the concentrate feed was carried out in the same way.

3.3.8 GC conditions for FAME analyses of milk, pasture and pellets

Milk FA was analyzed using the 120 m long GC capillary column. This column was selected due to superior resolution capacity and because milk is a complex sample with many *cis* and *trans* FA's. Pasture and feed concentrate FA content was analyzed using the 30 m short GC column. Pasture and feed FA's are not as complex to separate as milk FA's and therefore do not need the large separation area of the 120 m long column (see Appendix B for comparison of columns). The GC conditions and column specifications used to analyze milk and pasture samples are presented in Table 3.1 and Table 3.2.

Table 3.1: GC conditions

GC conditions	Milk (120m column)	Pasture (30m column)
Initial oven temp:	80°C	140 °C
Initial hold time:	8 min	1.5min
<u>Oven Program 1</u>		
Temp. ramp:	9.0°C/ min	6.0°C/ min
Oven holding temp:	175°C	200°C
Oven holding time:	52 min.	2 min.
<u>Oven Program 2</u>		
Temp. ramp:	9.0°C/ min	6.0°C/ min
Oven holding temp:	225°C	220°C
Oven holding time:	18 min.	5 min
Total run time:	94 min	22 min
Carrier gas:	Hydrogen	Hydrogen
Make-up gas:	Nitrogen	Nitrogen
FID Detector temp:	280 °C	260 °C
Injector/Inlet temp:	240 °C	240 °C
Injection mode:	Split 80:1	Split 20:1
Gas flow rates:	Hydrogen = 25 mL/min Oxygen = 250 mL/min Nitrogen = 25 mL/min Carrier/column flow = 2 mL/min	Hydrogen = 25 mL/min Oxygen = 250 mL/min Nitrogen = 25 mL/min Carrier/column flow = 2 mL/min

Table 3.2: GC column specifications

Column specifications	Milk (120m column)	Pasture (30m column)
Column	BPX 70	BPX 70
Column length	120m	30m
Internal diameter (ID)	0.25 mm	0.32 mm
Film thickness	0.25 μ m	0.25 μ m
Film/Stationary phase	70% cyanopropyl- polysilphenylene siloxane	70% cyanopropyl- polysilphenylene siloxane
Catalogue no.	054224 (SGE, USA)	054616 (SGE, USA)

3.4 Statistical Analysis

Data from the study was subjected to a two-way repeated measures of analysis of variance (ANOVA) using PROC MIXED Model of SAS Enterprise Guide 5.1 (SAS Institute Inc., 2012). The model included the treatment effect (breed), time effect of observation (test) and interaction effect of breed and test as fixed effects, whereas animal influence within treatments were specified as a random effect. The measured variables obtained at each test (i.e. every five week milk test, $n = 7$) during the lactation were considered as repeated observations of a particular Block.

The model was defined as follows:

$$Y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_{(ij)k} + \epsilon_{ijk} \text{ where}$$

μ = overall mean;

α_i = i th level of treatment factor (main effect, breed);

β_j = j th level of time factor (main effect, time);

$(\alpha\beta)_{ij}$ = interaction between level i of treatment and j of time (interaction effect);

$\delta_{(ij)k}$ = effect of the k th block effect in the i th treatment (variable effect);

ϵ_{ijk} = i, j, k th error term.

The assumptions were described as $\sum_i \alpha_i = 0$, $\sum_j \beta_j = 0$ and $\sum_i (\alpha\beta)_{ij} = \sum_j (\alpha\beta)_{ij} = 0$ with $\delta_{(ij)k} \sim N(0, \sigma^2)$ varying independently of ϵ_{ijk} . Differences between treatments were obtained using the pair wise comparison of Bonferroni t-test. Significance was declared at $P < 0.05$.

3.5 Results and Discussion

The average FA content of the pasture and pellets across seasons are presented in Table 3.3. The total SFA's decreased in season 3 and 4, while the same trend was observed for

the Total MUFA's, mainly due to decreased 18:1n-9 content. The pasture PUFA content, comprising of the omega-3 and -6 FA families, was significantly higher than both SFA's and MUFA's due to the high 18:3n-3 level. This resulted in a similar increasing trend for the PUFA's and Total omega-3 in season 3 and 4. Pasture is known to have a higher content of 18:3n-3 (omega-3 FA's), than 18:2n-6. The total omega-6 level was approximately 4-5 times lower than the total omega-3's and comprised mainly of 18:2n-6. A decrease in the level of 18:2n-6 at season 3 and 4 did not reflect strongly in the total omega-6 content. The N-6/N-3 and LA/ALA ratio can be considered to be low, due to the higher omega-3 FA content, in particular 18:3n-3. The most abundant FA in the pasture was 18:3n-3. These results correspond with those obtained by Mel'uchova *et al.* (2008).

Table 3.3 Mean ± SD fatty acid content of pasture and pellets

FA's	g FA/100 g fat				Pellets
	Autumn Season 1	Winter Season 2	Spring Season 3	Summer Season 4	All seasons
18:1n-9	0.40 ± 0.06	0.59 ± 0.18	0.13 ± 0.02	0.21 ± 0.06	1.56 ± 0.04
18:1n-7	0.02 ± 0.00	0.05 ± 0.03	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.01
18:2n-6 (LA)	2.32 ± 0.53	2.48 ± 0.39	1.15 ± 0.12	1.61 ± 0.50	2.53 ± 0.00
18:3n-6	0.01 ± 0.00	0.16 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	0.05 ± 0.04
18:3n-3(ALA)	9.32 ± 2.36	8.45 ± 3.76	10.93 ± 6.89	9.32 ± 3.07	1.41 ± 0.03
20:4n-6	0.01 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.01
22:6n-3	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0.00
Total SFA's	3.80 ± 0.78	3.94 ± 1.04	3.44 ± 0.16	3.23 ± 1.03	3.28 ± 0.03
Total MUFA's	0.84 ± 0.15	0.88 ± 0.26	0.29 ± 0.5	0.45 ± 0.12	1.96 ± 0.04
Total PUFA's	11.82 ± 2.91	11.32 ± 3.54	12.99 ± 7.55	12.22 ± 3.96	2.29 ± 0.01
Total omega-6	2.48 ± 0.56	2.84 ± 0.86	2.03 ± 0.68	2.85 ± 0.87	2.86 ± 0.05
Total omega-3	9.35 ± 2.36	8.48 ± 2.68	10.95 ± 6.90	9.37 ± 3.12	1.43 ± 0.04
N-6/N-3 ratio	0.27 ± 0.02	0.33 ± 0.01	0.32 ± 0.01	0.31 ± 0.03	2.00 ± 0.09
LA/ALA ratio	0.25 ± 0.01	0.29 ± 0.02	0.26 ± 0.01	0.17 ± 0.001	1.79 ± 0.04

LA = linoleic acid; ALA = alpha linolenic acid; SFA's = saturated fatty acids; MUFA's = mono-unsaturated fatty acids; PUFA's = polyunsaturated fatty acids (p<0.05).

The FA content of the pellets differed considerably from that of pasture regarding certain FA families. The SFA content was similar to pasture, however the MUFA content was higher in the pellets, mainly due to the FA 18:1n-9. In contrast, the pellet total PUFA was approximately 6 times lower, due to the lower omega-3 content, reflected by the low 18:3n-3 level. The omega-6 level in the pellets was similar to the omega-6 level in the pasture samples. The FA 18:2n-6 levels were in the pasture in seasons 1 and 2. These differences compared to pasture resulted in higher N-6/N-3 and LA/ALA ratios in the pellets. Considering the amount of pellets consumed per day compared to pasture consumption, i.e. 7 kg pellets versus 10 kg dry matter (DM) pasture per day, this could have a potential diluting effect on

the transfer of omega-3 FA from pasture to milk. The pasture constituted 59% of the total feed and the pellets 41% of the daily intake for both breeds. Pellets comprised a significant portion of the daily feed intake.

The effect of breed on the percentage of milk fat and FA content of cow's milk is presented in Table 3.4. No significant difference in the Total SFA's was observed between the two breeds. A significant ($P<0.05$) difference was observed in some FA's between breeds. FxJ cows produced significantly ($P<0.05$) more $c9,t11$ CLA isomer than J cows (0.584 ± 0.023 vs. 0.678 ± 0.018 g FA/100g fat). FxJ cows also produced significantly ($P<0.05$) more total CLA content than J cows (0.630 ± 0.024 and 0.728 ± 0.018 g FA/100g fat). The CLA content of the milk increased from early to late lactation for both breeds. CLA content ranged from 0.565 ± 0.040 to 0.847 ± 0.033 g FA/100 g fat in FxJ cows over the lactation period.

Table 3.4 Mean \pm SD milk fat percentage and fatty acid content (g FA/100 g fat)

FA's	Breeds		P-values
	Jersey	FxJ	
Milk fat %	4.141 \pm 0.094	3.767 \pm 0.072	0.0006
18:0	8.904 \pm 0.244	9.307 \pm 0.184	0.1227
Total MUFA's	16.148 ^a \pm 0.455	17.709 ^b \pm 0.343	0.0017
18:1n-9	14.120 ^a \pm 0.421	15.624 ^b \pm 0.319	0.0011
Total PUFA's	1.862 ^a \pm 0.056	2.088 ^b \pm 0.042	0.0003
LA (18:2n-6)	1.342 ^a \pm 0.043	1.506 ^b \pm 0.032	0.0005
ALA (18:3n-3)	0.263 \pm 0.014	0.278 \pm 0.010	0.3455
Total omega-6	1.537 ^a \pm 0.047	1.743 ^b \pm 0.035	0.0001
Total omega-3	0.325 \pm 0.016	0.344 \pm 0.012	0.2627
N-6/N-3 ratio	5.212 ^a \pm 0.151	5.558 ^b \pm 0.117	0.0418
Total CLA	0.630 ^a \pm 0.024	0.728 ^b \pm 0.018	0.0004
CLA ($c9,t11$ 18:2)	0.584 ^a \pm 0.023	0.678 ^b \pm 0.018	0.0003
<i>Trans</i> 18:1	0.402 ^a \pm 0.015	0.462 ^b \pm 0.011	0.0004

Means \pm SD with significant differences indicated by superscripts ($p<0.05$). SFA's = saturated fatty acids; MUFA's = mono-unsaturated fatty acids; PUFA's = polyunsaturated fatty acids; LA= linoleic acid (18:2n-6); ALA= alpha-linolenic acid (18:3n-3); CLA- conjugated linoleic acid.

The milk fat percentage differed ($P<0.05$) according to the lactation stage, although the test interaction was not significantly different. This indicated that the total milk fat percentage showed higher levels in the Jersey cows than the FxJ cows, while the fat percentage increased significantly with increased lactation stage in both breeds.

A number of studies have confirmed milk fat as one of the important energy components for humans and ruminants (Bauman *et al.*, 2003). Bauman (2002) stated that the important

compounds (minerals, proteins, FA and vitamins) that milk fat contains are known to have anti-carcinogenic properties. With regards to the two dairy breeds, Jersey cows are known to have a higher milk fat content than any other dairy breeds. The present results are in agreement with previous studies (Samkova *et al.*, 2012). Soyeurt and Gengler (2008) compared milk fat content produced by five different dairy breeds, i.e. in Jersey, Brown Swiss, Guernsey, Ayrshire and Holstein and found higher levels of milk fat content in the Jersey breed. This study also showed different milk fat levels in Jersey and FxJ cows with Jersey cows having higher milk fat content.

3.6 Individual FA group results

The analysis of each group of FA's in Table 3.4 showed significant differences in most groups. Total CLA was higher ($P<0.05$) in the FxJ cows than in the Jersey cows, due to an increase ($P<0.05$) in the main CLA isomer, namely *c9,t11* 18:2. The synthesis of CLA is dependent on site of formation, i.e rumen or tissues. Both 18:2n-6 and *trans* 18:1 were at higher levels in the FxJ crossbreeds. This is due to the inherently higher levels of CLA found in Fleckvieh breed than in any other dairy breeds (Lock and Bauman, 2004)

The effects of lactation stage on the total CLA are presented in Figure 3.2 below. Breed and lactation stage showed no interaction. There were significant differences ($P<0.05$) in lactation stage, with a similar pattern in both breeds for the total CLA and *c9,t11* isomer showing an increase from test 1 to 5 plateauing off at test 7 and 8. Levels in the FxJ cows were higher than in the J cows. Most published papers report that different dairy breeds (Jersey, Brown Swiss and Holstein) influence the CLA content in milk (Song and Kennelly, 2003), but Kelsey *et al.* (2003) stated that little information is available on the difference between breeds with regards to the CLA content of dairy cow milk. Grega *et al.* (2005) found that percentages of CLA in the total FA content of the Fleckvieh was the highest with 69% when compared to other breeds.

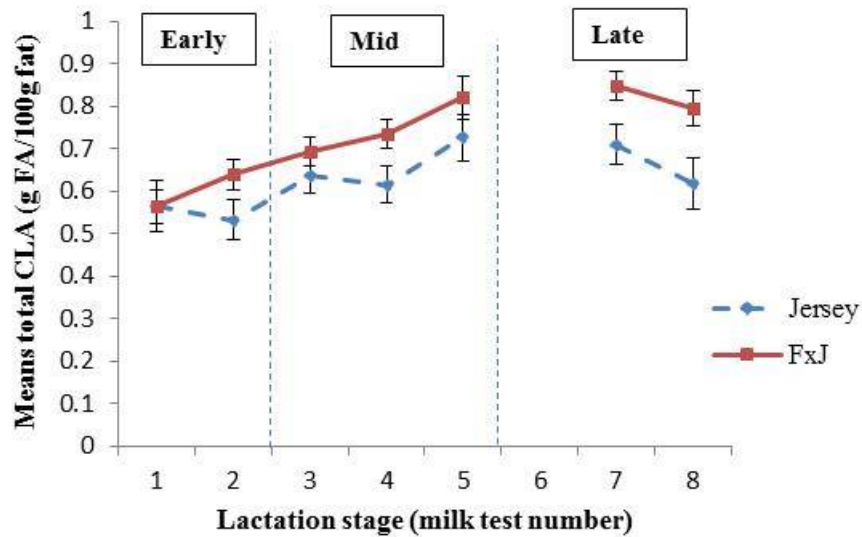


Figure 3.2. The effect of lactation stage and breed on total CLA content of the milk from Jersey and FxJ cows. (CLA = conjugated linoleic acid; FxJ = Fleckvieh x Jersey crossbred cows)

Increases in total CLA and the *c9,t11* CLA isomers in Jersey and FxJ cows followed the same trend, with an increase from the beginning of the early lactation stage to the end of the lactation stage. Higher levels in the crossbred ranged from 0.565 ± 0.040 to 0.847 ± 0.033 g FA/100 g fat and for purebred from 0.566 ± 0.060 to 0.710 ± 0.047 g FA/100 g fat.

Lactation stage had an effect on total CLA and *c9,t11* as the total CLA content of Jerseys increased by 28% and FxJ cows by 45% from early lactation stage to late lactation stage. Both *18:2n-6* and *trans 18:1* were higher in the FxJ in comparison to Jersey cows. This resulted in higher total CLA levels in the FxJ in comparison to Jerseys. Brown *et al.* (2008) found the same results. The results of the present study are in agreement with other studies that utilising pure and crossbred dairy cows, i.e. comparing Holstein, Jersey, Fleckvieh, Brown Swiss and Montbeliarde cows on pasture grazing system (Chin *et al.*, 1992; Bauman *et al.*, 2004; Brown *et al.*, 2008).

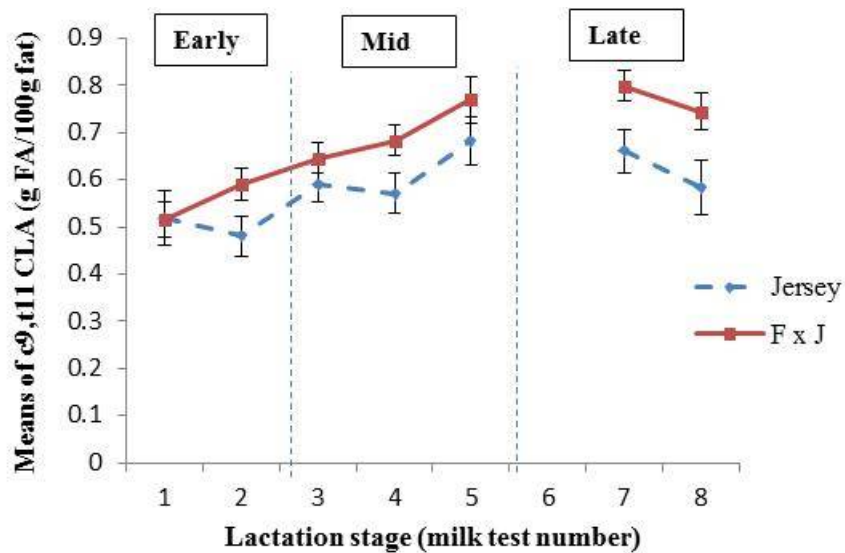


Figure 3.3 The effect of lactation stage and breed on c9,t11 CLA isomer content of the milk from Jersey and FxJ cows. (CLA = conjugated linoleic acid; FxJ = Fleckvieh x Jersey crossbred cows)

Analyzing the results in Table 3.4 for each group of FA indicated that there was no significant difference in the total SFA's when considering lactation stage. A number of studies have found no significant difference with regards to breed when looking at total SFA's. Similar results were found in this current study (Zapletal *et al.*, 2009). There was a difference ($P < 0.05$) in the individual SFA's (8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 22:0) with the lactation stage, with higher levels in the crossbred (Appendix D). There was no significant difference or interaction between breed and lactation stage.

The individual SFA's tended to be higher in the crossbred with no significant difference with breed. Subrt *et al.* (2006) reported that this could be associated with the ability of the breeds and cattle genotypes to deposit adipose tissue intramuscularly. This was confirmed by Esposito (2010) stating that milk fat is relatively more saturated than most plant oils, and could contribute to a negative consumer perception and a public health concern related to excessive intake of saturated fats.

The higher total PUFA's in this study correspond with results obtained in other studies. Parodi (1999) noted higher levels of PUFA's in cows' milk on pastures. A similar tendency was observed in the studies of Grega *et al.* (2005) and Kraft *et al.* (2003). Zapletal *et al.* (2009) also found the same results, although their study focused on comparing beef breeds (Montbeliarde and Czech Fleckvieh breed). A reason for this fact may be connected to high contents of PUFA's in grass (Zapletal *et al.*, 2009).

The total PUFA's were significantly ($P<0.05$) higher in FxJ cows. The increase in PUFA's in the FxJ breed, was mainly due to a higher ($P<0.05$) 18:2n-6 level in the omega-6 FA ($P<0.05$) component and not to a change in the omega-3 content, reflected by the unchanged 18:3n-3 level. There was a difference ($P<0.05$) in the total MUFA based on lactation stage, with higher levels in the FxJ cows. A higher MUFA was mainly due to an increased 18:1n-9 ($P<0.05$) and *trans* 18:1 ($P<0.05$) levels. There was no significant difference with regards to breed and test interaction. The major individual MUFA's (18:1n-9 and *trans* 18:1) showed the same pattern as for total MUFA with higher ($P<0.05$) levels in crossbred cows over the whole lactation period. The same trend for total PUFA's was similar to that of total MUFA's, however, there was no significant difference ($P<0.05$) in lactation stage. No interaction was recorded between breed and lactation stage.

A similar pattern as for the total PUFA was observed with the total omega-3 FA's, but again there was no significant difference ($P<0.05$) with regards to lactation stage. However, again the level initially tended to be slightly higher in the crossbred cows. No interaction was recorded between breed and lactation stage. No significant lactation differences for 18:3n-3 was observed between the breeds. There was also no difference in 22:5n-3 between lactation stages.

The higher omega-6 level in the milk from the FxJ cows resulted in a higher ($P<0.05$) N6/N3 ratio, approximately in the same order as observed for the pellet supplement discussed above. The total omega-6 followed the same pattern as for the total PUFA's, but again with no significant difference in lactation stage. The same pattern for 18:2n-6 (LA) is observed as for total PUFA, with differences ($P<0.05$) in lactation stage due to higher levels in the FxJ cows. No interaction was recorded between breed and lactation stage. Schroeder *et al.* (2005) showed higher 18:2n-6 levels in his study where he compared lactation stage with Holstein dairy breeds on pastures. These results are similar to those of the current study. Findings from a study by Samkova *et al.* (2012) regarding 18:2n-6, precursor for CLA, showed no effect was observed with lactation stage by cows on pastures.

3.7 Conclusion

The FA composition of the milk fat of dairy cows is influenced by several factors such as breed, diet, lactation stage and seasonal variations. This study has, for the first time, confirmed that FA composition is affected to a large degree by breed and lactation stage under South African conditions. Purebred Jersey cows showed higher levels of milk fat percentage. Some FA's differed between Jersey and FxJ cows, although not all differences were significant. In the current study, DIM or lactation stage had little effect on the total SFA's and PUFA's, although changes were observed within the different PUFA omega-FA families.

No significant effects on the omega-3 FA's were observed, however the omega-6 FA's (18:2n-6) were higher in the FxJ cows. Concomitantly, the FxJ cows showed higher levels of CLA (total and *c9,t11* isomer) than purebred Jersey cows, with an increase of 45% from early lactation to late lactation stage. This can be ascribed to the fact that Fleckvieh breeds are known for their high levels of CLA, possibly due to genetic differences between breeds and efficiency of converting 18:2n-6 and *trans* 18:1 to the CLA isomers.

Notwithstanding the higher total CLA content in the crossbred cows, it is worth considering the diluting effect of the high omega-6 PUFA content in the pellet supplement which can negatively impact on the additional health benefits of omega-3 FA present in milk.

References

- Atti, N., Rouissi, H. and Othmane, M. H. 2006. Milk production, milk fatty acid composition and conjugated linoleic acid (CLA) content in dairy ewes raised on feedlot or grazing pasture. *Livest. Sci.* 104:121-127.
- Bauman, D.E., Baumgard, L.H., Corl, B.A. and Griinari, J.M. 1999. Biosynthesis of conjugated linoleic acid in ruminants. *Proc. Amer. Soc. Anim. Sci.* 1999:1-15
- Bauman, D.E. 2002. Conjugated linoleic acid (CLA) and milk fat: A good news story. https://www.ansci.cornell.edu/bauman/cla/conference_proceedings/articles [25 July 2012].
- Bauman, D.E., Perfield, J.W., de Veth, M.J. and Lock, A.L. 2003. New perspectives on lipid digestion and metabolism in ruminants. https://ansci.cornell.edu/bauman/cla/conference_proceedings/articles/2003_cnc_bauman_et_al.pdf [25 July 2012].
- Bauman, D.E., Perfield, J.W. and Lock A.L. 2004. Effect of trans fatty acids on milk fat and their impact on human health. https://ansci.cornell.edu/bauman/cla/conference_proceedings/articles/2004_Bauman_Perfield_and_Lock_SWNC.pdf [20 July 2012].
- Bell, M.J., Cullen, B.R. and Eckard, R.J. 2011. The production of perennial ryegrass and kikuyu pastures in south-eastern Australia under warmer and drier future climate scenarios. *19th Int. Congr. Mod. Sim. Perth*, 12-16 December.
- Botha, P.R., Meeske, R. and Snyman, H.A. 2006. Forage quantity and quality from kikuyu pastures over-sown with ryegrass and clover. *J. Elsenburg* 1:2-4.
- Botha, P.R., Meeske, R. and Snyman, H.A. 2008. Kikuyu over-sown with ryegrass and clover: grazing capacity, milk production and milk composition. *Afr. J. Range For Sci.* 25:103-110.
- Brown, W., AbuGhazaleh, A.A. and Ibrahim, S.A. 2008. Milk conjugated linoleic acid

- response to fish oil and linseed oil supplementation of grazing dairy cows. *Asian-Aust. J. Anim. Sci.* 21:663-670.
- Chin, S.F., Liu, W., Storkson, J.M., Ha, Y.L. and Pariza, M.W. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Compos. Anal.* 5:185-197.
- Christie, W.W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *J. Lipid Res.* 23:1072-1075.
- Christie, W.W. 1993. Preparation of lipid extracts from tissues. In Christie, W.W. (Ed.). *Advances in Lipid Methodology*. Dundee, UK: The Oily Press: 195-213.
- Chouinard, P.Y., Corneau, L., Saebo, A. and Bauman, D.E. 1999. Milk yield and composition during abomasal infusion of CLA in dairy cows. *J. Dairy Sci.* 82:2737-2745.
- Collomb, M., Schmid, A., Sieber, R. and Wechsler, D. 2006. Conjugated linoleic acid in milk fat variation and physiological effects. *Int. Dairy J.* 16:1347-1361.
- Collomb, M., Bisig, W., Butikofer, U., Sieber, R., Bregy, M. and Etter, L. 2008. Fatty acid composition of mountain milk from Switzerland: comparison of organic and integrated farming system. *Int. Dairy J.* 18:976-982.
- Descalzo, A.M., Rossetti, L., Paez, R., Grigioni, G., Garcia, P.T., Costabel, L., Negri, L., Antonacci, L., Salado, E., Bretschneider, G., Gagliostro, G., Comeron, E. and Taverna, M.A. 2012. Differential characteristics of milk produced in grazing systems and their impact on dairy products. <http://cdn.intechopen.com/pdfs-wm/39316.pdf> [13 January 2013].
- Dewhurst, R.J., Shingfield, K.J., Lee, M. R. F. and Scollan, N. D. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim. Feed Sci. Tech.* 131:168-20.
- Esposito, G. 2010. Conjugated linoleic acid and dairy cows: metabolism, reproduction and product quality. PhD thesis, Universita' degli Studi di Napoli Federico II, Naples.
- Fulkerson, B., 2007. *Kikuyu grass (Pennisetum clandestinum)*. <http://www.frds.dairyaustralia.com.au> [21 August 2013]
- Gavino, V.C., Gavin, G., Leblanc, M.J. and Tuchweber, B. 1999. An isomeric mixture of conjugated linoleic acids but not pure *cis*-9, *trans*-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters. *J. Nutr.* 130:27-29.
- Grega, T., Sady, M., Najgebauer, D., Domagala, J., Pustkowiak, H. and Faber, B. 2005. Factors affecting the level of conjugated linoleic acid (CLA) in milk from different cow's breeds. *Biotechnol. Anim. Husb.* 21:241-244.
- Grogan, A., Wichham, B., Olori, V. and Cromie, A. 2005. Strategies of European Fleckvieh cattle breeding to optimize dual-purpose breeding particularly for beef production. *Stocarstvo.* 59(5):339-353.
- Hara, A and Radin, N.S. 1978. Lipid extraction of tissues with low-toxicity solvent. *Anal.*

- Biochem.* 90:420-426.
- Kaizer, A.G., Havilah, E.J., Chopping, G.D. and Walker, R.G., 1993. Feeding system during winter and spring. *Northem Dairy Feedbase 2001. Trop.Grasslands.* 27, 180-211.
- Khanal, R.C. and Dhiman, T.R., 2004. Biosynthesis of Conjugated Linoleic Acid (CLA): A Review. *Pak. J. Nutr.* 3, 72-81.
- Kelly, G.S. 2001. Conjugated Linoleic Acid: A review. *Altern. Med. Rev.* 6:367-382.
- Kelly, M.L., Kolver, E.S., Bauman, D.E., Van Amburgh, M.E. and Muller, L.D. 1998. Effect of intake of pasture on concentration of conjugated linoleic acid in milk of lactating cows. *J. Dairy Sci.* 81(6):1630-1636.
- Kelsey, J.A., Botha, B.A., Collier, R.J. and Bauman, D.E. 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *J. Dairy Sci.* 86(8):2588-2597.
- Kraft, J., Collomb, M., Mockel, P., Sieber, R. and Jahreis G. 2003. Differences in CLA isomer distribution of cow's milk lipids. *Lipids.* 38:657-664.
- Lawless, F., Stanton, C., Escop, P.L., Devery, R., Dillon, P. and Murphy, J.J. 1999. Influence Of breed on bovine milk *cis*-9, *trans*-11-conjugated linoleic acid content. *Livest. Prod. Sci.* 62:43-49.
- Lock, A.L. and Bauman, D.E. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids.* 39:1197-1206
- Mears, P.T. 1970. Kikuyu (*Pennisetum Clandestinum*) as a pasture grass: A review. *Trop. Grasslands.* 4(2): 139-152.
- Mel'uchova, B., Blasko, J., Kubinec, R., Gorova, R., Dubravaska, J., Margetin, M. and Sojak, L. 2008. Seasonal variations in fatty acid composition of pasture plants and CLA content in ewe milk fat. *Small Rumin. Res.* 78:56-65.
- Mitchell, L. and Mcleod, S. 2008. Conjugated linoleic acid and atherosclerosis: studies in animal models. *Biochem. Cell Biol.* 86(4):293-301.
- Miles, N., de Villiers, J.F. and Dugmore, T.J. 1995. Macro-mineral composition of kikuyu herbage relative to the requirements of ruminants. *J. S. Afr. Vet. Assoc.* 66:206-212.
- Muller, C.J.C., Botha, J.A., Meshelle, G., Van Pittius., Grupp, T., Swart, M.J. and Nesor, F.W.G. 2006. Preliminary results on the growth performance of Holstein and Fleckvieh x Holstein bull and heifer calves. *J. Elsenburg.* 3:1-12.
- Muller, C. J. C. & Botha, J. A. 2008. Preliminary results on crossbreeding Jerseys with Fleckvieh sires. *J. Elsenburg* 1: 2-3.
- Nichols, B.W., 1963. Separation of the lipid of photosynthesis tissue: improvements in analysis by thin-layer chromatograph. *Biochem. Biophys. Acta.* 70:417-422.
- Parodi, P.W. 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *J. Dairy Sci.* 82(6): 1339-1349.
- Quinlan, T J., Shaw, K.A. and Edgley, W.H.R. 1975. Kikuyu grass. *Queensland Agric. J.*

101:737-749.

- Qureshi, M.S., Jan, S., Mushtaq, A., Rahman, I.U., Jan, M. and Ikramullah. 2011. Effect of age on milk fatty acids in dairy buffaloes. *J. Anim. Plant Sci.* 22(2):108-112.
- Salter, A.M. 2005. The role of diet in preventing cardiovascular disease in the “post-statin” era. *Proc. Cornell Nutr. Conf.* 127-137.
- Samkova, E., Spicka, J., Pesek, M., Pelikanova, T. and Hanus, O. 2012. Animal factors affecting fatty acid composition of cow milk fat: A review. *S. Afr. J. Anim. Sci.* 42:83-99.
- Schroeder, G.F., Couderc, J.J., Bargo, F. and Rearte, D.H. 2005. Milk production fatty acid profile of milk fat by dairy cows fed a winter oats (*Avena sativa L.*) pasture only or a total mixed ration. *New Zealand J. Agric. Res.* 48:187-195.
- Smith, W.A., Cruywagen, C.W. and Maree, C. 1993. Dairy Production Systems. In: Maree, C and Casey, N.H. (eds). *Livestock Production Systems. Principles and Practice.* Brooklyn, Pretoria: Agri Development Foundation: 220-254.
- Smuts, C.M., Weich, H.F.H., Weight, M.J., Faber, M., Kruger, M., Lombard, C.J. and Benadé, A.J.S. 1994. Free cholesterol concentrations in the high-density lipoprotein subfraction-3 as a risk indicator in patients with angiographically documented coronary artery disease. *Coron. Artery Dis.* 5:331-338.
- Song, M.N. and Kennelly, J.J. 2003. Biosynthesis of Conjugated Linoleic Acid and its incorporation into ruminant's products. *Asian-Aust. J. Anim. Sci.* 16(2):306-314
- Soyeurt, H. and Gengler, N. 2008. Genetic variability of fatty acids in bovine milk. *Biotechnol. Agron. Soc. Environ.* 12:203-210.
- Steinmetz, K.A., Childs, M.T., Stimson, C., Kushi, L.H., McGovern, P.G., Potter J.D. and Yamanaka, W.K. 1994. Effects of consumption of whole milk and skim milk on blood lipid profiles in healthy men. *J. Amer. Clin. Nutr.* 59:612-618.
- Subrt, J., Filipcik, R., Zupka, Z., Fialova, M. and Drackova, E. 2006. The content of polyunsaturated fatty acids in intramuscular fat of beef cattle in different breeds and crossbreds. *Arch. Anim. Breed.* 49(4): 340-350.
- Tichelaar, H.Y., Benadé, A.J.S., Daubitzer, A.K., and Kotze, T.J. v.W. 1989. An improved rapid thin-layer chromatographic-gas-liquid chromatographic procedure for the determination of free fatty acids in plasma. *Clinica. Chimica. Acta.* 183(2):207-216.
- Zapletal, D., Chladek, G. and Subrt, J. 2009. Breed variation in the chemical and fatty acid compositions of the *Longissimus dorsi* muscle in Czech Fleckvieh and Montbeliarde cattle. *J. Livest.Sci.* 123:28-33.

CHAPTER 4

MILK FATTY ACID COMPOSITION AND CLA CONTENT OF HOLSTEIN AND FLECKVIEH X HOLSTEIN COWS IN A TOTAL MIXED RATION FEEDING SYSTEM

4.1 Abstract

The aim of the study was to analyze the effect of crossbreeding on the milk FA content and composition from 40 Holstein (H) and 28 Fleckvieh × Holstein (F×H) cows fed a total mixed ration (TMR) in a feedlot system. The study was conducted at the Elsenburg Research Farm of the Western Cape Department of Agriculture. Milk samples were collected and tested at 35-day intervals. The tests were repeated six times during the lactation period of each cow.

Samples were kept frozen at -20 °C until analysis. Concentration levels of FA were determined by gas liquid chromatography and 36 different FA's were detected in the milk. The variables that were measured during each test were the milk fat percentage, CLA isomers, total SFA's, total PUFA's, total MUFA's, essential FA's content as well as the ratio of LA to ALA and Omega 3 FA's and Omega 6 FA's. A total of n=477 milk samples were analysed and FA profiles compared between the breeds.

Statistical analysis was carried out using two-way repeated measures of analysis of variance (ANOVA) and a mixed model procedure. The model included the treatment effect (breed), time effect of observation (test) and interaction effect of breed and test as fixed effects. Animal influence within treatments were specified as a random effect. The measured variables obtained at each test (i.e. every five week milk test, n=6) during the lactation were considered as repeated observations of a particular block.

F×J cows produced more total omega-6, omega 3 and CLA per 100g fat, however these differences were not significant. The FA content significantly differed for both breeds ($P<0.05$) from test to test, indicating a lactation stage effect. Higher CLA values ($P<0.05$) were also found for both breeds at the end of the lactation, possibly due to the higher fat content in milk at the end of lactation.

4.2 Introduction

Dietary manipulation of FA composition in milk has become an important issue during the last few decades with the goal of obtaining healthier milk and dairy products. It is proposed that CLA might reduce the risk of certain diseases and at some point the evidence supporting

the benefits of CLA could be strong enough that it too, is included among the essential FA's for humans (Clancy, 2006).

Ruminants produce high levels of CLA in their meat and milk (Liebenberg, 2007). This FA has been shown to have potent anti-carcinogen, anti-diabetic and anti-atherosclerosis effects (Cruz-Hernandez, 2007; Mitchell and McLeod, 2008). Holstein dairy cows produce larger quantities of milk than other dairy breeds and are normally kept in TMR feeding systems (Underwood, 2002; White *et al.*, 2001). Improved income from dairy cattle is possible through crossbreeding, because it ensures improved cow fertility, cow health and calf survival (Caraviello, 2004).

Crossbreeding Holstein cows with the Fleckvieh may improve fertility, while milk yield would not be affected negatively. The Fleckvieh type dual-purpose breeds appear to contain higher levels of CLA, omega-3 and omega-6 FA's in their milk than purebred Holstein dairy cows (Lock *et al.*, 2005). Crossbreeding Holstein cows with Fleckvieh may therefore improve the CLA content in the crossbred progeny.

Other factors that affect the milk CLA level, other than breed, include diet and lactation stage (Lawless *et al.*, 1999). Samkova *et al.* (2012) reported lactation stage as one of the important factors influencing milk production and concentration of milk and its FA's. Qureshi *et al.* (2011) also found that CLA content in milk increases with advancing lactation stage. Diet has also been regarded one of the major factors affecting the level of CLA in dairy breed (Song and Kennelly, 2003 and Kelly *et al.*, 1998).

Mitchell and McLeod (2008) reported that the amount of CLA in cows' milk is affected mostly by their diet and healthy FA's (18:3n-3, 18:2n-6 and *trans* 18:1) increase when cows are on pasture or when feeds, such as extracted soy beans and cottonseed, are supplemented to TMR diets (Collomb *et al.*, 2006).

A TMR consists of a complete feed mix consisting of forages and concentrates, balanced to meet the nutrient requirements of cows (Cabrera, 2012). Often dairy cows in a TMR-based feeding system are divided into two groups, mainly first lactation cows and older cows. Each TMR feed contains the required level of nutrients (energy, protein, minerals and vitamins) needed by the average cow in the group (Mierlita, 2011). The advantage of a TMR is that it may be adapted throughout the year, while some dairy farms use it during the non-grazing months (Soriano *et al.*, 2001).

The aim of this study is to compare:

1. The milk FA's composition, particularly CLA content of Holstein (H) and Fleckvieh x Holstein (F×H) cows on a TMR based feeding system, as well as the effect of breed and lactation stage on CLA content.
2. Present a reference model of the CLA content of Holstein and F×H milk within a TMR feeding system prevalent in the Western Cape, South Africa.

4.3 Methods and material

4.3.1 Site description

The experimental location of the study was the Elsenburg Research Farm of the Western Cape Department of Agriculture. Elsenburg is situated approximately 50km east of Cape Town at an altitude of 177m, longitude 18° 50' and latitude 33° 51' and is situated in the winter rainfall region of South Africa. The area is characterized by a Mediterranean climate with cool wet winters and long dry summers. The long term average annual rainfall is 650mm.

4.3.2 Study cows

Cows used in the study form part of a long-term project by the Western Cape Department of Agriculture to investigate the production characteristics of Fleckvieh crossbred dairy cows and steers. Fleckvieh bull semen from Bavarian Fleckvieh Genetics (BFG) had previously been used to breed crossbred cows from purebred Holstein and Jersey at the Elsenburg experimental farm. The herd consisted of 280 Holstein, 350 Jersey, 180 F×H and 210 F×J cows at the beginning of the current study. A total of 40 pure Holstein (H) cows and 28 Fleckvieh × Holstein (F×H) cows were selected for the study. The experimental herd was divided into groups based on breed, and cows were randomly and independently selected from each group to form part of the current study. Data were collected as these cows calved down over a period from November 2011 to December 2013.

4.3.3 TMR feed

The TMR consisted of forages and concentrates mixed together to meet the exact requirements of the cows and fed in a feedlot twice per day. Cows were divided into two groups, first lactation cows (first parity cows) and older cows (multiparous cows). These two groups of cows were kept separately and fed different TMRs to prevent unfair competition for feed and over-feeding of young cows.

Table 4.1 TMR content

Feed consumed by cows/day	Multiparous cows (kg/cow/day)	First parity cows (kg/cow/day)
Oat Hay	7.8	7.8
Concentrate	7.8	6.6
Cotton seed oil cake meal	3.9	2.4
Lucerne hay	2.5	2.9
Maize	3.8	3.8
Salt	0.1	0.1
Lime	0.1	0.1
Total feed per day (kg)	26.0	22.0

4.3.4 Sample Collection

Samples of milk and TMR feed were collected and tested for FA content. Milk samples were collected at the evening and the next mornings' milking and pooled for each cow. A total of six milk FA tests were completed per cow over the course of lactation. The 100 mL milk samples were collected every 35 days. Samples were immediately cooled and frozen at -20 °C until laboratory analysis by gas liquid chromatography. A total of n=286 (H) and n=191 (F×H) milk samples were analyzed to compare the two breeds for FA content. Milk sample collection was the same for this study as described and illustrated in the preceding Chapter.

For the analysis of FA content of the milk, approximately 100 mL of milk was sampled from the pooled amounts proportional to the cow's milk yield at each milking session (morning and evening) making up a single day's milk sample. Milk samples collected were separated into two subsamples; i.e. one for FA analysis and another subsample preserved with Bronopo-B₂ preservative pills. The Bronopo-B₂ preserved milk samples were analyzed for fat, protein, lactose, milk urea nitrogen (MUN) and SCC with the aid of a Milk-O Scan 133B at the Animal Production Institute (ANPI) Dairy Lab of the Agricultural Research Council at Elsenburg for normal milk recording purposes. Only the data recorded for fat are reported in this study.

TMR concentrate feed was sampled according to the method followed by Schroeder *et al.* (2005). A sample was taken every month. Three 500g bags were collected and analyzed per season. Feed samples were collected from the beginning of the year 2012 until the end of 2012, representing the four seasons of the year. After collection, feed samples were stored at -20 °C until analysis at the PROMEC Unit of the Medical Research Council, Tygerberg, South Africa.

4.3.5 Data collection

Samples were taken for six milk recording events per lactation, *viz* the first to fourth and the seventh and eighth milk recording tests (test 1,2,3,4,7,8). Milk test 5 and 6 were not included in the study. The rationale of repeating the tests was to determine the effect of lactation stage on milk FA composition and content. Standard milk recording procedures were followed, i.e. the milk yield of cows was recorded at the evening and following morning's milking session.

The milk FA composition and CLA content of milk samples were analyzed by gas chromatography (GC) at the Program on Mycotoxins and Experimental Carcinogenesis (PROMEC) Unit of the Medical Research Council in Tygerberg, Western Cape, South Africa. Final milk composition was expressed based on weighted evening and morning's milk yields. The results from the analyses were compared for breed and lactation stage

4.3.6 Milk analyses

The milk FA composition and CLA content of milk samples were analyzed by gas chromatography (GC) at the PROMEC Unit of the Medical Research Council at Tygerberg in the Western Cape Province of South Africa. The results from the analyses were compared for breed and milk test (lactation stage).

All solvents and chemicals were obtained from Sigma-Aldrich: hexane, iso-propanol, chloroform, methanol, diethyl ether, butylated hydroxytoluene (BHT), anhydrous sodium sulphate, (Na_2SO_4), sodium methoxide (0.5M), oxalic acid, methyl acetate ($\text{CH}_3\text{CO}_2\text{CH}_3$). Triacylglyceride (TAG) 17:0 was obtained from Avanti Polar Lipids (USA). The FA standard mixture (4:0-24:1) for chromatogram peak identification was purchased from Nu-Chek Prep (USA).

The following solvents and solutions were used for milk FA extraction and methylation:

HIP solvent (Hexane: Isopropanol; 3/2, vol/vol) with 0.01% BHT as antioxidant, internal standard 17:0 TAG (300 mg TAG/2 mL chloroform), saturated anhydrous sodium sulphate solution (1 g anhydrous salt in 15 mL distilled H_2O), oxalic acid in diethyl ether (1g oxalic acid in 30 mL diethyl ether) and transmethylation reagent (methanol/18 M sulphuric acid; 95:5, v/v) for grass FA methylation and 0.5M sodium methoxide for milk FA methylation. All solvents are HPLC grade or double distilled.

All glassware were soaked for a minimum of 2 hours (or overnight) in a 2-5% phospholipid-free soap (Contrad, Merck) and rinsed in warm tap water (5x), distilled water (5x) and oven dried at 100 °C for 24 hours. Prior to use, all glassware were rinsed with distilled or high performance liquid chromatography (HPLC)-grade methanol and dried in a fume hood.

Milk samples were analyzed for fat % with the aid of a Milk-O Scan 133B at the ANPI dairy lab of the Milk Recording Scheme of the Agricultural Research Council at Elsenburg. For fatty acid determination, separation of milk fat was based on the method described by Chouinard *et al.* (1999). Milk (~500mL) stored at -20 °C was thawed at 36-38 °C after homogenization using a polytron. An aliquot of milk (~40 mL) was measured into a 50 mL Falcon tube and centrifuged at 4000 rpm for 30 minutes at 8 °C. The top fat layer (fat cake) was removed with a sterile/clean plastic spatula and placed in a Falcon tube (20 mL), then flushed with N₂ gas and stored at -20 °C.

The lipid from the isolated fat cake was extracted based on the method of Hara and Radin (1978). A 100 mg fat cake was removed and placed into a Teflon-capped extraction glass tube. HIP solvent (5.5 mL) was added to the internal standard 17:0 TAG (100 µl/15 mg). The tube was flushed with N₂ gas, closed, vortexed for 2 minutes and the mixture was filtered, under vacuum, through a sinterglass funnel lined with glass microfibre filter paper and rinsed with 3 x 0.6 mL HIP solvent. Saturated sodium sulphate solution (3.5 mL) was added and the sample vortexed for 1 minute and centrifuged at 500 rpm for 12 min at 8 °C (Sorvall model centrifuge). The top hexane-rich layer of the biphasic solution, containing lipid extract, was removed with a glass Pasteur pipette into Teflon-capped glass tube, flushed with N₂ gas and stored at -20 °C.

Transmethylation of FA's in the extracted lipids was done according to Christie (1982) and Christie (1993) with modifications based on Chouinard *et al.* (1999). An aliquot (2 mL) of the hexane lipid extract was removed and evaporated (37 °C) until dry under N₂ gas, in pre-weighed Teflon-capped glass tubes and the dried lipid weight recorded. Hexane (1 mL) and methyl acetate (40 µl) was added to the dried lipid and the mixture vortexed. Methylation reagent (40 µl, 0.5M sodium methoxide) was added, samples vortexed, flushed with N₂ gas and tubes tightly closed with a Teflon cap, and the methylation allowed to react for 15 minutes at 50 °C, thereafter the tubes were cooled to room temperature. The methylation reaction was terminated by adding oxalic acid/diethyl ether solution (60 µl; 1g acid/30mL) and vortexed after which the samples were centrifuged at 1000 rpm for 12 minutes. A one µl aliquot, containing FA methyl esters (FAMES), was removed for injection into a gas chromatograph analyses.

4.3.7 TMR analyses

After collection, TMR feed samples were stored at -20 °C at the PROMEC Unit for further analysis. One gram of feed from each sample collection bag was weighed into a Petri dish covered with a filter paper and dried in a microwave oven until there was no change in weight

recorded. Samples were cooled in a glass dessicator in between heating intervals. Thereafter, the dried samples were finely ground in liquid nitrogen for lipid extraction.

To prevent plant lipase activation and hydrolyzation of phospholipid glycolipids, the TMR lipids were extracted with isopropanol based on a modified method by Nichols (1963) and Hara and Radin (1978). An accurately weighed aliquot of 50 mg finely ground sample was transferred into a Teflon-capped glass tube. Six mL of hexane (12 volumes) to 4 mL (8 volumes) of isopropanol was added, the mixture was vortexed for 1 minute, and then centrifuged for 8 min at 500rpm using a Sorvall RC-3B centrifuge. The lipid extract was transferred to a new tube and the sample re-extracted with 3 mL HIP (7:2 hexane:isopropanol with 0.01% BHT). Two mL saturated sodium sulphate solution was added to the extract, vortexed and centrifuged for 12 minutes at 1000 rpm using a Sorvall RC-3B centrifuge. The top sodium sulphate phase was discarded and the bottom solvent lipid phase was flushed with N₂ and stored at 4 °C.

Sample lipid extracts were evaporated under N₂ gas until dry and redissolved in 1 mL transmethylating reagent (methanol/18 M sulphuric acid, 95:5, v/v) as described by Tichelaar *et al.* (1989) and Smuts *et al.* (1994) for 2 hours at 70 °C. Thereafter, samples were cooled and 2 mL hexane was added together with 1 mL distilled H₂O and vortexed. After phase separation, the top hexane layer containing the FAME was removed for analysis on a GC instrument (Varian 3300, USA). FA peaks were identified by a comparison of the retention times with pure standards of FAME and CLA purchased from Nu-Check Prep (MN, USA). The FA concentration of each FA was calculated with respect to an internal standard (TAG, 17:0) and expressed as g FA/100g fat.

4.3.8 GC conditions for FAME analyses in milk and TMR feed

Milk FA was analyzed using a long capillary column of 120 m due to the superior resolution compared to traditional shorter columns of 30-60 m. This was selected because milk is a complex sample containing many *cis* and *trans* FA's (Appendix A). The TMR FA analysis was done on a short capillary (30 m) GC column, as TMR is not a complex mixture of FA to separate (Appendix B). The GC conditions and column specifications used to analyze milk and TMR feed samples are presented in Table 4.2 and Table 4.3, respectively.

Table 4.2: Gas chromatography (GC) conditions

GC conditions	Milk (120m column)	TMR (30m column)
Initial oven temp:	80°C	140 °C
Initial hold time:	8 min	1.5min
<u>Oven Program 1</u>		
Temp. ramp:	9.0°C/ min	6.0°C/ min
Oven holding temp:	175°C	200°C
Oven holding time:	52 min.	2 min.
<u>Oven Program 2</u>		
Temp. ramp:	9.0°C/ min	6.0°C/ min
Oven holding temp:	225°C	220°C
Oven holding time:	18 min.	5 min
Total run time:	94 min	22 min
Carrier gas:	Hydrogen	Hydrogen
Make-up gas:	Nitrogen	Nitrogen
FID Detector temp:	280 °C	260 °C
Injector/Inlet temp:	240 °C	240 °C
Injection mode:	Split 80:1	Split 20:1
Gas flow rates:	Hydrogen = 25 mL/min Oxygen = 250 mL/min Nitrogen = 25 mL/min Carrier/column flow = 2 mL/min	Hydrogen = 25 mL/min Oxygen = 250 mL/min Nitrogen = 25 mL/min Carrier/column flow = 2 mL/min

Table 4.3 GC column specifications

Column specifications	Milk (120m column)	TMR (30m column)
Column	BPX 70	BPX 70
Column length	120m	30m
Internal diameter (ID)	0.25 mm	0.32 mm
Film thickness	0.25 µm	0.25 µm
Film/Stationary phase	70% cyanopropyl-polysilphenylene siloxane	70% cyanopropyl-polysilphenylene siloxane
Catalogue no.	054224 (SGE, USA)	054616 (SGE, USA)

4.4 Statistical analysis

Data from the study was subjected to a two-way repeated measures of analysis of variance (ANOVA) using PROC MIXED Model of SAS Enterprise Guide 5.1 (SAS Institute Inc., 2012).

The model included the treatment effect (breed), time effect of observation (test) and interaction effect of breed and test as fixed effects, whereas animal influence within treatments were specified as a random effect. The measured variables obtained at each test (i.e. every five week milk test, $n = 6$) during the lactation were considered as repeated observations of a particular Block.

The model was defined as follows:

$$Y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_{(ij)k} + \epsilon_{ijk} \text{ where}$$

μ = overall mean;

α_i = i th level of treatment factor (main effect, breed);

β_j = j th level of time factor (main effect, time);

$(\alpha\beta)_{ij}$ = interaction between level i of treatment and j of time (interaction effect);

$\delta_{(ij)k}$ = effect of the k th block effect in the i th treatment (variable effect);

ϵ_{ijk} = i, j, k th error term.

The assumptions were described as $\sum_i \alpha_i$, $\sum_j \beta_j$ and $\sum_i (\alpha\beta)_{ij} = \sum_i (\alpha\beta)_{ij}$ equal to zero with $\delta_{(ij)k} \sim N(0, \sigma^2)$ varying independently of ϵ_{ijk} . Differences between treatments were obtained using the pair wise comparison of Bonferroni t-test. Significance was declared at ($P < 0.05$).

4.5 Results and Discussion

The FA's in feeds used in the trial are presented in Table 4.4. Total SFA's tended to be higher in season 4 than the preceding seasons. The Total MUFA's and omega-6 displayed a similar pattern with season 1 and 4 being higher than season 2 and 3, mainly due to 18:1n-9 and 18:2n-6, respectively. In contrast, the Total omega-3 was higher in seasons 2 and 3 than in seasons 1 and 4 respectively, mainly because of a higher 18:3n-3 content. The contrasting levels of the omega-6 and -3 contents resulted in higher levels with regards to the N-6/N-3 ratio and LA/ALA ratio in seasons 1 and 4 and lower levels in seasons 2 and 3. The FA's in feeds differed between seasons, possibly due to seasonal maize content.

A number of studies have shown that TMRs often have high concentrations of the FA 18:2n-6 in the feed. This is probably due to a high level of maize in the TMR diet (Schroeder *et al.*, 2005). With regards to the current study, higher levels of 18:2n-6 were observed in seasons 1 and 4, probably because these two seasons had higher quantity of maize compared to the other two seasons, and also reflected by the higher Total omega-6 content.

Table 4.4 Mean ± SD fatty acid content of the total mixed ration

FA's	g FA/100 g of TMR DM			
	Autumn Season 1	Winter Season 2	Spring Season 3	Summer Season 4
18:1n-9	3.81 ± 0.79	1.66 ± 0.12	1.64 ± 0.14	3.45 ± 0.47
18:1n-7	0.03 ± 0.04	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.05
18:2n-6	4.89 ± 1.67	2.72 ± 0.23	2.66 ± 0.23	3.96 ± 0.81
18:3n-6	0.00 ± 0.00	0.05 ± 0.03	0.04 ± 0.03	0.01 ± 0.00
18:3n-3(ALA)	0.65 ± 0.13	1.53 ± 0.15	1.50 ± 0.16	0.48 ± 0.01
20:4n-6	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
22:6n-3	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Total SFA's	3.30 ± 0.61	3.38 ± 0.17	3.33 ± 0.17	4.10 ± 0.22
Total MUFA's	3.95 ± 0.82	2.10 ± 0.17	2.05 ± 0.17	3.63 ± 0.52
Total PUFA's	5.64 ± 1.78	4.62 ± 0.39	4.52 ± 0.41	4.57 ± 0.81
Total omega-6	4.97 ± 1.68	3.07 ± 0.24	3.00 ± 0.23	4.06 ± 0.81
Total omega-3	0.66 ± 0.13	1.55 ± 0.15	1.53 ± 0.18	0.51 ± 0.01
N6/N3 ratio	7.41 ± 1.65	1.98 ± 0.07	1.96 ± 0.09	7.99 ± 1.50
LA/ALA ratio	7.43 ± 1.60	1.78 ± 0.03	1.77 ± 0.04	8.25 ± 1.53

LA = linoleic acid; ALA = alpha linolenic acid; SFA's = saturated fatty acids; MUFA's = mono-unsaturated fatty acids; PUFA's = polyunsaturated fatty acids ($P < 0.05$).

Results of the milk FA test found in Table 4.5 show that milk fat percentage differed significantly ($P < 0.05$) between the H and FxH cows. In the milk, all FA's differed between the two breeds although only a few reached significance ($P < 0.05$), being higher in the FxH crossbred cows. No significant difference between breeds on Total SFA's or MUFA's was observed, however *trans* 18:1 was significantly higher ($P < 0.05$) in the crossbred cows. The Total PUFA's was significantly higher ($P < 0.05$) in the FxH due to a higher 18:2n-6 ($P < 0.05$) level as reflected by the increase in Total omega-6 ($P < 0.05$) content. No differences in the Total omega-3 content or 18:3n-3 level was observed. Kelly *et al.* (1998) and Schroeder *et al.* (2005) observed similar results for omega-6 FA. The study by Schroeder *et al.* (2005) with 12 Holstein cows on TMR and others on a pasture feeding system showed the same results with few SFA's showing significant difference and total PUFA's showing significant difference with regards to breed. Although significantly ($P < 0.05$) higher levels of *trans* 18:1 and 18:2n-6, both precursors for CLA in the milk of FxH cows were observed, the concentration of total CLA and the *c9,t11* isomer did not differ ($P > 0.05$) when the two breeds were compared. However, the FxH cows did show higher CLA levels than H cows. Schroeder *et al.* (2005) reported a significant difference with *c9,t11* and other CLA isomers.

Table 4.5 Mean \pm SD fatty acid content of milk

	Breeds		P <value
	Holstein	FxH	
Milk fat %	1.651 ^a \pm 0.039	1.807 ^b \pm 0.059	0.030
18:0	9.088 \pm 0.208	9.245 \pm 0.317	0.681
Total MUFA's	18.341 \pm 0.375	18.676 \pm 0.576	0.627
18:1n-9	16.193 \pm 0.346	16.546 \pm 0.533	0.579
Total PUFA's	1.941 ^a \pm 0.431	2.100 ^b \pm 0.064	0.043
LA (18:2n-6)	1.389 ^a \pm 0.036	1.519 ^b \pm 0.054	0.051
ALA (18:3n-3)	0.224 \pm 0.005	0.234 \pm 0.009	0.393
Total omega-6	1.651 ^a \pm 0.039	1.807 ^b \pm 0.059	0.030
Total omega-3	0.290 \pm 0.007	0.294 \pm 0.011	0.758
N-6/N-3 ratio	5.986 \pm 0.207	6.281 \pm 0.322	0.443
Total CLA	0.439 \pm 0.015	0.468 \pm 0.023	0.303
CLA (c9,t11 18:2)	0.408 \pm 0.014	0.440 \pm 0.023	0.244
Trans 18:1	0.332 ^a \pm 0.009	0.381 ^b \pm 0.014	0.005

All values except fat percentage are expressed as (g FA/100 g fat). Means \pm SD with different superscripts between breeds differ ($P < 0.05$). SFA's = saturated fatty acids; MUFA's = mono-unsaturated fatty acids; PUFA's = polyunsaturated fatty acids; LA= linoleic acid (18:2n-6); ALA= α -linolenic acid (18:3n-3); CLA= conjugated linoleic acid

Milk fat percentage showed a significant ($P < 0.05$) with lactation stage. Interaction between breed and lactation stage showed no significant difference. Both breeds displayed a similar pattern with milk fat peaking in test 7.

4.6 Individual FA group results

There was a significant difference ($P < 0.05$) between lactation stages for total CLA, as well as the main CLA isomer c9,t11. Figure 4.1 shows the increase in CLA content over the lactation period. Both these FA parameters increased over all three stages of the lactation period for both breeds, but appeared to be lower during late lactation for the H cows. No interaction was recorded with breed and lactation stage.

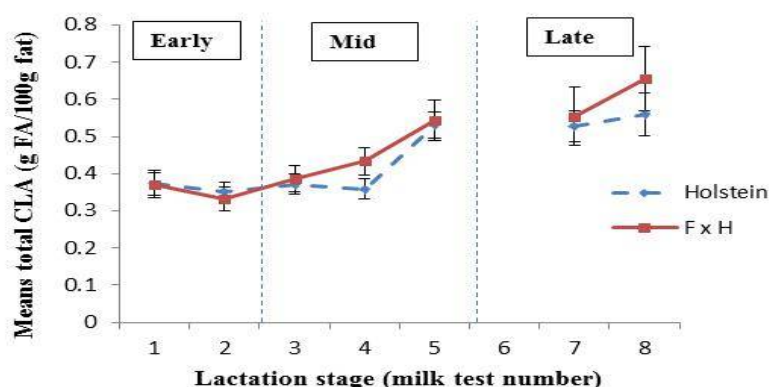


Figure 4.1 The effect of lactation stage and breed on total CLA content of the milk from Holstein and FxH cows. (CLA = conjugated linoleic acid; FxH = Fleckvieh x Holstein crossbred cows)

Although overall comparison between the two breeds did not show any significant increase in CLA (Total and isomer), there was a tendency for the FxH cows to have higher levels. This is borne out by the significant ($P<0.05$) higher levels of the main CLA precursors, *trans* 18:1 and 18:2n-6, in the FxH cows. Higher levels with regards to total CLA and the main *c9,t11* isomer in H cows and FxH cows ranged from 0.439 ± 0.015 to 0.468 ± 0.023 g/100 g fat and from 0.408 ± 0.014 to 0.440 ± 0.023 g/100 g fat, respectively. Overall, the *c9,t11* isomer represented 92% of the total CLA in the purebreds, while in the crossbreds it is 94%).

Total CLA content increased by 50% for H cows from early to late lactation, whereas it increased by 76% for FxH cows over the same period (Figure 4.2).

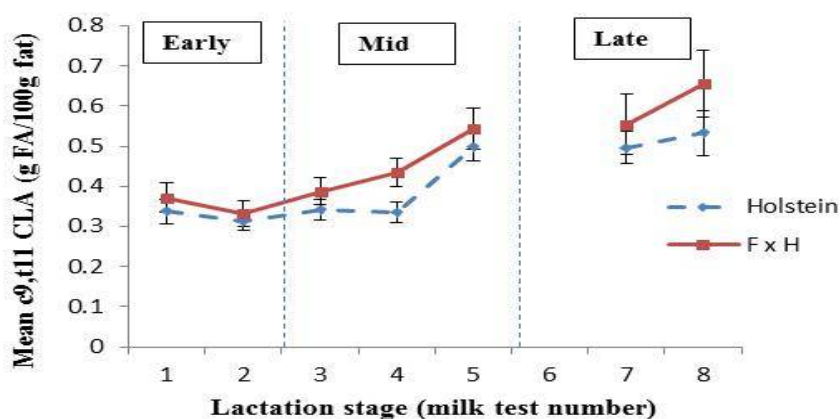


Figure 4.2 The effect of lactation stage and breed on *c9,t11* CLA isomer content of the milk from Holstein and FxH cows. (CLA = conjugated linoleic acid; FxH = Fleckvieh x Holstein crossbred cows)

There was no significant difference between lactation stage and breed interaction for total SFA's. A significant ($P<0.05$) difference with regards to breed interacting with lactation stage was shown for SFA 11:0 only. Similarly, Samkova *et al.* (2012) found a significant difference ($P<0.05$) for 18:0 between H and FxH cows on TMR, within their lactation stage. Schroeder *et al.* (2005) found comparable results with their study for Holstein cows on TMR feeding system.

Significant difference regarding lactation was observed in the total MUFA's. Levels tended to follow a similar pattern between the two breeds up to test 5, however from test 7 the crossbred cows showed an increase. Breed and lactations stage interaction showed no significant difference. For the individual MUFA's, there was a difference ($P<0.05$) between lactation stage with 14:1, 15:1, 16:1n-7 and 18:1n-9, while no significant difference was observed for *trans* 18:1. No significant differences were observed with total PUFA's regarding lactation stage. The omega-6 and omega-3 FA's followed the same pattern. No

interaction was recorded between breed and lactation stage. There was no significant difference with lactation stage for the total omega-3 PUFA, neither for 18:3n-3 and 22:5n-3, and the N-6/N-3 FA ratio.

4.7 Conclusion

Significant differences were found for several FA's with regards to breed and lactation stage, demonstrating that both parameters evidently affects the milk FA profile of cows under the present study conditions. CLA and c9,t11 had a higher percentage increase (50% vs 76%) in FxH compared to H cows, possibly due to crossbreeding with the Fleckvieh. The CLA profile is affected by lactation stage with both breeds demonstrating an increase from early lactation to late lactation.

References

- Cabrera, V.E. 2012. *DairyMGT: A Suite of Decision Support Systems in Dairy Farm Management*. http://www.intechopen.com/books/decision-support-systems_2012/dairymgt-a-suite-of-decision-support-systems-in-dairy-farm-management [12 June 2013].
- Caraviello, D.Z. 2004. Crossbreeding Dairy Cattle. <http://www.babcock.wisc.edu/node/508> [20 February 2012].
- Clancy, K. 2006. Greener Pastures: How grass-fed beef and milk contribute to healthy eating. Cambridge, Massachusetts: *Union of Concerned Scientists Publications*.
- Chouinard, P.Y., Corneau, L., Saebo, A. and Bauman, D.E. 1999. Milk yield and composition during abomasal infusion of CLA in dairy cows. *J. Dairy Sci.* 82:2737-2745.
- Christie, W.W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *J. Lipid Res.* 23:1072-1075.
- Christie, W.W. 1993. Preparation of lipid extracts from tissues. In Christie, W.W. (Ed.). *Advances in Lipid Methodology*. Dundee, UK: The Oily Press: 195-213.
- Collomb, M., Schmid, A., Sieber, R. and Wechsler, D. 2006. Conjugated linoleic acid in milk fat variation and physiological effects. *Int. Dairy J.* 16:1347-1361.
- Cruz-Hernandez, C., Kramer, J.K., Kennelly, J.J., Glim, D.R., Sorensen, B.M., Okine, E.K., Goonewardene, L. A. and Weselate, R.J. 2007. Evaluating the conjugated linoleic acid and *trans* 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. *Amer. Dairy Sci. Assoc.* 90:3786-3801.
- Hara, A. and Radin, N.S., 1978. Lipid extraction of tissues with low-toxicity solvent. *Anal. Biochem.* 90:420-426.
- Kelly, M.L., Kolver, E.S., Bauman, D.E., Van Amburgh, M.E. and Muller, L.D. 1998. Effect of intake of pasture on concentration of conjugated linoleic acid in milk of lactating dairy cows. *J. Dairy Sci.* 81(6):1630-1636.

- Lawless, F., Stanton, C., Escop, P.L., Devery, R., Dillon, P. and Murphy, J.J. 1999. Influence of breed on bovine milk *cis*-9, *trans*-11-conjugated linoleic acid content. *Livest. Prod. Sci.* 62:43-49.
- Liebenberg, L. 2007. Effect of nutrition on conjugated linoleic acid content of milk. MSc thesis, University of Stellenbosch, South Africa.
- Lock, A.L., Bauman, D.E. and Garnsworthy, P.C. 2005. Short communication: Effect of production variables on the *Cis*-9, *Trans*-11 conjugated linoleic acid content of cows' milk. *J. Dairy Sci.* 88:2714-2717.
- Mitchell, L. and Mcleod, S. 2008. Conjugated linoleic acid and atherosclerosis: studies in animal models. *Biochem. Cell Biol.* 86(4):293-301.
- Mierlita, D., Daraban, S.T. and Lup, F. 2011. Effects of breed on milk fatty acid profile in dairy ewes, with particular reference to *cis*-9, *trans*-11 conjugated linoleic acid. *S. Afr. J. Anim. Sci.* 41:224-231.
- Nichols, B.W. 1963. Separation of the lipid of photosynthesis tissue: improvements in analysis by thin-layer chromatograph. *Biochem. Biophys. Acta.* 70:417-422.
- Qureshi, M.S., Jan, S., Mushtaq, A., Rahman, I.U., Jan, M. and Ikramullah. 2012. Effect of age on milk fatty acids in dairy buffaloes. *J. Anim. Plant Sci.* 22(2):108-112.
- Samkova, E., Spicka, J., Pesek, M., Pelikanova, T. and Hanus, O. 2012. Animal factors affecting fatty acid composition of cow milk fat: A review. *S.Afr. J. Anim. Sci.* 42:83-99.
- Schroeder, G.F., Couderc, J.J., Bargo, F. and Rearte, D.H. 2005. Milk production fatty acid profile of milk fat by dairy cows fed a winter oats (*Avena sativa L.*) pasture only or a total mixed ration. *New Zealand J. Agric. Res.* 48:187-195.
- Smuts, C.M., Weich, H.F.H., Weight, M.J., Faber, M., Kruger, M., Lombard, C.J. and Benadé, A.J.S. 1994. Free cholesterol concentrations in the high-density lipoprotein subfraction-3 as a risk indicator in patients with angiographically documented coronary artery disease. *Coron. Artery Dis.* 5:331-338.
- Song, M.N. and Kennelly, J.J. 2003. Biosynthesis of Conjugated Linoleic Acid and its incorporation into ruminant's products. *Asian-Aust. J. Anim. Sci.* 16(2):306-314.
- Soriano, F.D., Polan, C.E. and Miller, C.N. 2001. Supplementing pasture to lactating Holstein fed a total mixed ration diet. *J. Dairy Sci.* 84:2460-2468.
- Tichelaar, H.Y., Benadé, A.J.S., Daubitzer, A.K., and Kotze, T.J. v.W. 1989. An improved rapid thin-layer chromatographic-gas-liquid chromatographic procedure for the determination of free fatty acids in plasma. *Clinica. Chimica. Acta.* 183(2):207-216.
- Underwood, J.P. 2002. Jersey versus Holsteins comparisons.
<http://www.livestocktrail.illinois.edu/dairynet/paperdisplay.cfm?contentid=351>.
 [20 July 2012]

White, S.L., Bertrand, J.A., Wade, M.R., Washburn, S.P., Green, J.T Jr. and Jenkins T.C., 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. *J. Dairy Sci.* 84(10): 2295-2301.

CHAPTER 5

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

For many years the nutritional image of milk fat has suffered because of the correlation between SFA's and chronic cardiovascular and coronary heart disease and diabetes (Kalac and Samkova, 2010; Butler *et al.*, 2007; McCrorie *et al.*, 2011; Kirchnerova *et al.*, 2013). Saturated FAs have therefore been considered an undesirable part of milk fat. However, this information is being questioned and scrutinized more closely, as more recent studies do not support the association between SFA's and cardiovascular diseases (Malhotra, 2013).

Milk contains FA's such as CLA, and omega-3 PUFA's, which may exert biological and physiological health benefits such as anti-carcinogenic, anti-diabetes, anti-adipogenic and anti-atherogenic effects. Recently, methods have been investigated to improve the composition of milk fat by modifying and supplementing the diet of dairy cows (Franklin *et al.*, 1999; Dhiman *et al.*, 2000; Rego *et al.*, 2004). In addition, crossbreeding dairy cows is a feasible method to modify composition (Lawless *et al.*, 1999; Wood *et al.*, 1980).

A number of factors can affect the level of CLA in milk, including diet, breed, lactation stage and seasonal variations. Several studies have compared the milk FA's and the CLA content in different dairy cows (White *et al.* 2001; Pesek *et al.*, 2005) and in ewes (Mihaylova *et al.*, 2004; Talpur *et al.*, 2009). The Fleckvieh dual-purpose breed is recognized for its fertility and higher CLA levels in both milk and beef (Muller *et al.*, 2013) therefore, crossbreeding with Fleckvieh bulls will enhance good quality crossbred products and lead to higher levels of CLA in milk and beef in crossbred animals.

The current study focused on the breed and lactation stage. Diet was not included as one of the factors affecting milk FA's, since two different feeding systems were utilised. Jersey and Holstein dairy cows were crossed with Fleckvieh dual-purpose sires, with J and FxJ cows being put on a pasture-based feeding system and the H and FxH cows being put on TMR feeding system.

5.2 Effect of pasture vs. TMR feeding systems on milk FA content

Regarding milk FA content, the Jersey, Holstein and their respective crossbreds could not be compared directly because both breeds were in different feeding systems. However, the FA content of the two feeding systems can be compared and an expected outcome on milk FA content projected (Table 5.1). Total SFA's in the pasture- and TMR-based feeding were

similar, which may be the reason similar levels in the milk content of all tested breeds were found. The levels of MUFA's tended to be higher in the TMR feeding system in comparison to the pasture-based system. Kelly *et al.* (1998) reported high levels of unsaturated FA's, particularly the PUFA's, in a pasture-based feeding system and in the milk of the cows on pastures. Similar PUFA levels in the milk from cows on the two different feeding schemes were observed. However, the high PUFA, mainly due to 18:3n-3, content in the pasture feed was not reflected in the milk of the Jerseys or their crossbreds.

Table 5.1 Comparison of the average FA's in feed and milk

FA's	Pasture	Pellets	TMR	Pasture		TMR	
				Jersey	FxJ	Holstein	FxH
Total SFA's	3.60	3.28	3.52	42.71	44.5	42.69	44.46
Total MUFA's	0.61	1.96	2.93	16.14	17.7	18.34	18.67
Total PUFA's	12.08	2.29	4.83	1.86	2.08	1.94	2.10
Total omega-6	2.55	2.86	3.77	1.65	1.80	1.53	1.74
Total omega-3	9.53	1.43	1.06	0.32	0.34	0.29	0.29
Total CLA	--	--	--	0.63	0.77	0.43	0.46
18:2n-6	1.89	2.53	3.55	1.34	1.50	1.38	1.51
18:3n-3	9.50	1.41	1.04	0.26	0.27	0.22	0.23
c9,t11	--	--	--	0.58	0.67	0.40	0.44
Trans 18:1	--	--	--	0.40	0.46	0.33	0.38

Values expressed as g FA/100 g fat (milk) and g FA/100g DM (pasture and TMR). Values represent means across the total lactation stage of the study.

In general, the crossbred cows showed higher levels than purebred cows with regard to total CLA and its main isomer (c9,t11), omega-3 (18:3n-3), omega-6 (18:2n-6) and *trans* 18:1 as shown in Table 5.1. Only 18:2n-6 showed a marginal difference in the FxH compared to their Holstein purebreds, although total CLA and its main isomer (c9,t11) as well as omega-3 (18:3n-3) tended to be higher compared to purebreds. *Trans* 18:1 showed a significant difference in the FxH.

Table 5.2 is a summary of the main FA's present in milk relevant to CLA between the milk FA profiles in the milk of Jersey and Fleckvieh x Jersey (FxJ) cows in a pasture-based feeding system and Holstein and Fleckvieh x Holstein (FxH) cows in a TMR-based feeding system.

Table 5.2 Comparison of the FA's from both feeding systems

FA	Pasture-based feeding			TMR-based feeding		
	Jersey	FxJ	P-value	Holstein	FxH	P-value
Total CLA	0.630 ^a ± 0.024	0.728 ^b ± 0.018	0.0004	0.439 ± 0.015	0.468 ± 0.015	0.3037
c9, t11	0.584 ^a ± 0.023	0.678 ^b ± 0.018	0.0003	0.408 ± 0.014	0.440 ± 0.023	0.2448
18:3n-3	0.263 ± 0.014	0.278 ± 0.010	0.3455	0.224 ± 0.005	0.234 ± 0.009	0.3937
18:2n-6	1.342 ^a ± 0.043	1.506 ^b ± 0.032	0.0005	1.389 ± 0.047	1.519 ± 0.035	0.0518
Trans 18:1	0.402 ^a ± 0.015	0.462 ^b ± 0.011	0.0004	0.332 ± 0.009 ^a	0.381 ± 0.014 ^b	0.0058

Values with different superscripts indicate significant differences ($p < 0.05$). Values are expressed as g FA/100 g fat.

It is evident from Table 5.2 that Total CLA and its isomer, c9,t11, are higher in the milk of the cows on the pasture-based feeding system than for the TMR-based fed cows. A number of reasons can be attributed to these results, other than the breed difference. The role of diet is significant and this is re-enforced by the evidence from several studies showing higher levels of CLA in a pasture feeding system than TMR system (Kolver and Muller, 1998; Kelly *et al.*, 1998; Jensen, 2002; Stockdale *et al.*, 2003; Kirchnerova *et al.*, 2013).

The results were also in agreement with those of Cabiddu *et al.* (2001) and Schroeder *et al.* (2005) who found low levels of PUFA's, particularly the CLA, on TMR and higher levels of CLA on winter oats (*Avena sativa L.*) pastures. Collomb *et al.* (2006) also stated that dairy cows on a TMR feeding system have lower CLA levels, and that dairy cows on pasture produce up to two or three times higher CLA levels (Atti *et al.*, 2006; Liebenberg, 2007).

Furthermore, the higher CLA in milk could be because 18:3n-3 is converted to *trans* 18:1 in the rumen and later converted to CLA in the tissues, i.e. 18:3n-3 is an additional source of CLA (Shingfield *et al.*, 2012). This could explain the lower than expected 18:3n-3 in the milk.

5.3 Pasture intake and pellet supplementation effect on milk FA profile

Jersey and FxJ cows were on pasture, consuming an estimated average of 10 kg dry matter (DM) pasture per day. In addition, they were fed 3.5 kg of pellets at the end of each morning and evening's milking sessions, for a total pellet intake of 7 kg/day. Table 5.3 indicates the average total feed plus pellet intake per day over the four seasons, i.e. season 1 (February-April), season 2 (May-July), season 3 (August-October) and season 4 (November-January). Pellets were supplemented to the dairy cows to increase the total daily energy and protein intake. Both breeds received a total of 16 - 17 kg DM feed per day consisting of a pasture intake of about 10 kg DM per day and about 6.3 kg DM as pellets. The pasture and pellet intake therefore constituted about 61 and 39%, respectively, of the total daily feed intake for both Jersey and FxJ cows.

Season 1 and 2 showed higher levels of 18:2n-6 in the diet as it was shown in milk that early lactation 18:2n-6 was slightly higher than mid lactation and late lactation stage showed an increase and higher levels were observed. Lower levels with the FA profile were observed in season 3 (spring), probably due to the fact that in spring the grass is in its early stages of growth after winter. As mentioned, the total omega-3 and 18:3n-3 levels in the milk did not reflect the overall pasture FA content. Comparing total omega-3 and 18:3n-3 in Table 5.3 with respective values in Table 5.4, the omega-3 is much lower than expected when only taking pasture into consideration, i.e. 9.53 and 9.50 g FA/100 g DM vs. the totals for omega-3 and 18:3n-3 in milk, respectively. In Table 5.4 values were 5.64 ± 1.38 and 5.62 ± 1.38 , 5.13 ± 1.57 and 5.10 ± 1.56 , 2.80 ± 0.32 and 2.80 ± 0.31 and 5.65 ± 1.83 and 5.62 ± 1.81 g FA/100 g DN for all seasons, respectively. If recalculated according to intake as described above, it is evident that supplementation with pellets diluted the omega-3 intake level. This could be the dilution effect of the 7 kg of pellets supplemented to the cows per day which contains high levels of omega-6. In addition, the level of 18:3n-3 could be further diluted by being used as an additional source of CLA, thereby decreasing 18:3n-3 availability for milk incorporation.

With regards to CLA in the milk profile, the *c9,t11* and *trans* 18:1 showed slightly higher levels in the pasture-based feeding system compared to TMR (Table 5.3), probably due to the conversion of 18:3n-3 to CLA in the rumen and tissues (udder). It is evident that dairy cows on pasture-based feeding system have a better chance of containing higher milk CLA, although CLA content of dairy cows on TMR feeding system could be increased through the manipulation of the diet with supplemental feeding of products containing high 18:3n-3 FA, i.e. with oilseeds such as canola oil.

Table 5.3 FA intake from pasture and pellets by Jersey and FxJ cows

FA's	Pasture with pellets FA daily intake								
	Season 1		Season 2*		Season 3		Season 4		Pellets
	Pasture	Total	Pasture	Total	Pasture	Total	Pasture	Total	
18:1n-9	0.24 ± 0.04	1.55 ± 0.18	0.35 ± 0.12	1.66 ± 0.17	0.07 ± 0.01	1.39 ± 0.16	0.19 ± 0.02	1.50 ± 0.16	1.31 ± 0.16
18:2n-6	1.37 ± 0.31	2.74 ± 0.38	1.46 ± 0.44	2.83 ± 0.49	0.68 ± 0.07	2.05 ± 0.21	0.94 ± 0.27	2.32 ± 0.40	1.37 ± 0.19
18:3n-3(ALA)	5.48 ± 1.39	5.62 ± 1.38	4.97 ± 1.43	5.10 ± 1.56	2.66 ± 0.30	2.80 ± 0.31	5.48 ± 1.65	5.62 ± 1.81	0.14 ± 0.01
Total SFA's	2.23 ± 0.46	3.43 ± 0.49	2.32 ± 0.56	3.51 ± 0.58	0.84 ± 0.06	2.04 ± 0.16	1.90 ± 0.55	3.09 ± 0.70	1.19 ± 0.10
Total MUFA's	0.49 ± 0.09	1.86 ± 0.20	0.52 ± 0.14	1.89 ± 0.19	0.17 ± 0.03	1.54 ± 1.57	0.27 ± 0.07	1.64 ± 0.20	1.37 ± 0.15
Total PUFA's	6.95 ± 1.71	8.53 ± 1.72	6.65 ± 1.90	8.24 ± 2.07	3.51 ± 0.42	5.09 ± 0.47	7.18 ± 2.13	8.77 ± 2.38	1.58 ± 0.17
Total omega-6	1.46 ± 0.33	2.90 ± 0.38	1.67 ± 0.46	3.11 ± 0.52	0.85 ± 0.09	2.29 ± 0.21	1.67 ± 0.47	3.11 ± 0.61	1.44 ± 0.18
Total omega-3	5.50 ± 1.39	5.64 ± 1.38	4.98 ± 1.44	5.13 ± 1.57	2.66 ± 0.32	2.80 ± 0.32	5.51 ± 1.67	5.65 ± 1.83	0.14 ± 0.01
N6/N3 ratio	0.16 ± 0.01	4.37 ± 0.68	0.20 ± 0.01	4.41 ± 0.67	0.19 ± 0.01	4.40 ± 0.68	0.18 ± 0.02	4.39 ± 0.69	4.21 ± 0.68
LA/ALA ratio	0.15 ± 0.01	4.25 ± 0.73	0.17 ± 0.01	4.27 ± 0.73	0.15 ± 0.01	4.25 ± 0.73	0.10 ± 0.01	4.20 ± 0.73	4.10 ± 0.73

Cows consumed an average 10 kg DM pasture per day and received 7 kg supplemented pellets per day. Pasture represents 59% (10 kg) and pellets 41% (7 kg) of the total feed intake of 17 kg per day of average intake. Pellets were supplemented after each milking session. Values expressed as g FA/100 g DM. Season 1 (February-April), Season 2 (May-July), Season 3 (August-October) and Season 4 (November-January)

*Season 2 (winter) supplemented with lucerne

5.4 CLA milk content of purebred and crossbred cows on two different feeding systems

The effect of lactation stage on the CLA content of the milk of Jersey and FxJ and Holstein and FxH cows is presented in Figure 5.1. Even though crossbred cows showed higher levels of total CLA compared to purebreds, all breeds displayed an increase for total CLA content from early lactation stage towards the late lactation stage.

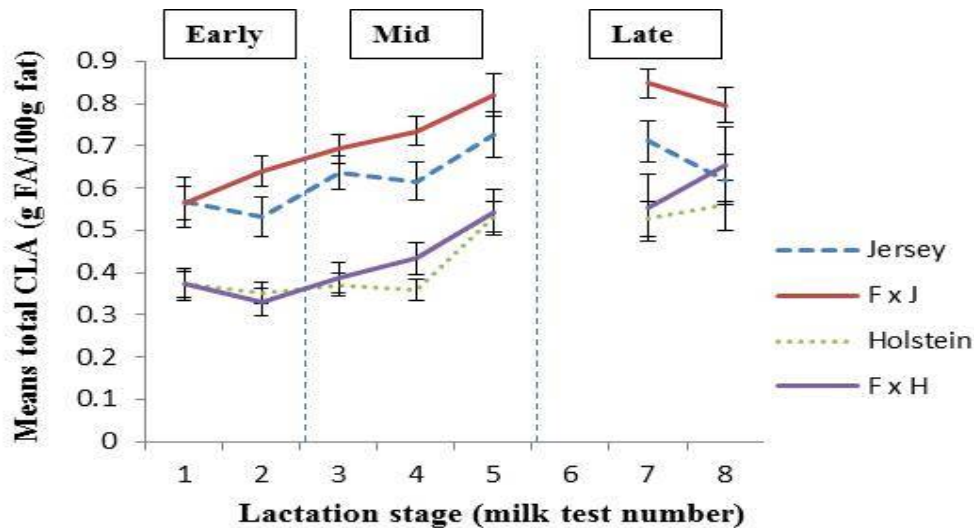


Figure 5.1 The effect of lactation stage on the means of total CLA content of the milk of Jersey and F x J on pasture-based feeding system and Holsteins and F x H in a TMR feeding system.

of the milk of Jersey and FxJ on pasture-based feeding system and Holsteins and FxH in a TMR feeding system.

Studies by Kelly *et al.* (1998) and Samkova *et al.* (2012) have also found similar results. This means that as the stage of lactation advances, the milk production slowly decreases and the total FA profile (including CLA) increases, i.e. a concentrating effect on milk FA content.

Results that were obtained in this study are similar to studies from other countries (Samkova *et al.*, 2012; Schroeder *et al.*, 2005; Kirchnerova *et al.*, 2013) which indicate that breed and lactation stage are significant factors affecting the CLA content of milk fat. A number of studies suggest a pasture feeding system as being more advantageous for dairy breeds, if the type of FA in the milk is considered. Cows grazing pasture produce higher CLA and omega-3 levels than in a TMR feeding system (Kalac and Samkova, 2010), potentially ensuring a healthier diet for consumers.

5.5 Conclusion

Milk is considered an important functional food, containing many biological components benefiting human health. These components have the potential for easy manipulation by modulation of feed content and by utilizing the appropriate breed or even by crossbreeding. This study serves as a reference that CLA content in milk fat of dairy cows can be increased through crossbreeding and pasture feeding. Better understanding of the mechanism of CLA biosynthesis will allow the design of optimal diets, which in turn will lead to enhanced CLA concentration in milk fat. Within the limits of the conditions of this study, the results indicated that breed and lactation stage have an effect on CLA in milk fat, implying that breed selection could be used as a tool to improve milk quality in terms of PUFA's and CLA content for human consumption. Crossbreeding with Fleckvieh could result in crossbreds that would ensure income through beef production in Jerseys and Holsteins, while not having any negative effect on the milk yield of crossbred cows and also improving the CLA levels of the two crossbreds.

Literature demonstrated that milk from Fleckvieh cows have a higher CLA content (Liebenberg, 2007). In this regard, crossbreeding is an economical and viable option for introducing the benefits of Fleckvieh in the milk production system on a dairy farm. Crossbreeding Jersey and Holstein cows with the Flechvieh breed has shown higher levels of CLA on pasture-based and TMR feeding system in comparison to the two pure breeds. This clearly demonstrates that Fleckvieh has higher levels of CLA. Holstein cows and their crossbreds on TMR showed lower levels than those on pastures. Although not directly comparable, it does appear to support the claims that have been made by other studies that cows on pastures produce higher levels of CLA than those on TMR.

CLA has been shown to have many biological activities, i.e. possessing anti-cancer activity, immune-enhancing activity, weight-reducing effects and possible anti-atherogenic properties, in animals and humans. Because of these biological properties of CLA, there has recently been a lot of interest in enriching dairy products for human consumption. However, since CLA is a mixture of several isomers, each isomer might have different activities. Currently, the c9,t11 CLA and t10,c12 CLA are the only isomers that have actually been shown to exert physiological effects in animal experiments. With the potential anti-carcinogenic properties and other health benefits that can be derived from consuming CLA, it should be a major focus that researchers and producers find feasible ways of increasing CLA in milk fat.

An opportunity to establish a niche market for the milk from FxJ and FxH cows is possible, because the crossbred cows were found to have higher levels of CLA in both groups. Of further interest is the potentially higher omega-3 FA milk content which showed higher levels

in the crossbreds and which is also associated with health benefits to humans. Further studies concerning the CLA content of milk products, such as cheese, and beef may further improve the financial situation of commercial and emerging farmers.

Recommendations

Based on the literature review and the results of the study, the following recommendations can be made:

1. Dairy producers interested in optimizing the levels of omega-3 and CLA content in milk should opt for pasture-based feeding systems..
2. The TMR feeding system has little effect on the improvement of CLA and the omega-3 content in the milk of dairy cows, except if alternative supplements are added to the feed.
3. In order to increase the omega-3 and CLA content in milk of dairy cows in a TMR feeding system, different types of oils may be added to the diet as supplements.

Follow-up studies

1. The effect of processing and storage of cheese and beef products on the CLA content.
2. Determining the seasonal variation in feed and how the milk CLA and FA profile changes with season.

References

- Atti, N., Rouissi, H. and Othmane, M. H. 2006. Milk production, milk fatty acid composition and conjugated linoleic acid (CLA) content in dairy ewes raised on feedlot or grazing pasture. *Livest. Sci.* 104:121-127.
- Butler, G., Stergiadis, S., Eyre, M., Leifert, C., Borsari, A., Canever, A., Slots, T. and Nielsen, J.H. 2007. Effect of production system and geographic location on milk quality parameters. *Proc. 3rd QLIF Congr.* Hohenheim, 20-23 March 2007.
- Cabiddu, A., Decandia, G., Molle, G., Piredda, G., Pirisi, A. and Addis, M. 2001. Effect of diet on conjugated linoleic acid (CLA) content of milk and sheep cheese. *Proc. Ass. Spec. Prof. Accr Congr.* Fireze, 12–15 June 2001.
- Collomb, M., Schmid, A., Sieber, R., Wechsler, D. and Ryhanen, E.L. 2006. Conjugated linoleic acid in milk fat variation and physiological effects. *Int. Dairy J.* 16:1347-1361.
- Dhiman, T.R., Satter, L.D., Pariza, M.W., Galli, M.P., Albright, K. and Tolosa, M.X. 2000. Conjugated linoleic acid content of milk from cows offered diets rich in linoleic and linolenic acid. *J. Dairy Sci.* 83(5):1016-1027.
- Franklin, S.T., Martin, K.R., Baer, R.J. and Schingoethe, D.J. 1999. Dietary marine algae

- (*Schizochytrium spp.*) increase concentrations of conjugated linoleic acid, conjugated linoleic acid, docosahexanoic acid and transvaccenic acid of milk in dairy cows. *J. Nutr.* 129:2048-2052.
- Jensen, R.G. 2002. The composition of bovine milk lipids. January 1995 to December 2000: A review. *J. Dairy Sci.* 85(2):295-350.
- Kalac, P. and Samkova, E. 2010. The effects of feeding various forages on fatty acid composition of bovine milk: A review. *Czech J. Anim. Sci.* 55:521-537.
- Kelly, M.L., Kolver, E.S., Bauman, D.E., Van Amburgh, M.E. and Muller, L.D. 1998. Effect of intake of pasture on concentration of conjugated linoleic acid in milk of lactating dairy cows. *J. Dairy Sci.* 81:1630-1636.
- Kirchnerová, K., Foltys, V. and Špička, J. 2013. Impact of lactation stage on milk fat fatty acids profile in grazing dairy cows. *J. Microbiol. Biotechnol. Food Sci.* 2:1164-1174.
- Kolver, E.S. and Muller, L.D. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. *J. Dairy Sci.* 81:1403-1411.
- Lawless, F., Stanton, C., Escorp, P.L., Devery, R., Dillon, P. and Murphy, J.J. 1999. Influence of breed on bovine milk *cis*-9, *trans*-11 conjugated linoleic acid. *Livest. Prod. Sci.* 62:43-49.
- Liebenberg, L. 2007. Effect of nutrition on conjugated linoleic acid content of milk. MSc thesis, University of Stellenbosch, South Africa.
- Malhotra, A. 2013. Saturated fat is not the major issue. *BMJ.* 347:f6340.
- McCrorie, T.A., Keaveney, E.M., Wallace, J.M, Binns, N. and Livingstone, M.B. 2011. Human health effects of conjugated linoleic acid from milk and supplements. *Nutr. Res. Rev.* 24:206-227.
- Mihaylova, G., Gerchev, G., Moeckel, P. and Jahreis, G. 2004. Comparative study on fatty acid content in milk of Tsigay and Karakachan sheep. *Bulgarian J. Vet. Med.* 7:181-187.
- Muller, C.J.C., Sasanti, B., Abel, S and Schmulian, A. 2013. The milk fatty acid composition and conjugated linoleic acid content of Jersey and Fleckvieh x Jersey cow milk in a pasture-based feeding system. *Fleckvieh World 2013:* 20-25.
- Pešek, M., Špička, J. and Samkova, E., 2005. Comparison of fatty acid composition in milk fat of Czech Pied and Holstein cattle. *Czech J. Anim. Sci.* 50, 122-128.
- Rego, O.A., Portugal, P.V., Sousa, M.B., Rosa, H.J.D., Vouzela, C.M., Borba, A.E.S. and Bessa, R.J.B. 2004. Effects of diet on the fatty acid pattern of milk from dairy cows. *Anim. Res.* 53:213-220.
- Samková, E., Špička, J., Pešek, M., Pelikánová, T. and Hanus, O. 2012. Animal factors affecting fatty acid composition of cow milk fat: A review. *S. Afr. J. Anim. Sci.* 42:83-99.
- Schroeder, G.F., Couderc, J.J., Bargo, F. and Rearte, D.H., 2005. Milk production fatty acid

- profile of milk fat by dairy cows fed a winter oats (*Avena sativa L.*) pasture only or a total mixed ration. *New Zealand J. Agric. Res.* 48:187-195.
- Shingfield, K.J., Kalrenius, P., Arola, A., Paillard, D., Muetzel, S., Ahvenjarvi, S., Vanhatalo, A., Huhtanen, P., Tivonen, V., Griinari, J.M. and Wallace, R.J. 2012. Dietary fish oil supplements modify ruminal biohydrogenation, alter the flow of fatty acids at the omasum, and induce changes in the ruminal *butyrivibrio* population in lactating cows. *J. Nutr.* 142(8):1437-1448.
- Stockdale, C.R., Walker, G.P., Wales, W.J., Dalley, D.E., Birkett, A., Shen, Z. and Doyle, P.T. 2003. Influence of pasture and concentrates in the diet of grazing dairy cows on the fatty acid composition of milk. *J. Dairy Res.* 70:267-276.
- Talpur, F.N., Bhangar, M.L. and Memon, N.N. 2009. Milk fatty acid composition of indigenous goat and ewe breeds from Sindh, *Pakistan. J. Food Comp. Anal.* 22: 59-64.
- White, S., Bertrand, J.A., Wade, M.R., Washburn, S.P., Green, J.T. and Jenkins, T.C. 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or total mixed ration. *J. Dairy Sci.* 84(10):2295-2301.
- Wood, J.D., Macfie, H.J., Pomeroy, R.W. and Twinn, D.J. 1980. Carcass composition of four sheep breeds: The importance of type of breed and stage of maturity. *Anim. Prod.* 30: 135-152.

APPENDICES

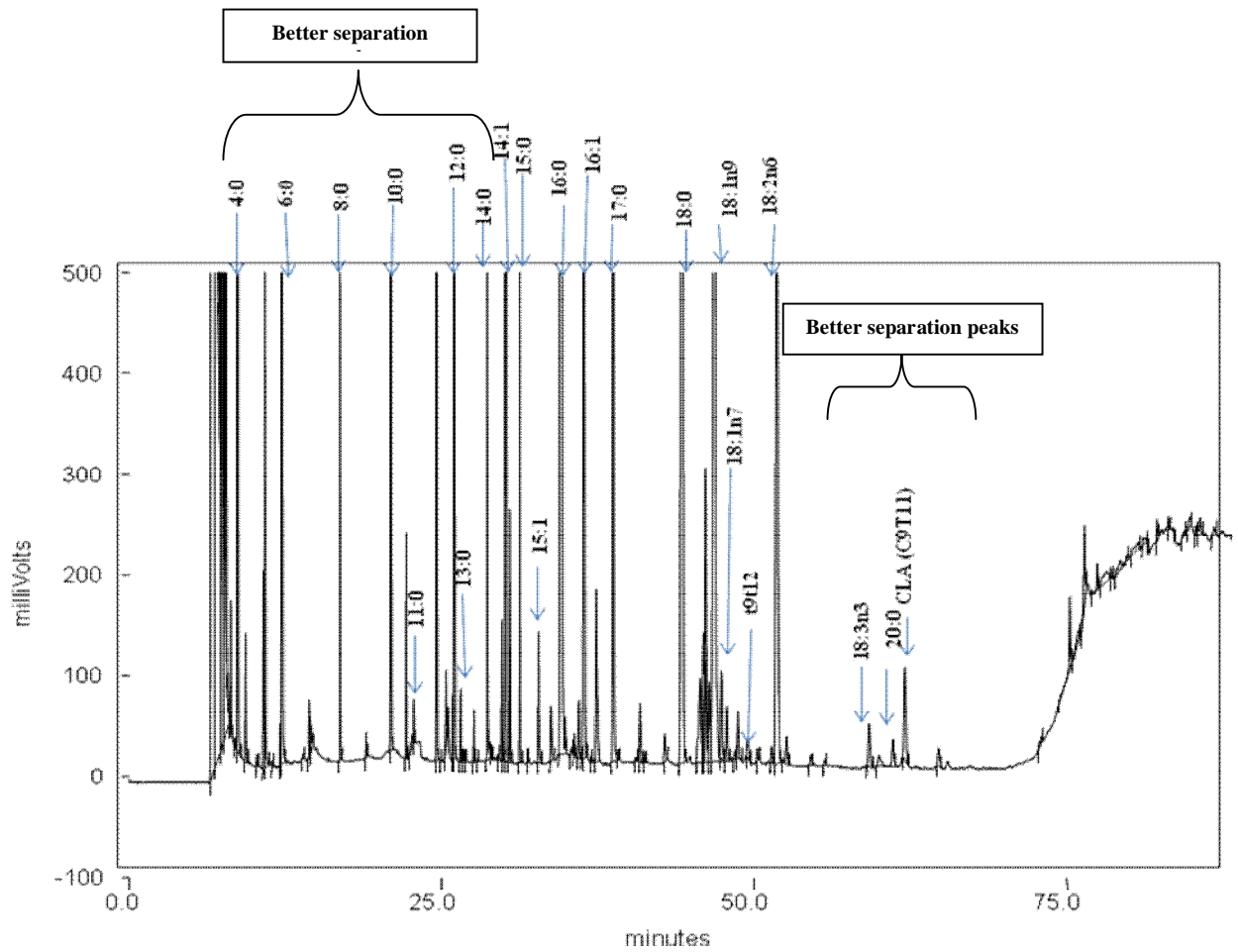
Appendix A: Long column chromatography (120 m column)

Gas chromatography is a chromatographic technique that can be used to separate volatile organic compounds (Collomb *et al.*, 2008). A gas chromatograph consists of a flowing mobile gas phase, an injection port, a separation column containing the stationary phase, a detector, and a data recording system (Appendix C). The organic compounds are separated due to differences in their partitioning behaviour between the mobile gas phase and the stationary phase in the column (Seppanen-Laasko *et al.*, 2002). The stationary phase of the SGE BPX-70 capillary column used in this study is 70% cyanopropyl polysilphenylene-siloxane. FA's in milk and feed were analyzed by using different GC column lengths and conditions as stipulated by the American Oil Chemists Society (AOCS), for ruminant animals (AOCS Official Method Ce 1j-07, 2009).

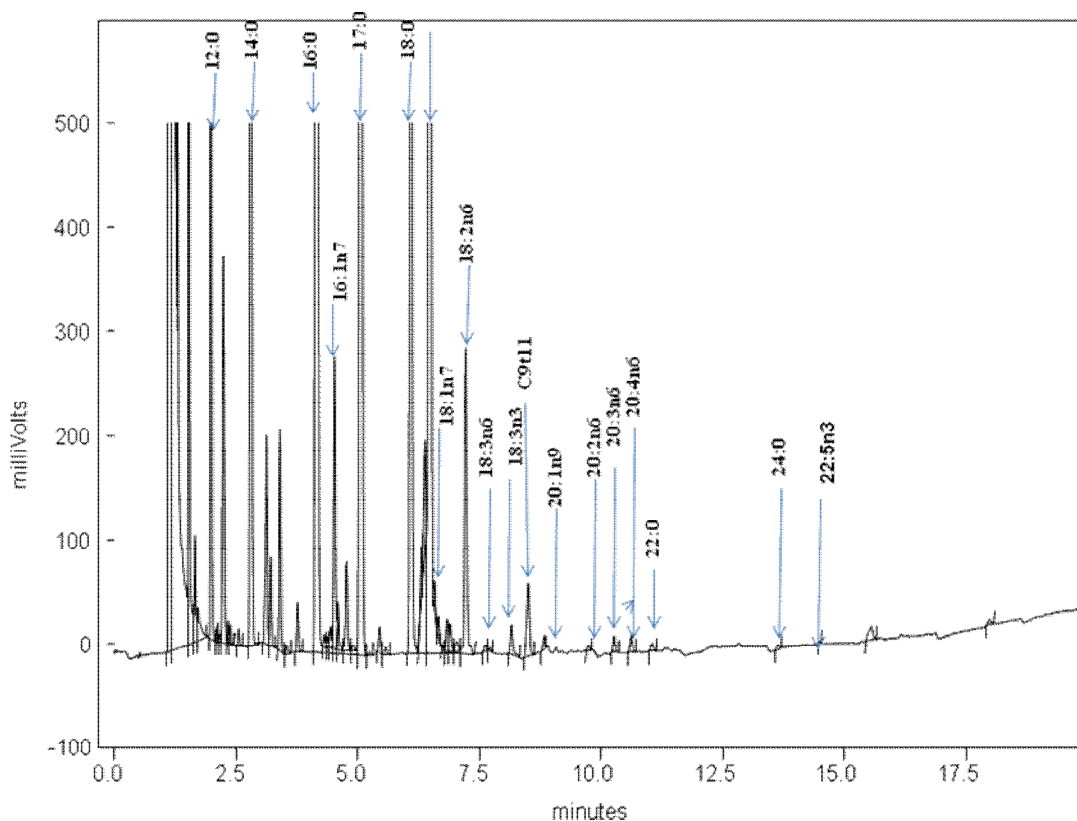
Gas chromatography analyses is still considered the method of choice for routine analysis of FA's and CLA isomers as endorsed by the AOCS to standardize methodology to determine FA and CLA in dairy samples (De La Fuente *et al.*, 2006; Christie, 1982: AOCS Official Method Ce 1j-07, 2009). For milk analysis, the use of a 100-120m polar column allows clear and reasonable separation of the different 18:2 isomers. The type of column used is also important, as the long flexible fused silica columns coated with highly polar polysiloxane stationary phase, such as the CP- Sil 88; SP 23810; SP 2560; SGE BPX-70, gives much better separation than the carbowax type. With columns that are generally 100 m long, 4 CLA peaks, i.e. in the order: c9,t11 18:2; t8,c10 18:2; c11,t13 18:2; t10,c12 18:2 are identifiable. These are then followed by the *cis/cis* configuration, and then the *trans / trans* groups. FA peaks are identified by a comparison of the retention times with methylated FA (FAME) standards.

Differences in FA analyses using a long capillary column vs a short column

Initially, samples were tested on 120m and 30m GC columns. The long column was selected as all individual FA peaks were separated and resolved well from 4:0 to 20:0, including the CLA, compared to the short 30 m column (fig 2). The long column could separate the major CLA peak, c9,t11 18:2, from 20:0, whereas the shorter 30m column could not.



Appendix B: Short column chromatography (30 m column)



Appendix B shows how the peaks were separated less clearly by the short GC capillary column (30m). This is of particular importance in the region where the c9,t11 CLA isomer appears, which may interfere/co-elute with the same region for 20:0. Therefore, the 120 m column was selected as all individual FA peaks would be separated better and well resolved than with 30 m column.

Appendix C: Gas Chromatograph Instrument

Varian 3300 for milk analysis and feed analysis, dual channel



The milk, feed and pasture FA analyses were done using the gas chromatograph (GC) instrument, a Varian model 3400.

Appendix D: Saturated FA's differed significantly between Jersey and Fleckvieh x Jersey (F×J) cows

FA's	Breeds		P-values
	Jersey	Fleckvieh x Jersey (F×J)	
4:0	1.630 ^a ±0.052	1.735 ^b ±0.059	0.020
8:0	0.748 ^a ±0.023	0.780 ^b ±0.018	0.052
10:0	1.745 ^a ±0.062	1.792 ^b ±0.047	0.005
12:0	2.050 ^a ±0.068	2.083 ^b ±0.052	0.002
13:0	0.059 ^a ±0.002	0.062 ^b ±0.001	0.002
14:0	6.999 ^a ±0.192	7.262 ^b ±0.001	0.001
15:0	0.697 ^a ±0.020	0.754 ^b ±0.015	0.001
16:0	18.601 ^a ±0.438	19.409 ^b ±0.328	0.039
22:0	0.047 ^a ±0.001	0.047 ^b ±0.001	0.008

^{ab}Values means ± SD with different superscripts between breeds differ at (p<0.05)

Appendix E: Saturated FA's differed significantly between Holstein and Fleckvieh x Holstein (F×H) cows

FA's	Breeds		P-values
	Holstein	F×H	
10:0	1.409 ^a ±0.032	1.604 ^b ±0.049	0.001
12:0	1.719 ^a ±0.038	1.890 ^b ±0.058	0.015
13:0	0.065 ^a ±0.001	0.067 ^b ±0.001	0.001
14:0	6.728 ^a ±0.115	7.115 ^b ±0.176	0.070
14:1	0.517 ^a ±0.016	0.474 ^b ±0.025	0.001
18:0	9.088 ^a ±0.2089	9.245 ^b ±0.317	0.039

^{ab}Values means ± SD with different superscripts between breeds differ at (p<0.05)

Individual FA's 10:0, 12:0 showed difference ($P<0.05$) with breed, lactation stage and 13:0, 14:0, 14:1 and 18:0 showed a difference ($P<0.05$) with lactation stage.

Appendix F: Study Location (Elsenburg dairy farm)

The Elsenburg Research Farm of the Western Cape Department of Agriculture is located on a fairly sloped area in Stellenbosch with many grape farms around which mainly produce wine. Elsenburg is situated approximately 50 km east of Cape Town in the winter rainfall region of South Africa. The area has a typical Mediterranean climate with short, cold, wet winters and long, dry summers (Muller *et al.* 2006). The annual rainfall is approximately 630mm per annum and average temperature is 30 to 35°C in summer and 11 to 20°C in winter. The rainfall occurs mainly in winter from May to August. The soils in the area are mainly from granite form; soil classification is categorized according to taxonomic criteria for South Africa. The reason behind this classification of soil types is to plant or cultivate a pasture that is correctly aligned with the soil type. This will ensure optimal usage of the pastures, ie optimal pasture growth and sustainability, for milk production from the dairy cows.

Elsenburg Map (study location)

