THE EFFECT OF DIETARY CONJUGATED LINOLEIC ACID SUPPLEMENTATION ON THE PHYSICOCHEMICAL, NUTRITIONAL AND SENSORY QUALITIES OF PORK

by

CARINA BOTHMA (*nee* MYBURG)

Submitted in fulfillment of the requirements
For the degree of

PHILOSOPHIAE DOCTOR

in the
Department of Microbial, Biochemical and Food Biotechnology
Faculty of Natural and Agricultural Sciences
University of the Free State
Bloemfontein, South Africa

Promoter: Prof. A. Hugo
Co-promoter: Prof. H.L. de Kock

February 2012

TABLE OF CONTENTS

CHAPTER	CHAPTER TITLE	PAGE
	ACKNOWLEDGEMENTS	iv
	LIST OF TABLES	vi
	LIST OF FIGURES	viii
	GLOSSARY OF ABBREVIATIONS	ix
	DEDICATION	xv
1.	**INTRODUCTION**	**1**
2.	**LITERATURE REVIEW**	**4**
	HISTORICAL INTRODUCTION	4
	CONSUMPTION PATTERNS OF PORK	4
	SOUTH AFRICAN CONSUMPTION OF PORK	7
	PORK QUALITY	8
	Lipid Quality	9
	Technological Quality	**10**
	Consumer Quality	**11**
	Fat Deposition in the Pig	**13**
	Fat Composition of the Pig	**16**
	Fatty Acid Composition of Adipose Tissue and Muscle in Pigs	*16*
	Fatty Acid Composition of Triacylglycerols (Neutral Lipids) and Phospholipids	*17*
	Effects of Fat Content on Fatty Acid Composition	*17*
	Genetic Effects on Fatty Acid Composition	*18*
	Diet Effects on Fatty Acid Composition	*19*
	WHAT IS CONJUGATED LINOLEIC ACID (CLA)?	21
	Background to Conjugated Linoleic Acid	**22**
	Controversy surrounding Conjugated Linoleic Acid	**25**
	Structure of Conjugated Linoleic Acid	**28**
	Origins of Conjugated Linoleic Acids in the Human Diet	**29**
	Formation of Conjugated Linoleic Acid Isomers	**31**
	Biosynthesis	*31*
	Chemical Synthesis	*32*
	Conjugated Linoleic Acid Pork Research	**32**
	Animal Performance	*33*
	Carcass Composition	*34*

Pork Quality 36
Fatty Acids 38
Sensory Analysis 40
CONCLUSIONS 42

3. MATERIALS AND METHODS 44
Animal Feeding Experiment 44
Diets 44
Oil and Feed Analysis 44
Slaughter and Carcass Measurements 45
Tissue Sampling 48
Intramuscular and Backfat Quality 49
Lipid Extraction and Fractionation 49
Fatty Acid Analysis 50
Differential Scanning Calorimetry 51
Other Fat Quality Parameters 51
Sensory Analysis 51
Training of Sensory Panel 51
Preparation of Sensory Samples 52
Physical Texture Analysis 54
Chemical and Oxidative Stability Studies 54
Accelerated Oxidation Test (Schaal Oven Test) 54
Colour and Lipid Stability of Fresh and Frozen Pork Chops 54
Oxidative Stability of Frozen Pork Patties 55
Quality and Oxidative Stability of Fermented Salami 55
Quality, Oxidative Stability and Consumer Preference of Bacon 57
Reagents 58
Statistical Analysis 59
Statistical Analysis of Quantitative Descriptive Analysis on Sensory Properties of Fresh Pork 59
Statistical Analysis of Sensory Data from Consumer Panel on Bacon 59
Statistical Analysis of Animal Production and Chemical Data 59

4. RESULTS AND DISCUSSION 60
Feed Composition 60
Growth Performance and Carcass Characteristics 66
Quality Characteristics of the *M. longissimus thoracis* measured between the 2nd and 3rd rib 68
Quality Characteristics of Backfat measured between the 2nd and 3rd last rib 70

Physical and Chemical Properties of Subcutaneous Fat **71**
Fatty Acid Composition of Subcutaneous Fat **77**
Saturated Fatty Acids *78*
Mono-unsaturated Fatty Acids *80*
Δ^9 *Desaturase Index* *83*
Polyunsaturated Fatty Acids *84*
Fatty Acid Ratios with Nutritional, Health and Fat Quality Implications *89*
Fatty Acid Ratios with Technological Implications *96*
Differential Scanning Calorimetry of Back- and Belly Fat **102**
Physical and Chemical Properties of Intramuscular Fat **112**
Fatty Acid Composition of Intramuscular Fat **117**
Saturated Fatty Acids *117*
Mono-unsaturated Fatty Acids *120*
Δ^9 *Desaturase Index* *121*
Polyunsaturated Fatty Acids *123*
Fatty Acid Ratios with Nutritional, Health and Fat Quality Implications *129*
Fatty Acid Ratios with Technological Implications *137*
Sensory Analysis **142**
Sensory Analysis of Pork Meat and Backfat Samples *142*
Chemical Stability Studies **147**
Accelerated Oxidation Test (Schaal Oven Test) *147*
Stability of Fresh and Frozen Pork Chops *148*
Lipid Stability of Frozen Pork Patties ***151***
Lipid Stability of Fermented Salami *153*
Lipid Stability of Bacon *156*

5. GENERAL DISCUSSION AND CONCLUSIONS **161**

6. REFERENCES **167**

7. SUMMARY / OPSOMMING **194**

ANNEXURE 1 198

Language and Style used in this Dissertation are in accordance with the requirements of Meat Science

ACKNOWLEDGEMENTS

This study would not have been possible without the assistance of the following persons:

* My study leader, Prof Arnold Hugo, of the Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for introducing me to this field of study, for his interest during the study, his contribution to the revision of the dissertation and most of all, for believing in me and giving me the chance to do the study;
* Prof Riëtte de Kock, Department of Food Science, University of Pretoria, for her advice on sensory analysis, for her constant interest during the study and for her constructive and invaluable criticism of the dissertation;
* Prof Gernot Osthoff, Head of the Food Science Division, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for a valuable discussion on the differential scanning calorimetry technique;
* Prof James du Preez, Head of the Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for his constant support and interest in the completion of my studies;
* Prof Jannie Swarts, Department of Chemistry, University of the Free State, and his laboratory, for the analysis of samples by differential scanning calorimetry;
* Mss Eileen Roodt and Arina Kemp and Mr Joseph Katongole, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for assisting me in a competent and enthusiastic manner with the chemical analysis;
* Mrss Celia Roets and Desire Harris, Grafic Design Division, University of the Free State, for their assistance with graphs and drawings;
* Miss Eileen Roodt, Food Science Division, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for her technical assistance with tables and figures and for keeping the laboratory running by ordering and purchasing reagents and for arranging for repair of defect equipment;
* Mr Sarel Marais, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for providing me with the graphic drawing of the CLA isomers;
* Ms Rosalie Hunt, Food Science Division, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State for keeping the laboratory running by ordering and purchasing reagents and for arranging for repair of defect equipment;

* Dr. Ina van Heerden and the staff of the ARC- Animal Nutrition and Animal Products Institute, Sensory Division, at Irene for the use of their panelists and facilities during the descriptive sensory analysis of the pork samples;
* Proff. Johan Greyling, Erhardt du Toit and Mr Kobus Grobler for making the facilities of the Animal Science Department of the UFS available;
* Mr Albert van Rensburg from SENWES Animal Feeds Division for formulating and mixing of experimental diets from Bothaville;
* The National Research Foundation for research funding;
* The Red Meat research and Development Trust, for their financial support;
* My colleagues, in particular Rosalie, Anita (postuum), Maryna and Ilze, at the Food Science Division of the Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for their never-ending support and interest throughout this study;
* Prof Celia Hugo and her family, for their patience and endurance throughout this study;
* My colleagues at the Department of Consumer Science, University of the Free State, for moral support and belief in me;
* My dearest friends, Gesine, Myra, Elaine, Trix, Alida, Henrihet, Sandra, Phildre, Mrss Fenwick and Lecuona, for their prayers and unconditional friendship during this study;
* Miss Esti Andrin Smith, for taking over my lecturing duties during my six months study leave months in 2010;
* My undergraduate and post-graduate students, as well as my student assistants, for their understanding and patience during my studies;
* My brothers, Jacobus and Gerrit, and their families, for their support and interest;
* My parents, for their constant interest, encouragement, support and love; and
* My Heavenly Father, for giving me the ability to undertake and complete this study.

LIST OF TABLES

NR.	DESCRIPTION	PAGE
2.1	Worldwide pork consumption 2006	5
2.2	Groups of pork quality characteristics	9
2.3	“Hidden” and “visible” pork quality characteristics	12
2.4	Fatty acid composition and content (g/100g total FA) in subcutaneous adipose tissue and muscle of loin chops in pigs	16
2.5	Fatty acid composition (%) of *M.longissimus* muscle triacylglycerol (neutral lipids) and phospholipids in Duroc pigs	17
2.6	Percentage C18:1c9 and C18:2 fatty acids of vegetable oils and fats	23
2.7	Reported health beneficial effects of CLA	26
2.8	Pork research from 1997-2011 at different CLA concentrations (%)	33
3.1	Composition (%) of the experimental diets on an air dry basis based on CLA inclusion	45
3.2	Formulated nutrient composition of the experimental diets on an air dry basis	46
3.3	Analyzed nutrient composition of the experimental diets on an air dry basis	47
3.4	The descriptions and definitions of each attribute as used by the members of the trained sensory panel in the evaluation of the pork fat and meat samples	53
3.5	Pork patty formulation	56
3.6	Salami formulation	56
4.1	Chemical properties, fatty acid composition and fatty acid ratios of the four diets used in this experiment	61
4.2	Lipid content and fatty acid composition of individual lipid-bearing feed components of the four experimental diets	62
4.3	Growth performance and feed efficiency of gilts in the four dietary groups	67
4.4	Carcass, muscle and backfat characteristics of gilts in the four dietary groups	69
4.5	Analysis of variance (ANOVA) for main effects and their interactions of the subcutaneous fat samples	72
4.6	Physical and chemical properties of subcutaneous fat from gilts from four dietary treatments	74
4.7	Correlation coefficients and significance levels between iodine value, physical and chemical properties, as well as fatty acid composition of subcutaneous fat	77
4.8	Saturated fatty acid content (%) of the subcutaneous fat from gilts from the four dietary treatments	79
4.9	Mono-unsaturated fatty acid content (%) of subcutaneous fat from gilts from the four diets	81
4.10	The Δ^9 desaturase index of subcutaneous fat from gilts from the four diets	83
4.11	Dienoic and trienoic polyunsatured fatty acid content (%) of subcutaneous fat from gilts from the four diets	85
4.12	Tetraenoic, pentaenoic and hexaenoic polyunsatured fatty acid content (%) of	88

subcutaneous fat from gilts from the four diets

4.13	Fatty acid ratios with nutritional and health implications of subcutaneous fat from gilts from the four diets	90
4.14	Fatty acid ratios with technological implications of subcutaneous fat from gilts from the four diets containing different levels of supplemented CLA	97
4.15	Positional distribution of major fatty acids in pig fat (mole%)	103
4.16	Setting and melting properties of back- and belly fat from the four dietary treatments	107
4.17	Analysis of variance (ANOVA) for main effects and their interactions of the intramuscular fat samples	113
4.18	Physical properties of intramuscular fat from gilts fed four dietary treatments containing varying levels of CLA	115
4.19	Saturated fatty acid content (%) of intramuscular fat from gilts from the four diets	118
4.20	Mono-unsaturated fatty acid content (%) of intramuscular fat from gilts from the four diets	120
4.21	The Δ^9 desaturase index of intramuscular fat from gilts from the four dietary treatments	122
4.22	Dienoic and trienoic polyunsatured fatty acid content (%) of intramuscular fat from gilts from the four diets	124
4.23	Tetraenoic, pentaenoic and hexaenoic polyunsatured fatty acid content (%) of intramuscular fat from gilts from the four diets	127
4.24	Fatty acid ratios with nutritional, health and fat quality implications of intramuscular fat from gilts from the four diets	130
4.25	Fatty acid ratios with technological implications of intramuscular fat from gilts from the four dietary treatments	138
4.26	Sensory properties of the pork *M. longissimus* muscle and fat samples from pigs receiving different dietary levels of CLA	143
4.27	Cooking data of the *M. longissimus* according to treatments	145
4.28	Results of accelerated oxidation test for backfat (Schaal oven test)	148
4.29	Chemical stability of fresh and frozen pork chops	149
4.30	Fresh belly fat firmness of gilts in the four dietary treatments	156
4.31	Demographic profile of consumer panel for bacon	158
4.32	Mean values for the preference of bacon samples from the four treatments (n = 75)	159

LIST OF FIGURES

NR.	DESCRIPTION	PAGE
2.1	South African pork production, consumption and imports	7
2.2	Principal component analysis plot showing countries as scores and pork chop attributes as factor loadings	14
2.3	Characteristic infrared absorption differences for the =C-H deformation vibration for isolated *trans* double bonds and conjugated *trans*, *trans* and *cis*/*trans* double bonds	24
2.4	Partial silver-iron high-performance liquid chromatography (Ag^{+}-HPLC) profile of the methyl esters of a commercial conjugated fatty acid mixture	24
2.5	The chemical structure of the c18:2, *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA	29
2.6	Biosynthesis of *cis*-9, *trans*-11 18:2	31
2.7	Chemical structure of *trans*-11 18:1 vaccenic acid	32
3.1	Sampling positions for subcutaneous fat	49
3.2	Sampling positions for intramuscular fat	49
3.3	Structured line scale	58
4.1	Iodine values of subcutaneous fat samples measured at different positions	76
4.2	Double bond index values of subcutaneous fat samples from the gilts fed four dietary treatments	101
4.3	The setting (top) and melting (bottom) thermograms of backfat from gilts fed four different concentrations of CLA	105
4.4	The setting (top) and melting (bottom) thermograms of the belly fat from gilts fed four different concentrations of CLA	106
4.5	Principal component analysis of the characteristics of backfat (position b) of gilts from the four dietary treatments	110
4.6	Principal component analysis biplot of the characteristics of belly fat (position f) of gilts for the four dietary treatments	111
4.7	Principal component analysis biplot of significant sensory and physical attributes for meat from pigs fed CLA at four different concentrations	146
4.8	Pattern and general trend of changes that took place in peroxide values of backfat of different treatments during 13 days storage, of extracted backfat at 63±0.5°C, with free access to air	147
4.9	Peroxide values of pork patties during frozen storage	152
4.10	Thiobarbituric acid reactive substance values of pork patties during frozen storage	153
4.11	Changes in peroxide values during salami manufacturing	154
4.12	Changes in thiobarbituric acid reactive substances during salami manufacturing	155
4.13	Peroxide values of bacons during refrigerated storage	157
4.14	Thiobarbituric acid reactive substance values of bacon during refrigerated storage	158
4.15	Frequency of consumers' ratings per treatment on the nine-point scale	159

GLOSSARY OF ABBREVIATIONS

a*	Colour ordinate – redness value
@	At
α	Alpha
ADG	Average daily gain
ADF	Acid detergent fibre
ADFI	Average daily feed intake
Ag^+	Silver
AI	Atherogenicity index
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ARC	Agricultural Research Centre
ASTM	American Society for Testing and Materials
b*	Colour ordinate – yellowness value
BC	Before Christ
BF	Backfat
BFAP	Bureau of Food and Agriculture Policy
BFT	Backfat thickness
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BW	Body weight
ca.	Approximately
CC	Carbonyl compounds
CD	Control diet
CGR	Carcass growth rate
CL	Control
CLA	Conjugated linoleic acid
cm	Centimeter
CVA	Canonical variant analysis
d	Day
DAFF	Department of Agriculture, Forestry and Fisheries
DBI	Double bond index
°C	Degrees Celsius
Δ	Delta

DFD	Dark, firm and dry
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DSC	Differential scanning calorimetry
EDTA	Ethylene diamino tetra-acetic acid
EFC	Extractable fat content
e.g.	For example
etc.	Etcetera
EPA	Eicosapentaenoic acid
EXPT	Exposure time
FA	Fatty acid
FAME	Fatty acid methyl ester/s

Individual FAME:

Abbreviation	*Common name*	*Complete formula*	*Systematic (IUPAC) name*
C12:0	Lauric	C12:0	Dodecanoic
C14:0	Myristic	C14:0	Tetradecanoic
C15:0	Pentadecylic	C15:0	Pentadecanoic
C16:0	Palmitic	C16:0	Hexadecanoic
C16:1	Palmitoleic	C16:1*c*9	*cis*-9-Hexadecenoic
C17:0	Margaric	C17:0	Heptadecanoic
C17:1	Heptadecenoic	C17:1*c*10	*cis*-10-Heptadecenoic
C18:0	Stearic	C18:0	Octadecanoic
C18:1c7	Vaccenic	C18:1*c*7	*cis*-7-Octadecenoic
C18:1t7	Octadecenoic	C18:1*t*7	*trans*-7-Octadecenoic
C18:1c9	Oleic	C18:1*c*9	*cis*-9-Octadecenoic
C18:1t9	Elaidic	C18:1*t*9	*trans*-9-Octadecenoic
C18:2	Linoleic	C18:2*c*9,12(*n*-6)	*cis*-9,12-Octadecadienoic
C18:3n-3	α-Linolenic	C18:3*c*9,12,15(*n*-3)	*cis*-9,12,15-Octadecatrienoic
C18:3n-6	λ-Linolenic	C18:3*c*6,9,12(*n*-6)	*cis*-6,9,12-Octadecatrienoic
C19:0	Nonadecanoic	C19:0	Nonadecanoic
C20:0	Arachidic	C20:0	Eicosanoic
C20:1	Eicosenoic	C20:1*c*11	*cis*-11-Eicosenoic
C20:2	Eicosadienoic	C20:2*c*11,14(*n*-6)	*cis*-11,14-Eicosadienoic
C20:3n-3	Eicosatrienoic	C20:3*c*11,14,17(*n*-3)	*cis*-11,14,17-Eicosatrienoic
C20:3n-6	Eicosatrienoic	C20:3*c*8,11,14(*n*-6)	*cis*-8,11,14-Eicosatrienoic
C20:4	Arachidonic	C20:4*c*5,8,11,14(*n*-6)	*cis*-5,8,11,14-Eicosatetraenoic
C20:5	Eicosapentaenoic	C20:5*c*5,8,11,14,17(*n*-3)	*cis*-5,8,11,14,17-Eicosapentanoic
C22:0	Behenic	C22:0	Docosanoic

C22:1	Erucic	C22:1*c*13	*cis*-13-Docosenoic
C22:2	Docosadienoic	C22:2*c*13,16(*n*-6)	*cis*-13,16-Docosadienoic
C22:5	Docosapentaenoic	C22:5*c*7,10,13,16,19(*n*-3)	*cis*-4,7,10,13,16-Docosapentaenoic
C22:6	Docosahexaenoic	C22:6*c*4,7,10,13,16,19(*n*-3)	*cis*-4,7,10,13,16,19-Docosahexanoic
C24:0	Lignoceric	C24:0	Tetracosanoic
C24:1	Nervonic	C24:1*c*15	*cis*-15-Tetracosenoic

FCR Feed conversion ratio
FFA Free fatty acids
FDA Food and Drug Administration
FB Fat blend
FFDM Fat free dry matter
FHM Fat hardness meter
FS Fat score

g Gram
g/d Gram/day
GC Gas chromatograph
GL Glycolipids

HDL High density lipoprotein
h Hour
HOSF High-oleic sunflower oil
HPLC High-performance liquid chromatography
Hz Hertz

i.e. That is
IM Intramuscular
IMF Intramuscular fat
IV Iodine value

Kcal Kilocalorie
kg Kilogram

l Litre
L* Colour ordinate – lightness value
LDL Low density lipoprotein
LMC Lean meat content
LO Linseed oil
LSD Least significant difference

m	Meter
MAP	Modified atmosphere packaging
max	Maximum
MDA	Malondialdehyde
ME	Metabolizing energy
mg	Milligram
mg/d	Milligram per day
mg/g	Milligram per gram
MJ/kg	Megajoule per kilogram
mm	Millimeter
milliequiv	Milliequivalent/s
min	Minimum
min.	Minute(s)
ml	Millilitre
MT	Muscle thickness
MUFA	Mono-unsaturated fatty acid/s
NaCl	Sodium chloride (salt)
n-3	Omega-3 fatty acid/s
n-6	Omega-6 fatty acid/s
ND	Not determined / detected
NDF	Neutral detergent fibre
NE	Nett energy
NF	No added fat
NIRS	Near infrared spectroscopy
NL	Neutral lipids
nm	Nanometer
NS	Not significant
NSA	Not statistically analysed
OB	Oil blend
OOO	Oleic-oleic-oleic
OPO	Oleic-palmitic-oleic
p	Significance level
%	Percentage
PC	Polar compound/s
PCA	Principal component analysis
pH_{45}	pH value 45 minutes post mortem

pH_{24}	pH value 24 hours post mortem
PI	Peroxidizability index
PL	Phospholipid/s
ppm	Parts per million (mg/kg)
PSE	Pale, soft and exudative
P:S	PUFA : SFA
PTG	Polymerized triglyceride/s
psi	Per square inch
PUFA	Polyunsaturated fatty acid/s
PV	Peroxide value
PVC	Polyvinyl chloride
QDA	Quantitative descriptive analysis
R	Rand
RA	Rumenic acid
RH	Relative humidity
RSE	Red, soft and exudative
SADC	South African Development Community
SAMIC	South African Meat Industry Company
SCD	Stearoyl CoA desaturase
SFA	Saturated fatty acid/s
SFO	Sunflower oil
SI	Saturation index
Sign	Significance
SL	Significance level
SLW	Slaughter weight
SPO	Stearic-palmitic-oleic
T	Tallow
TBARS	Thiobarbituric acid reactive substance/s
TBQH	Tertiary-butylhydroxyquinone
tr	Trace amounts
UFA	Unsaturated fatty acid/s
UK	United Kingdom
µl	Microlitre
USDA	United States Department of Agriculture

UV	Ultra-violet
V	Volts
VA	Vaccenic acid
vs	Versus
WHC	Water-holding capacity
WOF	Warmed-over flavour
<	Less than
>	More than

Opgedra aan GOD

Toe ek nie meer krag of tyd gehad het nie, het HY tyd, krag en berusting gegee.

"Moenie bang wees nie. Ek is by jou, moenie bekommerd wees nie. Ek is jou God. Ek versterk jou, Ek help jou. Ek hou jou vas, met my eie hand red ek jou." JESAJA 41:10

"… en hoe geweldig groot Sy krag is wat Hy uitoefen in ons wat glo. Dit is dieselfde kragtige werking van Sy mag wat Hy uitgeoefen het toe Hy Christus uit die dood opgewek het." EFESIëRS 1:18-20

"*Ek wil oor die Here sing, want Hy is geweldig groot. My krag en my sterkte kom van die Here.*" EKSODUS 15:1-2

CHAPTER 1
INTRODUCTION

Since the 1970's, consumers have become more aware of a healthy lifestyle and are presently more aware of diet, health and nutritional concerns than ever before (Verbeke, Van Oeckel, Warnants, Viaene, & Boucqué, 1999). Pork meat was often controversial in the past, because consumers considered it to contain an excess of fat, saturated fatty acids (SFAs) and cholesterol (Hernández, Navarro, & Toldrá, 1998). The main response of the global meat industry, to meet consumer demands for leaner and healthier pork, was to start producing leaner pigs by utilizing modern pig breeding and feeding, as well as altered management techniques (Blanchard, 1995).

In pigs and other monogastric animals, the fatty acid (FA) composition of the fat tissue triglycerides can be changed by altering the FA composition of dietary fat, which are absorbed intact from the small intestine and incorporated directly into the fat tissue (Rhee, Davidson, Cross, & Ziprin, 1990). There is currently considerable interest in the modification of the FA composition of animal tissues, in an attempt to produce new "designer" or "functional" foods. Adding different lipid products to an animal's diet can successfully alter the FA profile of the tissue from that animal (Wood, Sheard, Enser, Nute, Richardson, & Gill, 1999). It seems perfectly possible to utilize dietary manipulation to design pigs with a healthier FA profile, which can improve the image of pork among consumers. Pork can then be marketed as nutraceuticals, which are foods with perceived medicinal or health benefits that may prevent, ameliorate or cure a disease.

If South Africa wishes to become a significant role player and improve its competitiveness in the global meat industry, where competition is fierce and quality is non-negotiable, it must take cognisance of these developments and keep on the forefront of research in this field.

One way of designing functional pork products, with superior health properties that can be marketed as nutraceuticals, is by means of dietary manipulation, by supplementing pig diets with conjugated linoleic acid (CLA). Conjugated linoleic acid is a collective term describing several forms of linoleic acid (C18:2). Linoleic acid (C18:2*c*9,12(*n*-6) has double bonds located at carbons 9 and 12, both in the *cis* configuration. Conjugated linoleic acid has either the *cis* or *trans* configuration or both, located on carbons 9 and 11, 10 and 12, or 11 and 13. The *cis* 9, *trans* 11 form of CLA is apparently the biologically active form that can be incorporated into phospholipids in the body (Pariza, Park, & Cook, 2001). Experiments, on laboratory animals in human medicine, indicate that CLA has beneficial effects on improving the immune function, preventing cancer, reducing the incidence of heart disease, improving blood sugar level, decrease blood cholesterol and reduce body weight (Migdal, Paściak, Wojtysiak, Barowicz, Pieszka, & Pietras, 2004).

The feeding of CLA to laboratory animals improved rate and efficiency of gain, and decreased fat deposition (O'Quinn, Smith, Nelssen, Tokach, Goodband, & Smith, 1998). In pigs, CLA has also shown to improve performance and reduce fat deposition, and increase lean meat content (Swan, Parrish, Wiegand, Larsen, Baas, & Berg, 2001; Wiegand, Parrish, Swan, Larsen, & Baas, 2001; D'Souza, & Mullan, 2002). Pigs fed CLA had less backfat (BF), more carcass lean, bigger loin muscle area and better feed conversion ratio (FCR) (Migdal *et al.*, 2004). With respect to meat quality, CLA increased the saturated/unsaturated fat ratio in adipose tissue and intramuscular fat, and improved belly firmness (Eggert, Belury, & Schinckel, 1998; Dugan, Aalhus, Jeremiah, Kramer, & Schaefer, 1999; Wiegand *et al.,* 2001; Joo, Lee, Ha, & Park, 2002).

Most researchers (Dugan *et al.*, 1999; Wiegand, Spark, Parrish, & Zimmerman, 2002; Corino, Spark, Parrish, & Zimmerman, 2003) found no detrimental effect of CLA supplementation on the eating and sensory quality of pork. D'Souza and Mullan (2002), however, found inferior eating quality, i.e. flavour, tenderness, juiciness and overall acceptability, as a result of CLA feeding. Except for the observation of increased belly firmness (Dugan *et al.,* 1999), no information is available regarding the effect of CLA supplementation on the technological properties of the fatty tissue. Nor is any information available on the oxidative stability of processed meat products manufactured from CLA supplemented pork.

The first aim of this study was to determine the optimum level of dietary CLA supplementation to deliver the required positive effects in pork, without having a negative effect on eating quality.

The following hypothesis was formulated:

A supplementation level of 0.5% CLA is advised by manufacturers (BASF, 2006) to improve the performance of the pigs in general. Lower and higher levels have also resulted in favourable results, in regard to carcass characteristics and meat quality. Most researchers found no detrimental effect on the eating and sensory quality of pork at 2% CLA supplementation, while D'Souza and Mullan (2002) found inferior eating quality at 0.5% CLA. A hypothesis for optimum level would thus be that from 0.5% CLA upwards, eating quality would be negatively influenced, compared to lower concentrations.

The second aim was to determine the effect of CLA supplementation on health and nutritional implications and the technological properties of pork fat and muscle tissue.

The following hypothesis was formulated:

The feeding of a CLA-supplemented diet, in the finishing period of pigs, has shown positive results towards nutritional and health implications, and the technological properties of pork fat from selected areas such as the loin, back and belly. Only a few researchers have done research on the intramuscular fat (IMF), concentrating mainly on *longissimus* and

semimembranosus muscles. A hypothesis for nutritional and health implications, and technological properties would thus be that CLA supplementation would have different effects on subcutaneous fat, due to different expression of FAs in different sampling positions. The effects for the subcutaneous fat would also differ from the effects for IMF, which, in turn, would also differ between different intramuscular sampling positions.

The third aim was to determine the oxidative stability of processed meat products manufactured from CLA supplemented pork.

The following hypothesis was formulated:

Conjugated linoleic acid possesses certain antioxidative properties (Ha, Storkson & Pariza, 1990), which are transferred to processed products, already susceptible to oxidation. However, with an increase in CLA concentration in the processed products, Flintoff-Dye and Omaye (2005) suggested that it is reverted to a pro-oxidant due to oxidative reactions, causing destruction of the conjugated double-bond system of CLA. The hypothesis for oxidative stability would thus be that processed meat products manufactured from pork, supplemented with CLA in the diet at 0.5 % or higher, would be less oxidative stable than products made from pork with lower CLA levels.

CHAPTER 2
LITERATURE REVIEW

HISTORICAL BACKGROUND

Pork is the culinary name for meat from the domestic pig (*Sus domesticus),* which is one of the oldest forms of livestock, having been domesticated from the wild boar as early as 5000 BC in the Near East or China (Anonymous, 2005). The adaptable nature and omnivorous diet of this creature allowed early humans to domesticate it much earlier than many other forms of livestock, such as cattle (Anonymous, 2005).

From the earliest times onwards pork featured on the menus of feasts and banquets. The following are examples of the extravagant ways in which it was served during seven course meals: at a marriage in 1368, "two gilded suckling-pigs spitting fire" were served as the first course, while at the coronation banquet of Henry VI in 1429, the menu included "boars' heads in castles of gold" (Strong, 2002).

During the same time, French tradesmen in the food production industry were regulated by local guilds. One such a guild was the *charcutiers.* The members of this guild produced a traditional range of cooked or salted and dried meats, mostly pork, which varied, sometimes distinctively, from region to region. This led to the development of a new branch of cookery, named *charcuterie* (Courtine, 1994). Products included bacon, ham, sausage, *terrines* (pork cooked in a deep dish with straight sides), *galantines* (a dish made from lean pieces of e.g. pork, mixed with a forcemeat containing eggs, spices and various other ingredients, and pressed into a symmetrical shape, after which it was cooked in an aspic stock and served cold), *pâtés* (a meat, game or fish preparation put into a dish (*terrine*) lined with bacon, cooked in the oven and served cold)*, confit* (a piece of pork cooked in its own fat and stored in a pot, covered in the same fat to preserve it), *rillettes* (a preparation of pork cooked in lard, pounded to a smooth paste, potted and served cold), trotters and head cheese/brawn (Courtine, 1994). Originally intended as a way to preserve meats before the advent of refrigeration, these foods are prepared today for their flavours that are derived from the preservation processes (Courtine, 1994).

CONSUMPTION PATTERNS OF PORK

Pork is the most widely eaten meat in the world, despite religious restrictions on the consumption of pork by certain groups and the prominence of beef production in the West (Raloff, 2003). For the past 30 years, pork consumption has been rising, both in actual terms and in terms of meat-market share. It provides about 38% of daily meat protein intake worldwide, although consumption varies from place to place (Raloff, 2003).

According to the United States Department of Agriculture's (USDA) Foreign Agricultural Service (2006), nearly 100 million metric tons of pork were consumed worldwide in 2006 (Table 2.1). Increasing urbanization and disposable income has led to a rapid rise in pork consumption in China, where 2006-consumption was 20% higher than in 2002, and a further 5% increase was projected in 2007 (USDA, 2006). It is consumed in many ways and is highly esteemed in Chinese cuisine (Tropp, 1982). There, pork is preferred over beef due to economic and aesthetic reasons; the pig is easy to feed and not used for labour. The colour of the meat and fat of pork is regarded as more appetizing, while the taste and smell are described as sweeter and cleaner. It is also considered easier to digest (Tropp, 1982).

Table 2.1: Worldwide pork consumption in 2006 (USDA Foreign Agricultural Service, 2006; Agricultural Statistics, 2009).

Region	**Metric tons (millions)**	**Per capita (kg)**
People's Republic of China	52.5	40.0
European Union	20.1	43.9
United States	9.0	29.0
Russian Federation	2.6	18.1
Japan	2.5	19.8
South Africa	2.1	4.4
Others	10.1	n/a
Total	98.9	

n/a = not applicable

As mentioned previously, religious restrictions on the consumption of pork, in both the Muslim and Jewish dietary laws, make it a taboo meat. Pork may, however, be imported or consumed in Hindu or Christian areas of Muslim countries where it is otherwise forbidden, such as Bali in Indonesia (Solomon, 1996). According to the Qur'an, pork may be consumed to avoid starvation (Khan, 1997). Pork is one of the most well known examples of non-kosher food and the basis for this prohibition is Leviticus 11:2-4, 7-8 and Deuteronomy 14:8 (Anderson, 1988). In Buddhism and Hinduism pork and beef are both prohibited.

Christianity has no food taboos (Bonne & Verbeke, 2008), however, the Seventh-day Adventists also consider pork taboo, along with other foods forbidden by Jewish law (Seventh-day Adventist Church, 2009). Many Eastern Orthodox and Oriental Orthodox groups also discourage pork consumption, although, with the exception of the Ethiopian Orthodox Church, the proscription is rarely enforced. The Rastafari too, avoid the consumption of pork, their basis also being the book of Leviticus (Rastafari Movement, 2009).

Man has taken the restriction on pork consumption further by looking for another reason behind the prohibition. Unlike many other forms of livestock, pigs are omnivorous scavengers, eating virtually anything they come across, including carrion, refuse, diseased and dead pigs in the same enclosure, and even their own young. The Hebrew word for "impure meat" is translated by words such as filthy, pungent and decayed - the same terminology used to describe human faeces and other revolting substances. The pig is thus, according to Rubin (2004), such a filthy animal, that not the body, meat or carcass should even be touched.

There is another physical reason why the pig does not feature on God's list of "clean" animals. "Clean" animals that bring up their cud have a digestive tract consisting of three stomachs, which process the clean, plant-based food consumed and refine it to "flesh" over a period of more than 24 hours (h). In contrast, the pig is a mono-gastric animal and many dietary components are consequently readily transferred from the feed to the muscle and fat tissues, which subsequently affect pork quality (Rosenvold & Andersen, 2003). According to Rubin (2004), four h after the pig has eaten pig feed, and other putrid and revolting filth, man can eat the same (pig feed), "secondhand from pork ribs" (Josephson as cited by Rubin, 2004).

Rubin (2004) further argues that pork is seen by many specialists to be the primary cause of bad health in America. This type of meat supposedly causes blood diseases, liver problems, eczema, tuberculosis, tumors, cancer and poor indigestion, amongst others. The reason for this argument is dual: firstly, scavenger meat (like pork) is already spoiled in a poisonous way, and secondly, byproducts that are formed after the digestion of such meat, are severely toxic. It is furthermore alleged that so-called death enzymes, which are important in the decay of any carcass, are present in the human body after digestion of pork and other so-called scavengers. Rubin failed to note that these naturally-occurring enzymes are also present in man himself, as well as in all other meats consumed by humans.

Mass production and re-engineering of pork started in the 20th century in Europe and North America. Over the last century research into factors of significance for pork quality commenced and dealt with aspects such as genotype, feeding, production systems, fasting, pre-slaughter handling, stunning methods and slaughter procedures (Rosenvold & Andersen, 2003). Even as early as between 1920 and 1930, studies in the United States already showed the dramatic effects of the FA composition of dietary fat, on the FA composition and quality of body fat in the pig (Ellis & Isbel, 1926a,b). Today, there is even a mention of pork as being a nutraceutical, with the incorporation of CLA into the feed (Wood *et al.*, 1999; Averette Gatlin, See, Larick, Lin, & Odle, 2002b).

SOUTH AFRICAN CONSUMPTION OF PORK

The South African pork industry is relatively small in terms of the overall South African agricultural sector and contributes around 2.15% to the primary agricultural sector (DAFF, 2010). Despite this, pork is not a popular meat choice in South Africa; however, it was reported in 2009 (Pigprogress, 2009) that the consumption of pork had overtaken that of lamb/mutton. Pig consumption showed a rapid growth of 65%, and was 20.8 million kg in 2007/2008 (Agricultural Statistics, 2009), which is mainly due to population growth and economic development (Pigprogress, 2009). Furthermore, pork consumption is expected to grow by 14% until 2014 (BFAP, 2010). Beef, though, remains South Africa's favourite red meat and was traditionally followed by lamb, but over the last decade pork consumption has overtaken lamb. In 2008, the average South African consumed 17.9 kg beef, 4.4 kg pork (Agricultural Statistics, 2009) and 3.4 kg lamb (Pigprogress, 2009). Pig numbers decreased with roughly 10%, from 1.78 to 1.62 million in 2009. Yet, the number slaughtered rose from 2 million to 2.6 million in 2008 (Pigprogress, 2009), followed by a slight decline to over 2.4 million in 2009 (DAFF, 2010), which accounts for less than 0.2% of the world's pork production. Pork production increased sharply (59%) from 1999 to 2008, from 119 200 tons to 189 900 tons in 2008 (Pigprogress, 2009), followed by a slight decline to 170 000 tons in 2010 (BFAP, 2010). Since the growth in consumption (14%) marginally outpaces the projected growth (11%), pork imports will increase to approximately 22 000 tons by 2019 (Figure 2.1) (BFAP, 2010).

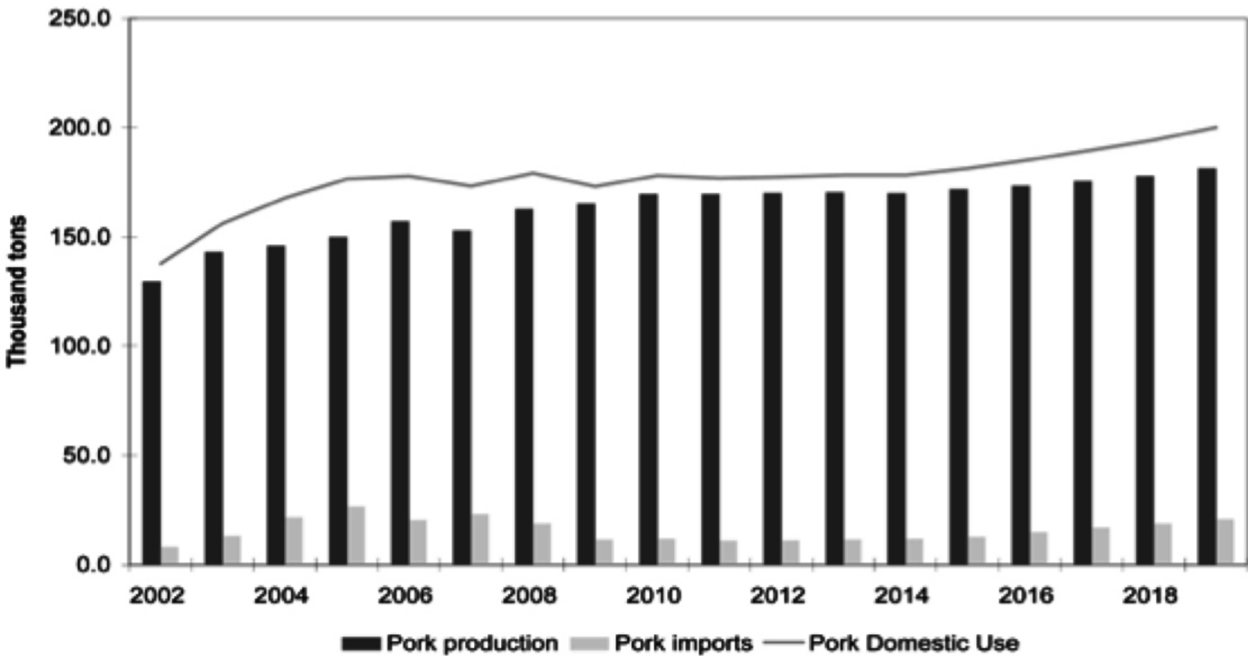


Figure 2.1: South African pork production, consumption and imports (BFAP, 2010).

More pork is consumed in South Africa than is produced, which makes South Africa a net importer of pork (DAFF, 2010) and it is expected to remain so (BFAP, 2010). In 2008, 2.3 million kg of pork were exported by South Africa, including frozen ribs (56 036 kg), frozen hams and shoulders (84 144 kg), frozen carcasses and half carcasses (9 629 kg), fresh ribs (20 078 kg), and fresh hams and shoulders (46 532 kg) (SAMIC, 2009). South African pork is mainly exported to the SADC countries, with Zimbabwe commanding the highest share in 2009 (DAFF, 2010). From January to November 2011, South Africa imported 29.8 million kg of pork, consisting mainly of ribs (18.4 million kg), hams and shoulders (1.9 million kg), carcasses (49.46 tons) and other unspecified frozen cuts (9.4 million kg) (Sapork, 2011).

It is estimated that around half of all South African pork is utilized by the meat processing industry to manufacture bacon, sausages, hams and other meat products. This pork is produced by 400 commercial producers, in an industry that employs 10 000 people. There are around 4 000 non-commercial producers who slaughter 350 000 pigs annually, mainly for domestic consumption. The balance of the commercial production is used for fresh pork consumption, in products such as pork chops, roasts and gammons. The market is estimated to be worth in excess of R1.5 billion annually, with volumes of around 120 000 tons. Polonies and viennas hold a market share of 40% and 30%, respectively, of the processed meat market, with the balance being made up by bacon, sausages, hams, spreads and meat rolls (Eskort, 2011).

PORK QUALITY

The concept “pork quality” is continually being developed and includes, besides composition and size, also health, eating quality, nutritional quality, technological quality, hygienic quality and ethical quality (Table 2.2) (Andersen, 2000). Pork quality has different meanings to different people. For the pig producers, pork quality equals those properties which raise the most favourable price when selling the pig to the abattoir; therefore, pig producers only rear pigs which give lean meat with minimum production costs. At the abattoir, the main parameters for the evaluation of pork quality include the absence of pathogens, water holding capacity (WHC), composition of the meat, meat:bone ratio, microbial load, presence/absence of residues and contaminants, as well as specific physical/chemical properties of value in further sale. More or less the same parameters are valid in relation to quality requests made by the meat processing industry. Finally, the consumer can only differentiate between sensory quality parameters, such as tenderness, juiciness, flavour, and absence of off-flavours for the heated/processed product (Fortin, Robertson, & Tong, 2005). The consumer is also concerned about the safety aspects and appearance of the product (Andersen, 2000).

The customers of fresh pork are the meat processing industry and consumers, who respectively buy 65-80% and 20-35% of the pork produced as a whole (Andersen, 2000). Previously, practically all the emphasis has been on consumer-associated quality characteristics. However, characteristics demanded or needed by the food processing industry should have more focus in the future, since raw material quality requirements of food processors are becoming stricter by the day.

Table 2.2: Groups of pork quality characteristics (Andersen, 2000).

Group	Individual attributes
Eating quality	Appearance/colour Flavour Tenderness Juiciness
Nutritional quality	Protein content/composition Lipid content/composition Vitamins Minerals Digestibility
Technological quality	Water holding capacity pH value Protein content and its status Lipid content and its characteristics Content of connective tissue Cutting piece/size Anti-oxidative status
Hygienic quality	Microorganisms Residues Contaminants
Ethical quality	Organic farming Religion Outdoor rearing Welfare aspects (e.g. no use of growth promoters)

Lipid quality

Lipid characteristics are important for the technological quality of pork (Hugo & Roodt, 2007). Wood (1984) defined good quality fat in pigs as firm and white, while poor quality fat is soft, oily, wet, grey and floppy. If pork lipids became too unsaturated, the pork would not be suitable for sausage production, for example (Teye, Wood, Whittington, Stewart, & Sheard, 2006b) and products would become oxidatively unstable, accelerating rancidity problems.

Pertinent values for fat quality parameters are subject to great variation, because of the great interdependence with factors such as pig genotyp, sex, age, feeding conditions, commercial quality grade and fatty tissue localization within the carcass (Fischer, 1989a). The content of individual

FAs, combinations of FAs and ratios of FAs had extensively been used to predict fat quality (Wenk *et al.*, as cited by Hadorn, Eberhard, Guggisberg, Piccinali, & Schlichtherle-Cerny, 2008; Muchenje, Dzama, Chimonyo, Strydom, Hugo, & Raats, 2009). Increased polyunsaturated (PUFA) levels are associated with a higher occurrence of oxidation and rancidity, and together with mono-unsaturated fatty acids (MUFAs), a soft, greasy and oily texture of the fat (Wenk *et al.*, as cited by Hadorn *et al.*, 2008). Various maximum levels of PUFA had been proposed for good quality fat, ranging from < 15% (Houben & Krol, 1983), to even < 12% (Prabucki, as cited by Houben & Krol, 1983). Other FA parameters include: > 41 % SFA content (Hauser & Prabucki, 1990); > 12 % stearic acid (C18:0) content (Lizardo, van Milgen, Mourot, Noblet, & Bonneau, 2002); < 59 % unsaturated fatty acid (UFA) content (Prabucki, 1991); < 57 % MUFA; 12 - 15 % C18:2 content (Lizardo *et al.*, 2002); 11 % C18:2 content in salami and fermented sausages (Fischer, 1989b); < 15% % C18:2 content in bacon (Enser, 1983); and < 15 % to < 12% C18:2 in meat (Houben & Krol, 1983).

In larger Swiss abattoirs, fat quality is characterized by the fat score (FS), which is a measure of the number of double bonds in the outer layer of the BF. Its analytical determination refers to the iodine value (IV), which includes the attachment of iodine to the double bonds of the fat (Prabucki, 1991). The carcass fat quality recommended by Prabucki (1991) and since then demanded by the larger Swiss abattoirs is a FS < 62. Barton-Gade (1987) recommended a maximum IV = 70 as the cut-off point for good fat quality. A new method has been introduced to determine FS by using near-infrared spectroscopy (NIRS), which has been tested by Müller, Wenk and Schreeder (2008) for additional fat parameters in BF.

Another important physical quality parameter for fat is colour. Consumers, butchers and meat processors prefer pork BF to be white and yellow discolouration, caused by rancidity (Barton-Gade, 1983), will be rejected. Colour measurement equipment, like the Minolta chromometer or Hunter Labscan, may be used to determine BF colour L*, a* and b* values (Tischendorf, Schöne, Kirchheim, & Jahreis, 2002).

Technological quality

This term contains all the attributes of value in further processing of fresh pork (Ingr, 1989). Water holding capacity includes the ability of fresh pork to retain water in the meat and bind extra water. A higher WHC will increase the value of the pork for use in highly processed pork products (Andersen, 2000). Meat with lower WHC is associated with higher drip loss, higher cooking loss, lower juiciness and lower tenderness values (Sheard, Nute, Richardson, & Wood, 2005; Muchenje, *et al.*, 2009; Muchenje & Ndou, 2011). Initiatives to improve WHC of pork, such as the elimination of pale, soft and exudative (PSE), red, soft and exudative (RSE), and acid meat, have

consequently high priority in the pork industry (Barbut, Sosnicki, Lonergan, Knapp, Ciobanu, Gatcliffe, *et al*., 2008). The pH is likewise a compelling technological quality attribute, as pH_{24} of pork is highly correlated to the WHC of the meat (Andersen, 2000). A pH_{24} around 5.8 is preferable, due to acceptable processing quality (e.g. reasonable WHC and good sliceability of derived pork products) (Barbut *et al*., 2008). Even though higher pH results in better WHC of pork, it also results in inferior colour and flavour (Andersen, 2000).

The anti-oxidative status of the meat, e.g. content of vitamin E, is becoming an important technological quality attribute in pork, which is destined for use in the production of different kinds of convenience meat products (Lauridsen, Nielsen, Henckel, & Sørensen, 1999). Most of these products are pre-cooked, thereby having problems with the formation of an inferior flavour development upon re-heating, called warmed-over-flavour (WOF). Dietary supplementation of vitamin E to pigs is an effective tool in minimizing WOF (Andersen, 2000). Eating quality attributes are slowly becoming technological quality attributes in pork used in processing industries, specializing in convenient food products (Andersen, 2000).

Consumer quality

The consumer's experience of pork quality is much more complex and can be divided into "hidden" and "visible" quality characteristics, as shown in Table 2.3. The "hidden" quality characteristics include mainly safety and nutritional aspects, as well as image and reputation, which are critical attributes of pork (Andersen, 2000). Pork (fat) still has a negative image in the public eye (Wood, Enser, Fisher, Nute, Sheard, Richardson, *et al*., 2008), despite nutritionists proving the opposite, and periodically market shares falling due to adverse publicity, e.g. 'swine flu' (Doyle & Erickson, 2006) and the use of growth promoters (D'Souza & Mullan, 2002). Until recently, hidden quality characteristics have had only little effect as to whether pork is purchased or not, as they have been taken for granted. However, "soft" quality characteristics (animal welfare, environmental influence of production, organic and ethical production), which fall into the "hidden" category, are becoming more and more important in the consumer's choice of pork (Andersen, 2000).

Of the "visible" quality characteristics, the sensory properties of pork undoubtedly provide the most important reason for its acceptability, as most meat is eaten for pleasure. Appearance, tenderness, flavour and juiciness are known to be the most important factors in the consumer's acceptance of pork. Consumers look for colour, fluid retaining characteristics and fat content of pork, in the hope that they will indicate the eventual enjoyment of the product when it is eaten (Dransfield, 2008).

Flavour development mainly depends on constituents in the fresh meat, e.g. sugars, free amino acids, peptides, nucleotides (Campo, Nute, Wood, Elmore, Mottram, & Enser, 2003), fat

composition, glycogen concentration, vitamin content, especially thiamine and vitamin E, etc. (Andersen, 2000), and the heat treatment of the product (Aaslyng & Støier, 2004). Andersen (2000) stated that in recent years the intensity in pork flavour seemed to have decreased, most probably as a result of the production of pork with a minimal content of IMF. Boar taint and lipid oxidation are among conditions associated with off-flavors in pork. Boar taint is produced by a steroid (andostenone) and a degradation product of tryptophane, skatole, which are both deposited in the fat and released upon heating (De Kock, Heinze, Potgieter, Dijksterhuis, & Minnaar, 2001). Lipid oxidation, better known as rancidity, occurs in the unsaturated lipid fraction during prolonged storage (freezer) and upon re-heating of the pork (Andersen, 2000).

Table 2.3: "Hidden" and "visible" pork quality characteristic (Andersen, 2000).

Groups	Attributes	Expectations and assumptions
"Hidden" Pork Quality	Safety	Absence of : Pathogens, Toxins, Contaminants and Other harmful substances
	Nutritional value	Wholesome, Nourishing, Good protein source, Functional iron source
	Image / Reputation	Good, reliable
	Ethical	Organic, No use of growth promoters, Outdoor rearing, Good animal welfare, Ritual slaughtering
	Labeling	All correct
"Visible" Pork Quality	Appearance	Appealing
	Flavour	Expected meaty
	Tenderness	Good
	Juiciness	Good
	Convenience	Functional sensory properties satisfactory at time of consumption
	Price	Cheapest possible in relation to expected quality

In contrast to beef, tenderness is a somewhat neglected quality attribute in the eating quality of pork. In pigs, *post mortem* conditions might affect sarcomere length, creating differences in tenderness. Aging of fresh pork, often called conditioning, can be used to improve tenderness (Andersen, 2000). Juiciness of pork is associated with the amount of moisture present in the cooked product and the amount of IMF (Sheard *et al.*, 2005). Intramuscular fat can vary from <1% to >5%, though typical values in the UK are about 0.8% (Wood, 2001). Lower IMF values are

usually associated with lower tenderness and juiciness. A level of 1.5% IMF was found to be the minimum level necessary to ensure a pleasing eating experience in Canada (Fortin *et al*., 2005). Degree of doneness has a dramatic effect on the juiciness of pork. Increasing internal end point cooking temperature from 60 to 80 ºC results in a severe decrease in juiciness and moisture content of the heated pork (Andersen, 2000).

The importance of fat content to the appearance and choice of meats is incontrovertible. In a recent study in 23 countries, including South Africa, 12 590 consumers chose photographs of pork chops that showed variations in fat cover, colour of lean, marbling and drip. Results showed that the amount of pork BF and colour of lean were the most important factors, with marbling and drip less important (Figure 2.2). The figure shows the relationship (correspondence analysis) between the country and their preference for fat/lean and marbled/non-marbled meat (Ngapo, Martin, & Dransfield, 2007a). The majority of consumers, particularly in Poland, Finland and Mexico, preferred low fat cover. The majority of Irish consumers preferred light red, lean pork, with no marbling and no drip, and Australian consumers, light red, lean pork, again with no marbling. However, many Korean, Japanese and Taiwanese consumers, as well as South African consumers, preferred the more marbled and fatter pork. The results of a self-reported questionnaire showed that most socio-economic factors and eating habits were common across countries. Gender had the most consistent influence and, in all but one of the countries, a greater proportion of women than men chose the pork with less fat cover (Ngapo, Martin, & Drangsfield, 2007b).

Fat deposition in the pig

The metabolism of fat deposition in pigs can be regarded as a balance between two processes – lipogenesis (fat synthesis) and lypolysis (fat mobilization) (Farnworth & Kramer, 1987). Both processes are substantially influenced by hormones, such as adrenalin, glucagon, insulin and the thyroid hormones. The hormones involved in the control of lipolytic activity in the adipose tissue have glycogenolytic effects in the muscle (Müller, 1983).

Carcass fat is deposited in different anatomical locations as subcutaneous, visceral, intermuscular (between muscles) or intramuscular (within muscle) fat (Kouba & Bonneau, 2009). There is a gradient of increasing saturation from the outer layer of subcutaneous adipose tissue, to the inner layer, to intermuscular adipose tissue, to flare fat. The MUFA concentrations follow the same pattern (Monziols, Bonneau, Davenel, & Kouba, 2007).

The term FA refers to any aliphatic mono carboxylic acid that can be liberated by hydrolysis from naturally occurring fats. Saturated FAs and MUFAs are synthesized in the animal body from carbohydrates and proteins (Okuyama & Ikemoto, 1999). Like other mammals, pigs are unable to

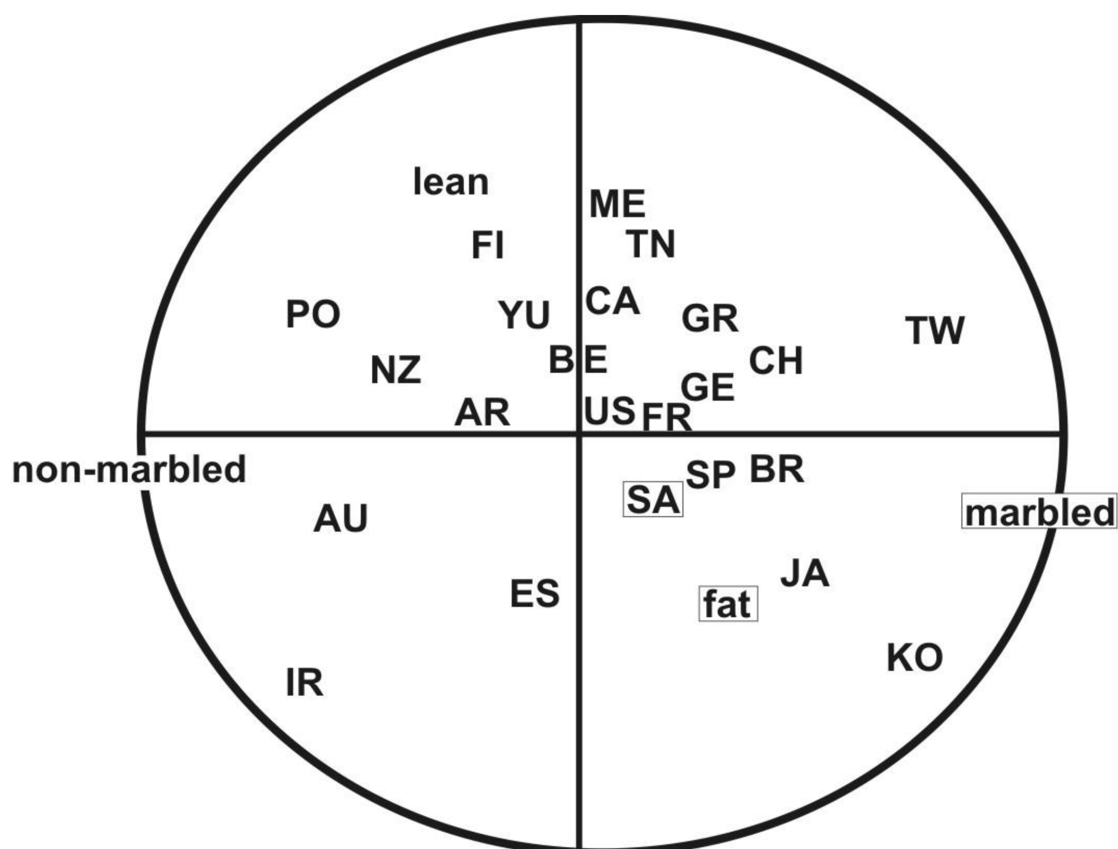


Figure 2.2: Principal component analysis plot showing countries as scores and pork fat attributes as factor loadings. AU(Australia); AR(Argentina); BE(Belgium); BR(Brazil); CA(Canada); CH(China); ES(Estonia); FI(Finland); FR(France); GE(Germany); GR(Greece); IR(Ireland); JA(Japan); KO(Korea); ME(Mexico); NZ(New Zealand); PO(Poland); SA(South Africa); SP(Spain); TN(The Netherlands); TW(Taiwan); US(United States); YU(Yugoslavia) (Ngapo *et al.*, 2007a).

synthesize the essential FA C18:2 and linolenic (C18:3*n*-3) acid (Christensen, 1985, as cited by Madsen, Jakobsen, & Mortensen, 1992) and these must therefore be supplied in their diet. Linoleic acid is essential for the maintenance of growth, reproductive physiology, maintenance of the brain and retinal functions, but the essential amount (1%) is relatively small (Okuyama & Ikemoto, 1999). Its proportion in pig adipose tissue declines as fat deposition proceeds and is an index of fatness (Wood et al., 2008).

The considerable anatomical variation in FA composition in the pig has been known for many years (Sink, Watkins, Ziegler, & Miller, 1964). It has been suggested that not all adipose tissues are similar, but that each shows specific development and metabolism (Mourot, Kouba, & Peiniau, 1995).

Intermuscular fat is described as an earlier maturing tissue than subcutaneous adipose tissue and consequently it would be expected to have a higher concentration of lipids (Fortin, Wood, & Whelehan, 1985). Total lipid contents do not differ significantly between the two layers of subcutaneous adipose tissue (66.8-67.8%) (Monziols *et al*., 2007). There is greater unsaturation and a higher deposition of C18:2 (essential FA for the pig, coming exclusively from the feed) in the outer than in the inner layer of subcutaneous adipose tissue, suggesting that lipid metabolism is lower in the outer than in the inner layer of subcutaneous adipose tissue (Christie, Jenkinson, & Moore, 1972). The inner layer exhibits larger *de novo* lipogenesis, with the result that PUFAs (particularly C18:2 of feed origin) are diluted with more endogenous FAs in the inner layer than in the outer layer (Christie, Jenkinson, & Moore, 1972).

The saturation degree of the fat depots in the pig follows a negative gradient from outside inwards. The outer layer is the most unsaturated, then the middle layer, the inner layer and the perirenal fat. Intermuscular adipose tissue fits into this pattern, its degree of unsaturation being lower than in subcutaneous, but higher than in flare fat (Villegas, Hedrick, Veum, Mcfate, & Bailey, 1973). A difference in composition between the fat layers could be due to an adaptation of adipose tissue to temperature, trying to maintain the physical fluidity of the lipids in the different adipose tissues (Dean & Hilditch, 1933).

There is also a difference in the MUFA contents between the different adipose tissues, along a gradient, with the highest concentration in the outer layer of the subcutaneous adipose tissue, then the inner layer, the IMF, and the flare fat (Bee, Gebert, & Messikommer, 2002). Oleic acid (C18:1*c*9) is the most abundant FA in pigs fat and is synthesized by stearoyl-CoA-desaturase (SCD) (Kouba, Mourot, & Peiniau, 1997). The activity of this enzyme could be different in the various adipose tissues, as was shown by Thompson & Allen (1969), where its activity was indeed higher in the subcutaneous adipose tissue than in perirenal fat, which could explain, at least partly, the difference in the content of MUFAs.

The concentration in C18:2 (exclusively from exogenous origin) is higher in lean pigs (Kouba *et al*., 1997). This could be explained by the fact that *de novo* lipogenesis is lower in lean pigs, with therefore less endogenous FAs, resulting in less dilution of exogenous C18:2. This could be the reason why contemporary pigs, selected against fatness, present high C18:2 concentrations (Monziols *et al*., 2007).

Kouba and Bonneau (2009) found that in Large White X Landrace castrated males, kidney fat grew more rapidly than subcutaneous or IMF. In the shoulder and loin, about one third of the total adipose tissue was in the intermuscular fraction. In the belly, there was as much IMF (in 30-110 kg body weight (BW) pigs), or more IMF (in 140 kg BW pigs) than subcutaneous adipose tissue. The intermuscular fraction of adipose tissue in the ham grew more slowly than the

subcutaneous fraction, so that it represented less than one fourth of total ham adipose tissue in 140 kg BW pigs. Intermuscular adipose tissue exhibited lower lipid content than the subcutaneous adipose tissue, whatever the body weight, but the differences in lipid content between the adipose tissues decreased with increasing weight.

Fat composition of the pig

Fatty acid composition of adipose tissue and muscle in pigs

The FA composition and total FA content of subcutaneous adipose tissue and *longissimus* muscle from loin chops of pigs, purchased at retail in the United Kingdom, are shown in Table 2.4. The data show that adipose tissue has a much higher FA content than the muscle, but that the FA composition of the two tissues is broadly similar. Pigs have high proportions of the major PUFA, C18:2, in both tissues, which are derived entirely from the diet (Enser, Hallet, Hewitt, Fursey, & Wood, 1996). It passes through the pig's stomach unchanged and is then absorbed into the blood stream in the small intestine and incorporated into the tissue (Nürnberg, Wegner, & Ender, 1998).

Table 2.4: Fatty acid composition and content (g/100 g total FA) in subcutaneous adipose tissue and muscle of loin chops in pigs (Enser *et al.*, 1996).

Fatty acid	**Adipose tissue**	**Muscle**
C14:0	1.6[a]	1.3[a]
C16:0	23.9[a]	23.2[a]
C16:1*cis*-9	2.4[a]	2.7[b]
C18:0	12.8[a]	12.2[a]
C18:1*cis-9*	35.8[b]	32.8[a]
C18:2*n*-6	14.3[a]	14.2[a]
C18:3*n*-3	1.4[b]	0.95[a]
C20:4*n*-6	0.2	2.21
C20:5*n*-3	ND *	0.31
Ratio *n*-6/*n*-3	7.6	7.2
P:S**	0.61	0.58
Total ***	65.3	2.2

Means with different superscripts in the same row are significantly different ($p<0.05$)

* not determined **polyunsaturated:saturated ***fatty acid content

The second most important PUFA is C18:3n, which is present in many concentrated feed ingredients, but at lower levels than C18:2. In pigs, the proportion is higher in the adipose tissue than in the muscle. The muscle contains significant proportions of long chain (C20-22) PUFAs, which are formed from C18:2 and C18:3n-3 by the action of Δ5 and Δ6 desaturase and elongase enzymes. Important products are arachidonic acid (C20:4) and eicosapentaenoic acid (C20:5),

which have various metabolic roles, including eicosanoid production. Greater incorporation of C18:2 into pig muscle FAs produces higher levels of C20:4 by synthesis and the net result is a high ratio of *n*-6:*n*–3 PUFAs (Wood *et al*., 2008).

Fatty acid composition of triacylglycerol (neutral lipid) and phospholipid

The major lipid class in adipose tissue (> 90%) is triacylglycerol or neutral lipids. In the muscle a significant proportion is phospholipids, which have a much higher PUFA content in order to perform its function as a constituent of cellular membranes (Wood, Nute, Richardson, Whittington, Southwood, Plastow, *et al*., 2004). Values for the FA composition of *longissimus* muscle neutral lipids and phospholipids from pigs, are shown in Table 2.5.

Table 2.5: Fatty acid composition (%) of *M.longissimus* muscle triacylglycerol (neutral lipids) and phospholipids in Duroc pigs (Wood *et al*., 2004).

Fatty acid	Neutral lipids	Phospholipids
C14:0	1.6	0.3
C16:0	23.8	16.6
C16:1	2.6	0.8
C18:0	15.6	12.1
C18:1*c*9	36.2	9.4
C18:2	12.0	31.4
C18:3*n*-3	1.0	0.6
C20:4	0.2	10.5
C20:5	ND *	1.0

*not determined

Oleic acid (C18:1*c*9*)* is the major FA in meat and is formed from C18:0 by the enzyme SCD. It was much more predominant in the neutral lipids, while C18:2 was much higher in the phospholipids. The proportion of C18:3*n*-3 acid was slightly higher in neutral lipid than phospholipid in pigs (Wood *et al*., 2004). Long chain *n*–3 and *n*–6 PUFAs are mainly found in phospholipids, but are detected in pig muscle neutral lipids and adipose tissue (Cooper, Sinclair, Wilkinson, Hallett, Enser, & Wood, 2004).

Effects of fat content on FA composition

As the fat content of the animal and meat increases from early life to time of slaughter, the proportions of FAs change. In pig subcutaneous adipose tissue, the C18 FAs, C18:0 and C18:1*c*9, increase in proportion, while C18:2 declines during this period. This could be ascribed to an

increase in *de novo* tissue synthesis of SFAs and MUFAs, and a relative decline in the direct incorporation of C18:2 from the diet (Kouba, Enser, Whittington, Nute, & Wood, 2003).

The inverse relationship between the concentrations of C18:2 in subcutaneous adipose tissue and backfat thickness (BFT), has been observed by Wood, Enser, Whittington, Moncrieff and Kempster (1989). In a study with 300 pigs, with 8 mm, 12 mm and 16 mm P_2 BFT, average values for C18:2 in the subcutaneous adipose tissue fell from 14.9% to 12.4% to 10.6%, respectively. Proportions of PUFAs tend to be high in subcutaneous adipose tissue from entire males, mainly due to their thinner BF. However, even at the same BFT, there was a higher proportion of C18:2 and a lower proportion of C18:1*c*9 in subcutaneous adipose tissue from entire males than from castrates and females. Also, at the same fat thickness as females, subcutaneous adipose tissue from entire males contained a higher proportion of water and a lower proportion of lipid, signifying a less mature tissue. This helps to explain why fat tends to be lower in entire male pigs than castrates and females (Wood *et al*., 1989).

The overall fat content of the animal and muscle have an important impact on the proportionate FA composition, because of the different FA compositions of the neutral lipids and phospholipids (Table 2.5) (Wood *et al*., 2004). Phospholipids are essential components of cell membranes, with the amount remaining fairly constant or increasing little as the pig fattens. In young, lean animals, genetically lean animals or animals fed a low energy diet, the lower C18:1*c*9 and higher C18:2 contents of the phospholipids have a major influence on the total muscle FA composition. However, as body fat increases, neutral lipids predominate the overall FA composition (Kouba *et al*., 2003).

Kouba *et al.* (2003) also found that age effects on neutral lipids, total lipids and FA proportions were statistically significant. There was an increase in the proportion of C18:1*c*9 and a decrease in the proportion of C18:2 in the neutral lipids, due to the increasingly important role of SCD. The importance of dietary fat as a source of muscle FAs declined as fat deposition accelerated in the muscle triacylglycerol and adipose tissue.

Genetic effects on FA composition

The Duroc breed is notable in having higher muscle lipid (marbling fat) content, in relation to subcutaneous fat, when compared to other breeds. In a study with purebred Berkshire, Duroc, Large White and Tamworth pigs, the two traditional breeds, Berkshire and Tamworth, grew slowly and were lighter and fatter than the two modern breeds, Duroc and Large White, at slaughter (Wood *et al*., 2004). The amount of phospholipid in the *longissimus* muscle was similar between the breeds, but the amounts of neutral lipids and total lipids were higher in Berkshire and Duroc than in Large White and Tamworth. Duroc had the highest ratio of muscle lipid to subcutaneous fat

thickness. The proportion of phospholipids in the total lipids was 18.8, 23.8, 31.7 and 38.9% in the Berkshire, Duroc, Tamworth and Large White, respectively. Values for the proportions of C18:1*c*9 and C18:2 in the total lipids were the same for all breeds, except for Duroc, where the proportion of C18:1*c*9 was lower and the proportion of C18:2 was higher than expected. A possible explanation for this is the slightly higher proportion of phospholipids in the Duroc *longissimus* muscle, which is also associated with their 'redder' muscle fibre type profile, in comparison with the other breeds (Chang, Da Costa, Blackley, Southwood, Evans, & Plastow, 2003). Thus, the FA profile would be expected to be closer to the *psoas* muscle than the *longissimus* muscle, with a higher C18:2 and a lower C18:1*c*9 proportion (Wood *et al*., 2004).

Diet effects on FA composition

The pig, being a monogastric species, is amenable to changes in the FA composition of adipose tissue and muscle, using diets containing different oils. Spectacular results can be achieved using diets with high levels of C18:2, which is a common FA in grains and oilseeds (Stanton, Murphy, McGrath, & Devery, 1999). In general, the proportion of this FA in tissues increases linearly as the dietary intake increases (Wood, 1984). In early studies by Ellis & Isbell (1926a,b), the proportion of C18:2 in the subcutaneous adipose tissue increased from 1.9% on a low fat diet, to over 30% on diets containing a high level of soybeans.

Other dietary lipid sources containing particular FAs can be used to influence meat FA composition. The study by Scheerder, Gläser, Eichenberger and Wenk (2000) showed that the dietary FA composition was reflected in the FA composition of the phospholipids in the *M. Longissimus* thoracis and *Triceps brachii* muscles. Swiss Landrace and Large White siblings were fed a diet supplemented with (i) 7% pork fat, 4.95% olive oil or 3.17% soybean oil, or (ii) 5% olein or stearine fraction of pork fat or hydrogenated fat. The unsaturated to saturated ratio was not affected by the dietary intake of PUFAs and was only slightly increased by the olive oil supplement. *Trans* FAs, including CLA, were only incorporated into phospholipids to a small extent.

Averette Gatlin, See, Hansen, Sutton and Odle (2002a) evaluated the effects of dietary fat sources and levels on carcass FA composition. Barrows and gilts were allocated to seven dietary treatments for the last six weeks of the finishing phase. Diets contained 0, 2.5 or 5% dietary fat, comprised of 100, 50 or 0% beef tallow. The balance was provided by an animal-vegetable blended fat. As the level of tallow increased, there was a linear decrease ($p<0.05$) in C18:2 content and the IV of the carcass fat. Conversely, C16:1 and C18:1*c*9 increased linearly ($p<0.05$) as the tallow increased. However, C16:1 decreased linearly ($p<0.05$) as the level of the fat increased. As the level of the tallow was increased, a greater reduction in C18:2 and the IV was observed in diets with 5% dietary fat, compared to diets with 2.5% fat ($p<0.05$). These results indicated that reduction of

dietary PUFA content had the desired effect of lowering C18:2 content and the IV of pork fat, and that significant alteration could be elicited in as little as six to eight weeks of feeding.

In a study by Nürnberg, Fischer, Nürnberg, Kuechenmeister, Klosowska, Eliminowska-Wenda, *et al*. (2005), the FA composition of porcine tissue was altered by accumulating essential FAs, without adversely affecting carcass composition, muscle structure or meat eating quality. A total of 13 female and 12 castrated Pietrain x German Landrace pigs were fed a basal concentrate diet, supplemented by 5% olive or 5% linseed oil, during the growing-finishing period. Feeding linseed oil to pigs increased the relative content of C18:3*n*-3 and long chain *n*–3 FAs in the lipids of the muscle, BF and heart, at the expense of C20:4. Oleic acid accumulated in the muscle, BF and heart lipids by feeding olive oil.

The incorporation of fat in the diets of heavy pigs may be necessary in order to increase their energy intake in the finishing period. Lard may be a good lipid source, but it contains 10-13% C18:2, which make the subcutaneous fat less suitable for long term curing of raw ham. Partial hydrogenation of lard decreased C18:2 content, but increased *trans* FA content. With this in mind, Bochicchio, Faeti, Marchetto, Poletti, Maranesi, Mordenti, *et al.* (2005) fed pigs, the last two months before slaughter, diets containing 3% lard or 3% partially hydrogenated lard. The hydrogenated lard contained about 10% *trans* FAs and 2.5% C18:2. The pigs fed partially hydrogenated lard showed a lower percentage of C18:2 in the BF (12.28% vs 13.04% in pigs fed lard) and a higher percentage of C18:1 *trans* FAs in both BF (0.5% vs 0.06%) and IMF (0.2% vs 0.04%).

The study of Teye, Sheard, Whittington, Nute, Stewart and Wood (2006a) and follow-up study by Teye *et al.* (2006b) evaluated the effects of palm kernel, palm and soybean oil on pork quality, especially for the processing of bacon and frankfurter-style sausages. Palm kernel oil significantly reduced the PUFA to SFA (P:S) ratio in the *M. longissimus* muscle ($p<0.001$). Palm kernel oil increased the concentrations of lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0) FAs and decreased C18:2. Palm kernel oil resulted in a poor P:S ratio (0.34). Palm oil had a FA composition closer to the soybean control and better P:S ratio than palm kernel oil (0.48). The results suggested that palm kernel and palm oil could be used in tropical developing countries as cheaper alternatives to soybean oil for the production of good quality and healthy pork, but the limits of inclusion need to be determined.

A diet with 5% sunflower oil (SFO) increased the incorporation of PUFA in adipose tissues, loin and liver, at the expense of the sum of SFA and MUFA (Mitchaothai, Yuangklang, Wittayakun, Vasupen, Wongsutthavas, Srenanul, *et al.*, 2007). Thirty-six castrated male growing pigs were fed diets containing either 5% beef tallow or 5% SFO. In particular, the SFO diet produced an increase in the content of C18:2 in the various tissues.

Realini, Duran-Montagé, Lizardo, Gispert, Oliver and Esteve-Garcia (2010) fed concentrated diets containing 10% tallow (T), 10% high-oleic sunflower oil (HOSF), 10% sunflower oil (SFO), 10% linseed oil (LO), 10% fat blend (FB) or 10% oil blend (OB) in finishing diets vs. a semi-synthetic diet with no added fat (NF). The diets rich in PUFA did not reduce fat deposition in separable fat depots, with respect to MUFA and SFA. Carcasses from gilts fed NF had a high degree of saturation (40.6% SFA), followed by carcasses of T- and FB-fed gilts. Feeding HOSF-, SFO- and LO- enriched diets elevated the percentages of MUFA (56.7%), *n*-6 (30.0%) and *n*-3 (16.6%) PUFA, respectively, whereas carcasses from gilts fed OB had greater percentages of *n*-3 FA (14.8% *n*-3, 0.9% C20:5, 1.0% C22:5, 3.1% C22:6) than gilts fed FB (6.72% *n*-3, 0.1% C20:5, 0.4% C22:5, 0.1% C22:6).

Dietary supplementation of CLA in pig diets has received attention in recent years, due to the positive effects on carcass and meat quality characteristics (Dugan, Aalhus, Schaefer, & Kramer, 1997; Ostrowska, Muralitharan, Cross, Bauman, & Dunshea, 1999; Thiel-Cooper, Parrish, Sparks, Wiegand, & Ewan, 2001), and for nutritional interest in CLA-enriched meat (Martín, Antequera, Muriel, Andres, & Ruiz, 2008a; Larsen, Wiegand, Parrish, Swan, & Sparks, 2009).

WHAT IS CONJUGATED LINOLEIC ACID (CLA)?

The term conjugated linoleic acid and its acronym CLA, refer generically to the class of positional and geometric conjugated dienoic isomers of the FA, C18:2. Two of these isomers, *cis*-9*, trans*-11 and *trans*-10*, cis*-12 CLA, are known to possess biological activity (Müller, Kirchgessner, Roth, & Stangl, 2000; Pariza *et al*., 2001; Eynard & Lopez, 2003).

Unsaturated FAs are named after the parent unsaturated hydrocarbons. The terminal *enoic* indicates unsaturation, and the *di*, *tri* and so on, represent the number of double bonds present, e.g. octadecatrienoic for C18:3 (Nawar, 1996). The simplest way to specify the location of the double bonds, is to put one number for each unsaturated linkage before the name of the acid. Oleic acid, for example, with one double bond between carbons 9 and 10, is named 9-octadecenoic acid. In certain cases it is convenient to distinguish UFAs, by the location of the first double bond from the methyl end of the molecule. Linoleic acid (9,12-octadecadienoic acid) is therefore an 18:2ω6 (or *n*-6) acid (Nawar, 1996).

The geometric configuration of double bonds is usually designated by the use of *cis* (on the same side), and *trans* (across), indicating whether the alkyl groups are on the same or opposite sides of the molecule. The *cis* configuration is the naturally occurring form, but the *trans* configuration is thermodynamically favoured. Linoleic acid, with both double bonds in the *cis* configuration, is named *cis*-9, *cis*-12-octadecadienoic acid (Nawar, 1996).

The number, position and geometry of double bonds affect the rate of oxidation. Relative rates of oxidation for C20:4, C18:3*n*-3, C18:2 and C18:1*c*9 are approximately 40:20:10:1, respectively (Nawar, 1996). *Cis* acids oxidize more readily than their *trans* isomers, and conjugated double bonds are more reactive than non-conjugated. At room temperature oxidative rancidity of unsaturates becomes detectable.

Fats in the group of C18:1*c*9-C18:2 are the most abundant (Table 2.6). The oils are all of vegetable origin and contain large amounts of C18:1*c*9 and C18:2 and < 20% SFAs. The most important members of this group are cottonseed, corn, peanut, sunflower, safflower, olive, palm and sesame oils (Nawar, 1996), while the highest concentrations of C18:2 are found in cucumber, grapeseed, flaxseed, poppy, safflower and walnut (Reaney, Liu, & Westcott, 1999). Although lipid components of most fruit and vegetables are normally present at relatively low levels, their metabolism is important during post-harvest storage of plant tissues, especially when handling and processing conditions are not ideal. Changes in membrane lipids are also important in stress response, ripening and senescence. The aging process in plant tissues is associated with a decline in PUFAs, and such changes apparently accompany autolysis of membranes and loss of cell integrity (Nawar, 1996). Biosynthesis of fruit and vegetable flavours involves many different reactions that can include the metabolism of UFAs.

Background to conjugated linoleic acid

The history of CLAs started in the 1930s, when it was found that dairy fats contained compounds that absorbed UV radiation at around 230 nm (Moore, 1939, as cited by Kramer, Cruz-Hernandez, Deng, Zhou, Jahreis, & Dugan, 2004). In the 1950s, it was discovered that the *cis/trans* structure of the conjugated double bonds had an unique doublet in the *trans* infrared region (Figure 2.3), which has subsequently been used for their specific identification (Jackson, Paschke, Tolberg, Boyd, & Wheeler, 1952). The introduction of very long highly polar capillary columns in gas chromatography (GC) exposed the complexity of the CLA mixtures (Kramer, Blackadar, & Zhou, 2002). It was recently found that silver ion Ag^{+-} HPLC columns resolved both geometric (Figure 2.4), as well as positional CLA isomers and this became a powerful analytic tool to complement GC, for the complete analysis of the CLA isomer profile (Kramer *et al*., 2004).

Prior to 1987, scientific interest in CLA was largely confined to rumen microbiologists, who studied the *cis-9, trans-11*-CLA isomer as an intermediate in the biohydrogenation of C18:2 (Pariza *et al*., 2001). Rumen bacteria produce CLA and MUFAs as intermediates of PUFAs, specifically C18:2 and C18:3*n*-3 (Kepler, Hirons, McNeill, & Tove, 1966; Hughes, Hunter, & Tove, 1982). Two major groups of rumen bacteria have been identified that isomerizes either the *cis*-12 bond to *trans*-11, eg, *Butyrivibrio fibrisolvens*, or the *cis*-9 bond to *trans*-10, eg, *Megasphaera elsdenii*

(Kim, Liu, Rychlik, & Russell, 2002). It was not until 1997, after the use of CLA as a dietary supplement began that Christie, Dobson and Gunstone (1997) demonstrated that commercial CLA was a blend of positional isomers. In response to this discovery, new commercial CLA products

Table 2.6: Percentage C18:1*c*9 and C18:2 fatty acids of vegetable oils and fats (Nawar, 1996; Reaney *et al*., 1999; Stanton *et al*., 1999).

Source		C18:1c9	C18:2
Cereals			
	Corn	44[a]	48[a]/57[b]
	Wheat	31[a]	57[a]
	Rye	18[a]	35[a]
	Rice	35[a]	39[a]
Pulses			
	Soybean	20[a]/23[c]	64[a]/51[b]/51[c]
	Peanut	51[a]/53[c]	26[c]
	Chickpeas	35[a]	51[a]
	Lentils	36[a]	21[a]
Tropical			
	Palm	15[a]	1[a]
	Copra	8[a]	1[a]
	Cocoa	28[a]	
	Coconut	6[a]	
Vegetables			
	Cucumber		72[b]
	Squash (pumpkin)		60[b]
Other			
	Olive	75[a]/74[c]	10[a]/9[c]
	Cottonseed	32[a]/17[c]	45[a]/53[b]/53[c]
	Canola	55[a]/59[c]	20[a]/23[c]
	Sunflower	31[a]/22[c]	57[a]/64[b]/69[c]
	Rapeseed	59[c]	23[c]
	Grapeseed		70[b]
	Linola flaxseed		72[b]
	Poppy		77[b]
	Safflower	14[c]	75[b]/75[c]
	Walnut		62[b]
	Linseed	20[c]	18[c]

Note: Values are percentage of total lipid
[a] Nawar, 1996 [b] Reaney *et al.,* 1999 [c] Stanton, *et al.,* 1999

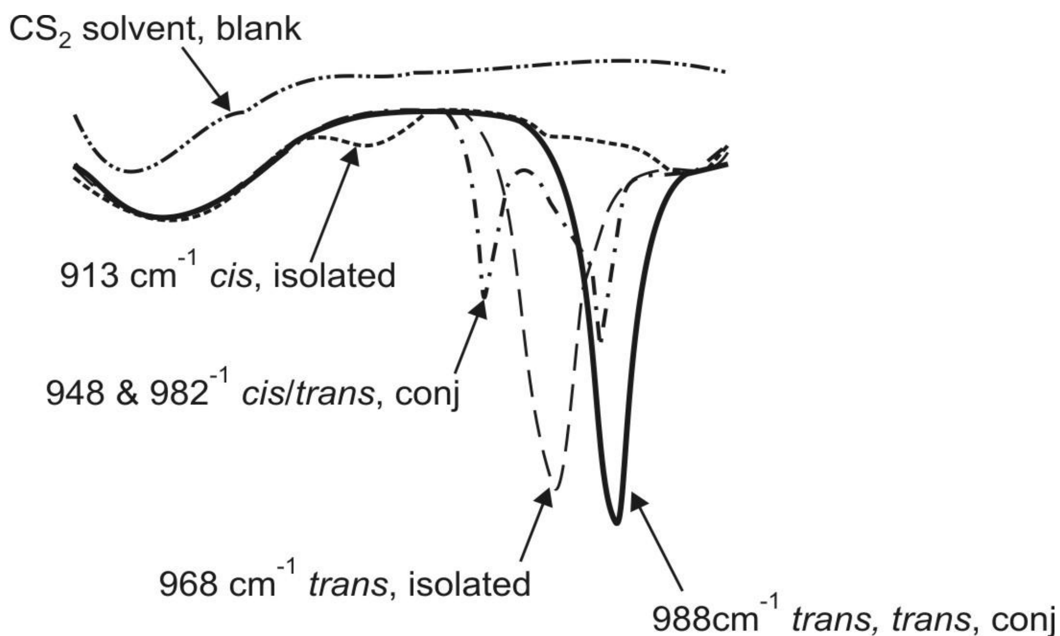


Figure 2.3: Characteristic infrared absorption differences for the =C-H deformation vibration for isolated *trans* double bonds, and conjugated *trans, trans* and *cis/trans* double bonds (Kramer, Sehat, Fritsche, Mossoba, Eulitz, Yurawecz, *et al*., 1999).

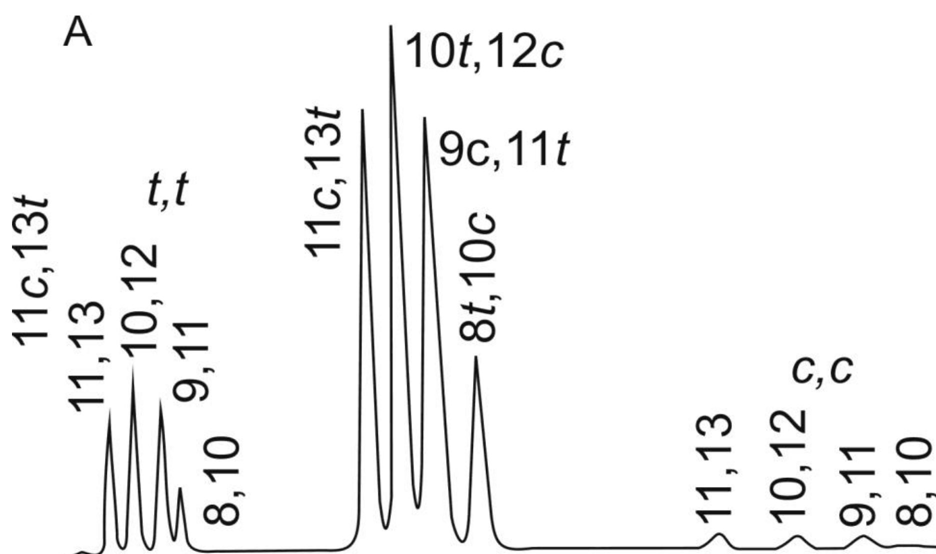


Figure 2.4: Partial silver-ion high-performance liquid chromatography (Ag^+-HPLC) profile of the methyl esters of a commercial conjugated fatty acid mixture (Kramer *et al*., 1999).

have been introduced that have comparatively high levels (±28% of each) of the preferred isomers, i.e. *cis*-9*, trans*-11 and *trans*-10*, cis*-12-CLA. In spite of the improvements, all currently available

commercial CLA products still contain some level of the less desirable isomers and other components that may or may not be desirable (Reaney *et al.*, 1999).

Controversy surrounding conjugated linoleic acid

A nutraceutical is a product isolated or purified from foods that are generally sold in a medicinal form, not usually associated with foods. The term initially arose by combining "nutrition" and "pharmaceutical", and was defined as a food that provided medical or health benefits. A nutraceutical has to be demonstrated to possess protective action against chronic diseases or to have physiological benefit. The concept generally refers to a dietary supplement that contains a concentrated form of bioactive substance originally derived from food (Foster, Amason, & Briggs, 2005). Numerous research studies have been done worldwide over the past 27 years to test the alleged health benefits that have been ascribed to CLA (Table 2.7).

During the first 15 years it was found that although *n*-3 and certain *n*-6 PUFA showed potent effects in animal models of cancer (Cave, 1992), retarded the progression of experimental vascular disease in many species (Leaf & Kang, 1998), and improved diabetic control in rats (Storlien *et al.*, 1987), their use did not yield impressive results in cancer patients (Karmali, 1996), did not alter progression of vascular disease in one human model of atherosclerosis, angioplasty restenosis (Leaf *et al.*, 1994), and worsened rather than improved glucose intolerance in human diabetics (Borkman *et al.*, 1989). Considerable evidence, however, showed that *n*-3 PUFA are essential for optimal brain development in neonates (Innis *et al.*, 1999) and are added to infant formula in a number of countries (Koletsko & Sinclair, 1999). Despite considerable governmental support and thousands of publications from many research groups, the only significant clinical use in adults for *n*-3 FAs was for hypertriglyceridemia in patients who were intolerant of the first-line agents (niacin and the fibrate drugs) or who had an inadequate response to them (Borkman *et al.*, 1989).

In human populations, there is little evidence for a cancer-preventing (primary breast) effect of CLA, if it is valid to extrapolate the consumption of dairy products to CLA intake (Knekt & Järvinen, 1999). A significant decrease in mammary tumor yields in rats was produced by as little as 0.1% CLA (Ip, 1994), indicating that a 350 g rat would consume ~0.015 g CLA/d. If this was extrapolated directly to a 70 kg human, an amount of 3 g CLA would have to be consumed to receive a similar benefit (Ip *et al.*, 1994). A more appropriate extrapolation would be on the basis of metabolic size (body weight$^{0.75}$), meaning that 0.8 g CLA/d would be protective in humans. Therefore, on the basis of the anticancer benefit of CLA in rats as animal models, daily consumption of 0.8-3.0 g of CLA would provide a significant health benefit to humans (Parish, Wiegand, Beitz, Ahn, Du, & Trenkle, 1999).

Table 2.7: Reported health beneficial effects of CLA.

Property	Reference
Anti-cancer	Cave, 1992; Ip, Singh, Thompson, & Scimeca, 1994; Leaf, Jorgensen, Jacobs, Cote, Schoenfeld, Scheer *et al.*, 1994; Karmali, 1996; Knekt & Järvinen, 1999; Chajes, Lavillonniere, Maillard, Giraudeau, Jourdan, Sebedio *et al.*, 2003; Rissanen, Knekt, Jarvinen, Salminen, & Hakulinen, 2003; Field & Schley, 2004; McCann, Ip, Ip, Mcguire, Muti, Edge *et al.*, 2004; Weiler, Austin, Fitzpatrick-Wong, Nitschmann, Bankovic-Calic, Mollard *et al.*, 2004 Watkins, Li, Lippman, Reinwald, & Seifert, 2004; Rastmanesh, 2011
Anti-atherosclerosis	Leaf & Kang, 1998; Kritchevsky, 1999; Mcleod, LeBlanc, Langille, Mitchell, & Currie, 2004; Herrera, Shababuddin, Ersheng, Wei, Garcia, & Lopez-Jaramillo, 2005; Attar-Bashi, Weisinger, Begg, Li, & Sinclair, 2007; Iwata, Kamegai, Yamauchi-Sato, Ogawa, Kasai, Aoyama *et al.*, 2007; Mooney, McCarthy & Belton, 2012
Anti-obesity	Storlien, Kraegen, Chisholm, Ford, Bruce, & Pascoe, 1987; Borkman, Chisholm, Furler, Storlein, Kraegen, Simons *et al.*, 1989; Atkinson, 1999; Belury & Vanden Heuvel, 1999; Gaullier, Halse, Høye, Kristiansen, Fagertun, Vik *et al.*, 2004; Lamarche & Desroches, 2004; Terpstra 2004; Tiikkainen, Bergholm, Rissanen, Aro, Salminen, Tamminen *et al.*, 2004; Wang & Jones, 2004; Park, Albright, Storkson, Liu, & Pariza, 2007; Park & Pariza, 2007
Bone health	Brownbill, Petrosian, & Illich, 2005; Doyle, Jewell, Mullen, Nugent, Roche, & Cashman, 2005; Jewell, Cusack, & Cashman, 2005; Park, Pariza, & Park, 2008
Modulation of immunity	Cook, Devoney, Drake, Pariza, Whigham, & Yang, 1999; Innis, Sprecher, Hachey, Edmond, & Anderson, 1999; Koletsko & Sinclair, 1999; Aminot-Gilchrist & Anderson, 2004; Field & Schley, 2004; O'Shea, Bassaganya-Riera, & Mohede, 2004; Taylor & Zahradka, 2004; Tricon, Burdge, Kew, Banerjee, Russell, Grimble *et al.*, 2004; Song, Grant, Rotondo, Mohede, Sattar, Heys *et al.*, 2005; Turpeinen, Ylonen, Von Willebrand, Basu, & Aro, 2008; MacRedmond & Dorscheid, 2011

Dietary records, food-frequency questionnaires and food intake surveys suggested that typical CLA intake in the United States of America ranged from 52 to 135 mg/d, with lactating women consuming up to 227 mg/d. Studies in Germany and Finland indicated that Germen men consumed amounts ranging up to 430 mg daily, German women, 350 mg daily and Finnish men and women, 310 mg daily. More consumption of dairy products caused the higher intakes of CLA by Germans and Finns than by Americans (McGuire, McGuire, Ritzenthaler, & Shultz, 1999). It can thus be concluded that the typical intake of all test subjects were insufficient, when using the lower value (800 mg/d) of the daily amount necessary for beneficial health effects (Parish *et al*., 1999).

Furthermore, it must be taken into consideration that the human diet is made up of food items, not individual FAs, and that the many compounds, co-ingested with CLA in food products,

could certainly obscure to some degree, any effect this mixture of FAs might have in specific individuals. This data, however, does not rule out the useful preventive effect of CLA against breast cancer in women (Knapp, 1999).

The few human studies on effects of CLA on body composition, reported from 1985 – 1999, have provided a disappointing contrast to the many animal studies that showed increased lean body mass and did not show significant effects on body mass composition (Atkinson, 1999). It was noted that 2-3 g/d of a FA failed to alter body composition, during the course of weight loss in obese individuals. From a practical standpoint, it seemed unlikely that a similar mixture, presented as a food item from the grocery store, would exert beneficial effects in individuals, who had a low likelihood of losing weight or exerting dietary modification for any prolonged period on their own (Knapp, 1999).

Furthermore, despite considerable evidence of the potential for *n*-3 and certain *n*-6 FAs to be useful therapy for several aspects of cardiovascular disease, this health claim was denied by the U.S. Food and Drug Administration (FDA) as being premature (Wallinford & Yetty, 1991). Again, it was pointed out by the FDA reviewers that people eat foods, not specific FAs (Knapp, 1999). Also, the failure of *n*-3 PUFA to become a useful therapy for human diseases, in which they had potent activity in analogous animal models, could be due to species differences. Another concern of clinicians about the work done thus far in CLA and cancer was that studies had by necessity been conducted with impure mixtures and inadequate analytical methods (Knapp, 1999).

Over the last 12 years research has intensified on the discrepancy between animal and human studies, involving again the existing alleged health benefits for body fat reduction, cardiovascular diseases, cancer, immune and inflammatory responses and bone health (Table 2.7).

The safety of commercial CLA preparations has also been evaluated in numerous clinical trials. Most commercial CLA preparations intended for human use, consist > 90% of the two biologically active isomers, in approximately equal amounts (Gaullier, Berven, Blankson, & Gudmundsen, 2002). There is no evidence to indicate that these preparations of CLA, when consumed at 3-6 g/d, will have adverse effects in healthy humans (Gaullier *et al*., 2004). However, there are still concerns regarding the potential safety of therapeutic CLA used in humans for liver functions, milk fat depression, glucose homeostasis and oxidative markers (Riserus, Basu, Jovinge, Fredrikson, Arnlov, & Vessby, 2002; Kelley & Erickson, 2003; Larsen, Toubro, & Astrup, 2003; Tricon & Yaqoob, 2006). Also, cancer patients are already prone to weight loss and their energy intake is lowered due to the disease or treatments. Because cancer patients are anorexic and have lower appetite and have chemosensory changes, it is possible that CLA might indeed reduce appetite further and lead to weight loss (Rastmanesh, 2011).

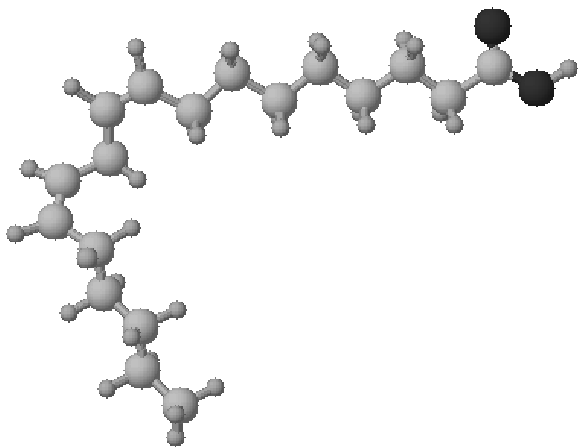
A review of the literature on CLA and loss of body fat or body weight in humans was conducted by Plourde, Jew, Cunnane and Jones (2008), to explore the reasons for the discrepancy between animal and clinical trials. It indicated that the incongruity between human and animal data is largely related to methodological differences in the experimental design, including age and gender and, to a lesser extent, CLA dose and isomers. The relatively unknown metabolic fate of CLA in humans may also be a contributing factor, that helps explain the lack of consistency for CLA efficacy across studies.

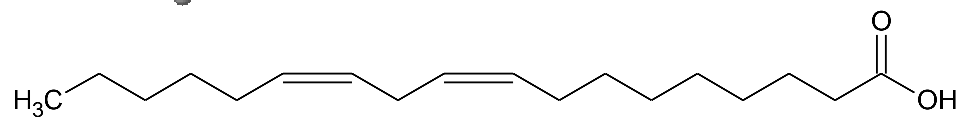
On the other hand, studies on atherosclerosis, involving CLA isomers, indicated potentially beneficial effects through inhibition of monocyte migration, inflammatory mediator expression and foam cell formation. Importantly, the results provide significant evidence that both monocytes and macrophages are critical cellular targets of CLA, providing new avenues of investigation which may elucidate the mechanism of CLA induced regression of atherosclerosis. In addition, studies also showed that both vaccenic acid (VA) and *cis*-9, *trans*-11 CLA possess independent bioactivity in reducing major cardio vascular disease risk and normalising metabolic abnormalities associated with dyslipidemic and pre-diabetic conditions (Wang, Jacome-Sosa, & Proctor, 2011). For some diseases, like asthma, CLA may be considered to fulfill the criteria for “tolerable”, and may be used as a complementary and alternative treatment (MacRedmond & Dorscheid, 2011).

In summary it can be said that, despite all the promising findings, definitive conclusions about the usefulness of CLA in humans, as a nutriceutical in the treatment or prevention of chronic diseases, are limited. Recent results of human studies are variable and therefore unconvincing (Terpstra, 2004). Whether this variability is due to selection bias, cohort size or doses, is not known, but it implies design flaws. Thus, the importance of appropriately powered clinical trials cannot be overstated and they are essential to the validation of CLA’s efficacy. Until that validation is achieved, judgment must be reserved, caution exercised in promoting CLA as a therapeutic product in the management of patients, and continuing research encouraged in this nascent area of inquiry (Angel, 2004).

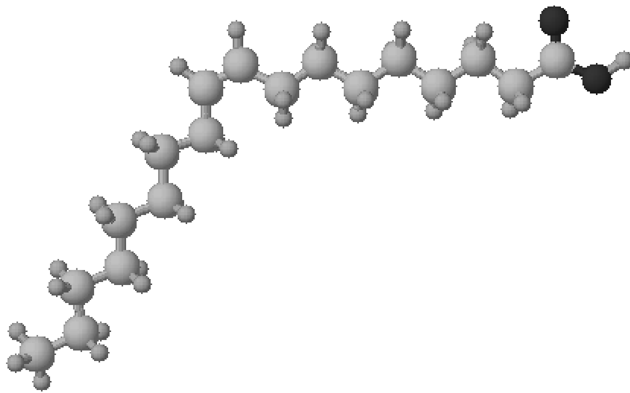
Structure of conjugated linoleic acid

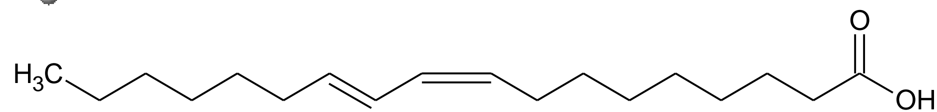
Conjugated linoleic acids are a series of positional and geometric isomers of C18:2 (Figure 2.5). One or both of the double bonds are either in the *cis* or the *trans* configuration and are transposed to different positions along the acyl chain, with the bonds separated by a simple carbon-carbon linkage, rather than by the normal methylene group (Wahle *et al.,* 2004). Many CLA isomers have been identified in natural products, mainly in fats from ruminants, ranging form 7,9 to 12,14 CLA. Each of the positional isomers occurs as four geometric isomers (*cis,trans; trans,cis; cis,cis; trans,trans*), making up a total of 24 (Kramer *et al.*, 2004). The most abundant CLA isomer in



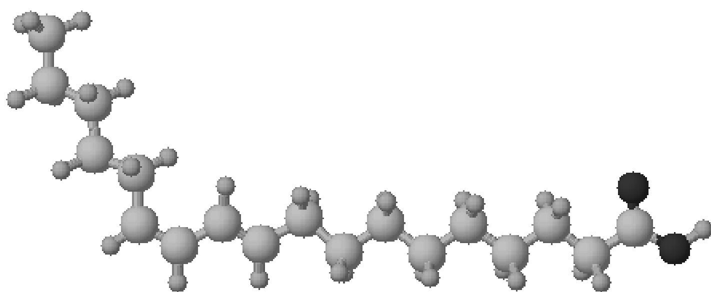


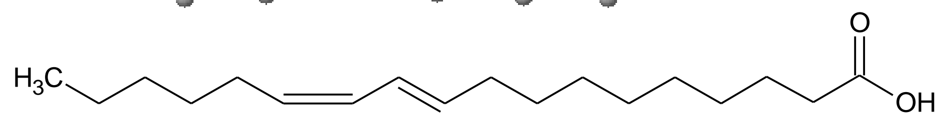
C18:2 c9 c12 - Linoleic acid





C18:2 c9 t11- *cis*-9, *trans*-11-CLA/rumenic acid





C18:2 t10 c12 - *trans*-10, *cis*-12-CLA

Figure 2.5: The chemical structure of the C18:2, *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA (Pariza, 1999).

normal dairy and beef fats is *cis*-9, *trans*-11 (Mir, McAllister, Scott, Aalhus, Baron, McCartney, *et al*., 2004), with smaller levels of *trans*-7, *cis*-9 and *trans*-11, *cis*-13, depending on the diet; the remaining CLA isomers are generally present in low concentrations.

Origins of CLAs in the human diet

The major sources of CLAs are predominantly the fat tissues of ruminant animals or those with ruminant-like, fermentative digestive processes, like wallabies and kangaroos. The main food source of CLAs in the Western diet is thus meat and dairy products derived from cows, sheep, goats and deer. The rumen of these animals is like a large anaerobic fermentation vat, containing microbes capable of biohydrogenating the ingested PUFAs, derived largely from forage, but also from other feed sources, natural or otherwise (Wahle *et al.,* 2004). The bacterial population is influenced by dietary factors to produce either *trans*-11 or *trans*-10 containing FAs. High fat diets, particularly containing high concentrations of soybean, substantially increased the CLA content in milk. This reaffirmed that *cis*-9, *trans*-11 CLA was the principal isomer that was modulated (Pariza *et al.*, 2001), with minor but significant proportions of *trans*-10, *cis*-2 CLA (Parodi, 1997). Parodi (1997) also described the seasonal fluctuation of CLA in cow's milk. The amounts in spring and summer, when cows were pastured, were substantially higher than in fall and winter, when cows were stall-fed. Intriguingly, the highest natural levels of CLA observed in nature occur in wallaby milk (Parodi, 1997), the reason for which is unclear at present, but may reflect the animal's diet.

The CLA content of dairy products, such as cheese and yoghurt, is largely dependent on the CLA content of the milk they are derived from, since processing appears to have little or no effect on the final CLA content (Parodi, 1997). Meat from ruminant animals, particularly the fat associated with meat, is also an important source of CLA, contributing in the region of 25-30% of the total intake in Western populations (Parodi, 1997).

Partial hydrogenation of edible oils, as used in the production of margarines and shortenings, produces a wide spectrum of *cis* and *trans* isomers, including *trans*-11-octadecenoic acid [vaccenic acid (VA)] and CLAs. Studies supported the hypothesis that dietary *trans* FAs in hydrogenated oils were detrimental to human health and had particularly adverse effects on the risk of coronary heart disease (Katan, Zock, & Mensink, as cited by Wahle *et al*., 2004). The edible oil industry responded to these criticisms and developed methods which virtually eliminated the partially hydrogenated isomers from its products, so that modern margarines and shortenings contain negligible quantities of *trans* acids, including C18:1*c*7 and CLA (Wahle & James, 1993).

Formation of CLA isomers

1. Biosynthesis

The *cis*-9*, trans*-11 CLA isomer is produced during the microbial biohydrogenation of C18:2 and C18:3, by the isomerisation and transportation of the Δ^{12} double bond (Wahle *et al.*, 2004), in the rumen of ruminant and rumen-like animals (Figure 2.6). This is the most abundant and natural isomer present in ruminant tissue fats (> 90% of total CLA) and has been termed rumenic acid (RA) (Parodi, 2003).

After formation, *cis*-9*, trans*-11 CLA may be directly absorbed or further metabolized by rumen microorganisms. *Trans*-11-octadecenoic acid (*trans*-11-18:1 VA) (Figure 2.7) is formed, which is the major *trans* MUFA present in the fats of ruminant food products (milk, yoghurt, cheese, butter and meats) (Pariza *et al.*, 2001; Wahle *et al.*, 2004). Following its absorption, VA may then be converted by SCD, within mammalian cells, back to *cis*-9*, trans*-11 CLA. This appears to be a major pathway in the formation of *cis*-9*, trans*-11 CLA in cow's milk (Griinari &Bauman, 1999).

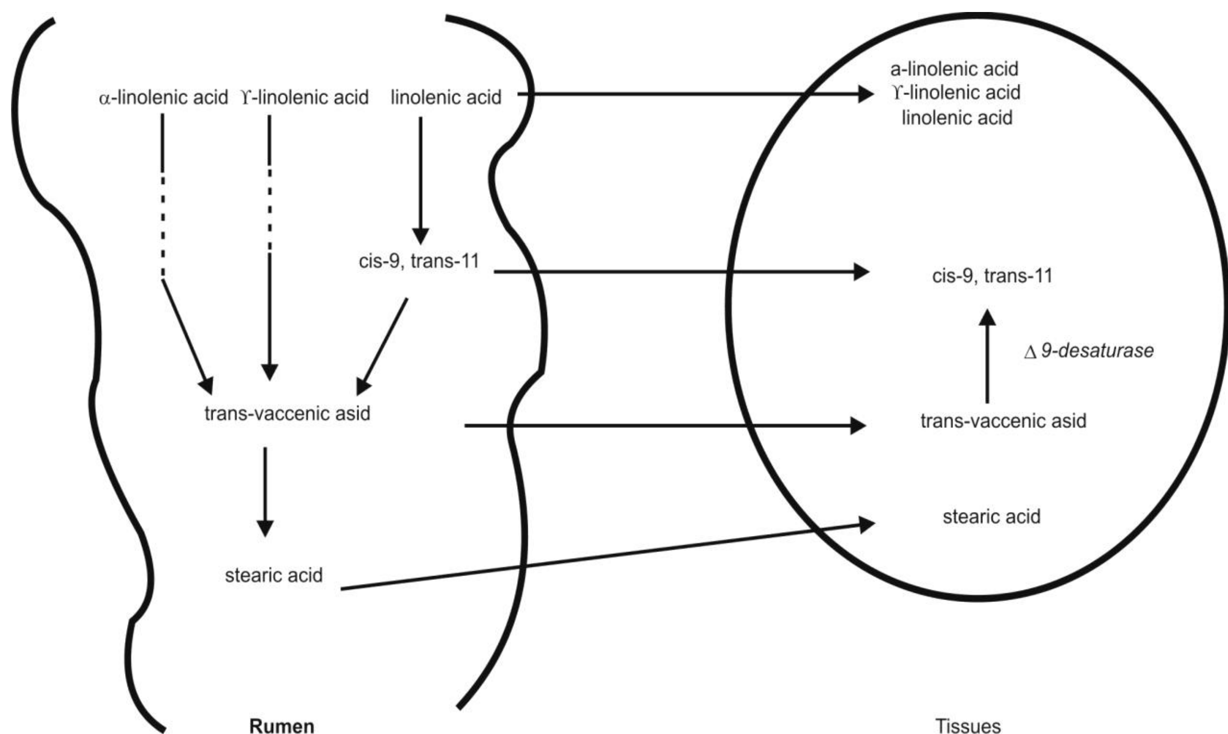


Figure 2.6: Biosynthesis of cis-9, *trans*-11 18:2 (Griinari & Bauman, 1999).

Ongoing research has suggested that individual CLA isomers might act differently in biological systems and contribute differently in their potential side effects. The cis-9, trans-11 CLA was found to be more effective to enhance growth (Pariza *et al.*, 2001) and was preferred over *trans*-10*, cis*-12 CLA to be incorporated into cellular lipid fractions (Yu, Donkin, & Watkins, 1998)

and tissue lipids (Martin, Grégoire, Siess, Genty, Chardigny, Berdeaux. *et al*., 2000). In contrast, *trans*-10*, cis*-12 CLA might be more effective in inducing apoptosis and reducing incorporation of FAs into cell triglycerides (Evans, Geigerman, Cook, Curtis, Kuebler, & Mcintosh, 2000). This isomer also showed a greater potency in changing body composition (Park, Albright, Storkson, Liu, & Pariza, 1999).

As mentioned before, a wide spectrum of minor geometrical and positional isomers of CLA is produced during rumen biohydrogenation by bacterial isomerases. These isomers range from *trans*-6, *trans*-8 CLA to *trans*-13, *trans*-15 CLA, with a number of *cis*-*trans*, *trans*-*cis* and *cis*,*cis* positional isomers in between the extreme positions on the acyl chain. Little is known about any possible beneficial or detrimental metabolic effects of these minor components in a natural CLA mixture (Parodi, 1997).

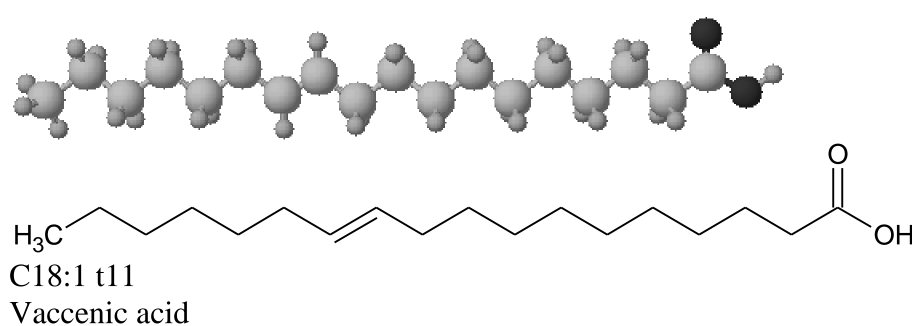


Figure 2.7: Chemical structure *of trans*-11-18:1 vaccenic acid.

2. Chemical synthesis

The aim of chemical synthesis should be to produce a fully characterized CLA composition, with maximal biological activity. Total CLA content and CLA isomeric distribution are two critical quality parameters of commercial CLA products. Accordingly, laboratory methods have been developed to convert C18:2 into CLA, consisting mainly of the *cis*-9*, trans*-11 and *trans*-10*, cis*-12 isomers. The Luta-CLA®60 that was used for the experimental studies in this dissertation consisted of C18:2 (min 56%), *cis*-9*, trans*-11 CLA (min 28%) and *trans*-10*, cis*-12 CLA (min 28%) isomers (BASF, 2003).

Conjugated linoleic acid pork research

Numerous seemingly beneficial physiological effects have been attributed to CLA, including improvements in body composition and increases in both muscle marbling fat and fat hardness. Both of these characteristics have the potential to increase carcass value (Dugan, Aalhus, & Kramer, 2004).

Pigs are monogastric animals and the stomach contains few microorganisms relative to ruminant animals, e.g. cattle. In addition, stomach contents in pigs have a greater rate of passage, relative to ruminants, which further limits the potential for gastric hydrogenation of FAs and the production of CLA. Consequently only a small amount of CLA is produced by way of bacterial biohydrogenation and pork therefore contains a limited amount of CLA (0.1-0.2 mg/g FAs) (Dugan *et al.*, 2004).

When feed-grade CLA became available in 1996-1997 (±28% of each isomere) (Dugan *et al.*, 2004), research began to determine whether feed-added CLA could improve production economics, by improving animal performance and carcass composition, and also to determine whether CLA would affect any aspect of pork quality (Table 2.8).

Table 2.8: Pork research from 1997-2011 at different CLA concentrations (%).

CLA concentration (%) used	Reference
0.12	Ramsay, Evock-Clover, Steele, & Azain, 2001; Thiel-Cooper *et al*., 2001
0.15	Szymczyk, 2005
0.25	Ramsay, *et al*., 2001;Thiel-Cooper *et al*., 2001; Corino *et al*., 2003; Lo Fiego, Macchioni, Santoro, Pastorelli, & Corino, 2005
0.3	Szymczyk, 2005
0.5	Ramsay, *et al*., 2001; Thiel-Cooper *et al*., 2001; D'Souza & Mullan, 2002; Corino *et al*., 2003; Lauridsen, Mu, & Henckel, 2005; Intarapichet, Maikhunthod, & Thungmanee, 2008; Han, Feng, Yu, Tang, Bamikole, Tan *et al*., 2011
0.61	Szymczyk, 2005; Richard, Wiegand, Pompeu, Hinson, Gerlemann, Disselhorst *et al*., 2011
0.75	Wiegand *et al*., 2002
0.92	Szymczyk, 2005
1	Eggert *et al.,* 1998; Carroll, Eggert, Schinckel, & Richert, 1999; Stahl, Eggert, Richert, Gerrard, Forrest, Bowker *et al*., 1999; Heckart, Eggert, Schinckel, Mills, & Donkin, 2000; Schinckel, Eggert, Richert, & Carroll, 2000; Weber, Decamp, Bowers, Herr, Knoll, Richert *et al*., 2000; Eggert, Belury, Kempa-Steczko, Mills, & Schinckel, 2001; Ramsay *et al*., 2001; Thiel-Cooper *et al*., 2001; Weber, Enright, Richert, & Schinckel, 2001; Wiegand *et al*., 2001; Averette Gatlin, See, Larick, Lin & Odle, 2002b; Joo *et al*., 2002; Wiegand *et al*., 2002; Cordero, Isabel, Menoyo, Daza, Morales, Piñeiro *et al*., 2010; Martin *et al*., 2008a; Martin, Antequera, Muriel, Perez-Palacios, & Ruiz, 2011; Martin, Muriel, Gonzalez, Viguera, & Ruiz, 2008b; Han, *et al*., 2011
1.25	Wiegand *et al*. (2001); Wiegand *et al*. (2002)
1.5	King, Behrends, Jenschke, Rhoades, & Smith, 2004; Han, *et al*., 2011
2	Dugan *et al*., 1997;Müller *et al*., 2000; Dugan *et al*., 1999; Tischendorf *et al*., 2002; Dugan *et al*., 2004; Migdal *et al*., 2004; Sun, Zhu, Qiao, Fan, & Li, 2004; Martin, Antequera, Muriel, Perez-Palacios, & Ruiz, 2011
2.5	Joo *et al*., 2002
3	Müller *et al*., 2000; Demaree, Gilbert, Mersmann, & Smith, 2002
4	Sun *et al.,* 2004
5	Joo *et al*., 2002

Animal performance

Thiel-Cooper *et al*. (2001) supplemented the diets of 40 crossbred pigs with low CLA concentrations (0, 0.12, 0.25, 0.5, and 1.0%), to determine its effect on animal and carcass performance. Average daily gain (ADG) increased linearly as the level of CLA increased, while

average daily feed intake (ADFI) was not affected by the concentration of CLA in the diet. A linear increase in FCR was thus observed. Other researchers found that 0.5% CLA supplementation improved the feed utilization by 4.7% and 4.3% for barrows slaughtered at 100 and 130 kg, respectively, and ADG tended to increase (Lauridsen *et al*., 2005). Although 0.75% CLA did not affect ADG and ADFI (Wiegand *et.al*., 2002), FCR responded quadratically (p=0.05) over the entire body weight gain (28 to 115 kg). Han *et al*. (2011) found that feeding 0.5, 1 and 1.5% CLA to 48 pigs did not affect the animals' growth performance and carcass characteristics.

Studies done at a concentration of 1% supplemented CLA had contradicting results. Gilts fed 1% CLA diets, had greater ADG and were more feed efficient during week six to eight than gilts not fed CLA. Schinckel *et al*. (2000), Weber *et al*. (2000) and Martín *et al.* (2008b) found no differences due to dietary CLA at this level, on ADG, ADFI and feed conversion effciciency. In a follow-up study by Weber *et al*. (2001), it was found that feeding 1% CLA diets increased feed efficiency, but had no effect upon ADG or ADFI. Other researchers even found that CLA supplementation at this level decreased ADFI, with no adverse effects on overall growth or feed conversion (Carroll *et al*., 1999), while it was also found to decrease the ADG in lean-genotype gilts (Eggert *et al*., 1998). When Wiegand *et al*. (2001) and (2002) increased the CLA concentration to 1.25%, ADG and ADFI were not affected, but FCR responded quadratically (p=0.05) over the entire BW gain (28 to 115 kg).

Contradicting results were also evident in studies done at 2% CLA supplementation. Average daily weight gain, ADFI and FCR were not influenced by 2% CLA (Tischendorf *et al.*, 2002), and had no influence on fattening results (Migdal *et al*., 2004). In contrast, improved ADG and ADFI of 54 barrows, fed CLA at this concentration, were reported by Sun *et al.* (2004). Müller *et al*. (2000) found in their research with 20 growing gilts (German Landrace) that at 3% CLA, ADG was unaffected.

Fifty-four barrows (Duroc x Landrace x Large White) were fed a 4% CLA diet for six weeks, resulting in improved ADG and ADFI. In another experiment, 54 barrows were fed a diet supplemented with 4% CLA for three to six weeks before slaughter. Average daily gain and ADFI were higher when CLA was fed for six versus three weeks (Sun *et al*., 2004).

Carcass composition

Diets of 40 crossbred pigs were supplemented with CLA at different concentrations (0.12, 0.25, 0.5, 1%). Carcasses from animals fed CLA had decreased 10th rib BF thickness, while ultrasound measurement and carcass measurements showed less fat depth over the loin eye at the 10th rib. When bellies were measured for firmness, either lean side up or down, hardness/firmness increased linearly, as the concentration of CLA in the diet increased (Thiel-Cooper *et al.*, 2001). In another

experiment, 40 barrows and 40 gilts were randomly assigned to five dietary groups containing 0.0, 0.15, 0.30, 0.61 and 0.92% CLA (65% CLA isomers), respectively. No significant differences were found in the pH_{45} and pH_{24} of meat (Szymczyk, 2005), between the treatments.

Corino *et al*. (2003) fed 36 Large White pigs (18 barrows and 18 gilts) either 0.25 or 0.5% CLA diets (65% CLA isomers). No differences were observed in dressing percentage, loin and ham weight, pH, and colour of *longissimus* and *semimembranosus* muscle. D'Souza and Mullan (2002) found that the 10th rib BFT tended to be lower in carcasses from CLA-fed pigs. At an inclusion concentration of 0.75% CLA, Wiegand *et al*. (2002) reported a linear increase in loin muscle area, along with a linear increase in weight gain, while feeding 0.75% CLA to 92 barrows. In accordance with Corino *et al*. (2003), the 10th rib, and also the first and last rib depth, decreased linearly. Subjective quality measures on loin muscles from this experiment increased linearly for marbling and tended to increase for firmness, with increasing weight gain.

Experiments using 1% CLA supplement to pigs' diets, showed contradictory results. Conjugated linoleic acid did not affect the percentage of fat-free lean (Averette Gatlin *et al*., 2002b) or tended to increase it (Carroll *et* al., 1999). Outer layer 10th rib BF depth was decreased (Eggert *et al*., 1998; Carroll *et* al., 1999; Weber *et al*., 2001) and total 10th rib BF tended to decrease in gilts fed 1% CLA. Carroll *et* al. (1999) found that CLA did not affect the inner or middle layers, or the overall 10th rib fat depth (Averette Gatlin *et al*., 2002b; Martín *et al*., 2008b). Gilts also had less last rib fat depth (Weber *et al*., 2001) or it was unaffected (Carroll *et* al., 1999). There was a tendency for loin eye area to be increased (Weber *et al*., 2001), or not affected (Averette Gatlin *et al*., 2002b), increased subjective belly firmness measurements (Eggert *et al*. 1998; Carroll *et* al., 1999; Weber *et al*., 2001), increased belly weights (Averette Gatlin *et al*., 2002b) and numerically greater marbling scores (Weber *et al*., 2001; Averette Gatlin *et al*., 2002b) have all been reported. Eggert *et al*. (1998) also reported no effect on dressing percentage, pH_{24}, loin eye area, or subjective evaluations of loin colour, firmness or marbling. In a follow-up experiment by Eggert *et al.* (2001), 30 genetically lean gilts were allowed *ad libitum* access to a corn-soybean meal diet, supplemented with 1% CLA. Results again showed no effect on pH_{24}, drip loss or subjective quality evaluations of the *longissimus* muscle, but bellies were subjectively evaluated to be firmer. Schinckel *et al*. (2000) found that supplementary dietary CLA at 1% improved colour, firmness, and marbling scores. They confirmed substantial increases in belly firmness scores, predicted fat-free lean, reduced midline last rib BFT and reduced 10th rib BF depth. Less purge loss was also observed with samples from pigs at this concentration (Joo *et al*., 2002). No differences due to 1% dietary CLA were found on carcass yield and loin weight in research by Martín *et al*. (2008b). At a concentration of 1.25%, CLA-supplemented pigs exhibited less 10th rib fat depth and last rib fat depth than control pigs, while loin muscle area was not affected (Wiegand *et al.*, 2001). Dietary

enrichment with 1and 2% CLA increased the loin weight (p<0.01) and the combined weights of the hams + forelegs + loins (p<0.02) (Cordero *et al*., 2010).

Results from a study where 54 pigs (27 gilts and 27 barrows) were fed 2% CLA, no effect on postmortem *M. longissimus thoracis* glycogen utilization, lactate accumulation or pH decline was shown. The pigs had slightly higher *M. longissimus thoracis* temperature at 3 h post mortem, but subsequent *M. longissimus thoracis* shear force, drip loss and soluble protein levels were unaffected. Diet did not affect subjective *M. longissimus thoracis* scores for structure or colour. *M. longissimus thoracis* from CLA fed pigs also had increased subjective marbling scores and increased petroleum-ether-extractable IMF (Dugan *et al*., 1999). Sun *et al.* (2004) found that at this concentration, loin muscle area and IMF increased, while 10th rib fat and last rib fat thickness decreased. In another study, carcass lean was found to increase significantly (Tischendorf *et al*., 2002). No differences were found on carcass yield, loin weight, loin pH and loin colour (Martín *et al*., 2008b).

Intramuscular fat content was increased by 2.5% dietary CLA (Joo *et al*., 2002) and less purge loss was observed There were no effects, at 3% CLA, on the mass of dissected skin, bone, muscle or adipose tissue of the 7th to 9th thoracic rib sections (Demaree et al., 2002) and no difference in fat deposition (Müller *et al*., 2000). Intramuscular fat content was increased by dietary CLA at 4% (Sun *et al.,* 2004) and 5% (Joo *et al*., 2002), while loin muscle area increased at 4% (Sun *et al.,* 2004). Tenth rib fat and last rib fat thickness decreased with increasing dietary CLA at 4% (Sun *et al.,* 2004) and less purge loss was observed at 5% (Joo *et al*., 2002).

Pork quality

Several studies were done where diets were supplemented with CLA at fairly low concentrations to determine its effects on pork quality. Thiel-Cooper *et al*. (2001) added 0.12, 0.25, 0.5 or 1.0% to the feed of 40 crossbred pigs. Conjugated linoleic acid produced a quadratic treatment effect both for less ITF and less subcutaneous fat, and a linear increase for bone. In another study, no significant difference and only a tendency towards increased colour lightness (L*), redness (a*) and yellowness (b*) were found in the meat of pigs fed 0.1, 0.2 or 0.4% CLA. In the group receiving 0.6% CLA, a considerably lighter meat colour was observed. Technologically, the most beneficial WHC capacity was characteristic of the meat of pigs given 0.1 and 0.2% CLA. The 0.4% CLA group's WHC was better than the control group (Szymczyk, 2005).

A dietary treatment of 0.25% CLA had no influence on lipid content of *biceps femoris* muscle and subcutaneous adipose tissue, but increased the content of both isomers, more markedly in the latter tissues (Lo Fiego *et al*., 2005). Lauridsen *et al*. (2005) found that 0.5% CLA had no effect on meat quality responses, such as pH, temperature and WHC. It was also found that 0.5%

CLA tended to reduce the intramuscular cholesterol, but had no influence on the total content of IMF. Recently it was found that loins from pigs fed 0.5% CLA had lighter colour, while ham portions showed darker colour (Intarapichet *et al.*, 2008). Cooked loins from pigs fed CLA supplements gave higher shear forces, while cooked hams had lower forces in spite of higher forces, observed in the raw state (Intarapichet *et al.*, 2008). Subjective scores for colour were not affected by feeding 0.75% CLA, however, subjective marbling scores were increased and subjective firmness scores were higher, as well as highly correlated with marbling scores (Wiegand *et al.*, 2001). Wiegand *et al.* (2002) used a supplement of 0.75% CLA and found that objective Hunter colour values for loin chops were higher for b* values, while lipid oxidation values of loin muscle tissue were lower. Iodine values decreased significantly ($p<0.05$) in fresh pork, containing 0.6% CLA (Richard *et al.*, 2011).

Chops from gilts (n=78) fed 1% CLA (60% CLA) received higher subjective colour evaluations 24 h postmortem and improved colour evaluations, after being displayed for three days, based on the change from day one to three. At 24 h postmortem, the L* values were slightly lower for CLA treated loins, while a* values were higher. Following frozen storage, CLA had a negative effect on colour scores, with a* values being significantly lower in the chops of CLA fed pigs (Stahl *et al.*, 1999). Heckart *et al.* (2000) used 60 gilts and also supplemented their diet with 1% CLA (60% CLA). Feeding CLA decreased the rate of lipogenesis in the average lean line, but did not appreciably alter lipogenesis in the high lean line. Conjugated linoleic acid was found to be most effective in reducing lipogenesis through 45 kg (100 lbs) of body weight and appeared most effective for average lean pigs. There was a tendency for decreased fat cell size in pigs consuming the diet containing CLA. Feeding CLA to the average lean line, decreased cell size and the greatest response to CLA was observed at 45 kg (100 lbs), where adipocytes from average lean pigs fed CLA were smaller than the control (Heckart *et al.*, 2000).

Intarapichet *et al.* (2008) found that loins from pigs fed 1% CLA, had lighter colour, while the ham portions showed darker colour. Similarly to their results for the 0.5% CLA treatment group, the cooked 1% CLA loins gave higher shear forces, while cooked hams had lower forces, in spite of higher forces observed in the raw state. At 1% CLA, Carroll *et al.* (1999) reported that subjective colour scores increased with prolonged CLA feeding, but the L*, a* and b* scores did not reflect this trend. Pork quality scores like marbling and firmness also increased with prolonged CLA supplementation. Martin *et al.* (2008a) found that, after feeding 1 and 2% CLA to 288 finishing gilts, L* values in refrigerated loins were reduced by dietary CLA, whereas a* and b* values were unaffected by dietary supplements.

At 1.5% added CLA, the Δ^9desaturase index and SCD enzyme activity were decreased in the muscle of 18 crossbred barrows. The decrease was expressed as nanomoles of palmitate

converted to palmitoleate and nanomoles of palmitate converted to palmitoleate. This data provided the first direct evidence that dietary CLA depressed SCD enzyme activity in porcine adipose tissue, which may in part be responsible for the depression of adiposity by CLA (Smith, Hively, Cortese, Han, Chung, Casteñada, *et al*., 2002).

Pigs fed 2% CLA deposited less subcutaneous fat and gained more lean (Dugan *et al.,* 1997). Grain soybean meal diets, with 2% CLA, were fed to 80 crossbred pigs. The CLA content of the preparation amounted to 54.0% *cis/trans* + *trans/cis*, 8.7% *cis/cis* and 32.7% *trans/trans* isomers. The male-castrated pigs in this study showed a stronger CLA effect than the female pigs. Not only was the carcass lean significantly increased by 2%, but BFT was significantly decreased by 2.8 mm, i.e. 11%. No effect of the CLA could be detected on pH_{45}, impedance, colour criteria, IMF, drip loss or shear force (Tischendorf *et al.,* 2002). Twenty crossbred fatteners were fed 2% CLA. The only significant differences were observed in dry matter content, meat flavour, L*, b* and hue angle values. Furthermore, the dietary CLA supplementation significantly increased both the number and diameter of white fibres, and correspondingly reduced the number and diameter of red fibres. The percentage and diameter of intermediate fibres were, however, unaffected (Migdal *et al*., 2004). Dietary CLA (1 and 2%) treatment significantly ($p<0.001$) increased IMF content in the *longissimus* muscle and a linear response was observed by Cordero *et al*. (2010).

At higher concentrations, like 4% CLA, researchers reported that the number of cells in subcutaneous adipose tissue was not affected, while adipocyte volume decreased with longer feeding time (6 vs 3 weeks) on dietary CLA (Sun *et al*., 2004). Five percent supplemented CLA improved the colour stability of pork loin during cold storage, for after 7 days, L* and b* values for the CLA fed group were significantly lower than those of the control group (Joo *et al*., 2002).

Fatty acids

The gradual increase of the level of CLA (0.12 0.25, 0.5 to 1.0%) in pigs' feed, were investigated by several rearchers (Ramsay *et al*., 2001; Thiel-Cooper *et al*., 2001; Szymczyk, 2005). With increase in CLA concentration, thiobarbituric acid-reactive substances (TBARS) linearly decreased in meat after 6 month storage at -20ºC. The CLA supplement exerted a strong influence on the composition of FAs in both the lipids of the *M. longissimus* muscle and in the BF. For the *M. longissimus* muscle, a significant increase in the level of SFAs (mainly C16:0 and C18:0) accompanied a decrease in MUFAs. There were no statistical differences between the levels of PUFAs in the meat. The increasing level of CLA in the diets caused a linear increase of CLA isomers in the total FAs (Szymczyk, 2005), as well as in the subcutaneous fat and lean tissue (Thiel-Cooper *et al*., 2001). In the case of BF, the incorporation of CLA isomers was more effective than

in the meat. The *cis*-9, *trans*-11 isomer was incorporated more efficiently into the fatteners' tissues than the *trans*-10, *cis*-12 isomer (Szymczyk, 2005; Han *et al*., 2011).

A steady elevation in the feeding of CLA from 0.25, 0.5, 1 to 2% of the diet, increased the percentage of C18:0, while the percentages of C18:1*c*9 and C18:3*n*-3 were reduced in the *lattisimus* muscle. The dietary CLA also increased the percentages of C16:0 and C18:0 in subcutaneous adipose tissue, while reducing the percentages of C18:1*c*9, C18:2, C18:3*n*-3 and C20:4 (Ramsay *et al*., 2001). CLA supplementation at 0.25% in pigs' feed increased C14:0, C16:0, C16:1 and total SFAs, and decreased C18:1*c*9 and C20:1 in the *M.biceps femoris* and subcutaneous adipose tissue. In the adipose tissue, C18:0 increased (Lo Fiego *et al*., 2005).

Corino *et al*. (2003) fed 0.25 and 0.5% CLA to pigs and found that the oxidative stability of *M.longissimus* muscle was greater, but only at the longer (300 min) oxidation time. Acetyl-CoA carboxylase activity in adipose tissue was also reduced. The composition of ham fat was markedly affected by the dietary CLA, with higher SFAs, lower MUFAs, and higher CLA in the fat of CLA fed pigs.

Pigs fed 0.5 and 1% CLA supplements contained higher moisture, less fat, and lower MUFA:SFA, PUFA:SFA and *n*-6/*n*-3 ratios. Fatty acid compositions were noticeably changed in the loin meats, but not in the hams. The CLA contents of meats increased with elevation of CLA concentration, the *cis*-9*, trans*-11 CLA isomer being the highest in concentration (Intarapichet *et al*., 2008).

Twenty gilts were fed a diet containing 0, 1, 2.5 or 5% CLA for 4 weeks, which reduced the concentration of C18:2 and increased CLA concentration in the IMF of the loins. The concentration of CLA in the muscles, e.g. the *M.longissimus*, and the BF (Han *et al*., 2011) was increased with dietary CLA level and did not change during storage. Thiobarbituric acid-reactive substance values of the CLA fed groups were higher than that of the control group (Joo *et al*., 2002).

Pigs fed 1 % CLA deposited more CLA in their loins and bellies (Eggert *et al*., 1998; Eggert *et al*., 2001; Averette Gatlin *et al.*, 2002b), and deposited an amount which was similar to what they were fed. Their loins contained more SFAs and less UFAs, which resulted in a higher saturated:unsaturated ratio. The belly fat also had more SFAs and less UFAs, resulting in lower IVs (Eggert *et al*., 1998; Eggert *et al*., 2001). Pigs fed 1% CLA had a greater concentration of C18:0 and less C18:1*c*9 in various fat depots, suggesting a reduction in Δ^9desaturase activity (Averette Gatlin *et al.*, 2002b).

Conjugated linoleic acid (1.25%) was fed to 92 growing-finishing barrows. Increasing the period of weight gain, while feeding CLA, linearly increased SFAs and CLA isomers in loin tissue,

and linearly increased SFAs and CLA isomers in subcutaneous adipose tissue (Wiegand *et al.*, 2002).

In a study with 18 crossbred barrows, 1.5% CLA was fed for 5 weeks, resulting in a significant ($p<0.05$) increase in the concentration of total SFAs, especially C16:0, and the isomers of CLA in the adipose tissue lipids. However, the total MUFAs, especially C18:1*n*-9, decreased significantly ($p<0.05$) (King *et al.*, 2004).

Two levels (1% and 2%) of CLA were combined with three levels (zero, low and high) of MUFAs for the feeding of 288 pigs. A significant increase in IMF content and SFAs, a decrease in MUFAs (Martín *et al.*, 2008b; Cordero *et al.*, 2010; Martin *et al.*, 2011), in both the BF and *M.longissimus* muscle (Han et al., 2011), and desaturase indices were found as a consequence of dietary CLA (Martín *et al.*, 2008b).

Meat from pigs supplemented with 2% (Migdal *et al.*, 2004; Martin *et al.*, 2008a) and 4% CLA (Sun *et al.*, 2004) had a significantly higher CLA content. These concentrations also increased C14:0, C16:0 and C18:0 levels, while C18:1*c*9, C18:2, C20:4 (Migdal *et al.*, 2004; Sun *et al.*, 2004) and C18:3*n*-3 decreased in loin muscle and subcutaneous adipose tissue (Sun *et al.*, 2004). Polyunsaturated FAs and the ratio of *n*-6: *n*-3 PUFAs also decreased, with no statistically significant influence on cholesterol (Migdal *et al.*, 2004).

Twenty growing pigs received isoenergetic rations from 0 or 3% CLA, on the basis of metabolic bodyweight. Blood serum lipoproteins exhibited few CLA-induced changes, the greatest effects being observed in the triacylglycerols, which were raised. The ratio of cholesterol-low-density lipoprotein to cholesterol-high-density lipoproteins rose by 17% as a result of the CLA. Serum concentrations of albumin, creatinine, urea and glucose were unchanged (Müller *et al.*, 2000). Twenty four early weaned piglets were fed different diets, supplemented with 3% CLA. Microsomes accumulated < 50% of the concentration of *trans*-10, *cis*-12, *cis*-11, *trans*-13, and *cis*-9, *trans*-11 CLA, as membrane and nonmembrane fractions of adipose tissue and *M.longissimus* muscle. There was no evidence of preferential incorporation of any CLA isomer into any of the subcellular fractions. Addition of CLA to the diets reduced adipose tissue nonmembrane MUFAs, while total SFAs were increased in this lipid fraction. This resulted in a reduced Δ^9desaturase index [MUFA/(SFA + MUFA)] in the nonmembrane lipid fraction. Thus, in spite of marked effects on FA composition and the Δ^9desaturase index, CLA had no effect on adiposity in early weaned piglets, fed high fat diets (Demaree *et al.*, 2002).

Sensory analysis

Limited research has been done on the effect of different concentrations of CLA on the eating quality of pork (Dugan *et al.*, 1999; Thiel-Cooper, Wiegand, Parrish, & Love, 1999; Wiegand,

Parish, & Sparks, 1999; Weber *et al*., 2001; Wiegand *et al*., 2001; D'Souza & Mullan, 2002; Tischendorf *et al.,* 2002; Corino *et al*., 2003; Martin *et al*., 2011) and results from these studies showed great contradictions.

M. Longissimus thoracis chops from crossbred pigs, fed 2% CLA, were broiled in a commercial oven at 174ºC and heated to an internal temperature of 72ºC. Six cubes (1.3 cm) from each chop were randomly assigned to a six member semi-trained sensory panel. Each sample was evaluated for initial and overall tenderness, amount of perceptible connective tissue, juiciness and flavour intensity, using a 9-point descriptive scale (9 = extremely tender, no perceptible connective tissue, extremely juicy and extremely intense pork flavour; 1 = extremely tough, abundant connective tissue, extremely dry and extremely bland pork flavour). In addition, each sample was evaluated for flavour desirability and overall palatability, using a 9-point hedonic scale (9 = extremely desirable, 1 = extremely undesirable). Distilled water and unsalted soda crackers were provided to cleanse the palate of residual flavours between samples. Diet did not affect any measured palatability characteristics, i.e. initial and overall tenderness, juiciness, flavour desirability, flavour intensity, connective tissue amount and overall palatability (Dugan *et al*., 1999).

Subjective firmness scores (range 1 to 5) were assigned to the bellies from pigs on a 1% CLA diet. A score of 5 was assigned to the firmest bellies and a score of 1 to the softest bellies. It was found that subjective belly firmness measurements were increased, following feeding CLA (Weber *et al*., 2001). Dietary treatment, containing 1 and 2% CLA, did not affect the cooking losses, lipid oxidation, volatile profile and sensory traits of cooked loin, evaluated by 17 trained assessors (Martin *et al*., 2011). They did, however, find that the content of the CLA isomers of the fresh meat decreased after the cooking process.

Conjugated linoleic acid was supplemented to crossbred growing-finishing barrows at 1.25%. Sensory evaluation was determined by a ten member panel. Panelists evaluated 1 cm^2 samples of loin chops that were cooked in a General Electric broiler, set at 176ºC. Chops were cooked to an internal temperature of 71ºC. Panelists evaluated samples for tenderness, juiciness and flavour intensity based on an 8-point descriptive scale. The characteristics of tenderness, juiciness and flavour intensity were not affected by the CLA supplementation (Wiegand *et al*., 2001). These results verify previous studies, in which no differences were observed in sensory characteristics with CLA supplementation (Dugan *et al*., 1999; Thiel-Cooper *et al*., 1999; Wiegand *et al*., 1999).

A total of 360 steaks were used for consumer taste panel assessment, after feeding 0.5% CLA to crossbred pigs, to determine the effect of management strategies on pork quality. The *M. longissimus thoracis* steaks were cooked for approximately 5 minutes to a standardised degree of

doneness (medium/well-done, 190ºC until an internal temperature of 75ºC was reached). Each steak was halved and tasted by two consumers. Forty-eight consumers assessed the steaks for odour, tenderness, juiciness, flavour and overall acceptance, using a line scale where 1 = dislike extremely to 100 = like extremely. Control pigs tended to have better flavour, tenderness, juiciness and overall acceptability, compared to pork from CLA fed pigs (D'Souza & Mullan, 2002).

For determination of eating quality, loin slices from 80 pigs, supplemented with 2% CLA, were fried to a core temperature of 70ºC and evaluated by a trained sensory panel. Samples of the two standardised cooked loin steaks per pig were randomly allocated to three panelists. Each sample was assessed on a scale of 1-6 (1 = insufficient, 2 = sufficient, 3 = satisfactory, 4 = good, 5 = very good, 6 = excellent) for the criteria tenderness, juiciness and flavour. It was found that the added CLA had no effect on the sensory attributes (Tischendorf *et al.,* 2002).

Corino *et al*. (2003) prepared 36 hams from pigs which were fed a cereal-based diet, containing 2% CLA. Quantitative descriptive analyses (QDA) were used by the panel of six male and six female assessors. After training to acquire a descriptive lexicon for ham, the following terms were selected as descriptors during sensory evaluation: muscle colour; fat colour; presence of IMF (marbling); rancid flavor; seasoned flavor; saltiness; and mouth consistency (tenderness, brittleness, melting quality). The panelists rated the samples on an anchored intensity scale, ranging from 0 (minimum intensity) to 10 (maximum intensity). Although panelists scored dry-cured hams from the CLA-fed pigs higher for melting quality, the sensory-evaluated colour, aroma, taste, mouth and tactile consistencies of the Parma ham were affected by the inclusion of CLA in the swine diet (Corino *et al*., 2003). This study's results are in contrast with the rest of the findings on the effect of feeding CLA to pigs. It can be ascribed to the fact that it was a highly trained panel of 12 assessors that did the evaluation, in contrast to the consumer panel, semi-trained panel and small trained panel of three members that were used in the other studies. Also, the other studies were done on cooked pork, whilst this result dealt with matured ham.

CONCLUSIONS

Interest in the fortification of human foods with CLA is growing and may provide benefits as neutraceuticals, based on research, evaluating CLA as an anticarcinogen, immune modulator, antiatherogenic agent and a body composition modulator. This tendency also includes pork, providing the pork industry with an opportunity to provide lean, value-added, healthful meat products for human consumption. In addition to the marketing advantages of CLA-enriched or "heart-healthy" pork, CLA has the potential to directly increase profitability to both producers and processors.

As the pork industry continues to select for leanness, pork quality could decline. Conjugated linoleic acid improved the pork quality of genetically lean pigs. The beneficial effects on pork quality and carcass composition measurements such as loin eye area, fat depth, subjective color and marbling, tended to increase in magnitude as the duration of CLA supplementation increased.

Feeding CLA-supplemented diets may provide means by which feed intake can be decreased, while increasing FCR. Backfat can be decreased and percentage lean can be increased, without affecting growth rate. Increased loin muscle area, decreased fat depth, and improvements in marbling and belly firmness could result in improved profitability of pork production systems. Water-holding capacity of pork loin may increase, with an increase in IMF content. Colour stability of pork can be improved, with inhibition of lipid oxidation and changing of FA composition, by dietary CLA.

Results indicated that dietary CLA altered lipid metabolism, producing lower concentrations of MUFAs and increased concentrations of CLA isomers in the fat of heavy pigs. It was demonstrated that feeding CLA to growing pigs decreased adipocyte cell size and the rate of lipogenesis in adipose tissue. Thus, feeding CLA to growing pigs may effectively decrease body fat when pigs are not genetically predisposed to lower adipose accretion and therefore are limited in their responsiveness to agents that depress adipose accretion. On the other hand, feeding CLA may be beneficial in increasing desirable carcass characteristics, related to adipose tissue composition. Part of the effects of CLA, despite little or no effect on gross lipogenesis, may be due to the modification of the nature of the FAs, deposited as adipose tissue, through changes in the expression of the genes for lipid metabolism. Although this was a minimal effect of CLA to reduce lipid synthesis in the high lean pigs, the data indicate potential benefits to pork quality, despite already low carcass lipid content.

Conjugated linoleic acid supplementation had a large effect on increased belly firmness, which is of practical significance, because it will increase the sliceability of lean bellies. Genetically lean gilts often have very soft fat. Lean pigs rely on dietary sources of fat for fat tissue growth, rather than *de novo* fat synthesis. *De novo* fat synthesis yields more saturated fat, which is much firmer than unsaturated fat. Feeding a higher percentage of dietary fat usually decreases *de novo* synthesis, because the pig is acquiring adequate FAs from dietary sources. Under these conditions, the pig will deposit the same type of fat as consumed from its diet. Conjugated linoleic acid, a PUFA, alters this process in some way, since the pig deposits a greater percentage of SFAs, when supplemented with dietary CLA.

CHAPTER 3
MATERIALS AND METHODS

Animal feeding experiment

Fourty-eight Large White x Duroc gilts weighing on average ± 35 kg, were randomly divided into four groups of twelve pigs each. Gilts were used for the following reasons: due to a high percentage of boars being castrated, gilts make up the largest proportion of slaughter pigs, and gilts also have meat quality intermediate to that of boars and barrows (BartonGade, 1987; Babol & Squires, 1995; Xue, Dial, & Pettigrew, 1997). The groups were then randomly assigned to each of four dietary treatments, that consisted of a control diet (CD) containing 1% sunflower oil (SFO), a diet containing 0.75% SFO + 0.25% CLA-60, a diet containing 0.5% SFO + 0.5% CLA-60 and a diet containing 1% CLA-60. The pigs were individually penned in a facility of the Animal Science Department of the University of the Free State in Bloemfontein, and provided *ad libitum* access to feed and water. Feed intake was measured daily, by subtracting feed refused from feed given, and weight was recorded weekly. Pigs were fed until their average live weight was ± 95 kg. The carcass growth rate was expressed as live-weight gain, divided by the days in the trial. Feed conversion ratio was calculated as the total feed intake, divided by the total live-weight gain. The experiment was approved by the Control Committee on Animal Experiments of the University of the Free State (Animal Experiment Number 05/2010).

Diets

The formulation and nutrient composition of the diets are shown in Tables 3.1, 3.2 and 3.3. Diets were formulated to be isocaloric and isonitrogenous (Table 3.2). Fresh SFO was obtained from Chipkins, Bloemfontein, South Africa, in 20 ℓ containers. Luta-CLA® 60, manufactured by BASF, was imported from BASF in Germany by Advit (Johannesburg, South Africa), in a 175 kg container. The CLA mixture contained 60% total CLA, consisting mainly of the two active ethyl-ester isomers, *cis*-9, *trans*-11 and *cis*-10, *trans*-12. All experimental diets were mixed in 2 000 kg batches, at Nutrifeeds, Viljoenskroon. A commercial antioxidant, Oxiban® P, (a mixture of BHA, BHT, ethoxyquin and trisodium citrate) was included in all diets, at a concentration of 0.0125%, to protect it against oxidation during storage. Feed was packaged in 50 kg bags and transported to Bloemfontein, where it was stored in the dark, at room temperature, until used.

Oil and feed analysis

Proximate analysis on the feed was performed by the laboratories of Nutrifeeds in Viljoenskroon. Lipid extraction and fatty acid methyl ester (FAME) analysis of the SFO, CLA, all four diets, as

well as individual fat containing feed ingredients, were determined as described by Folch, Lees and Sloane-Stanley (1957), and Park, Albright, Cai and Pariza (2001). Analysis on the feed was performed on samples from 12 randomly selected bags from each treatment.

Table 3.1: Composition (%) of experimental diets on an air dry basis based on % CLA inclusion.

Component	Control (CL)	0.25% CLA	0.5% CLA	1% CLA
Yellow Maize / Fine	67.87	67.87	67.87	67.87
Maize Gluten / 20 %	3.00	3.00	3.00	3.00
Soyabean Oilcake	20.00	20.00	20.00	20.00
Sunflower Oilcake	3.00	3.00	3.00	3.00
Fish Meal	2.00	2.00	2.00	2.00
Limestone Powder / Fine	1.45	1.45	1.45	1.45
Mono-calcium phosphate	0.47	0.47	0.47	0.47
Fine salt	0.42	0.42	0.42	0.42
Natuphos 500 (Phytase 500- High Inclusion)	0.10	0.10	0.10	0.10
Liquid Choline	0.02	0.02	0.02	0.02
Lysine	0.14	0.14	0.14	0.14
L-Threonine	0.01	0.01	0.01	0.01
Premix L	0.40	0.40	0.40	0.40
Oxiban® P	0.02	0.02	0.02	0.02
Mycosorb	0.10	0.10	0.10	0.10
Sunflower oil (SFO)	1.00	0.75	0.50	0.00
Luta-CLA® 60	0.00	0.25	0.50	1.00
Total	100.00	100.00	100.00	100.00

Slaughter and carcass measurements

At an average live weight of ± 95 kg, the pigs were weighed and their feed removed approximately 12 h before slaughter. Slaughter weights were determined after a 12 h fasting period, just before slaughter. The pigs were transported to the Bloemfontein abattoir, where they were electrically stunned (400 V @ 60 Hz), stuck, scalded (61ºC) and dressed, following commercial procedures. The Hennessey Grading Probe was used to measure the BFT and the thickness of the *M. longissimus thoracis* muscle, 4.5 cm off the carcass midline, between the second and third last rib, within 30 – 40 min of stunning. The % lean meat content (LMC) in each carcass was calculated, according to the formula currently used by the South African meat industry (Bruwer, 1992): % LMC = 72.5114 - 0.4618F + 0.0547M [F = Fat thickness (mm) and M = Muscle thickness (mm) at

45 mm from the carcass midline, between the second and third last rib]. Pigs were then classified according to lean meat content (LMC) into one of six classification groups (PORCUS), namely P = ≥ 70.1 % LMC; O = 68.1 – 70.0% LMC; R = 66.1 – 68.0% LMC; C = 64.1 – 66.0 % LMC; U = 62.1 – 64.0 % LMC; S = ≤ 62.0% LMC (Bruwer, 1992). The number of pigs for each classification group, per treatment, was annotated.

Table 3.2: Formulated nutrient composition of the experimental diets on an air dry basis.

Composition	Unit	Control (CL)	0.25% CLA	0.5% CLA	1% CLA
Protein	%	18.01	18.01	18.01	18.01
Fat	%	4.05	4.05	4.05	4.05
Moisture	%	10.79	10.79	10.79	10.79
Dry Matter	%	88.73	88.73	88.73	88.73
NE	Kcal/kg	2 300.60	2 300.60	2 300.60	2 300.60
ME	Kcal/kg	3221.69	3221.69	3221.69	3221.69
NDF	%	9.12	9.12	9.12	9.12
ADF	%	4.21	4.21	4.21	4.21
Fibre	%	2.85	2.85	2.85	2.85
Ash	%	5.05	5.05	5.05	5.05
Calcium	%	0.79	0.79	0.79	0.79
Phosphorous (P)	%	0.49	0.49	0.49	0.49
Digestible P	%	0.27	0.27	0.27	0.27
Ca / P Ratio	%	1.62	1.62	1.62	1.62
Chlorine	%	0.36	0.36	0.36	0.36
Sodium	%	0.18	0.18	0.18	0.18
Potassium	%	0.67	0.67	0.67	0.67
Magnesium	%	0.18	0.18	0.18	0.18
Sulfur	%	0.21	0.21	0.21	0.21
Isoleucine	%	0.73	0.73	0.73	0.73
Lysine	%	1.02	1.02	1.02	1.02
Methionine	%	0.33	0.33	0.33	0.33
Threonine	%	0.67	0.67	0.67	0.67
Tryptophane	%	0.20	0.20	0.20	0.20
Valine	%	0.86	0.86	0.86	0.86
CYS EQVL	%	0.59	0.59	0.59	0.59

CYS EQVL = Total Cysteine equivalents

Commercial warm carcass weights were also obtained at this time. To identify the PSE and DFD conditions in pigs, pH values were measured 45 min. (pH_{45}), as well as 24 h (pH_{24}) post mortem in the *M. longissimus thoracis,* between the second and third last rib. In this study, a pH_{45} value of ≤

6.00 was used to differentiate PSE from normal pork, whereas a pH_{24} value ≥ 6.00 indicated whether the DFD condition existed in the carcasses.

Table 3.3: Analyzed nutrient composition of the experimental diets on an air dry basis.

Composition	Unit	Control (CL)	0.25% CLA	0.5% CLA	1% CLA
Moisture	%	10.69	10.71	10.94	10.61
Fat	%	4.02	4.04	4.04	4.01
Fibre	%	5.89	6.29	6.28	6.09
Protein	%	19.42	19.55	19.49	19.56
Ash	%	4.66	4.85	4.59	4.44
Phosphorous	%	0.44	0.44	0.44	0.43
Sodium	%	0.15	0.16	0.18	0.17
Magnesium	%	0.16	0.17	0.16	0.16
Potassium	%	0.77	0.75	0.75	0.73
Chlorine	%	0.28	0.28	0.34	0.33
Calcium	%	0.71	0.69	0.64	0.69
Chromium	ppm	4.42	4.65	4.13	4.31
Manganese	ppm	69.5	59.4	68.4	71.6
Iron	ppm	123.1	120.4	112.2	126.9
Cobalt	ppm	<1	<1	<1	<1
Nickel	ppm	3.95	3.93	3.57	3.72
Copper	ppm	98.6	76.8	122.5	121.6
Zink	ppm	148.2	111.8	153.8	174.2
Mercury	ppm	<1	<1	<1	<1
Cadmium	ppm	<0.03	<0.03	<0.03	<0.03
Molibdenum	ppm	2.36	2.89	2.74	2.19
Selenium	ppm	1.43	1.58	1.10	0.75

After a 24 h chilling period in a cold room at approximately 1ºC, cold carcass weights were recorded and carcasses were transported to the meat technology laboratory of the University of the Free State. Heads were removed, carcasses split (between the second and third last rib) and the right loin portion from each carcass (last three ribs) was removed. After the fat was shaved and smoothed, the firmness of the subcutaneous fat was measured with a fat hardness meter MK2 (FHM) on the cross sectional surface, at the position between the second and third last rib. These values were obtained from the average of three readings, adjusted to 1ºC, using the equation: FHM = M – 18(1ºC - TºC), with FHM being the temperature-corrected meter reading, M the actual reading and T the actual fat temperature (Sather, Jones, Robertson, & Zawadski, 1995). Backfat colour and muscle (*M. longissimus thoracis)* colour (L*, a* and b* values) was determined at the

same position, after a 30 min bloom time, with a Minolta CR-200 tristimulus colour analyzer. Saturation index (SI), which is related to the colour intensity of the meat, was calculated according to the formula: $SI = (a^{*2} + b^{*2})^{0.5}$, for both BF and muscle (Lanari, Schaefer, & Scheller, 1995). Hue angle was calculated according to the formula $\tan^{-1}(b^*/a^*)$ (Rippoll, Joy, & Munoz, 2011). To measure the area of the *M. longissimus thoracis* muscle between the second and third last rib, the muscle was traced off directly onto transparant film (Edwards, Cannell, Garrett, Savell, Cross, & Longnecker, 1989). The trace outline was scanned with a scale bar and the eye muscle area measured, using a video image analysis system (Soft Imaging System: Analysis® 3.0). The video image analysing system was calibrated with the scale bar.

Water-holding capacity (WHC) was determined by calculating the ratio of meat area and liquid area, after pressing a 400–600 mg meat sample on a filter paper (Whatman 4, Whatman International Limited, Maidstone, England), sandwiched between two perspex plates, and pressed at constant pressure for 5 min., according to the method described by Grau and Hamm (1953). The areas were measured by means of a Video Image Analyser (analySIS Life Science system), described by Irie, Izumo and Mohri (1996) and WHC was expressed as the area of the meat, divided by the area of the moisture (including meat area). Drip loss was measured on duplicate slices of pork. Fifty grams of fresh meat (24 h post mortem) was sliced into cubes of 10 × 10 × 20 mm and were hung on a pin inside a sample bottle (200 ml), ensuring that the meat did not touch the sides of the bottle. The samples were stored for 3 days at 4°C ± 2°C. The amount of drip, measured as the difference between the sample mass before and after, was expressed as a percentage of the starting mass.

Tissue sampling

Twenty-four h post mortem, a core (±1 g) sample of both layers of subcutaneous fat (position B on Figure 3.1) and *M. longissimus thoracis* (± 5 g) was taken, 45 mm from the mid-dorsal line, between the second and third last rib, on the left side of the carcass. Subcutaneous fat samples (±1 g) were also removed from both layers, as close as possible to the middle of the areas marked A, C, D, E, F and G (Figure 3.1) and muscle samples (5g) of the *M.* s*emimembranosus, M. longissimus thoracis, M. biceps femoris*, *M.* t*riceps brachi* and *M. supra spinatus* (Figure 3.2), were also removed from the left side of each carcass. Samples intended for lipid extraction were stored in Nunc cryotubes in liquid nitrogen, while samples for general chemical analysis were stored in Nunc cryotubes at – 20°C. A 60 g BF sample was removed from position B, on the right side of the carcass, and used for the accelerated oxidation test.

The right loin was removed, cut into 1.5 cm thick chops, frozen and used for stability tests of fresh and frozen pork, and sensory analysis. The left loin, including the last three ribs, was

removed and deboned, while the BF and lean were separated. The lean, as well as BF of the 12 pigs from every dietary treatment, were pooled, minced through a 13 mm Okto mincer plate, vacuum sealed and stored at –18°C, until used for the preparation of pork patties and salami.

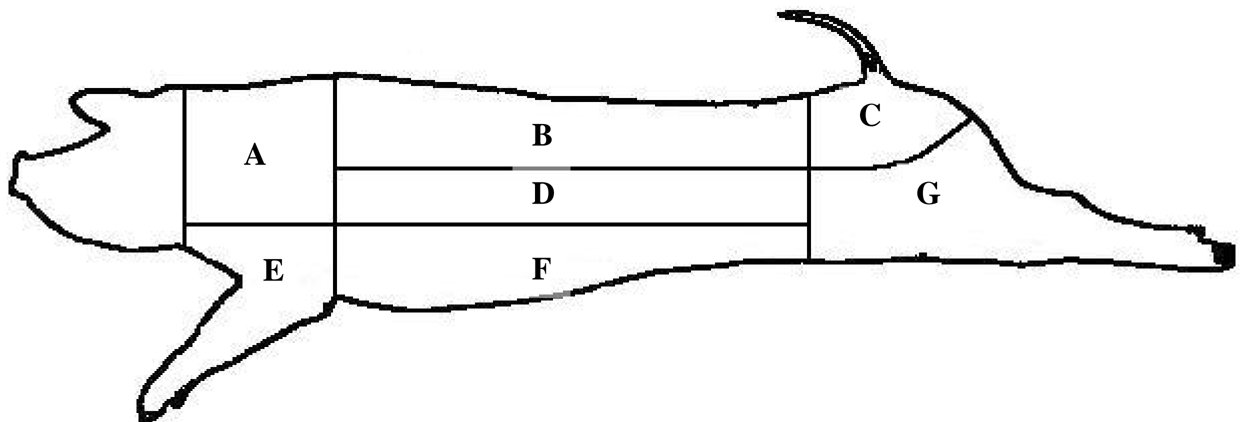


Figure 3.1: Sampling positions for subcutaneous fat (Barton-Gade, 1983).

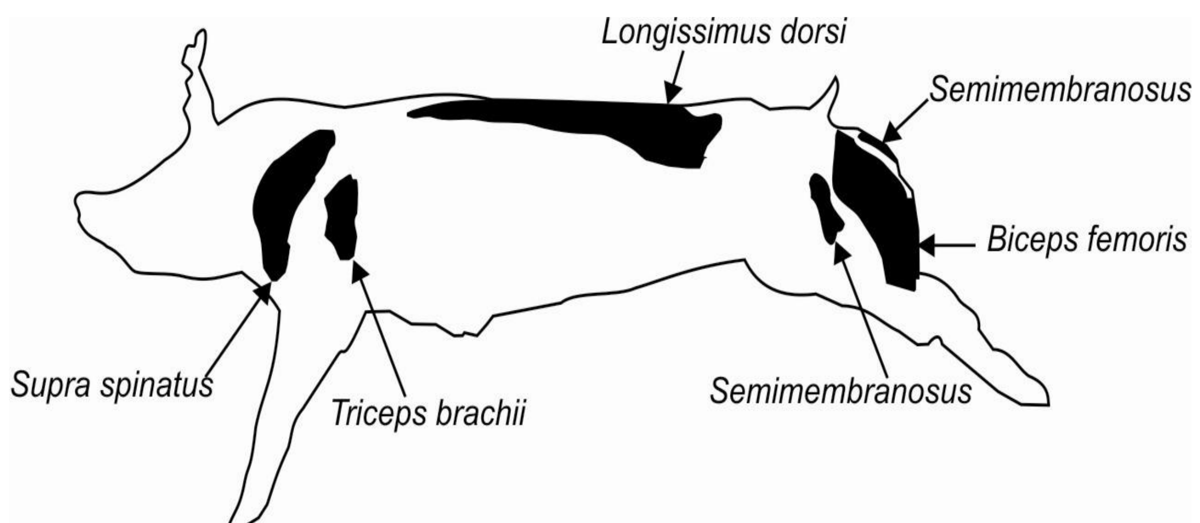


Figure 3.2: Sampling positions for intramuscular fat (Pork.org, 2005).

Intramuscular and backfat quality

Lipid extraction and fractionation

Extraction of total lipids from the muscle (±5 g) and the subcutaneous fat (±1g) was performed quantitatively, according to Folch *et al.* (1957), using chloroform and methanol in a ratio of 2:1. Butylated hydroxytoluene (BHT) was added to the chloroform:methanol mixture as an antioxidant, at a concentration of 0.001%. The extracts were dried under vacuum in a rotary evaporator and

further dried in a vacuum oven at 50º C for 3 h, with phosphorus pentoxide as moisture adsorbent. Total extractable fat content (EFC) was determined by weighing and expressed as % fat (w/w) per 100g tissue. The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for Folch extraction after drying. By determining the difference in weight, the FFDM could be expressed as % FFDM (w/w) per 100g tissue. The moisture content of the muscle and BF was determined by subtraction (100% -% lipid - % FFDM) and expressed as % moisture (w/w) per 100 g of tissue. The extracted fat from muscle and subcutaneous fat samples were stored in polytops (glass tube, with push-in top) under a blanket of nitrogen and frozen at –20ºC, pending FA analysis.

Fatty acid analysis

Total lipids (±30 mg) from IMF, as well as subcutaneous fat, were converted to methyl esters, by base-catalysed transesterification with sodium methoxide (0.5 M solution in anhydrous methanol), in order to avoid CLA isomerisation, during 2 h at 30°C (Park *et al*., 2001). Fatty acid methyl esters (FAME) were quantified, using a Varian 430 flame ionization gas chromatograph (GC), with a fused silica capillary column (Chrompack CPSIL 88, 100 m length, 0.25 mm ID, 0.2 μm film thicknesses). Analysis was performed using an initial isothermic period (40°C for 2 min.). Thereafter, temperature was increased at a rate of 4°C/min. to 230°C. Finally an isothermic period of 230°C for 10 min. followed. Fatty acid methyl esters, in n-hexane (1 μl), were injected into the column, using a Varian CP-8400 autosampler. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas and nitrogen was employed as the makeup gas. Galaxy Chromatography Data System Software recorded the chromatograms. Identification of sample FAME was made by comparing the relative retention times of FAME peaks from samples, with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Conjugated linoleic acid standards were obtained from Matreya Inc. (Pleasant Gap, Unites States). These standards included: *cis*-9, *trans*-11and *trans*-10, *cis*-12 C18:2 isomers. Nonadecanoic acid (C19:0) (SIGMA N553377 – 1G) was used as the internal standard to improve quantitative FAME estimation.

Fatty acid data were used to calculate the following ratios of FAs: C16:0+C18:0; (C16:1+C18:1*c*9)/(C16:0+C18:0); C16:0/C18:2; C18:0/C18:2; total MUFAs; total dienoic FAs; total trienoic FAs; total tetraenoic FAs; total pentaenoic FAs; total hexaenoic FAs; total pentaenoic + total hexaenoic FAs; total SFAs; total PUFAs; total unsaturated FAs (UFA); MUFA/SFA; PUFA/SFA; Δ^9 desaturase index (C18:1*c*9/C18:0); and the ratio of omega-6 to omega-3 (*n*-6)/(*n*-3) FAs. Double bond index (DBI) was calculated as: DBI = Σ % of UFA x number of double bonds of each UFA (Alam & Alam, 1986). Peroxidizability index (PI) was calculated as: PI = [(%

monoenoic x 0.025) + (% dienoic x 1) + (% trienoic x 2) + (% tetraenoic x 4) + (% pentaenoic x 6) + (% hexaenoic x 8)] (Pamplona, Portero-Otín, Riba, Ruiz, Prat, Bellmut *et al.*, 1998). Atherogenicity index (AI) was calculated as: AI = (C12:0 + 4 x C14:0 + C16:0)/(MUFA + PUFA) (Chilliard, Ferlay, Rouel, & Lambere, 2003). Iodine value (IV) for IMF was calculated from FA data, according to Ham, Shelton, Butler and Thionville (1998).

Differential scanning calorimetry

Differential scanning calorimetry (DSC), on subcutaneous fat samples from positions B and F (Figure 3.1), was performed on a Mettler Toledo DSC 822e/700, utilizing sample sizes of approximately 8 mg, in a 40 µl aluminium crucible, that was hermitically sealed. Experiments were performed in triplicate, under a nitrogen atmosphere, utilizing a nitrogen flowrate of 3.5 cm^3 min^{-1}. The crystallization history was destroyed at 80°C for 1 min., followed by cooling to -50°C, at a cooling rate of 10°C min^{-1}. Thereafter, the temperature was cycled three times from -50°C to 85°C and back, with an isothermal break of 1 min. between each temperature apex. Finally, the temperature was returned to 25°C (Sasaki, Mitsumoto, Nishioka, & Irie, 2006). Differential scanning calorimetry thermograms of the first cooling and second heating cycle will be presented. Tempering of the fat was accomplished by destroying the crystallization history, followed by one cooling and heating cycle, after which the fats were kept at 22°C or 4°C for one day or two weeks. Normally, only one heating and cooling rate is chosen (Svenstrup, Brüggemann, Kristensen, Risbo & Skibsted, 2005; Osthoff, Hugo, Joubert & Swarts, 2011).

Other fat quality parameters

A sample of 0.5 g lipid, extracted by Folch extraction from subcutaneous fat, was used to determine the Hanus IV (AOAC method nr. 920.158, 2000). Iodine value was expressed as the number of gram iodine absorbed by 100 g fat, which indicates the level of unsaturation of the fat.

Descriptive analysis of sensory properties of fresh pork

Training of sensory panel

A 12 member trained sensory panel was used for the sensory analysis of the pork. The purpose of the descriptive analysis was to determine how the samples from the different treatments differed in specific sensory characteristics. The 12 panellists were selected to participate based on their participation in previous descriptive sensory panels, taste and smell acuity, interest, ability to discriminate between the four basic tastes and availability for the entire study. They were previously involved in numerous sensory evaluations on various food products, including meat and

meat products (pork, beef, lamb and chicken), fats and oils, dairy products, beverages and many more.

Evaluations were performed in individual sensory booths. Samples were evaluated under red light conditions to mask colour differences. The sensory analysis facilities conform to the American Society for Testing and Materials (ASTM) (1996) design guidelines for sensory facilities. The analyses were conducted over an eight day period (including training). Samples from all treatments were randomly assigned to three sessions (25 min. apart) per day. All samples were coded with random three digit codes. Water and carrot rings at room temperature were served as palate cleanser in between evaluation sessions.

During the training sessions, panelists were exposed to the samples to be evaluated, in order to develop relevant terminology. The 12 panelists received a representative sample of pork meat and fat from the four dietary treatments and were then trained to increase sensitivity and ability to discriminate between specific samples and sensory attributes. In order to ensure that panelists were not influenced in any way, no information, with regard to the nature of the samples, was provided. A clear definition of each attribute was developed to describe the specific attribute to be evaluated. Panelists were instructed to give a detailed description of the aroma, flavour and aftertaste attributes of the pork meat and fat samples (Table 3.4). An eight-point intensity scale was used for scoring the different characteristics of the pork meat and fat from the different treatments (Annexure 1).

Preparation of sensory samples

The frozen pork cuts (at -20°C), were thawed over a 24 h period at 5°C before cooking. The cuts were prepared and evaluated according to the American Meat Science Association and National Live Stock and Meat Board (Chicago, Illinois, 1996) research guidelines, for the cookery and sensory evaluation measurements of fresh meat. The methodology was applied consistently and monitored, and the samples were cooked in identical electric ovens (Mielé H217) to a specific internal temperature (75-77ºC). A hand-held digital probe was used to record internal temperatures at the geometric centre of the meat. Cooking losses during preparation, total cooking time and end temperatures were recorded.

Meat that is low in connective tissue is usually roasted as steaks by consumers. The boneless *M.longissimus* portion was cut into steaks of 25 mm thickness. Steak location within a muscle was standardized and steaks from the anterior end of the muscle were assigned to sensory analysis, and steaks from the other end were assigned to shear-force tests. The steaks were cooked, according to an oven-broiling method, using direct radiant heat. An equal number of steaks from a single treatment were placed on a rack, which was positioned over a pan, to allow meat juices to drain during cooking. The steaks were positioned 9 cm below the pre-heated element of the

Table 3.4: Descriptions and definitions of attributes used by members of the trained sensory panel to evaluate pork fat and meat samples.

DESCRIPTOR	DEFINITION
AROMA – FAT	
Fresh Pork Fat	Aroma associated with fresh pork fat (cooked).
Roasted Pork Fat (caramel)	Aroma associated with cooked, roasted pork meat, meat extract (browned meat, caramel, sweet).
Chemical	Aroma associated with many different types of compounds, such as solvents, cleaning compounds, and hydrocarbons.
Sour	Aroma reminiscent of sour fruit.
Piggy (Old, Musty)	Aroma associated with a live pig or its habitat, or wet pig hair also associated with closed air spaces such as attics, closets (dry,) and basements and turned soil (wet).
AROMA – MEAT	
Roasted Pork Meat	Aroma associated with cooked, roasted pork meat, meat extract (browned meat, caramel, sweet).
Cooked Pork Meat (Fresh Pork)	Aroma associated with freshly boiled pork meat.
Musty	Aroma associated with closed air spaces such as attics, closets (dry), and basements and turned soil (wet).
Livery (Metallic/Bloody)	Aroma associated with the inside of an empty can, tinny. Regarded as a negative attribute.
JUICINESS OF MEAT	
Initial impression	The amount of fluid exuded on the cut surface when pressed between thumb and forefinger.
Sustained impression	The impression of juiciness that is formed when chewing, either dry or moist.
First bite	The impression of toughness of the meat formed on the first bite.
Tough	The impression of toughness of the meat whilst chewing
Tender	The impression of tenderness of the meat when biting into the meat: whether the meat breaks easily between the teeth (tender) or has become tough / difficult to bite through.
FLAVOUR – MEAT	
Meaty	Presence or absence of flavour.
Metallic (Tin/Aluminium)	A flavour associated with slightly oxidized metal, e.g. the inside of an empty can, tinny. Regarded as a negative attribute.
Cooked Pork	Flavour associated with freshly cooked pork meat.
Sour	Flavour reminiscent of citric acid in water.
AFTER TASTE (Off-flavour of meat)	
Metallic	An aftertaste associated with the inside of an empty can, tinny. Regarded as a negative attribute.

ovens (260°C). As the heat radiated form one direction, the steaks were turned during cooking. The steaks were cooked to an internal temperature of 35°C, then turned and finished to an average internal temperature of 75-77°C. The hot steaks were prepared immediately for sensory evaluation. Each panelist received a standardized size of each sample. Cubes measuring 12 mm x 12 mm x

12mm of the cooked steak cuts were used. For the fat, individual cubes measuring 12 mm x 12 mm x 12 mm were used. Only the centre cubes were used and the dryer outsides were avoided. The meat cubes were wrapped in three-digit coded foil squares (90 x 90 mm) and presented (55°C) on pre-warmed plates to the panel. The pieces of fat were each placed into pre-heated glass beakers (60°C), covered with similar pre-coded aluminium foil, placed on a pre- heated sand bath at 120°C and presented simultaneously with the matching meat sample to the panel. The meat sample was tasted first, followed by the smelling of the fat sample.

Physical texture analysis

Physical texture analysis was performed with the Instron Universal Testing Machine, to correlate the results with the findings of the taste panel. After cooking the steaks or intact muscle, the posterior end of the muscle were cooled down at room temperature for at least 5 h, before shear force measurements. Cylindrical samples, with a 12.7 mm core diameter, were taken parallel to the grain of the meat and sheared perpendicular to the fiber direction, using a Warner Bratzler shear device, mounted on a Universal Instron apparatus (cross head speed = 200 mm/minute; one shear in the the centre of each core; 8 cores / sample). The reported value in kg represents the average of the peak force measurements of each sample.

Chemical and oxidative stability studies

Accelerated oxidation test (Schaal oven test)

An accelerated oxidation test was carried out on fresh subcutaneous fat, sampled for this purpose. Fifty ml of melted fat was kept at 63 ± 0.5ºC in a 250 ml low form glass beaker, with free access to air (McGinley, 1991). Peroxide value was determined daily, in duplicate, on each of the six BF samples from each dietary group. Peroxide value was determined on 0.5 g fat, using the AOAC (2000) method nr. 920.158. The keeping quality, from a PV point of view, was determined as the number of days needed until the PV was 100 milliequiv./kg fat (Hertzman, Göransson, & Rudérus, 1988).

Colour and lipid stability of fresh and frozen pork chops

Twelve loin chops from each pig from each treatment group were placed individually in polystyrene trays, containing absorbent pads and over wrapped with oxygen-permeable polyvinyl chloride (PVC) meat stretch wrap. Six loin chops from each pig was stored at 4°C, under fluorescent light, for fresh meat stability studies and the remaining six was stored at –18°C, in the dark, for frozen storage stability studies. These chops were stored in the overwrapped form, to create abusive storage conditions, in terms of exposure to atmospheric oxygen.

On days 0, 4 and 8, two loin chops from each pig stored at 4°C, were opened and colour (L^*, a^* and b^* values) of both muscle and fatty tissue were assessed in duplicate after 30 min., using a Minolta chromometer. Saturation index (SI), which is related to the colour intensity of the meat, was calculated according to the formula: $SI = (a^{*2} + b^{*2})^{0.5}$, for both BF and muscle (Lanari *et al*., 1995). According to Joo, Kauffman, Kim and Kim (1995), SI was better correlated with visual perception of pork colour than a*. Hue angle was calculated according to the formula $\tan^{-1}(b^*/a^*)$ (Rippoll *et al*., 2011). Two 5 g samples of lean were removed from the middle of each loin chop and used for thiobarbituric acid reactive substances (TBARS) analysis, using the aqueous acid extraction method of Raharjo, Sofos and Schmidt (1992), to determine lipid oxidation. A 2 g sample of BF (inner + outer layer) was also removed for the lipid extraction, using the Folch *et al.* (1957) method. To assess lipid oxidation in BF, the PV was determined in duplicate on 0.5 g of the extracted lipid, using the AOAC (2000) method nr. 965.33

To assess oxidative stability during frozen storage, lean and BF were sampled on day 0, at 3 months and 6 months, and TBARS (Raharjo *et al.*, 1992) and PVs (AOAC, 2000; method nr. 965.33) were determined.

Oxidative stability of frozen pork patties

The minced lean and BF, earmarked for the manufacturing of processed meat products, were used to manufacture a batch of 1 kg pork patties for each dietary treatment, according to the formulation outlined in Table 3.5. Lean meat, BF and salt were mixed and then minced through a 4.5 mm plate of a nr. 32 Okto mincer. Eighteen 50 g pork patties, from each of the four treatments, were then prepared by using a handmodel patty press. Each patty was individually packaged in a polystyrene tray and wrapped with an oxygen permeable PVC neat stretch-wrap. The packed pork patties were stored at -18°C until sampling, which took place on day 0, week 8 and week 16 of frozen storage. Similarly to the chops, the patties were stored in the over wrapped form, to create abusive storage conditions. On day 0, twelve 5 g samples (two from each patty) were taken from each treatment for Folch *et al.* (1957) lipid extraction and another twelve 5 g samples (two from each patty) from each treatment, for TBARS determination. Peroxide value was determined on 0.5 g of extracted lipid, using the AOAC (2000) method nr. 965.33, to assess lipid oxidation. This sampling procedure was repeated on weeks 8 and 16.

Quality and oxidative stability of fermented salami

The minced lean meat and BF, earmarked for the manufacturing of processed meat products, were used to manufacture a batch of 7 kg salami for each dietary treatment, according to the formulation outlined in Table 3.6. Lean meat and BF were stored frozen overnight at -18ºC in polythene bags,

Table 3.5: Pork patty formulation.

Ingredient	Inclusion (%)
Lean Pork	80.00
Backfat	18.00
Salt	2.00
Total	100.00

prior to manufacturing the salami. Exactly 50% of the frozen lean pork was placed into a bowl cutter (OKTO 20L Bowl Cutter) and chopped to 10 mm particle size, before addition of the spice mixture (45.95% dextrose, 45.95% sucrose, 4.60% white pepper, 2.75% garlic powder and 0.75% nutmeg) and starter culture (0.25 g of freeze dried starter culture per kg product). The starter culture used was Bactoferm TD-66, CHR Hansen, consisting of a mixture of *Staphylococcus carnosus* (good flavour development and stable red colour) and *Lactobacillus plantarum* (medium to fast initiation of acidification, which results in a medium to low final pH). After the addition of the starter culture and spice mixture, the lean pork was chopped fine. The other 50% of frozen lean pork was then added and chopped to 20 mm particle size. The BF and curing salt (99.31% sodium chloride, 0.30% sodium nitrate and 0.39% sodium nitrate) were finally added and the mixture chopped to the required consistency (± 4-5 mm particle size), at slow bowl cutter speed. The curing salt was formulated to deliver 92 mg/kg sodium nitrate and 120 mg/kg sodium nitrite in the final product. The sausage mix was kneaded into a piston filler (Trespade) to exclude air and filled into Colpak Fibrous Bak 65/50 casings. Twenty-one salamis (250 ± 10 g) were prepared from each of the four batches.

Table 3.6: Salami formulation.

Ingredient	Inclusion (%)
Lean Pork	74.72
Backfat	20.00
Curing Salt	3.05
Spice Mixture	2.18
Freeze-dried Starter culture	0.05
Total	100.00

Three salamis were removed from each batch, weighed and marked before fermentation. These sample weights were used as initial weights to monitor the moisture loss of the rest of the batch, throughout the production period. The pH of these models were also determined at the same intervals as for the weight loss determinations, with a Orion (model 3 STAR pH benchtop) digital pH meter, equipped with a probe (model MA 920). The salamis were transferred to a fermentation

room at 22 ± 0.5ºC, with 90% relative humidity (RH) and fermented for 48 h. After fermentation, the salamis were oak smoked in a smoking chamber (Crown Mills) at 18-22ºC for 10 min., and then transferred to a ripening room at 12 ± 0.5 ºC and 75% RH. During production, the marked salamis were weighed every two days, to monitor moisture loss. The drying and ripening process was considered complete when a 20% minimum weight loss was obtained. Six salamis were drawn from each batch, directly after stuffing (day 0), after fermentation and smoking (2 days), and at the end of ripening (after 20% weight loss). For the purpose of analysis, the casings were removed, the salamis minced, and thoroughly mixed and stuffed (± 5 g) into 5 ml Nunc Cryotubes. If not analyzed immediately, the samples were stored in liquid nitrogen. All salamis were sampled in duplicate.

To determine oxidative stability, TBARS analysis was determined on a 5 g sample, in duplicate, on each of the six salamis from all four batches, using the aqueous acid extraction method of Raharjo *et al.* (1992). Folch *et al.* (1957) extracted lipids from each salami were used to determine PV (AOAC method nr. 965.33, 2000), as well as FFAs (Pearson, 1968). Fatty acid analysis was also performed on all salamis, using the same methodology as described for subcutaneous and IMF.

Quality, oxidative stability and consumer preference of bacon

One pork belly with bone and skin, from each experimental animal in the four treatment groups, was deboned and prepared for brine injection. Before injection, the fat from all four sides of each belly was shaved and smoothed, and the firmness of the subcutaneous fat was measured with a fat hardness meter MK2, on the cross sectional area. These values were obtained from the average of four readings, adjusted to 1ºC, using the equation: FHM = M – 18(1ºC - TºC), with FHM being the temperature-corrected meter reading, M the actual reading and T the actual fat temperature (Sather *et al*., 1995).

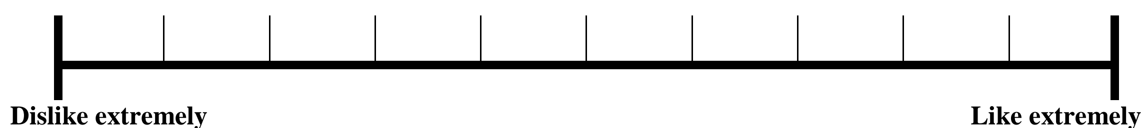
The brine was prepared for a 15% injection level, to deliver 160 mg/kg sodium nitrite, 0.5% sodium tripolyphosphate, 2% salt and 500 mg/kg ascorbic acid into the final product. The brine solution was injected to the desired injection level and the meat left overnight in a cool room at 4°C, to allow for even brine distribution. Bellies were then dried at 60-65°C in a smoking chamber (Crown Mills), whereafter it was oak smoked at 65.5-68.5°C, until golden brown. The smoked meat was frozen, tempered, cut and vacuum packaged in 250 g packages, as for retail purposes. The packed bacons were stored at 4°C until sampling, which took place on day 0, week 3 and week 6 of refrigerated storage. Bacon from each treatment group was also stored at -18°C, until used for sensory analysis. On day 0, 5 g samples were taken from every pack of bacon in the four treatment groups for Folch *et al.* (1957) lipid extraction, as well as for TBARS determination. Peroxide value

was determined on 0.5 g of extracted lipid, using the AOAC (2000) method nr. 965.33, to assess lipid oxidation. This sampling procedure was repeated on weeks 3 and 6.

Packets of bacon from the four treatments were removed from the freezer and defrosted in a refrigerator at 4°C, one day before it was to be evaluated. The bacon from each dietary treatment were separately pan-fried and kept warm in stainless steel containers on hot trays. Care was taken not to cook the bacon any further or let it dry out on the hot plate, during waiting periods. One rasher, with fat intact, from each treatment, measuring about 40 mm, was served to the panelists. Each sample was place in a pre-heated 20 ml glass bowl, covered with a square of aluminum foil. Samples in glass bowls, ready to be served to the panel, were kept warm in a *bain marie*, filled with hot water (60ºC). The four bacon samples were coded with randomized, three-digit codes and rotated to prevent bias. Diluted apple juice [Dairy Belle's *Real Juice* apple (100% apple juice), Tiger Food Brands limited, 3010 William Nicol drive, Bryanston 2191], at room temperature, was served as palate cleanser to prevent fat build-up during tasting. This specific brand was chosen for its bland taste.

Seventy-five regular bacon consumers convened at the sensory facility of the Food Science Division, at specified times, to taste/evaluate and give their opinion on the acceptability of the bacon samples from the four treatments. The panel was compiled of students and staff from the Agricultural Faculty of the University of the Free State. The questionnaire consisted of a 110 mm structured line scale (Figure 3.2), with word anchors "dislike extremely' and 'like extremely" on opposite sides. Respondents were asked to respond to the question "how much do you like or dislike the sample?" and make an X in the appropriate box. Testing was done in individual booths, under red lights, to mask any colour differences, and at an ambient temperature of 20-22°C.

Sample nr:



Dislike extremely **Like extremely**

Figure 3.2: **S**tructured line scale.

Reagents

All reagents were of analytical grade and obtained from Merck, Halfway House, South Africa, unless indicated otherwise.

Statistical analysis

Statistical analysis of animal production and chemical data

Differences between treatments were determined by using factorial analysis of variance (ANOVA) procedure for balanced data. Where applicable, interactions were determined. The Tukey-Kramer multiple comparison test ($\alpha=0.05$) was used to determine differences between treatment means (NCSS, 2007). Differences between treatments for animal production, physical chemical attributes and FA profiles were determined by using an ANOVA procedure for balanced data.

Statistical analysis of quantitative descriptive analysis of sensory properties of fresh pork

The descriptive data obtained from the sensory panel were entered on a spreadsheet, using Microsoft Excel (2000). Data were statistically analysed using the GenStat for Windows (2000) statistical computer program. The significance of all the sensory attributes, measured for each meat and fat sample, was tested by means of an ANOVA, which tested the main effects of the sample at a 5% level of significance. If the sample main effect was significant, the Fisher protected-, t-test Least Significant Difference (LSD) was applied to separate the sample means. Multivariate analysis techniques, principal component analysis (PCA) and canonical variant analysis (CVA), were performed to reduce the large set of variants into a smaller set, to explain most of the variations in the entire data set. Both PCA and CVA are graphical presentations of the data and are used to better classify and group samples, using the sensory variables (Shaw, Moshonas, Buslig, Barros, & Widmer, 1999). A correlation matrix was constructed to show the correlation (positive or negative) between the sensory attributes measured.

Statistical analysis of sensory data from consumer panel on bacon

The significance of the overall acceptance measured for each treatment was tested by means of a one-way ANOVA. When applicable, Fisher's LSD-test ($p\leq0.05$) was applied to determine the direction of the differences between mean values (NCSS, 2007).

CHAPTER 4
RESULTS AND DISCUSSION

Feed composition

The composition of the four diets was the same, except for the decrease in the concentration of the sunflower oil (SFO) from treatment Control (1%), 0.25 % CLA (0.75%), 0.5 % CLA to 1 % CLA (0%), with a corresponding increase in the CLA concentration (0%, 0.25%, 0.5% and 1%) (Table 3.1). Table 4.1 illustrates the fat content, FA composition and FA ratios of the different diets, and Table 4.2, the lipid content and FA composition of individual lipid bearing feed components, which differ in their contribution to the two experimental diets. No significant differences were observed in the fat content of the different diets (Figure 4.1). That also explains to a large extent the similar formulated energy values for the different diets, depicted in Table 3.1.

The control and 0.25% CLA diets had significantly higher SFA contents than the 0.5 and 1% CLA diets (Table 4.1). The reason for this was mainly due to the higher C16:0 contents, which was a result of the SFO in the control diet (Table 3.1). Sunflower oil contained 6.63 % C16:0 compared to the 6.06% of Luta CLA 60® (Table 4.2). Lauric acid (C12:0) and C14:0 were also significantly ($p<0.001$) higher in the control and 0.25% CLA diets than in the 0.5 and 1% CLA diets. The significantly ($p<0.001$) higher percentages of C16:0 and C18:0 in the control and 0.25% CLA diets, also resulted in higher C16:0+C18:0 and significantly ($p<0.001$) higher C18:0/C18:2 contents in these two diets. The C16:0/C18:2 ratio of the 0.25% CLA diet was also significantly ($p<0.01$) higher than in the 1% CLA diet. Furthermore, C20:0, C22:0 and C24:0 were also significantly ($p<0.001$) higher in the control and 0.25% CLA treatment diets than in the 0.5% and 1% CLA treatment diets.

Palmitoleic acid (C16:1) was significantly ($p<0.001$) higher in the control diet than in the CLA-containing diets. That may be attributed to the presence of C16:1 in SFO and the absence of C16:1 in Luta CLA 60® (Table 4.2). The major source of C16:1 in all diets was fishmeal (Table 4.2). The CLA diets had a significantly ($p<0.01$) higher content of C18:1*c*9 than the control (Table 4.1). For C18:1*c*7, the 1 % CLA diet had significantly ($p<0.05$) higher content than the other diets. The reason for this was mainly a 1.47% higher C18:1*c*9 and 0.22% higher C18:1*c*7 content of the CLA diets, compared to the control diet (Table 4.2). Eicosenoic acid (C20:1*c*11) content was significantly ($p<0.01$) lower for the 1% CLA diet treatment than the control and 0.25% CLA diets.

Table 4.1: Chemical properties, fatty acid composition and fatty acid ratios of the four diets used in this experiment.

Dietary Groups	Control n=12	0.25% CLA n=12	0.5% CLA n=12	1% CLA n=12	Sign. level
Chemical properties					
% Fat	4.02 ± 0.11	4.04 ± 0.39	4.04 ± 0.28	4.01 ± 0.39	NS
IV	123.23 ± 0.63^{ab}	121.84 ± 3.32^{a}	123.67 ± 1.66^{ab}	124.62 ± 1.48^{b}	*
Fatty acid composition(%)					
C12:0	0.29 ± 0.02^{c}	0.14 ± 0.06^{b}	0.05 ± 0.08^{a}	0.01 ± 0.02^{a}	***
C14:0	0.44 ± 0.02^{c}	0.33 ± 0.04^{b}	0.29 ± 0.04^{ab}	0.27 ± 0.06^{a}	***
C16:0	11.57 ± 0.18^{b}	11.58 ± 0.60^{b}	11.23 ± 0.44^{ab}	10.91 ± 0.56^{a}	**
C16:1*c*9	0.37 ± 0.02^{b}	0.31 ± 0.03^{a}	0.29 ± 0.02^{a}	0.28 ± 0.06^{a}	***
C17:0	0.10 ± 0.01	0.10 ± 0.01	0.08 ± 0.03	0.09 ± 0.01	NS
C18:0	3.77 ± 0.04^{c}	3.68 ± 0.13^{c}	3.43 ± 0.14^{b}	3.21 ± 0.12^{a}	***
C18:1*c*9	27.14 ± 0.24^{a}	28.08 ± 1.25^{b}	28.14 ± 0.46^{b}	28.16 ± 0.48^{b}	**
C18:1*c*7	0.75 ± 0.02^{a}	0.76 ± 0.03^{a}	0.76 ± 0.03^{ab}	0.79 ± 0.05^{b}	*
C18:2*c*9,12 (*n*-6)	51.69 ± 0.42^{d}	47.48 ± 1.44^{c}	44.98 ± 0.41^{b}	38.15 ± 1.04^{a}	***
C18:2*c*9*t*11(*n*-6) (CLA)	ND	1.80 ± 0.41^{a}	3.64 ± 0.48^{b}	7.35 ± 1.28^{c}	***
C18:2*t*10*c*12(*n*-6) (CLA)	ND	1.79 ± 0.41^{a}	3.63 ± 0.48^{b}	7.34 ± 1.30^{c}	***
C18:3*c*9,12,15 (*n*-3)	1.40 ± 0.04^{b}	1.37 ± 0.11^{ab}	1.30 ± 0.04^{a}	1.39 ± 0.06^{b}	**
C20:0	0.41 ± 0.01^{b}	0.42 ± 0.04^{b}	0.40 ± 0.02^{ab}	0.39 ± 0.02^{a}	***
C20:1*c*11	0.22 ± 0.01^{b}	0.23 ± 0.01^{b}	0.22 ± 0.01^{ab}	0.21 ± 0.01^{a}	**
C20:5*c*5,8,11,14,17 (*n*-3)	0.45 ± 0.03^{b}	0.31 ± 0.06^{a}	0.34 ± 0.02^{a}	0.34 ± 0.08^{a}	***
C22:0	0.33 ± 0.01^{b}	0.35 ± 0.01^{c}	0.31 ± 0.01^{a}	0.31 ± 0.01^{a}	***
C22:5*c*7,10,13,16,19 (*n*-3)	0.12 ± 0.04^{ab}	0.20 ± 0.08^{b}	0.14 ± 0.12^{ab}	0.08 ± 0.06^{a}	**
C22:6*c*4,7,10,13,16,19 (*n*-3)	0.73 ± 0.05^{c}	0.50 ± 0.09^{a}	0.58 ± 0.05^{b}	0.56 ± 0.07^{ab}	***
C24:0	0.18 ± 0.01^{bc}	0.19 ± 0.01^{c}	0.17 ± 0.01^{ab}	0.16 ± 0.01^{a}	***
Fatty acid ratios					
SFA (%)	17.09 ± 0.24^{b}	16.77 ± 0.74^{b}	15.97 ± 0.72^{a}	15.35 ± 0.79^{a}	***
MUFA (%)	28.49 ± 0.25^{a}	29.38 ± 1.27^{b}	29.42 ± 0.51^{b}	29.45 ± 0.59^{b}	**
Dienoic (%)	51.69 ± 0.42	51.26 ± 2.19	52.25 ± 1.21	52.83 ± 1.56	NS
Trienoic (%)	1.40 ± 0.04^{b}	1.37 ± 0.11^{ab}	1.30 ± 0.04^{a}	1.39 ± 0.06^{b}	**
Pentaenoic (%)	0.57 ± 0.05^{c}	0.51 ± 0.06^{bc}	0.48 ± 0.10^{ab}	0.42 ± 0.10^{a}	***
Hexaenoic (%)	0.73 ± 0.05^{c}	0.50 ± 0.09^{a}	0.58 ± 0.05^{b}	0.56 ± 0.07^{ab}	***
PUFA (%)	54.40 ± 0.43	53.64 ± 2.35	54.61 ± 1.20	55.20 ± 1.36	NS
UFA (%)	82.89 ± 0.24^{a}	83.02 ± 1.16^{a}	84.03 ± 0.72^{b}	84.65 ± 0.79^{b}	***
C16:0+C18:0 (%)	15.34 ± 0.21^{c}	15.26 ± 0.71^{bc}	14.66 ± 0.57^{ab}	14.13 ± 0.68^{a}	***
C16:1 + C18:1/C16:0+C18:0	1.84 ± 0.02^{a}	1.91 ± 0.03^{b}	1.99 ± 0.05^{c}	2.07 ± 0.06^{d}	***
C18:0/C18:2	0.07 ± 0.01^{c}	0.07 ± 0.01^{c}	0.07 ± 0.01^{b}	0.06 ± 0.01^{a}	***
C16:0/C18:2	0.22 ± 0.01^{ab}	0.23 ± 0.02^{b}	0.22 ± 0.01^{ab}	0.21 ± 0.01^{a}	*
C18:2/C18:1	1.85 ± 0.03	1.78 ± 0.15	1.81 ± 0.07	1.83 ± 0.09	NS
MUFA/SFA	1.67 ± 0.02^{a}	1.75 ± 0.03^{b}	1.84 ± 0.06^{c}	1.92 ± 0.06^{d}	***
DBI	143.34 ± 0.75^{ab}	141.56 ± 3.87^{a}	143.72 ± 1.91^{ab}	144.75 ± 1.63^{b}	*
PI	64.50 ± 0.74^{b}	61.80 ± 2.97^{a}	63.13 ± 1.22^{ab}	63.35 ± 0.71^{ab}	**
PUFA/SFA	3.18 ± 0.07^{a}	3.21 ± 0.26^{ab}	3.43 ± 0.22^{bc}	3.61 ± 0.26^{c}	***
n-6 (%)	51.69 ± 0.42	51.26 ± 2.19	52.25 ± 1.21	52.83 ± 1.56	NS
n-3 (%)	2.71 ± 0.10^{b}	2.38 ± 0.21^{a}	2.36 ± 0.07^{a}	2.37 ± 0.22^{a}	***
n-6/*n*-3	19.11 ± 0.70^{a}	21.63 ± 1.42^{b}	22.15 ± 0.92^{b}	22.51 ± 2.48^{b}	***

Means with different superscripts in the same row differ significantly.
ND = Not detected; NS = Not significant; * = p<0.05; ** = p<0.01; *** p<0.001

Table 4:2: Lipid content and fatty acid composition of individual lipid-bearing feed components of the four experimental diets

	Soya Oilcake n=3	**Sunflower Oilcake** n=3	**Yellow Maize** n=3	**Gluten 20** n=3	**Fishmeal** n=3	**Sunflower Oil** n=3	**Luta CLA60[R]** n=3
Fat Content of Feed Components (%)	1.72 ± 0.52	1.37 ± 0.59	3.51 ± 0.20	1.78 ± 0.52	10.42 ± 2.13	98.82 ± 0.22	98.58 ± 0.21
% Ingredient in Control Diet	20.00	3.00	67.87	3.00	2.00	1	0.00
% Ingredient in 0.25% CLA Diet	20.00	3.00	67.87	3.00	2.00	0.75	0.25
% Ingredient in 0.5% CLA Diet	20.00	3.00	67.87	3.00	2.00	0.50	0.50
% Ingredient in 1% CLA Diet	20.00	3.00	67.87	3.00	2.00	0.00	1.00
Contribution of Feed Components to the Total Fat Content of Diets							
Fat to Control Diet with 4.02% fat (%)	8.56	1.02	59.33	1.33	5.18	24.58	0.00
Fat to 0.25% CLA Diet with 4.04% fat (%)	8.51	1.02	59.54	1.32	5.16	18.35	6.10
Fat to 0.5% CLA Diet with 4.04% fat (%)	8.51	1.02	59.56	1.32	5.16	12.23	12.20
Fat to 1% CLA Diet with 4.01% fat (%)	8.58	1.02	59.29	1.33	5.20	0.00	24.58
Fatty Acid Composition (%)							
C12:0	ND	ND	ND	ND	0.01 ± 0.03	ND	ND
C14:0	ND	0.02 ± 0.05	ND	ND	5.54 ± 1.46	0.02 ± 0.03	ND
C14:1*c*9	ND	ND	ND	ND	0.04 ± 0.08	ND	ND
C15:0	ND	ND	ND	ND	0.51 ± 0.08	ND	ND
C16:0	13.99 ± 0.73	6.91 ± 0.37	10.54 ± 0.49	14.95 ± 5.19	20.23 ± 2.32	6.63 ± 0.38	6.06 ± 0.40
C16:1*c*9	0.04 ± 0.06	0.02 ± 0.04	0.03 ± 0.04	0.19 ± 0.07	6.25 ± 0.77	0.05 ± 0.01	ND
C17:0	0.05 ± 0.06	0.03 ± 0.05	0.03 ± 0.04	0.03 ± 0.05	0.52 ± 0.16	0.04 ± 0.01	ND
C17:1*c*10	ND	ND	ND	ND	0.26 ± 0.16	ND	ND
C18:0	4.49 ± 0.09	5.31 ± 0.21	2.42 ± 0.11	2.33 ± 0.05	5.09 ± 1.58	4.73 ± 0.15	3.67 ± 0.05
C18:1*t*9	ND	ND	ND	ND	0.36 ± 0.37	ND	ND
C18:1*c*9	17.09 ± 1.57	23.30 ± 1.71	34.68 ± 1.06	24.49 ± 5.36	14.75 ± 5.96	23.56 ± 0.12	25.03 ± 0.17
C18:1*c*7	1.16 ± 0.56	0.11 ± 0.02	ND	0.30 ± 0.21	3.99 ± 0.75	0.55 ± 0.01	0.77 ± 0.01

Table 4:2: Continued

	Soya Oilcake n=3	**Sunflower Oilcake** n=3	**Yellow Maize** n=3	**Gluten 20** n=3	**Fishmeal** n=3	**Sunflower Oil** n=3	**Luta CLA60[R]** n=3
C18:2*t*9,12	ND	ND	ND	ND	0.03 ± 0.05	ND	ND
C18:2*c*9,12(*n*-6)	53.95 ± 0.95	61.44 ± 1.73	49.93 ± 1.12	52.56 ± 1.23	1.69 ± 0.42	63.19 ± 0.29	1.62 ± 0.01
C18:2*c*9,*t*11(*n*-6)(CLA)	ND	ND	ND	ND	ND	ND	30.70 ± 0.54
C18:2*t*10,*c*12(*n*-6) (CLA)	ND	ND	ND	ND	ND	ND	30.59 ± 0.57
C 18:3*c*6,9,12(*n*-6)	ND	ND	ND	ND	0.09 ± 0.06	ND	ND
C18:3*c*9,12,15(*n*-3)	7.68 ± 0.28	0.38 ± 0.17	1.11 ± 0.11	3.35 ± 0.39	0.99 ± 0.14	0.15 ± 0.03	0.17 ± 0.01
C20:0	0.36 ± 0.02	0.47 ± 0.05	0.57 ± 0.02	0.64 ± 0.11	0.36 ± 0.19	0.30 ± 0.03	0.24 ± 0.02
C20:1*c*11	0.13 ± 0.05	0.15 ± 0.02	0.25 ± 0.02	0.20 ± 0.04	4.78 ± 2.32	0.03 ± 0.05	ND
C20:2*c*11,14(*n*-6)	0.01 ± 0.02	0.01 ± 0.03	ND	ND	0.48 ± 0.12	ND	ND
C20:3*c*8,11,14(*n*-6)	ND	ND	ND	ND	0.23 ± 0.05	ND	0.06 ± 0.02
C20:3*c*11,14,17(*n*-3)	ND	ND	ND	ND	0.07 ± 0.06	ND	ND
C20:4*c*5,8,11,14(*n*-6)	ND	ND	ND	ND	1.09 ± 0.40	ND	ND
C20:5*c*5,8,11,14,17(*n*-3)	0.01 ± 0.04	0.04 ± 0.06	ND	ND	11.95 ± 4.75	0.05 ± 0.09	ND
C21:0	ND	ND	ND	ND	ND	ND	0.30 ± 0.35
C22:0	0.58 ± 0.06	0.83 ± 0.05	0.20 ± 0.03	0.38 ± 0.04	0.19 ± 0.07	0.61 ± 0.04	0.58 ± 0.04
C22:1*c*13	ND	ND	ND	ND	0.43 ± 0.50	ND	ND
C22:2*c*13,16(*n*-6)	ND	ND	ND	ND	0.47 ± 0.26	ND	ND
C22:5(*n*-3)	ND	ND	ND	ND	1.96 ± 0.73	ND	ND
C22:6*c*4,7,10(*n*-3)	0.01 ± 0.04	ND	ND	ND	16.66 ± 4.77	ND	ND
C23:0	0.07 ± 0.07	0.02 ± 0.05	ND	0.04 ± 0.07	ND	0.01 ± 0.02	ND
C24:0	0.28 ± 0.05	0.28 ± 0.03	0.22 ± 0.02	0.36 ± 0.02	0.18 ± 0.13	0.13 ± 0.09	0.16 ± 0.01
C24:1*c*15	0.02 ± 0.05	0.20 ± 0.08	ND	ND	0.70 ± 0.49	ND	ND

Table 4.2: Continued

	Soya Oilcake n=3	**Sunflower Oilcake** n=3	**Yellow Maize** n=3	**Gluten 20** n=3	**Fishmeal** n=3	**Sunflower Oil** n=3	**Luta CLA60**[R] n=3
Fatty Acid Ratios							
MUFA (%)	18.44 ± 1.42	23.78 ± 1.73	34.96 ± 1.07	25.18 ± 5.13	31.55 ± 8.71	24.18 ± 0.11	25.79 ± 0.18
Dienoic (%)	53.95 ± 0.95	61.46 ± 1.71	49.93 ± 1.12	52.56 ± 1.23	2.66 ± 0.57	63.19 ± 0.29	62.91 ± 1.11
Trienoic (%)	7.68 ± 0.28	0.38 ± 0.17	1.11 ± 0.11	3.35 ± 0.39	1.37 ± 0.19	0.15 ± 0.03	0.23 ± 0.03
Tetraenoic (%)	ND	ND	ND	ND	1.09 ± 0.40	ND	ND
Pentaenoic (%)	0.01 ± 0.04	0.04 ± 0.06	ND	ND	13.91 ± 4.49	0.05 ± 0.09	ND
Hexaenoic (%)	0.01 ± 0.04	ND	ND	ND	16.66 ± 4.77	ND	ND
Penta- + Hexaenoic (%)	0.03 ± 0.08	0.04 ± 0.06	ND	ND	30.58 ± 8.83	0.05 ± 0.09	ND
C16:0+C18:0 (%)	18.48 ± 0.80	12.23 ± 0.50	12.95 ± 0.49	17.28 ± 5.14	25.32 ± 3.55	11.35 ± 0.23	9.73 ± 0.34
C16:1+C18:1/C16:0+C18:0	0.99 ± 0.11	1.92 ± 0.19	2.68 ± 0.15	1.57 ± 0.66	1.02 ± 0.31	2.13 ± 0.05	2.65 ± 0.07
C18:0/C18:2	0.08 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	3.20 ± 1.20	0.07 ± 0.01	0.06 ± 0.01
C16:0/C18:2	0.26 ± 0.01	0.11 ± 0.01	0.21 ± 0.01	0.28 ± 0.10	12.34 ± 3.14	0.10 ± 0.01	0.10 ± 0.01
SFA (%)	19.84 ± 0.83	13.88 ± 0.62	13.97 ± 0.52	18.73 ± 5.13	32.62 ± 5.22	12.46 ± 0.18	11.02 ± 0.62
UFA (%)	18.44 ± 1.42	23.78 ± 1.73	34.96 ± 1.07	25.18 ± 5.13	31.55 ± 8.71	24.18 ± 0.11	25.79 ± 0.18
MUFA/SFA	0.93 ± 0.11	1.72 ± 0.18	2.51 ± 0.13	1.45 ± 0.59	1.01 ± 0.40	1.94 ± 0.03	2.35 ± 0.12
DBI	149.54 ± 1.68	148.03 ± 1.85	138.14 ± 1.22	140.36 ± 6.19	214.92 ± 41.27	151.27 ± 1.01	152.31 ± 2.13
PI	69.96 ± 1.35	63.05 ± 1.49	53.02 ± 0.99	59.90 ± 2.00	227.36 ± 61.63	64.39 ± 0.75	64.02 ± 1.16
PUFA (%)	61.66 ± 1.06	61.88 ± 1.71	51.04 ± 1.06	55.91 ± 1.61	35.71 ± 8.67	63.39 ± 0.34	63.14 ± 1.14
PUFA/SFA	3.11 ± 0.14	4.46 ± 0.21	3.66 ± 0.17	3.12 ± 0.76	1.12 ± 0.29	5.09 ± 0.09	5.75 ± 0.43
n-6 (%)	53.95 ± 0.95	61.46 ± 1.71	49.93 ± 1.12	52.56 ± 1.23	4.08 ± 0.73	63.19 ± 0.29	62.97 ± 1.13
n-3 (%)	7.71 ± 0.33	0.42 ± 0.15	1.11 ± 0.11	3.35 ± 0.39	31.72 ± 8.78	0.20 ± 0.08	0.17 ± 0.01
n-6/*n*-3	7.01 ± 0.30	162.51 ± 54.29	45.53 ± 5.16	15.78 ± 1.40	0.14 ± 0.06	349.98 ± 116.44	375.41 ± 14.62

ND=not detected

The MUFA contents of all the CLA diets were significantly ($p<0.01$) higher than that of the control diet (Table 4.1), since CLA contained 1.61% more MUFA content than SFO (Table 4.2). The MUFA/SFA ratio increased significantly ($p<0.001$), with an increase in the concentration of CLA in the diets. This can be attributed to the MUFA/SFA ratio of 1.94 for SFO, compared to the MUFA/SFA ratio of 2.35 for CLA 60® (Table 4.2).

The higher C16:1+C18:1/C16:0+C18:0 ratio of Luta CLA 60®, compared to SFO, was also reflected in the higher C16:1+C18:1/C16:0+C18:0 ratios of the CLA-containing diets, compared to the control diet.

The trienoic acid contents of the control and 1% CLA diets were significantly ($p<0.001$) higher than the 0.5% CLA diet, while the *n*-3 content for the contro1 diet was significantly ($p<0.001$) higher than the three CLA diets ($p<0.001$). The pentaenoic acid contents were significantly ($p<0.001$) higher for the control than the 0.5 and 1% CLA diets, while the 0.25% CLA diet also had significantly ($p<0.001$) higher eicosapentaenoic acid (C20:5) contents than the 1% CLA treatment diet. No C20:5 were detected in the Luta CLA 60®, with low amounts in the SFO. The main contributer of C20:5 to all the diets was fishmeal (Table 4.2). The control diet was significantly ($p<0.001$) higher for C20:5 than the three CLA-containing diets (Table 4.1). The 0.25% CLA diet was significantly ($p<0.001$) higher for docosapentaenoic acid (C22:5) than the 1% CLA-containing diet. The docosahexaenoic acid (C22:6) was significantly ($p<0.001$) higher in the control than in the CLA-containing diets, resulting in a significantly ($p<0.001$) higher hexaenoic content in the control and a significantly ($p<0.001$) lower *n*-6/*n*-3 percentage in the control diet. This was reflected in the 25.43% higher *n*-6/*n*-3 ratio of Luta CLA 60®, compared to SFO (Table 4.2). Neither C22:5 nor hexaenoic FAs were detected in either SFO or Luta CLA 60® (Table 4.2). The major source of C22:6 and hexaenoic FAs was the fishmeal (Table 4.2).

Linoleic acid [C18:2 (*n*-6)] decreased significantly ($p<0.001$) from the control diet to the 1% CLA diet, along with the increase in CLA concentration (Table 4.1). Sunflower oil contained 61.57% more C18:2 than Luta CLA 60® (Table 4.2). The contents of C18:2*c*9*t*11(*n*-6) (CLA) and C18:2*t*10*c*12(*n*-6) (CLA) in the diets increased significantly ($p<0.001$) with an increase in CLA concentration, with no CLA detected in the control diet. Luta CLA 60® contained approximately 30% each of both the CLA isomers (Table 4.2). The control and 1% CLA diets had significantly ($p<0.01$) higher C18:3 (*n*-3). That explains the higher trienoic content of these two diets (Table 4.1). The total PUFA contents, C18:2/C18:1 ratios and total *n*-6 contents did not differ significantly between the diets (Table 4.1). The PUFA contributions from SFO and Luta CLA 60® were almost the same (± 63%) (Table 4.2). The total UFA ratio of the 0.5 and 1% CLA-containing diets were significantly ($p<0.001$) higher than that of the control and 0.25% CLA diets. The PUFA/SFA ratio of the 1% CLA diet was significantly ($p<0.001$) higher than that of the control diet, whereas the

PUFA/SFA ratio of the 0.25 and 0.5% CLA diets did not differ significantly. This could be explained by the fact that the UFA content and PUFA/SFA ratio of Luta CLA 60® were respectively 1.61% and 0.66 times higher than that of SFO (Table 4.2).

The calculated IV and DBI were both significantly ($p<0.05$) higher for the 1% CLA diet than for the 0.25% CLA diet, which could be attributed to the increase in the level of the two CLA isomers (Table 4.1). The PI of SFO was 0.37 times higher than that of Luta CLA 60® (Table 4.2), which was reflected in the PI being significantly ($p<0.001$) higher for the control than for the 0.25% CLA diet (Table 4.1). The UFA content of the 0.5 and 1% CLA-containing diets were significantly ($p<0.001$) higher than the 0.25% CLA and control diets. That was because Luta CLA 60® contained 1.61% more UFA than SFO (Table 4.2).

Growth performance and carcass characteristics

As indicated in Table 4.3, no significant differences were observed in the average initial weight of the pigs in the four dietary groups, when entering the experiment. Slaughter weight (SLW) was also not significantly influenced by the increase in CLA concentration from 0 to 1%. Since there were no significant differences in SLW, no significant differences were observed in the total weight increase, for all treatments. Average daily gain, as well as ADFI, also showed no significant differences between the four treatments. Other researchers observed contradicting results. Some found, at the same concentrations of CLA as used in this study, that ADG increased linearly as the level of CLA increased (Thiel-Cooper *et al*., 2001), while others (Wiegand *et.al*., 2002) witnessed no effect on ADG. Average daily feed intake was not affected by the CLA concentration in studies by Thiel-Cooper *et al*. (2001) and Wiegand *et.al*. (2002).

Although not statistically significant, the FCR showed a gradual decrease, with increase in CLA concentration (Table 4.3). Thiel-Cooper *et al*. (2001) found a linear increase in FCR, while Wiegand *et.al*. (2002) reported that the FCR responded quadratically. Schinckel *et al*. (2000), Weber *et al*. (2000) and Martin *et al.* (2008b) found no differences due to 1% dietary CLA inclusion for ADG, ADFI and FCR. Other researchers even found that CLA supplementation at this level decreased ADFI, with no adverse effects on overall growth or FCR (Carroll *et al*., 1999), while it was also found to decrease the ADG in lean-genotype gilts (Eggert *et al*., 1998). Amongst such contradictions, the lack of significance in ADFI between the dietary groups in this experiment, implicated that the increase in CLA concentration to 1% did not have a negative effect on the acceptability of food to the animals. Although diet did not have a significant effect on FCR, Table 4.3 indicates that pigs on the 1% CLA diet, need 0.17 kg less feed to increase 1 kg in weight.

There were no significant differences, between the four treatment groups, for the slaughter characteristics such as warm carcass weight, cold carcass weight and dressing percentage (Table

4.4). Carcass characteristics such as BFT and muscle thickness (MT) were also not significantly influenced by the dietary treatment. As LMC is calculated from BFT and MT, LMC was also not significantly influenced by dietary treatment. Although not statistically significant, there was a trend for LMC to increase, with increased dietary CLA content (Table 4.4). Tischendorf *et al.* (2002) found that CLA supplementation resulted in increased LMC. According to Davenel, Riaublanc, Marchal and Gandemaer (1999), LMC should be < 57% for good technological fat properties. The LMC of all the pigs in the present study were > 57 %.

Table 4.3: Growth performance and feed efficiency of gilts in the four dietary groups.

Dietary Groups	Control n=12	0.25% CLA n=12	0.5% CLA n=12	1% CLA n=12	Sign Level
Initial weight (kg)	40.30 ± 5.03	40.40 ± 4.16	40.23 ± 2.09	40.50 ± 4.45	NS
Slaughter weight (kg) (SLW)	91.99 ± 10.87	90.70 ± 5.23	90.15 ± 5.83	91.02 ± 8.92	NS
Weight increase (kg)	51.69 ± 6.30	50.30 ± 4.92	49.92 ± 6.85	50.52 ± 5.54	NS
Average daily gain (kg) (ADG)	0.85 ± 0.10	0.82 ± 0.08	0.82 ± 0.11	0.83 ± 0.09	NS
Average daily feed intake (kg) (ADFI)	2.67 ± 0.30	2.58 ± 0.17	2.53 ± 0.30	2.46 ± 0.21	NS
Feed conversion ratio	3.17 ± 0.37	3.15 ± 0.25	3.11 ± 0.33	2.98 ± 0.19	NS

Means with different superscripts differ significantly. NS = Not significant; * = p<0.05; ** = p<0.01; *** p<0.001

Averette Gatlin *et al.* (2002b) similarly, did not find any effects of 1% CLA on quantitative carcass measurements (Schinckel *et al.*, 2000; Weber *et al.*, 2000; Martin *et al.,* 2008b). The average BFT of the pigs from all the treatments in the present study, except the 1% CLA treatment (14.33 mm), exceeded the minimum value of 15 mm BFT, as proposed by Davenel *et al.* (1999) for good fat quality. None of the other dietary groups (all < 16.70 mm) reached the minimum BFT of 18 mm, which was proposed by Prabucki (1991) for good fat quality.

There were thus a lack of significant differences in pig performance and growth traits (weight increase, ADG, ADFI, FCR), and slaughter characteristics (SLW, hot carcass weight, cold carcass weight, dressing percentage, BFT, MT and LMC) between pigs in all four treatment groups.

In South Africa, BFT and eye muscle thickness are used to classify pigs into one of six classification groups (SAMIC, 2009). Groups P (< 12 mm BF and > 70 % lean meat) and O (< 18mm BF and > 69% lean meat) are the leanest groups; R, C, U, and S, are the fatter groups. Lean classification groups are more desirable, as producers get paid more for these pigs. Table 4.4 indicates that 66.7% of the pigs in the control and 0.25% CLA groups, obtained P and O classifications, compared to 83.3% of pigs in the 0.5% CLA and 100% of pigs in the 1% CLA groups. This clearly illustrated that CLA supplementation improved the lean classification of pigs

and supported the findings of Eggert *et al*. (1998) and Migdal *et al*. (2004). This finding is of practical significance for the South African pig producer, since more pigs being classified as P and O means more profitable pig production.

Quality characteristics of the *M. longissimus thoracis* measured between the 2nd and 3rd last rib

Dietary supplementation of CLA did not result in a difference in the *M. longissimus thoracis* (eye muscle) area for the four treatments (Table 4.4). This confirmed the findings of Wiegand *et al.* (2001) and Averette Gatlin *et al*. (2002b), who found a tendency for the loin eye area to be unaffected by the addition of CLA to the pigs' feed. Characteristics like drip loss and WHC were also not significantly influenced by dietary treatment (Table 4.4), although there was a gradual increase in WHC, with increase in concentration of CLA. Eggert *et al.* (2001) and Tischendorf *et al*. (2002) showed that dietary CLA had no influence on WHC of pork meat. This was confirmed by Joo *et al.* (2002), who used 5% CLA in the diet and still observed no effect on drip loss. When feeding 0.5% CLA to as many as 100 barrows, Lauridsen *et al*. (2005) also did not find any dietary effect on WHC. Furthermore, there were no differences between the four treatments for pH_{45} and pH_{24}, which were in accordance with the results of Eggert *et al.* (2001) and Martín *et al.* (2008b).

Two of the muscle colour ordinates showed significant differences between the four treatments. The b* values for the control differed significantly ($p<0.01$) from the 0.5 and 1% CLA treatments, but not from the 0.25% CLA treatment. There was a gradual decrease in the value of the L* values, from the control to the 1% CLA treatment. Stahl *et al*. (1999) also found that the L* values were slightly lower for CLA treated loins. The lower L* values of the eye muscle area, from the 1% CLA fed group for the present study, indicated less lightness of pork, which may be related to the observed tendency of increased WHC. The L* value measures luminosity, which increases when light scattering increases, generally as a result of the increase in free water, due to an increase in protein damage. Therefore, as pH approaches the iso-electric points of the various water-binding muscle proteins, free water increases, scatters more light and the tissue appears "lighter" (Brewer, Zhu, Bidner, Meisinger, & Mckeith, 2001). Furthermore, Migdal *et al*. (2004) found that dietary CLA supplementation significantly increased both the number and diameter of white fibres, and correspondingly reduced the number and diameter of red fibres. The colour b* values (yellowness) also differed significantly ($p<0.05$) between the four dietary treatments. The control treatment differed significantly ($p<0.05$) from the 1% CLA treatment, but not from the 0.25 and 0.5% CLA treatments. The 0.25, 0.5 and 1 % CLA treatments did not differ significantly ($p<0.05$) from one another. Again there was a decrease in b* values from the control to the 1% CLA treatment. This was supported by previous reports, indicating that feeding CLA at higher concentrations ($\geq$ 1%),

Table 4.4: Carcass characteristics and muscle and backfat characteristics of gilts in the four dietary groups.

Dietary Groups	Control n=12	0.25% CLA n=12	0.5% CLA n=12	1% CLA n=12	Sign level
Carcass Characteristics					
Warm Carcass Weight (kg)	76.73 ± 8.86	74.67 ± 4.77	73.97 ± 4.49	74.20 ± 7.57	NS
Cold Carcass Weight (kg)	74.43 ± 8.59	72.42 ± 4.63	71.77 ± 4.35	71.98 ± 7.34	NS
Dressing Percentage	83.03 ± 0.99	82.30 ± 1.02	82.08 ± 2.02	81.50 ± 0.64	NS
Backfat Thickness (BFT) (mm)	16.67 ± 5.54	16.00 ± 2.76	16.50 ± 3.89	14.33 ± 2.66	NS
Muscle Thickness (MT) (mm)	54.08 ± 6.14	59.17 ± 3.49	58.58 ± 6.67	55.83 ± 4.20	NS
Lean Meat Content (LMC) (%)	67.77 ± 2.43	68.36 ± 1.38	68.10 ± 1.82	68.95 ± 1.27	NS
Number of Pigs with P Classification	0	0	0	4	NSA
Number of Pigs with O Classification	8	8	10	8	NSA
Number of Pigs with R Classification	2	4	0	0	NSA
Number of Pigs with C Classification	2	0	2	0	NSA
Conformation score (CF)	3.33 ± 0.52	3.67 ± 0.52	3.50 ± 0.55	3.67 ± 0.52	NS
% Pigs with P and O Classification	66.7	66.7	83.3	100.0	NSA
Quality Characteristics of Eye Muscle measured between the 2nd and 3rd last Rib					
Eye Muscle Area (mm²)	3802.09 ± 763.34	3938.86 ± 328.96	4060.99 ± 459.48	3813.80 ± 667.70	NS
Drip Loss (%)	3.09 ± 0.67	2.94 ± 0.55	3.04 ± 0.48	3.15 ± 0.49	NS
Water holding Capacity (WHC) (Area1/Area2)	0.35 ± 0.06	0.36 ± 0.05	0.37 ± 0.07	0.39 ± 0.06	NS
pH_{45min}	6.36 ± 0.27	6.36 ± 0.34	6.49 ± 0.21	6.42 ± 0.24	NS
$pH_{24hours}$	5.50 ± 0.05	5.47 ± 0.03	5.55 ± 0.11	5.51 ± 0.06	NS
Colour L*- Value	57.54 ± 3.22^{b}	54.66 ± 2.35^{ab}	50.63 ± 4.05^{a}	51.30 ± 2.07^{a}	**
Colour a*- Value	8.00 ± 2.62	7.33 ± 1.81	7.18 ± 2.08	6.07 ± 1.11	NS
Colour b*- Value	9.36 ± 2.03^{b}	8.11 ± 1.36^{ab}	6.88 ± 1.66^{ab}	6.77 ± 0.94^{a}	*
Chroma	12.35 ± 3.19	10.96 ± 2.13	9.99 ± 2.49	9.15 ± 1.25	NS
Hue Anngle	50.17 ± 4.39	.48.33± 4.14	44.04 ± 5.89	48.21 ± 4.79	NS
Quality Characteristics of backfat measured between the 2nd and 3rd last Rib					
Iodine Value (IV)	68.31 ± 2.38^{b}	62.08 ± 2.63^{a}	58.14 ± 5.05^{a}	57.59 ± 2.52^{a}	***
Fat Hardness	773.46 ± 102.85^{a}	985.30 ± 22.15^{b}	996.64 ± 60.40^{b}	1026.06 ± 43.21^{b}	***
Colour L*- Value	75.16 ± 2.21	76.37 ± 2.01	75.64 ± 0.62	75.75 ± 1.63	NS
Colour a*- Value	2.59 ± 1.58	1.85 ± 1.83	3.47 ± 1.55	3.37 ± 1.45	NS
Colour b*- Value	10.05 ± 0.77^{ab}	9.74 ± 0.89^{a}	10.53 ± 0.45^{ab}	11.11 ± 0.40^{b}	*
Chroma	10.49 ± 1.17	10.05 ± 1.25	11.17 ± 0.88	11.71 ± 0.76	NS
Hue Angle	76.15 ± 7.28	72.63 ± 12.42	72.19 ± 6.89	73,44 ± 6.42	NS

Means with different superscripts differ significantly. NS = Not significant; NSA = Not statistically analyzed;* = p<0.05; ** = p<0.01; *** p<0.001, SI = $(a^{*2} + b^{*2})^{0.5}$

P = < 12 mm BF and > 70 % LMC; O= < 18mm BF and 68- 69% LMC; R= 18-22mm BF and 66-67% LMC; C= 23-27mm BF and 64-65% LMC; CF: very flat=1; flat=2; medium=3; round=4; very round=5

could lead to a decline in b* values (Joo *et al.,* 2002; Migdal *et al.*, 2004). It could be possible that the lower b* values (less yellow colour) of the 1% CLA group, was due to the improved oxidative stability of IMF in pork. Chan, Faustman and Renerre (1996) reported that changes in meat colour were related to lipid oxidation. Colour a* values (redness), the colour saturation index (chroma) and Hue angle did not differ between the four treatments in the present study, although they decreased from the control to the 1% CLA treatment groups. Dugan *et al.* (1999) reported slightly higher chroma values in loins from CLA-fed pigs. According to Migdal *et al.* (2004), muscle pigment is the major factor affecting the a* and b* coordinates and the observed differences were most likely related to a lower concentration of pigment in the treated animals. Colour measurements observed in this study were within the normal ranges normally observed for pork (Dugan *et al.,* 1999).

Quality characteristics of Backfat measured between the 2nd and 3rd last rib

Iodine value (IV) is a parameter widely used to evaluate adipose tissue softness and gives an overall estimate of FA saturation. The higher the IV, the softer the fat (Fischer *et al*., 1989a). Hanus IV of BF was influenced by dietary treatment, with BF from the CLA treatments having significantly ($p<0.001$) lower IVs, compared to the BF from the control group (Table 4.4). However, IVs of the BF of pigs from the different CLA treatments did not differ, although it decreased with an increase in CLA content. The IV for the control treatment (68.31) was higher than the maximum value of 66, set by Hart as cited by Houben & Krol (1983), or the very strict maximum value of 65, set by Mortensen, Madsen, Bejerholm and Barton-Gade (1983), but lower than the maximum value of 70, proposed for good technological quality (Girard, Bout & Salort, 1988). The IVs for the CLA treatments were even lower than the very strict maximum value of 65, namely 62.08 (0.25% CLA), 58.14 (0.5% CLA) and 57.59 (1% CLA). Van den Berg, Cook and Tribble (1995) showed that dietary CLA reduced C20:4, C18:2 and C18:1*c*9 content in fat, shifting the whole FA composition to the more saturated side.

Significant differences were observed in FHM measurements of BF from the different dietary groups (Table 4.4). Backfat from the 1% CLA treatment had the hardest fat and differed significantly ($p<0.001$), together with the 0.25 and 0.5% CLA treatments, from the control group. The 0.25, 0.5 and 1 % CLA treatments did not differ from one another. The harder fat in the CLA groups could be attributed to the more SFA profile (as indicated by the lower IVs) of BF from these groups, compared to BF of pigs from the control group, which received SFO in the diet (Dugan *et al*., 2004). From these findings it became clear that elevated CLA levels in subcutaneous fat, resulted in improved technological properties of the BF, as were demonstrated by a decrease in IV and an increase in BF firmness. This finding is of special importance for manufacturers of

processed products, like bacon and salami. A firmer BF will result in less cutting losses during bacon packaging (Enser, 1983), as well as salami of better quality and higher stability (Hauser & Prabucki, 1990).

Only one of the colour measurements of the BF showed a significant difference between the four treatments. Colour b* values (yellowness) differed significantly ($p<0.05$) between the 0.25 and 1% CLA treatments. Flintoff-Dye and Omaye (2005) suggested that CLA acted as a pro-oxidant, antioxidant, and subsequently reverted to a pro-oxidant again, as enrichment levels increased. Oxidation in the BF of pigs from the 1% CLA treatment probably led to an increase in the yellowness of the fat. The control and 0.5% CLA did not differ significantly. Furthermore, no differences were observed between the control, 0.25, 0.5 and 1 % CLA groups for colour L* and a* values, as well as the colour saturation index.

According to Maw, Fowler, Hamilton and Petchey (2003), regression analyses identified C16:0, C18:1*c*9, C18:2 and C18:3*n*-3, as the FAs which best explained the variation in yellow colouration of fat. Cameron and Enser (1991) have also linked C18:2 (and other PUFAs) to poor fat quality and increasing colouration. Yet, like all aliphatic FAs, pure C16:0, C18:1*c*9, C18:2 and C18:3*n*-3 are colourless (O'Connor, 1960), thus implying that the colour cannot come from the acids themselves. However, natural fats and oils, extracted from plant and animal organs, contain varying amounts of associated pigments (O'Connor, 1960). Since C18:2 and C18:3*n*-3 cannot be synthesized by mammalian tissue, any of these FAs present in the BF must come from the diet. Thus, the relationship between increased yellow colour and increased percentages of C18:2 and C18:3*n*-3 is most likely due to an increase in the concentrations of the pigments (carotenoids), associated with these acids (Maw *et al*., 2003). Obviously, as one FA increases, the percentage of the others decreases. As the percentage of C18:2 and C18:3*n*-3 increases, there is a simultaneous decrease in the percentage of C16:0 and C18:1*c*9, and this accounts for C16:0 and C18:1*c*9 having significant ($p<0.05$) regression models for yellow colour (Maw *et al*., 2003).

Physical and chemical properties of subcutaneous fat

Figure 3.1 gives the locations of the various subcutaneous fat samples that were taken in this study. Barton-Gade (1983) did not name the areas in the figure, with the exeption of position B, which represents both layers of the BF, 45 mm from the carcass midline, between the second and third last rib. The following descriptions of the positions were used: A=neck; B=BF; C=chump; D=rib area; E=shoulder; F=belly and G=leg.

Dietary treatment and sampling position had a significant (at least $p<0.01$) effect on all physical and chemical properties of the subcutaneous fat (Table 4.5). The interaction between

dietary treatment and sampling position had a significant effect on FFDM ($p<0.001$) and Hanus IV ($p<0.05$) (Table 4.5).

Table 4.5: Analysis of variance (ANOVA) for main effects and their interactions of the subcutaneous fat samples.

	Dietary Treatment	Sampling Position	Dietary Treatment X Sampling Position
Physical and Chemical Properties			
Extractable Fat (%)	***	***	NS
FFDM (%)	***	**	***
Moisture (%)	**	***	NS
IV (Hanus)	***	***	*
Fatty Acid Composition (%)			
C10:0	NS	NS	NS
C12:0	***	NS	NS
C14:0	***	***	NS
C16:0	***	***	NS
C16:1*c*9	***	***	NS
C17:0	***	***	NS
C18:0	***	***	*
C18:1*t*9	***	NS	**
C18:1*c*9	***	***	NS
C18:1*c*7	***	***	NS
C18:2*c*9,12 (*n*-6)	***	***	NS
C18:2*c*9*t*11 (*n*-6)	***	***	**
C18:2*t*10*c*12 (*n*-6)	***	***	***
C18:3*c*9,12,15 (*n*-3)	***	***	NS
C20:0	***	***	NS
C20:1*c*11	***	***	NS
C20:2*c*11,14 (*n*-6)	***	***	NS
C20:3*c*11,14,17 (*n*-3)	***	***	NS
C20:4*c*5,8,11,14 (*n*-6)	***	***	NS
C22:5*c*7,10,13,16,19 (*n*-3)	NS	***	NS
C22:6*c*4,7,10,13,16,19 (*n*-3)	***	***	NS
Fatty Acid Ratios	***	***	NS
SFA (%)	***	***	*
MUFA (%)	***	***	NS
Dienoic (%)	*	***	NS
Trienoic (%)	***	***	NS
Tetraenoic (%)	***	***	NS
Pentaenoic (%)	NS	***	NS
Hexaenoic	***	***	NS
Penta + Hexaenoic	***	***	NS
PUFA (%)	NS	***	NS
UFA (%)	***	***	*
C16:0+C18:0 (%)	***	***	*
C16:1+C18:1/C16:0+C18:0	***	***	*
C18:0/C18:2	***	***	*
C16:0/C18:2	***	***	*
MUFA/SFA	***	***	*
DBI	***	***	NS
PI	***	***	NS
PUFA/SFA	***	***	NS
Atherogenicity index	***	***	NS
n-6 (%)	NS	***	NS
n-3 (%)	*	***	NS
n-6/*n*-3	**	***	NS
C18:1*c*9/C18:0	***	***	*

NS=Not Significant; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

No significant differences were found in extractable fat content for the same position, for different dietary treatments. Position A from the control and 0.5% CLA treatment had the highest extractable fat content (83.26 and 83.04%, respectively), while the lowest % extractable fat was found at position F from the 1% CLA treatment (72.88) (Table 4.6). For the control, only position A had a significantly ($p<0.001$) higher extractable fat content than position F. Major significant ($p<0.001$) differences occurred for some sampling positions in the 0.5% CLA treatment: position A had significantly higher ($p<0.001$) extractable fat content than positions D, F and G, while position B had higher extractable fat content than positions F and G. For the 0.5% CLA treatment, position C had significantly higher ($p<0.001$) extractable fat content than position F. At 1% dietary CLA inclusion level, position A had a significantly ($p<0.001$) higher extractable fat content than positions F and G. Although not statistically significant, Table 4.6 clearly illustrates a decrease in the extractable fat content of the belly fat in the CLA treatment groups. The belly fat from the control group had 76.99% extractable fat, compared to 76.79% for the 0.25% CLA treatment, 73.95% for the 0.5% CLA treatment and 72.88% for the 1% CLA treatment. This is in agreement with the findings of Sun *et al*. (2004), who found that CLA supplementation results in decreased lipid content of fat. According to Prabucki (1991), good quality subcutaneous fat should not have empty fat tissue, in other words, it should contain 84 - 90% lipid. No treatment group from the present study conformed to that criterion, although subcutaneous fat from position A from the control and 0.5% CLA treatment approached that value (Table 4.6).

For FFDM content, the only significant dietary treatment effect was for the 1% CLA treatment, which had a significantly ($p<0.001$) higher FFDM content at position F, compared to position F of the control group. Also for the 1% CLA treatment, the extractable fat content of position F was significantly ($p<0.001$) lower than that of position A (Table 4.6). This indicates an inverse relationship between the extractable fat content and FFDM content. The decrease in extractable fat content, with increased dietary CLA content (Table 4.6), was accompanied by a similar increase in FFDM content, with increased dietary CLA content. This confirmed the findings of Migdal *et al*. (2004), who fed 20 crossbred fatteners 2% CLA and observed a significant increase in FFDM of the subcutaneous fat.

Only two significant sampling position differences were observed for FFDM content. Within the 1% CLA treatment, the FFDM content of positions B and F were significantly ($p<0.001$) lower than that of position A. Fat free dry matter for position G was also significantly ($p<0.001$) higher than position A, for the 0.5% CLA treatment. The highest FFDM was found at position F (9.25%) for the 1% CLA treatment and the lowest (3.83%) at position A, for the 0.5% CLA treatment.

Table 4.6: Physical and chemical properties of subcutaneous fat from gilts from the four dietary treatments.

Dietary Treatment	Position	Extractable Fat (%)	FFDM (%)	Moisture (%)
Control (n = 12)	A	83.26 ± 1.74^{f}	5.91 ± 2.21^{abcd}	12.05 ± 2.62^{a}
	B	79.1± 5.07^{bcdef}	6.92 ± 2.76^{abcd}	13.93 ± 5.89^{abcd}
	C	80.68 ± 3.58^{ef}	6.90 ± 3.32^{abcd}	12.42 ± 3.35^{ab}
	D	78.87 ± 3.18^{bcdef}	6.69 ± 2.14^{abcd}	15.00 ± 2.32^{abcde}
	E	79.99 ± 3.85^{def}	6.01 ± 1.56^{abcd}	13.99 ± 3.59^{abcd}
	F	76.99 ± 4.84^{abcde}	4.91 ± 3.22^{ab}	18.10 ± 4.14^{cde}
	G	79.14 ± 2.00^{bcdef}	5.18 ± 2.29^{ab}	15.68 ± 2.39^{abcde}
0.25% CLA (n = 12)	A	80.55 ± 5.40^{ef}	5.18 ± 2.84^{ab}	14.28 ± 4.72^{abcd}
	B	80.97 ± 2.73^{ef}	5.75 ± 2.33^{abc}	13.28 ± 2.91^{abcd}
	C	81.09 ± 1.95^{ef}	5.97 ± 1.31^{abcd}	12.94 ± 2.37^{abc}
	D	79.06 ± 3.63^{bcdef}	6.78 ± 2.45^{abcd}	15.48 ± 5.57^{abcde}
	E	77.60 ± 3.37^{abcde}	7.16 ± 2.41^{abcd}	15.24 ± 2.68^{abcde}
	F	76.79 ± 4.63^{abcde}	6.48 ± 1.45^{abcd}	16.63 ± 4.46^{abcde}
	G	78.60 ± 3.29^{bcdef}	6.33 ± 2.36^{abcd}	15.07 ± 3.30^{abcde}
0.5% CLA (n = 12)	A	83.04 ± 1.40^{f}	3.83 ± 1.88^{a}	13.13 ± 1.64^{abcd}
	B	80.58 ± 2.83^{ef}	5.41 ± 3.29^{ab}	14.01 ± 3.36^{abcd}
	C	79.40 ± 2.42^{cdef}	5.90 ± 2.42^{abcd}	14.70 ± 3.42^{abcd}
	D	77.55 ± 5.56^{abcde}	5.40 ± 2.91^{ab}	17.04 ± 3.79^{abcde}
	E	78.29 ± 3.44^{bcdef}	5.74 ± 1.41^{abc}	15.97 ± 2.24^{abcde}
	F	73.95 ± 3.24^{ab}	5.95 ± 2.01^{abcd}	20.12 ± 2.63^{e}
	G	74.52 ± 3.95^{abc}	7.65 ± 1.53^{bcd}	17.06 ± 3.50^{abcde}
1% CLA (n = 12)	A	80.49 ± 2.24^{ef}	5.02 ± 0.85^{ab}	14.50 ± 2.15^{abcd}
	B	77.61 ± 4.10^{abcde}	8.94 ± 2.00^{cd}	13.45 ± 3.93^{abcd}
	C	76.14 ± 4.48^{abcde}	6.17 ± 0.96^{abcd}	17.69 ± 3.83^{bcde}
	D	77.17 ± 2.72^{abcde}	6.20 ± 1.72^{abcd}	15.22 ± 3.44^{abcde}
	E	76.18 ± 2.00^{abcde}	7.59 ± 1.80^{bcd}	16.24 ± 2.35^{abcde}
	F	72.88 ± 3.40^{a}	9.25 ± 1.33^{d}	17.02 ± 4.43^{abcde}
	G	74.67 ± 3.47^{abcd}	6.86 ± 3.16^{abcd}	18.47 ± 4.54^{de}
Significance Level		$p<0.001$	$p<0.001$	$p<0.001$

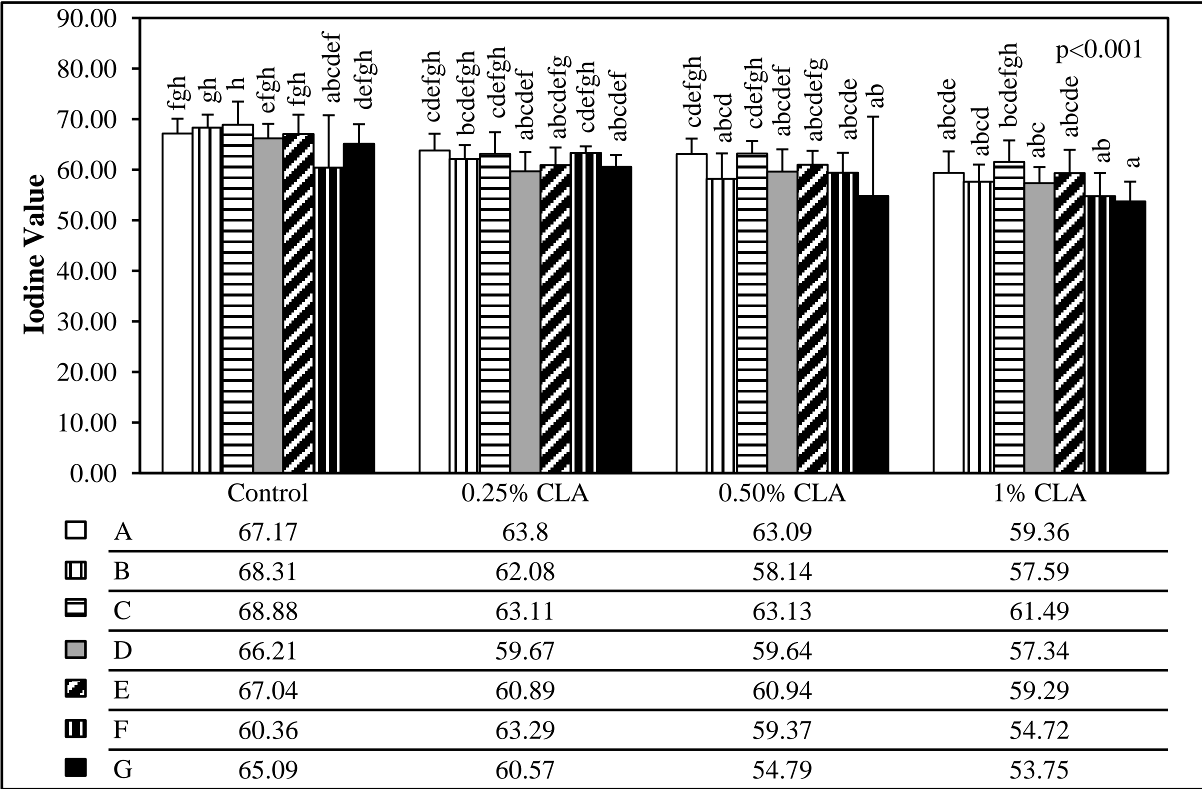
Means with different superscripts in the same column differ significantly

No statistically significant dietary effect was observed for moisture content of the subcutaneous fat (Table 4.6). Statistically significant sampling position differences were observed within some dietary treatments. Position A from the control had the lowest moisture content of 12.05% and differed significantly ($p<0.001$) from position F (Table 4.6). The moisture content of positions A, B and C, from the 0.5% CLA treatment, were significantly ($p<0.001$) lower than that of position F. The moisture content of the subcutaneous fat in position F (belly) did not differ significantly between the dietary treatments.

Iodine value, a parameter widely used to evaluate adipose tissue softness, gives an overall estimate of FA unsaturation (Davenel *et al.*, 1999) and has been used to control fat firmness (Lea, Swoboda & Gatherum, 1970). The higher the IV (the more unsaturated the fat), the softer the fat tissue (Fischer *et* al., 1989a). Various IVs have been suggested for good fat quality, ranging from < 70 (Barton-Gade, 1983; Girard *et al.*, 1988) to < 66 (Hart, as cited by Houben & Kroll, 1983; Ten Cate, as cited by Fischer, 1989b) and < 65 (Mortensen *et al.,* 1983; Warnants, Van Oeckel & Boucqué, 1996), and even < 60, in the case of firm cutting sausages, like salami (Fischer, 1989b). Subcutaneous fat from the control group generally had higher IVs than subcutaneous fat from all CLA treatments (Figure 4.1). Subcutaneous fat at position E of the control had a significantly ($p<0.001$) higher IV than position E of the 1% CLA treatment.

Subcutaneous fat at position G of the control group had a significantly ($p<0.001$) lower IV than position G from the 0.5 and 1% CLA treatments. This is in agreement with the findings of Van den Berg *et al*. (1995), who reported that dietary CLA reduced C20:4, C18:2 and C18:1*c*9 contents in fat, shifting the whole FA composition to the more saturated side. Iodine values from all treatments in the present study were < 70, with the highest being 68.88 (position C; control) and the lowest being 53.75 (position G; 1% CLA treatment). Significant sampling position differences were also observed for IV. For the control, the IV from position F was significantly ($p<0.001$) lower than positions B and C. In the case of the 0.5% CLA treatment, the IV of position G was significantly ($p<0.001$) lower than positions A and C. The IV for position G, from the 1% CLA treatment, was also significantly ($p<0.001$) lower than position C. Very low IVs were generally found at position F (belly), especially for the 0.5 and 1% CLA treatments. It was previously also reported that, with a dietary CLA inclusion level of 1%, the belly fat had more SFAs and less UFAs, resulting in lower IVs (Eggert *et al*., 1998; Eggert *et al*., 2001). These findings are in agreement with that of Barton Gade (1983), who also found positions F and G to have generally low IVs, and positions A, B and C to have generally high IVs. Barton-Gade (1983) ascribed the differences in IV for anatomical position, mainly to be due to differences in three FAs, namely C16:0, C18:0 and C18:1*c*9. Table 4.7 confirms these findings by clearly indicating that IV had

good correlation coefficients with C16:0, C18:0 and C18:1c9. Furthermore, Table 4.7 also indicates an additional good correlation between IV and C20:2.



	Control	0.25% CLA	0.50% CLA	1% CLA
A	67.17	63.8	63.09	59.36
B	68.31	62.08	58.14	57.59
C	68.88	63.11	63.13	61.49
D	66.21	59.67	59.64	57.34
E	67.04	60.89	60.94	59.29
F	60.36	63.29	59.37	54.72
G	65.09	60.57	54.79	53.75

Means with different superscripts differ significantly.

Figure 4.1: Iodine values of subcutaneous fat samples measured at different positions.

Data from the present study clearly show that CLA supplementation resulted in improved technological quality of subcutaneous fat, as demonstrated by reduced IVs in the subcutaneous fat of CLA supplemented pigs. A good example is the fact that the subcutaneous fat from all sampling positions, except position C, of the pigs from the 1% CLA supplemented group, had IVs < 60. According to Fischer (1989b), subcutaneous fat with an IV < 60, conforms to the very strict fat quality requirements for fat tissue, used in firm cutting sausages, like salami (Fischer, 1989b). Fat free dry matter and IV demonstrated significant ($p<0.001$ and $p<0.05$) interactions between dietary treatment and sampling position (Table 4.5). This interaction implies that the feeding of different concentrations of CLA resulted in different expressions of FFDM and IV for different sampling positions, depending on dietary CLA inclusion level. For the control, 0.25 and 0.5% CLA treatments, no significant sampling position differences were observed, within dietary treatment groups, whereas for the 1% CLA treatment group, position F had significantly ($p<0.001$) higher FFDM content than position A (Table 4.6). For IV, no sampling position differences were observed within the 0.25% CLA treatment group, whereas sampling position differences were observed within the contro1, 0.5 and 1% CLA treatment groups (Table 4.6).

Table 4.7: Correlation coefficients and significance levels between iodine values, physical and chemical properties, as well as fatty acid composition of subcutaneous fat.

Physical and chemical properties	Correlation coefficients
Fat (%)	0.2125***
FFDM (%)	-0.0554NS
Moisture (%)	-0.2185***
Fatty Acid Composition (%)	
C10:0	-0.0158NS
C12:0	-0.3698***
C14:0	-0.5247***
C16:0	-0.6450***
C16:1*c*9	-0.1500**
C17:0	-0.0102NS
C18:0	-0.6114***
C18:1*t*9	-0.3265***
C18:1*c*9	0.5218***
C18:1*c*7	0.3643***
C18:2*c*9,12 (*n*-6)	0.4589***
C18:2*c*9*t*11 (*n*-6)	-0.4902***
C18:2*t*10*c*12 (*n*-6)	-0.4593***
C18:3*c*9,12,15 (*n*-3)	0.1918***
C20:0	0.0678NS
C20:1*c*11	0.3909***
C20:2*c*11,14 (*n*-6)	0.6154**
C20:3*c*11,14,17 (*n*-3)	0.3827***
C20:4*c*5,8,11,14 (*n*-6)	0.4405***
C22:5*c*7,10,13,16,19 (*n*-3)	0.3160***
C22:6*c*4,7,10,13,16,19 (*n*-3)	0.4432***

NS = Not Significant; * = p<0.05; ** = p<0.01; *** = p<0.001

Fatty acid composition of subcutaneous fat

The FA composition of the four experimental diets (Table 4.1) was reflected in the FA profiles of the subcutaneous fat (Tables 4.8 – 4.14). Table 4.5 indicates that nearly all the individual FAs and FA ratios were significantly (at least $p<0.05$) influenced by dietary treatment and sampling position. The interaction between dietary treatment and sampling position had a significant (at least $p<0.05$) effect on C18:0, C181*t*9, C181*c*7, C18:2*c*9*t*11 and C18:2*t*10*c*12 FA content. The FA ratios SFA, UFA, C16:0+C18:0, C16:1+C19:1/C16:0+C18:0, C18:0/C18:2, C16:0/C18:2, C18:2/C18:1, MUFA/SFA and C18:1*c*9/C18:0 were also significantly (at least $p<0.05$) influenced by the dietary treatment X sampling position interaction.

Saturated fatty acids

Only trace amounts of C10:0 was detected in the subcutaneous fat from all dietary treatments (Table 4.8). Therefore, neither dietary treatment, nor sampling position had a significant effect on C10:0 content of the subcutaneous fat tissue (Table 4.5). For the same sampling position, dietary treatment had a significant ($p<0.001$) effect on all other SFAs. Although dietary treatment had a significant ($p<0.001$) effect on C17:0 and C20:0 content, the effect was not very clear (Table 4.8). Subcutaneous fat from positions A, C and D had a significantly ($p<0.001$) higher C17:0 contents in the 0.5% CLA treatment, compared to the control and 0.25% CLA treatment (Table 4.8). For position A and C the C20:0 content was significantly ($p<0.001$) lower for the 0.5% CLA treatment, compared to the control treatment (Table 4.8). For positions D, F and G, the control also had significantly ($p<0.001$) higher C20:0 content than the 0.5% CLA treatment (Table 4.8).

Lauric acid and the other more prominent SFAs (C14:0, C16:0 and C18:0) showed a significant ($p<0.001$) increase in the subcutaneous fat, with increased dietary CLA supplementation (Table 4.8). Other researchers have also observed an increase in SFAs, in subcutaneous BF, due to increased dietary CLA level (Bee, 2001; Eggert *et al.*, 2001; Ramsay *et al.*, 2001; Averette Gatlin *et al.*, 2002b; Demaree *et al.*, 2002; Smith *et al.*, 2002; Martín, Antequera, González, López-Bote, & Ruíz 2007). Ostrowska, Cross, Muralitharan, Bauman and Dunshea (2003) found that the concentration of C16:0 in subcutaneous fat, increased in a linear fashion with increasing dietary CLA. In agreement with this, Cordero and co-workers (2010) also reported that increase in CLA concentration in feed (0.5, 1 and 2%), had an increasing effect on the C12:0, C14:0, C16:0 and C18:0 in subcutaneous fat, while C20:0 was not affected. In the present study the observed increase in SFA (C12:0, C14:0, C16:0 and C18:0) content of the subcutaneous fat, with increased dietary CLA content, could not be ascribed to an increase in these FAs in the diets of the animals. There was, in fact, a statistically significant ($p<0.001$) decrease in these FAs in the animal feed, with increased dietary CLA content (Table 4.1). A similar increase in SFAs and decrease in PUFAs, as a result of dietary CLA supplementation, were observed by Han *et al.* (2011), who attributed this phenomenon to the inhibition of $^{\Delta}9$ desaturase activity and mRNA expression by CLA.

As far as sampling position was concerned, few significant differences were observed between sampling positions, within the same dietary treatment (Table 4.8). Position C had a significantly ($p<0.001$) higher C14:0 content than position A, for both the 0.5 and 1% CLA treatments. For the control, position G had a significantly ($p<0.001$) lower C17:0 content than position A. For the 0.5% CLA treatment, position G had a significantly ($p<0.001$) lower C17:0 content than positions A, B and C. Similarly, for the 1% CLA treatment, position G had a significantly ($p<0.001$) lower C17:0 content than positions A and B. For the 0.25% CLA treatment, positions C and E had significantly ($p<0.001$) lower C18:0 content than positions A and D. For the

Table 4.8: Saturated fatty acid content (%) of the subcutaneous fat from gilts from the four dietary treatments.

Dietary Treatment	Position	C10:0	C12:0	C14:0	C16:0	C17:0	C18:0	C20:0
Control	**A**	0.02 ± 0.02	0.02 ± 0.02[abcd]	1.31 ± 0.12[a]	24.56 ± 0.82[a]	0.30 ± 0.08[bcdef]	13.37 ± 2.03[abcd]	0.20 ± 0.03[defg]
(n = 12)	**B**	0.02 ± 0.02	0.04 ± 0.02[abcde]	1.35 ± 0.08[a]	24.85 ± 0.70[a]	0.27 ± 0.08[abcd]	13.25 ± 0.72[abc]	0.21 ± 0.03[fg]
	C	0.01 ± 0.02	0.02 ± 0.02[abcd]	1.40 ± 0.12[a]	23.75 ± 0.69[a]	0.26 ± 0.06[ab]	11.60 ± 1.12[a]	0.19 ± 0.02[cdefg]
	D	0.01 ± 0.02	0.01 ± 0.02[ab]	1.32 ± 0.11[a]	24.97 ± 0.73[ab]	0.24 ± 0.06[ab]	13.29 ± 1.10[abc]	0.21 ± 0.04[g]
	E	0.01 ± 0.02	0.02 ± 0.02[abc]	1.32 ± 0.11[a]	24.02 ± 0.77[a]	0.24 ± 0.05[ab]	11.91 ± 1.15[ab]	0.18 ± 0.03[abcdefg]
	F	0.01 ± 0.02	0.02 ± 0.02[abc]	1.33 ± 0.12[a]	24.68 ± 0.85[a]	0.26 ± 0.07[ab]	13.19 ± 2.20[abc]	0.22 ± 0.03[g]
	G	0.01 ± 0.01	0.01 ± 0.01[a]	1.28 ± 0.13[a]	23.96 ± 0.55[a]	0.20 ± 0.06[a]	12.32 ± 1.17[ab]	0.21 ± 0.02[fg]
0.25% CLA	**A**	0.01 ± 0.01	0.05 ± 0.02[abcdef]	1.80 ± 0.21[bc]	26.78 ± 0.87[cd]	0.29 ± 0.04[abcdef]	15.56 ± 0.94[efghij]	0.19 ± 0.02[cdefg]
(n = 12)	**B**	0.01 ± 0.02	0.06 ± 0.03[bcdefg]	1.77 ± 0.17[b]	27.81 ± 1.32[cdef]	0.29 ± 0.03[abcdef]	17.08 ± 0.88[hijkl]	0.19 ± 0.02[bcdefg]
	C	0.02 ± 0.02	0.05 ± 0.03[abcdef]	2.00 ± 0.16[bcdef]	26.59 ± 0.94[bc]	0.25 ± 0.02[ab]	13.77 ± 1.67[bcde]	0.18 ± 0.04[abcdefg]
	D	0.02 ± 0.02	0.05 ± 0.03[abcdef]	1.77 ± 0.21[b]	27.55 ± 1.06[cde]	0.26 ± 0.04[abc]	16.74 ± 1.28[ghijk]	0.22 ± 0.03[g]
	E	0.02 ± 0.03	0.05 ± 0.03[bcdefg]	1.87 ± 0.23[bc]	26.97 ± 1.30[cd]	0.27 ± 0.06[abcd]	14.59 ± 0.81[cdef]	0.16 ± 0.03[abcde]
	F	0.02 ± 0.02	0.07 ± 0.02[efgh]	1.84 ± 0.16[bc]	27.13 ± 0.83[cd]	0.27 ± 0.03[abcd]	15.44 ± 1.38[defghi]	0.21 ± 0.02[fg]
	G	0.01 ± 0.02	0.05 ± 0.04[abcdef]	1.82 ± 0.34[bc]	26.79 ± 1.18[cd]	0.20 ± 0.03[a]	14.67 ± 1.19[cdefg]	0.19 ± 0.03[cdefg]
0.5% CLA	**A**	0.01 ± 0.02	0.06 ± 0.03[cdefg]	1.94 ± 0.19[bcd]	26.98 ± 0.68[cd]	0.41 ± 0.06[g]	16.26 ± 1.50[fghij]	0.15 ± 0.03[ab]
(n = 12)	**B**	0.01 ± 0.02	0.07 ± 0.03[efgh]	1.96 ± 0.19[bcde]	28.43 ± 1.13[def]	0.38 ± 0.05[fg]	17.59 ± 1.97[jklm]	0.18 ± 0.03[abcdefg]
	C	0.01 ± 0.02	0.07 ± 0.03[efgh]	2.25 ± 0.20[efgh]	27.47 ± 0.93[cd]	0.40 ± 0.05[g]	14.69 ± 1.29[cdefg]	0.14 ± 0.03[a]
	D	0.01 ± 0.01	0.05 ± 0.03[bcdefg]	1.96 ± 0.23[bcde]	28.41 ± 1.46[def]	0.37 ± 0.06[efg]	16.74 ± 1.81[ghijk]	0.17 ± 0.03[abcdef]
	E	0.02 ± 0.02	0.05 ± 0.03[bcdefg]	1.94 ± 0.19[bcd]	27.61 ± 1.16[cde]	0.33 ± 0.07[bcdefg]	15.63 ± 1.49[efghij]	0.15 ± 0.04[abc]
	F	0.01 ± 0.02	0.06 ± 0.04[bcdefg]	2.09 ± 0.14[cdefg]	28.36 ± 1.06[def]	0.36 ± 0.07[defg]	15.95 ± 1.23[fghij]	0.17 ± 0.03[abcdef]
	G	0.02 ± 0.02	0.06 ± 0.04[cdefg]	2.05 ± 0.22[bcdef]	27.83 ± 1.62[cdef]	0.28 ± 0.09[abcde]	15.14 ± 1.31[cdefgh]	0.16 ± 0.03[abcd]
1% CLA	**A**	0.01 ± 0.01	0.06 ± 0.03[defgh]	2.24 ± 0.24[defgh]	28.04 ± 1.04[cdef]	0.35 ± 0.05[cdefg]	17.13 ± 1.65[hijkl]	0.16 ± 0.01[abcde]
(n = 12)	**B**	0.01 ± 0.01	0.08 ± 0.03[fgh]	2.35 ± 0.22[ghi]	29.38 ± 1.39[f]	0.35 ± 0.08[cdefg]	18.91 ± 0.91[lm]	0.18 ± 0.03[abcdefg]
	C	0.01 ± 0.01	0.11 ± 0.04[h]	2.56 ± 0.25[i]	28.12 ± 1.85[cdef]	0.33 ± 0.07[bcdefg]	17.29 ± 1.48[ijklm]	0.19 ± 0.02[bcdefg]
	D	0.01 ± 0.02	0.08 ± 0.03[efgh]	2.29 ± 0.26[fghi]	29.27 ± 1.61[ef]	0.32 ± 0.08[bcdefg]	19.38 ± 1.26[m]	0.20 ± 0.03[efg]
	E	0.01 ± 0.01	0.08 ± 0.02[efgh]	2.38 ± 0.19[ghi]	28.49 ± 1.17[def]	0.32 ± 0.08[bcdefg]	17.62 ± 1.31[jklm]	0.17 ± 0.02[abcdef]
	F	0.02 ± 0.03	0.07 ± 0.04[efgh]	2.45 ± 0.26[hi]	29.52 ± 1.31[f]	0.31 ± 0.08[bcdefg]	19.14 ± 1.43[lm]	0.20 ± 0.03[fg]
	G	0.01 ± 0.02	0.09 ± 0.03[gh]	2.47 ± 0.23[hi]	29.22 ± 1.51[ef]	0.25 ± 0.07[ab]	18.48 ± 1.50[klm]	0.18 ± 0.03[abcdefg]
Significance level		NS	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

Means with different superscripts in the same column differ significantly

0.5 % CLA treatment, position C had a significantly ($p<0.001$) lower C18:0 content than position B. For the 1% CLA treatment, position D had significantly ($p<0.001$) higher C18:0 content than position A. For the 0.25% CLA treatment, position E had significantly ($p<0.001$) lower C20:0 content than position D and F. For the 1% CLA treatment, position F had significantly ($p<0.001$) higher C20:0 content than position A.

A statistically significant ($p<0.05$) interaction between dietary treatment and sampling position was observed for C18:0. This can be ascribed to the absence of sampling position differences within the control group, whereas all other dietary treatment groups had sampling position differences within them.

Häuser and Prabucki (1990) proposed a C18:0 content > 12% as an indicator of good fat quality. In this study subcutaneous fat from all sampling positions, from all the CLA treatment groups, as well as subcutaneous fat from most sampling positions from the control treatment, conformed to this quality requirement (Table 4.8). The exceptions were the subcutaneous fat samples from positions C and E from the control group, which had a C18:0 content < 12 % (Table 4.8). An important observation is the fact that the C18:0 content of the subcutaneous fat increased with increased dietary CLA content (Table 4.8). That is important, since it implicates a better subcutaneous fat quality, with increased dietary CLA content.

Mono-unsaturated fatty acids

Table 4.9 summarizes the MUFA composition of the subcutaneous fat, from the gilts fed the four dietary treatments. Oleic acid, C18:1*c*7 and eicosenoic acid (C20:1) showed significant ($p<0.001$) decreases with increased dietary CLA inclusion level, for most sampling positions (Table 4.9). This is agreement with the findings of Ramsay *et al*. (2001), who found a steady elevation in the feeding of CLA, from 0.25, 0.5, 1.0 (Averette Gatlin *et al.*, 2002b) to 2.0 (Migdal *et al*., 2004) and even 4% (Sun *et al.*, 2004) of the diet, led to a reduction in C18:1*c*9 content in the subcutaneous adipose tissue. Averette Gatlin *et al.* (2002b) ascribed this to a reduction in $^{\Delta 9}$desaturase activity. Lo Fiego *et al*. (2005) also found that CLA supplementation of 0.25% in pigs' feed increased C16:1, and decreased C18:1*c*9 and C20:1*c*11 in the subcutaneous adipose tissue. For C16:1, the situation in the present study was less clear. The C16:1 content of all CLA treatments was generally higher than that of the control group. Furthermore, the C16:1 content of the CLA treatments showed no statistical difference for the same position, between the CLA dietary treatment groups. Elaidic acid showed a statistically significant ($p<0.001$) increase, with increased dietary CLA level. The lack of effect of increasing dietary CLA levels on C16:1 content and the decline in C18:1*c*7 content of the CLA treatments, observed in the present study, confirmed the findings of Cordero and co-workers

Table 4.9: Mono-unsaturated fatty acid content (%) of subcutaneous fat from gilts from the four dietary treatments.

Dietary Treatment	Position	C16:1*c*9	C18:1*t*9	C18:1*c*9	C18:1*c*7	C20:1*c*11
Control (n = 12)	**A**	1.92 ± 0.36^{a}	0.04 ± 0.03^{ab}	39.75 ± 2.10^{mn}	2.58 ± 0.31^{ghijkl}	0.69 ± 0.07^{fgh}
	B	1.95 ± 0.27^{a}	0.03 ± 0.03^{ab}	39.21 ± 1.44^{lm}	2.54 ± 0.19^{efghijk}	0.64 ± 0.07^{efg}
	C	2.18 ± 0.33^{abcde}	0.03 ± 0.03^{ab}	42.29 ± 1.63^{op}	3.02 ± 0.25^{n}	0.78 ± 0.06^{h}
	D	1.95 ± 0.34^{a}	0.03 ± 0.03^{ab}	39.77 ± 2.05^{mn}	2.65 ± 0.32^{ghijklmn}	0.70 ± 0.09^{fgh}
	E	2.11 ± 0.34^{abcd}	0.02 ± 0.02^{a}	42.10 ± 1.32^{nop}	2.90 ± 0.30^{klmn}	0.68 ± 0.08^{fgh}
	F	2.00 ± 0.39^{ab}	0.03 ± 0.02^{ab}	40.87 ± 2.28^{mno}	2.69 ± 0.35^{hijklmn}	0.72 ± 0.10^{gh}
	G	2.08 ± 0.49^{abc}	0.03 ± 0.03^{a}	44.33 ± 1.27^{p}	2.97 ± 0.25^{mn}	0.78 ± 0.10^{h}
0.25% CLA (n = 12)	**A**	2.51 ± 0.29^{bcdefg}	0.03 ± 0.03^{ab}	34.59 ± 1.19^{ghijk}	2.52 ± 0.15^{defghij}	0.59 ± 0.08^{bcdef}
	B	2.18 ± 0.27^{abcde}	0.04 ± 0.02^{ab}	32.27 ± 1.64^{defgh}	2.20 ± 0.21^{abcdef}	0.50 ± 0.07^{abcd}
	C	2.80 ± 0.35^{fg}	0.03 ± 0.03^{a}	36.86 ± 1.53^{kl}	2.94 ± 0.33^{lmn}	0.65 ± 0.09^{efg}
	D	2.37 ± 0.47^{abcdefg}	0.04 ± 0.03^{ab}	33.46 ± 1.33^{efghij}	2.36 ± 0.26^{cdefgh}	0.59 ± 0.08^{bcdef}
	E	2.81 ± 0.35^{fg}	0.03 ± 0.03^{a}	35.64 ± 2.20^{jk}	2.76 ± 0.22^{ijklmn}	0.54 ± 0.09^{abcde}
	F	2.69 ± 0.42^{efg}	0.05 ± 0.03^{abc}	34.85 ± 1.63^{ijk}	2.56 ± 0.30^{fghijk}	0.59 ± 0.10^{bcdef}
	G	2.71 ± 0.29^{efg}	0.02 ± 0.02^{a}	38.38 ± 2.45^{lm}	2.79 ± 0.33^{jklmn}	0.62 ± 0.12^{cdefg}
0.5% CLA (n = 12)	**A**	2.33 ± 0.33^{abcdef}	0.15 ± 0.07^{cde}	32.41 ± 1.49^{defghi}	2.29 ± 0.14^{bcdefg}	0.53 ± 0.06^{abcde}
	B	2.23 ± 0.33^{abcde}	0.11 ± 0.06^{abcde}	30.44 ± 1.92^{bcd}	2.07 ± 0.21^{abc}	0.49 ± 0.07^{ab}
	C	2.64 ± 0.22^{defg}	0.11 ± 0.03^{abcde}	33.91 ± 1.28^{fghij}	2.77 ± 0.27^{ijklmn}	0.59 ± 0.05^{bcdef}
	D	2.53 ± 0.52^{cdefg}	0.09 ± 0.05^{abcde}	32.17 ± 1.37^{defg}	2.34 ± 0.20^{bcdefgh}	0.54 ± 0.11^{abcde}
	E	2.59 ± 0.30^{cdefg}	0.09 ± 0.06^{abcde}	34.71 ± 1.63^{hijk}	2.64 ± 0.27^{ghijklm}	0.55 ± 0.13^{abcde}
	F	2.88 ± 0.41^{g}	0.10 ± 0.04^{abcde}	33.20 ± 1.66^{efghij}	2.60 ± 0.25^{ghijklm}	0.54 ± 0.05^{abcde}
	G	2.86 ± 0.32^{g}	0.07 ± 0.05^{abcd}	36.88 ± 2.60^{kl}	2.90 ± 0.26^{klmn}	0.60 ± 0.12^{bcdefg}
1% CLA (n = 12)	**A**	2.33 ± 0.34^{abcdef}	0.15 ± 0.08^{cde}	30.19 ± 1.28^{abcd}	2.18 ± 0.20^{abcde}	0.49 ± 0.05^{abc}
	B	1.99 ± 0.20^{ab}	0.18 ± 0.15^{def}	27.93 ± 1.40^{a}	1.88 ± 0.19^{a}	0.45 ± 0.07^{a}
	C	2.44 ± 0.38^{abcdefg}	0.14 ± 0.08^{bcde}	31.19 ± 1.82^{cde}	2.41 ± 0.25^{cdefghi}	0.62 ± 0.04^{defg}
	D	2.12 ± 0.23^{abcd}	0.28 ± 0.20^{f}	28.53 ± 0.94^{ab}	1.97 ± 0.13^{ab}	0.50 ± 0.06^{abcd}
	E	2.51 ± 0.34^{bcdefg}	0.14 ± 0.12^{bcde}	31.27 ± 0.86^{cde}	2.32 ± 0.24^{bcdefgh}	0.50 ± 0.06^{abcd}
	F	2.39 ± 0.35^{abcdefg}	0.12 ± 0.10^{abcde}	29.19 ± 0.63^{abc}	2.16 ± 0.14^{abcd}	0.50 ± 0.07^{abcd}
	G	2.63 ± 0.39^{defg}	0.19 ± 0.13^{ef}	31.85 ± 1.62^{def}	2.48 ± 0.22^{defghij}	0.54 ± 0.06^{abcde}
Significance level		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

(2010). They found that an increase of CLA concentration in the feed (0.5, 1 and 2%) had a negative influence on C18:1*c*7 content in BF, whereas the C16:1 were not affected.

As far as sampling position was concerned, a large number of significant differences were observed between sampling positions, within the same dietary treatment (Table 4.9). Within the 0.25% CLA treatment, positions C and D had significantly ($p<0.001$) higher C16:1 content than positions A and B. For the 0.5% CLA treatment, positions F and G had significantly ($p<0.001$) higher C16:1 content than positions A and B. In the 1% CLA treatment, position G had a significantly ($p<0.001$) higher C18:1*c*9 content than position B. As far as C18:1*t*9 content was concerned, the only significant sampling positional difference occurred within the 1% CLA treatment, where position D had significantly ($p<0.001$) higher C18:1*t*9 content than positions A, C, E and F. For C18:1*c*9, quite a few sampling position differences occurred within dietary treatments. Within the control treatment, positions C and G had significantly ($p<0.001$) higher C18:1*c*9 content than positions A, B and D. Position E had a significantly ($p<0.001$) higher C18:1*c*9 content than position B. Within the 0.25% CLA treatment, position C had significantly ($p<0.001$) higher C18:1*c*9 content than positions B and D. Position G had significantly ($p<0.001$) higher C18:1*c*9 content than positions A, B, D, E and F

For the 0.5% CLA treatment, position G had significantly ($p<0.001$) higher C18:1*c*9 content than positions A, B, C, D and F. Positions E and F had significantly ($p<0.001$) higher C18:1*c*9 contents than position B. For the 1% CLA treatment, position B had a significantly ($p<0.001$) lower C18:1*c*9 content than positions C, E and G.

Within the control group, sampling position C and G had significantly ($p<0.001$) higher C18:1*c*7 content than positions A and B. For the 0.25% CLA treatment, position C had statistically ($p<0.001$) higher C18:1*c*7 content than positions A, B and D. Position G also had statistically ($p<0.001$) significantly higher C18:1*c*7 content than positions B and D. For the 0.5% CLA treatment, positions C and G had statistically significantly ($p<0.001$) higher C18:1*c*7 content than positions A, B and D. For the 1% CLA treatment, positions C, E, F and G had significantly ($p<0.001$) higher C18:1*c*7 content than positions B and D. Within the control group, position C had significantly ($p<0.001$) higher C20:1*c*11 content than position B. Within the 1% CLA treatment group, sampling position C also had significantly ($p<0.001$) higher C20:1*c*11 content than positions A and B. A statistically significant ($p<0.01$) interaction between dietary treatment and sampling position was observed for C18:1*c*7. This can be ascribed to different expressions of C18:1*c*7 in different sampling positions, due to different dietary treatments.

Δ^9 Desaturase index

The Δ^9 desaturase index (C18:1*c*9/C18:0) showed a significant ($p<0.001$) decrease with increased dietary CLA level (Table 4.10). It decreased from ± 3 in the control to ± 1.6 in the 1% CLA treatment (Table 4.10). For the C18:1*c*9/C18:0 ratio, all sampling positions from the control had significantly ($p<0.001$) higher values than the corresponding sampling positions from all the CLA treatment groups. With the exception of position B, all sampling positions from the 0.5 and 1% CLA treatments had significantly ($p<0.001$) lower C18:1*c*9/C18:0 ratios than the corresponding sampling positions from the control group. This explain the increase in SFA content (C14:0, C16:0 and C18:0), observed in Table 4.8 and the decrease in MUFA content (C18:1*c*7 and C18:1*c*9), observed in Table 4.9. Other researchers also found that dietary CLA supplementation resulted in increased SFAs and decreased MUFAs of the subcutaneous fat and attributed it to a reduction in Δ^9 desaturase activity (Ramsay *et al.*, 2001; Averette Gatlin *et al.*, 2002b; Migdal *et al.*, 2004; Sun *et al.*, 2004; Cordero *et al.*, 2010).

Table 4.10: The $^{\Delta}$9 desaturase index of subcutaneous fat from gilts from the four diets.

Dietary Treatment	Position	C18:1*c*9/C18:0
Control (n = 12)	**A**	3.04 ± 0.52^{jk}
	B	2.96 ± 0.16^{jk}
	C	3.68 ± 0.41^{m}
	D	3.02 ± 0.33^{jk}
	E	3.56 ± 0.29^{lm}
	F	3.19 ± 0.60^{kl}
	G	3.63 ± 0.36^{m}
0.25% CLA (n = 12)	**A**	2.23 ± 0.16^{defgh}
	B	1.90 ± 0.17^{abcde}
	C	2.73 ± 0.46^{ij}
	D	2.01 ± 0.15^{cdef}
	E	2.45 ± 0.21^{ghi}
	F	2.28 ± 0.30^{efgh}
	G	2.64 ± 0.32^{hij}
0.5% CLA (n = 12)	**A**	2.00 ± 0.15^{cdef}
	B	1.75 ± 0.21^{abc}
	C	2.33 ± 0.25^{fghi}
	D	$1.94 \pm 0.0.19^{bcdef}$
	E	2.24 ± 0.23^{defgh}
	F	2.09 ± 0.21^{cdefg}
	G	2.46 ± 0.32^{ghi}
1% CLA (n = 12)	**A**	1.78 ± 0.26^{abc}
	B	1.48 ± 0.13^{a}
	C	1.82 ± 0.21^{abcd}
	D	1.48 ± 0.10^{a}
	E	1.79 ± 0.16^{abc}
	F	1.53 ± 0.12^{ab}
	G	1.74 ± 0.19^{abc}
Significance level		$p<0.001$

Means with different superscripts in the same column differ significantly

Polyunsaturated fatty acids

Table 4.11 summarizes the dienoic and trienoic PUFA composition of the subcutaneous fat of the gilts, from the four dietary treatments. Although C18:2, C18:3*n*-3, C20:2 and C20:3*n*-3 showed a significant (p<0.001) decline in the subcutaneous fat, with increased dietary CLA level, the difference were mostly not significant for the same position, between dietary treatments (Table 4.11). The exception was C20:2, since all the subcutaneous fat positions from the control had significantly (p<0.001) higher C20:2 content than the corresponding positions for the CLA treatments (Table 4.11). The levels of the two CLA isomers increased significantly (p<0.001) at all sampling positions, with increased CLA content in the three CLA dietary treatments. Linoleic acid is the major PUFA in pig's adipose tissue and is derived entirely from the diet (Enser *et al.*, 1996). It passes through the pig's stomach unchanged and is then absorbed into the blood stream in the small intestine and incorporated into the tissue (Nürnberg *et al.*, 1998). α-Linolenic acid is the second most important PUFA and in pigs, the proportion is higher in the adipose tissue than in the muscle (Wood *et al.*, 2008). The results from the present study (Table 4.11) support the findings of other researchers, that dietary CLA decreased the percentages of C18:2 and C18:3*n*-3 in the subcutaneous adipose tissue (Ramsay *et al.*, 2001; Joo *et al.*, 2002; Migdal *et al.*, 2004; Sun *et al.*, 2004).

In accordance with the composition of the diets (Table 3.1), the subcutaneous fat from the control did not contain any CLA (*cis-9, trans-11* or *trans*-10, *cis*-12). The two CLA isomers showed a significant (p<0.001) increase with increased dietary CLA level (Table 3.1 and Table 4.11). Other researchers also reported that the increasing level of CLA in the diets caused a linear increase of CLA isomers in the total FAs, as well as in the subcutaneous fat (Thiel-Cooper *et al.*, 2001; Joo *et al.*, 2002; Wiegand *et al.*, 2002; Migdal *et al.*, 2004; Sun *et al.*, 2004; Szymczyk, 2005; Cordero *et al.*, 2010). This was also confirmed by Corino *et al.* (2003), who concluded that by feeding 0.25 and 0.5% CLA to pigs, the acetyl-CoA carboxylase activity in the adipose tissue was reduced. In the present study, the *cis-9*, *trans*-11 isomer occurred at a concentration of roughly double that of the *trans*-10, *cis*-12 isomer (Table 4.11), at all sampling positions in the subcutaneous fat. Similarly to the findings of the present study, a previous study also found that the *cis*-9, *trans*-11 isomer was incorporated more efficiently into the tissues than the *trans*-10, *cis*-12 isomer (Szymczyk, 2005). The reason for the difference between the *cis*-9*, trans*-11 and *trans*-10, *cis*-12 contents in the tissues might be due to their dissimilar deposition efficiencies in the pig. Czanderna, Kowalczyk, Niedźwiedzka, Wąsowska, Pastuszewska, Bulska, *et al*. (2004) concluded that the *trans*-10, *cis*-12 isomer is more efficiently driven through β-oxidation in the cells of the muscles and adipose tissue of rats, and their homologues. For pigs, the varied deposition

Table 4.11: Dienoic and trinoic polyunsaturated fatty acid content (%) of subcutaneous fat from gilts from the four diets.

Dietary Treatment	Position	C18:2*c*9,12 (*n*-6)	C18:2*c*9*t*11 (*n*-6) (CLA)	C18:2*t*10*c*12 (*n*-6) (CLA)	C18:3*c*9,12,15 (*n*-3)	C20:2*c*11,14(*n*-6)	C20:3*c*11,14,11(*n*-3)
Control (n = 12)	**A**	13.64 ± 0.66^{hi}	ND	ND	0.47 ± 0.07^{cdefg}	0.57 ± 0.06^{i}	$0.04 \pm .03^{bc}$
	B	14.04 ± 1.14^{i}	ND	ND	0.49 ± 0.08^{cdefgh}	0.57 ± 0.06^{hi}	0.05 ± 0.01^{c}
	C	12.80 ± 1.40^{efghi}	ND	ND	0.43 ± 0.06^{bcd}	0.58 ± 0.06^{i}	0.04 ± 0.03^{c}
	D	13.36 ± 1.60^{ghi}	ND	ND	0.44 ± 0.05^{bcde}	0.55 ± 0.06^{ghi}	0.02 ± 0.03^{abc}
	E	12.91 ± 1.17^{efghi}	ND	ND	0.44 ± 0.04^{bcde}	0.54 ± 0.07^{fghi}	0.03 ± 0.03^{abc}
	F	12.48 ± 1.59^{defghi}	ND	ND	0.42 ± 0.06^{bc}	0.54 ± 0.08^{fghi}	0.04 ± 0.03^{abc}
	G	10.62 ± 1.18^{abc}	ND	ND	0.34 ± 0.03^{a}	0.50 ± 0.07^{efgh}	0.01 ± 0.02^{abc}
0.25% CLA (n = 12)	**A**	13.28 ± 1.21^{fghi}	0.24 ± 0.03^{a}	0.07 ± 0.03^{a}	0.51 ± 0.04^{defgh}	0.48 ± 0.05^{defg}	0.03 ± 0.03^{abc}
	B	13.84 ± 0.62^{hi}	0.30 ± 0.06^{ab}	0.11 ± 0.02^{ab}	0.52 ± 0.05^{efgh}	0.45 ± 0.05^{bcde}	0.04 ± 0.02^{abc}
	C	12.13 ± 0.63^{cdefgh}	0.24 ± 0.07^{a}	0.06 ± 0.07^{a}	0.47 ± 0.03^{cdefg}	0.46 ± 0.05^{bcde}	0.04 ± 0.03^{abc}
	D	12.90 ± 1.06^{efghi}	0.26 ± 0.10^{ab}	0.09 ± 0.06^{a}	0.48 ± 0.05^{cdefgh}	0.47 ± 0.05^{bcdef}	0.04 ± 0.03^{abc}
	E	12.59 ± 1.05^{defghi}	0.29 ± 0.13^{ab}	0.09 ± 0.09^{a}	0.47 ± 0.04^{cdefg}	0.43 ± 0.05^{abcde}	0.03 ± 0.03^{abc}
	F	12.45 ± 0.92^{defghi}	0.25 ± 0.03^{a}	0.09 ± 0.03^{a}	0.48 ± 0.04^{cdefgh}	0.45 ± 0.03^{bcde}	0.04 ± 0.02^{bc}
	G	10.42 ± 1.08^{abc}	0.22 ± 0.14^{a}	0.07 ± 0.11^{a}	0.38 ± 0.06^{ab}	0.40 ± 0.04^{abc}	0.02 ± 0.02^{abc}
0.5% CLA (n = 12)	**A**	13.91 ± 1.60^{i}	0.71 ± 0.25^{d}	0.35 ± 0.13^{de}	0.53 ± 0.06^{gh}	0.47 ± 0.04^{cdef}	0.04 ± 0.03^{abc}
	B	13.70 ± 1.81^{hi}	0.64 ± 0.11^{cd}	0.30 ± 0.06^{cd}	0.52 ± 0.07^{fgh}	0.42 ± 0.03^{abcd}	0.02 ± 0.03^{abc}
	C	12.69 ± 0.65^{efghi}	0.53 ± 0.10^{cd}	0.24 ± 0.07^{bcd}	0.47 ± 0.02^{cdefg}	0.46 ± 0.03^{bcde}	0.03 ± 0.03^{abc}
	D	12.57 ± 1.43^{defghi}	0.62 ± 0.13^{cd}	0.27 ± 0.13^{cd}	0.45 ± 0.05^{bcdef}	0.43 ± 0.06^{abcde}	0.02 ± 0.02^{abc}
	E	11.79 ± 1.37^{cdefg}	0.46 ± 0.14^{bc}	0.18 ± 0.10^{abc}	0.44 ± 0.06^{bcd}	0.41 ± 0.05^{abc}	0.03 ± 0.02^{abc}
	F	11.64 ± 0.84^{cdef}	0.51 ± 0.07^{c}	0.23 ± 0.05^{bcd}	0.44 ± 0.04^{bcde}	0.41 ± 0.04^{abc}	0.02 ± 0.02^{abc}
	G	9.52 ± 0.70^{ab}	0.46 ± 0.14^{bc}	0.19 ± 0.13^{abc}	0.34 ± 0.04^{a}	0.37 ± 0.05^{a}	0.01 ± 0.01^{a}
1% CLA (n = 12)	**A**	13.48 ± 0.84^{ghi}	1.25 ± 0.27^{f}	0.64 ± 0.14^{g}	0.54 ± 0.04^{gh}	0.42 ± 0.04^{abcd}	0.02 ± 0.02^{abc}
	B	13.06 ± 0.84^{fghi}	1.31 ± 0.17^{f}	0.69 ± 0.07^{g}	0.55 ± 0.04^{h}	0.41 ± 0.04^{abc}	0.01 ± 0.02^{a}
	C	11.24 ± 0.77^{cde}	1.18 ± 0.09^{f}	0.61 ± 0.06^{g}	0.49 ± 0.05^{cdefgh}	0.47 ± 0.04^{cdef}	0.03 ± 0.03^{abc}
	D	11.85 ± 1.03^{cdefg}	1.28 ± 0.07^{f}	0.69 ± 0.06^{g}	0.49 ± 0.04^{cdefgh}	0.42 ± 0.03^{abcd}	0.02 ± 0.02^{abc}
	E	11.28 ± 0.96^{cde}	1.17 ± 0.14^{f}	0.57 ± 0.08^{g}	0.47 ± 0.07^{cdefg}	0.40 ± 0.02^{abc}	0.02 ± 0.02^{abc}
	F	10.96 ± 1.20^{bcd}	1.16 ± 0.14^{ef}	0.61 ± 0.06^{g}	0.47 ± 0.06^{cdefg}	0.40 ± 0.03^{ab}	0.01 ± 0.02^{abc}
	G	9.24 ± 1.00^{a}	0.96 ± 0.16^{e}	0.45 ± 0.16^{ef}	0.38 ± 0.05^{ab}	0.36 ± 0.04^{a}	0.01 ± 0.01^{ab}
Significance level		$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

Means with different superscripts in the same column differ significantly.
ND = Not Detected

efficiencies of the CLA isomers are possibly attributed to their variations in absorption, transportation and catabolism (β-oxidation, etc.) or due to the competitive inhibition by the CLA *cis-9, trans-11* isomer of the CLA *trans*-10*, cis*-12 isomer incorporation (Han *et al*., 2011).

As far as sampling position was concerned, a large number of significant differences were observed between sampling positions, within the same dietary treatment. For the control and 0.25% CLA treatment, position G had significantly ($p<0.001$) lower C18:2 content than the other six sampling positions. Positions E, F and G had significantly ($p<0.001$) lower C18:2 content than positions A and B for the 0.5% CLA treatment. For the 1% CLA treatment position G had significantly ($p<0.001$) lower C18:2 content than all other sampling positions. Positions C, E and F, also from the 1% CLA treatment group, had significantly ($p<0.001$) lower C18:2 content than positions A and B (Table 4.11). No significant sampling position differences were found for both the CLA isomers, within the 0.25% CLA treatment (Table 4.11). For both CLA isomers, sampling positions E, F and G had significantly ($p<0.001$) lower CLA content than sampling position A from the 0.5% CLA treatment. For the 1% CLA treatment, positions G had significantly ($p<0.001$) lower *cis-9, trans-11* content, compared to sampling positions A, B, C, D and E; position G also had significantly ($p<0.001$) lower *trans*-10*, cis*-12 content, compared to the same five sampling positions. As mentioned before and in agreement with other researchers (Szymczyk, 2005; Intarapichet *et al*., 2008), the *trans*-10, *cis*-12 isomer was always occurring at lower levels than the *cis*-9 *trans*-11 isomer. Although not always statistically significant, there was a tendency for sampling positions on the dorsal (A, B, C) and lateral (D) sides of the carcass, to have higher CLA content than sampling positions on the ventral (E, F and G) side of the carcass.

For the C18:3*n*-3 content, all four dietary groups showed significantly ($p<0.001$) lower values for position G, compared to all the other sampling positions (Table 4.11). The 0.5 and 1% CLA treatments showed additional significant ($p<0.001$) differences between positions. Position A and B had significantly ($p<0.001$) higher C18:3*n*-3 contents than positions D, E, F and G, for the 0.5% CLA treatment group. For the 1% CLA treatment group sampling position B had significantly ($p<0.001$) higher C18:3*n*-3 content than positions E, F and G (Table 4.11).

The C20:2 content of sampling position A was significantly ($p<0.001$) higher than sampling position G, for the control, 0.25 and 0.5% CLA treatments. For the 0.5% CLA treatment, sampling position C also had significantly ($p<0.001$) higher C20:2 content than sampling position G. Also for the 1% CLA treatment, sampling position C had significantly ($p<0.001$) higher C20:2 content than sampling positions F and G. For C20:3, no significant sampling position differences were found within dietary treatment groups.

The only PUFAs that exhibited statistically significant (at least $p<0.01$) interactions between dietary treatment and sampling position, were the two CLA isomers (Table 4.5). This can be

ascribed to the different expressions of the two CLA isomers in the different sampling positions, due to different dietary treatments. At the 0.25% dietary CLA inclusion level, no significant differences were observed between sampling position for both CLA isomers, whereas at higher inclusion levels, significantly ($p<0.001$) different sampling position differences were observed.

Table 4.12 summarizes the tetraenoic, pentaenoic and hexaenoic PUFA composition of the subcutaneous fat from the gilts fed the four dietary treatments. A significant ($p<0.001$) decline was found for C20:4 content, with increased dietary CLA level. This confirms the findings from previous studies, that feeding CLA at concentrations of 2% (Ramsay *et al*., 2001; Migdal *et al*., 2004) and 4% (Sun *et al.*, 2004), led to a reduction in the percentage of C20:4. Although C22:5 showed a statistically significant ($p<0.001$) decline in the subcutaneous fat, with increased dietary CLA level, the difference was not significant for the same position, between dietary treatments (Table 4.12). A decline was also observed in C22:6 content, with increases in dietary CLA inclusion level. Sampling position E had a significantly ($p<0.001$) lower C22:6 content for the 1% CLA treatment group, compared to the control group.

Although no significant differences were found between sampling positions, within dietary treatments for C20:4, a few significant ($p<0.001$) sampling position differences were found for C22:5 and C22:6 (Table 4.12). For C22:5, the 0.5% CLA treatment showed significantly ($p<0.001$) higher C22:5 values for positions A and C, compared to positions D and G. For the 1% CLA treatment, only position C had significantly ($p<0.001$) higher C22:5 values than position G (Table 4.12). Significant ($p<0.001$) sampling position differences were also observed for C22:6. For the 1% CLA treatment, higher values were observed for position C, compared to positions B, D, E and G.

Only one fat quality requirement for processing was reported in the literature for an individual PUFA, namely C18:2*c*9,12. Various maxima were proposed for C18:2 to ensure subcutaneous fat of good quality. Ellis & Isbell (1926ab), Houben & Krol (1983), Enser (1984), Wood (1984) and Whittington, Prescott, Wood & Enser (1986) proposed a C18:2 content < 15 % for good quality subcutaneous fat. Other researchers (Girard *et al.*, as cited by Fischer, 1989b) proposed an even stricter C18:2 content of between 12 – 15%, for good fat quality. All samples from this study had C18:2 contents < 15%, however, some were as low as 9.24% (position G; 1% CLA group). This implies that CLA supplemented pigs produced subcutaneous fat more suitable for use in processed meat products.

Table 4.12: Tetra-, penta- and hexaenoic polyunsaturated fatty acid content (%) of subcutaneous fat from gilts from the four diets.

Dietary Treatment	Position	C20:4*c*5,8,11,14 (*n*-6)	C22:5*c*7,10,13,16,19 (*n*-3)	C22:6*c*4,7,10,13,16,19 (*n*-3)
Control (n = 12)	**A**	0.13 ± 0.04^{ef}	0.16 ± 0.08^{abcd}	0.24 ± 0.11^{bcde}
	B	0.13 ± 0.04^{f}	0.14 ± 0.08^{abcd}	0.23 ± 0.12^{bcde}
	C	0.13 ± 0.04^{def}	0.19 ± 0.08^{bcd}	0.30 ± 0.12^{e}
	D	0.12 ± 0.02^{cdef}	0.14 ± 0.04^{abcd}	0.22 ± 0.08^{abcde}
	E	0.13 ± 0.02^{f}	0.16 ± 0.04^{abcd}	0.26 ± 0.04^{de}
	F	0.13 ± 0.02^{f}	0.15 ± 0.05^{abcd}	0.23 ± 0.07^{abcde}
	G	0.09 ± 0.05^{abcdef}	0.09 ± 0.06^{ab}	0.18 ± 0.12^{abcde}
0.25% CLA (n = 12)	**A**	0.11 ± 0.02^{bcdef}	0.15 ± 0.04^{abcd}	0.22 ± 0.05^{abcde}
	B	0.07 ± 0.06^{abcd}	0.11 ± 0.07^{abcd}	0.18 ± 0.09^{abcde}
	C	0.10 ± 0.05^{bcdef}	0.15 ± 0.08^{abcd}	0.23 ± 0.10^{abcde}
	D	0.08 ± 0.05^{abcdef}	0.10 ± 0.08^{abc}	0.16 ± 0.10^{abcde}
	E	0.10 ± 0.04^{bcdef}	0.13 ± 0.06^{abcd}	0.16 ± 0.07^{abcde}
	F	0.12 ± 0.03^{cdef}	0.17 ± 0.04^{abcd}	0.24 ± 0.04^{cde}
	G	0.06 ± 0.05^{abc}	0.07 ± 0.07^{a}	0.10 ± 0.10^{abc}
0.5% CLA (n = 12)	**A**	0.10 ± 0.05^{bcdef}	0.18 ± 0.09^{bcd}	0.20 ± 0.13^{abcde}
	B	0.11 ± 0.05^{bcdef}	0.13 ± 0.07^{abcd}	0.20 ± 0.11^{abcde}
	C	0.11 ± 0.04^{bcdef}	0.20 ± 0.08^{cd}	0.22 ± 0.13^{abcde}
	D	0.07 ± 0.05^{abcde}	0.08 ± 0.07^{a}	0.10 ± 0.10^{abc}
	E	0.09 ± 0.05^{abcdef}	0.13 ± 0.06^{abcd}	0.17 ± 0.11^{abcde}
	F	0.09 ± 0.03^{abcdef}	0.17 ± 0.02^{abcd}	0.18 ± 0.10^{abcde}
	G	0.06 ± 0.03^{abc}	0.09 ± 0.06^{ab}	0.11 ± 0.06^{abcd}
1% CLA (n = 12)	**A**	0.08 ± 0.04^{abcdef}	0.14 ± 0.08^{abcd}	0.12 ± 0.12^{abcd}
	B	0.07 ± 0.04^{abcd}	0.11 ± 0.05^{abcd}	0.09 ± 0.08^{ab}
	C	0.10 ± 0.02^{bcdef}	0.20 ± 0.06^{d}	0.26 ± 0.09^{de}
	D	0.06 ± 0.03^{abc}	0.12 ± 0.05^{abcd}	0.11 ± 0.05^{abc}
	E	0.06 ± 0.04^{ab}	0.14 ± 0.07^{abcd}	0.11 ± 0.10^{abc}
	F	0.06 ± 0.04^{ab}	0.12 ± 0.08^{abcd}	0.12 ± 0.15^{abcd}
	G	0.03 ± 0.03^{a}	0.09 ± 0.07^{ab}	0.08 ± 0.09^{a}
Significance level		$p<0.001$	$p<0.001$	$p<0.001$

Means with different superscripts in the same column differ significantly

Fatty acid ratios with nutritional, health and fat quality implications

Table 4.13 is a summary of the FA ratios, with nutritional, health and fat quality implications. Dietary treatment had a significant (at least $p<0.05$) effect on all ratios, except for the PUFA and *n*-6 contents (Table 4.5). Increased dietary CLA level resulted in a significant ($p<0.001$) increase in SFA, AI and *n*-6/*n*-3 contents and a significant ($p<0.001$) decrease in MUFA, UFA, MUFA/SFA, PI and *n*-3 content. According to Table 4.5, sampling position had a significant ($p<0.001$) effect on all the ratios. Significant ($p<0.001$) sampling position differences, within dietary treatments, were found for all FA ratios with nutritional, health and fat quality implications (Table 4.13).

For SFA, all sampling positions from the control treatment had significantly ($p<0.001$) lower SFA content than the corresponding sampling positions from all the CLA treatment groups. Within the CLA treatment groups, all sampling positions of the 0.25% CLA treatment had significantly ($p<0.001$) lower SFA content than all sampling positions of the 1% CLA treatment. Sampling positions C, D, E, F and G from the 1% CLA treatment group had significantly ($p<0.001$) higher SFA content than the same sampling positions from the 0.5% CLA treatment.

Sampling positions B and D had significantly ($p<0.001$) higher SFAs than sampling position C, for the control and 0.25% CLA treatment. For the 0.5% CLA treatment, sampling position B had significantly ($p<0.001$) higher SFA content than sampling positions A, C, E and G. For the 1% CLA group, sampling position B and D had significantly higher SFA content than sampling position A.

The increase in SFA content of the subcutaneous fat, with increased dietary CLA content in the diet, confirmed the findings of other researchers, that dietary CLA supplementation result in increased *de novo* tissue synthesis of SFAs (Eggert *et al*., 1998; Eggert *et al*. 2001; Averette Gatlin *et al*., 2002b; Wiegand *et al*., 2002; Corino *et al*., 2003; Kouba *et al*., 2003; Lo Fiego *et al*., 2005). From a health point of view, the usual recommendation is to reduce SFA content in meat (Siri-Tarino, Sun, Hu, & Krauss, 2010), but from a fat quality point of view, a SFA content > 41 % (Hauser & Prabucki, 1990) has been proposed for good quality fat. In the present study, all the CLA containing dietary groups showed SFA values well in excess of this value (Table 4.13), while the subcutaneous fat from the control pigs could not conform to this standard. This is an important finding and implies that dietary CLA supplementation results in subcutaneous fat of better quality, from a meat technology point of view. The fact that subcutaneous fat, at some positions on the carcass, had higher SFA content than other, is also important. Sampling positions B (BF) and D (rib) generally had higher SFA contents than other sampling positions. This implies that subcutaneous fat from these areas may, e.g. be more suitable for the manufacture of products like salami and bacon, which is more sensitive to fat quality.

Table 4.13: Fatty acid ratios with nutritional, health and fat quality implications of subcutaneous fat from gilts fed four diets containing different levels of CLA.

Dietary Treatment	Position	SFA	MUFA	UFA	MUFA/SFA	PI	PUFA	Atherogenicity Index	PUFA/SFA	*n*-6	*n*-3	*n*-6/*n*-3
Control	A	39.77 ± 2.28^{ab}	44.98 ± 2.49^{kl}	60.23 ± 2.29^{kl}	1.14 ± 0.12^{k}	19.74 ± 1.84^{def}	15.94 ± 0.81^{defghi}	0.49 ± 0.03^{a}	0.40 ± 0.03^{ij}	14.34 ± 0.69^{defgh}	0.91 ± 0.25^{de}	17.55 ± 8.01^{abc}
(n = 12)	B	39.99 ± 0.76^{b}	44.38 ± 1.34^{jkl}	60.01 ± 0.76^{k}	1.11 ± 0.05^{ijk}	19.97 ± 2.16^{ef}	16.27 ± 1.22^{efghi}	0.50 ± 0.02^{a}	0.41 ± 0.03^{j}	14.73 ± 1.14^{efgh}	0.90 ± 0.25^{de}	17.64 ± 5.98^{abc}
	C	37.23 ± 1.31^{a}	48.30 ± 1.83^{no}	62.77 ± 1.31^{l}	1.30 ± 0.08^{m}	19.56 ± 2.76^{def}	15.24 ± 1.64^{defghi}	0.46 ± 0.02^{a}	0.41 ± 0.05^{j}	13.50 ± 1.46^{def}	0.96 ± 0.25^{e}	14.89 ± 4.42^{ab}
	D	40.05 ± 1.05^{b}	45.10 ± 2.16^{klm}	59.95 ± 1.05^{k}	1.13 ± 0.08^{jk}	18.99 ± 2.15^{def}	15.55 ± 1.69^{defghi}	0.50 ± 0.02^{a}	0.39 ± 0.04^{hij}	14.04 ± 1.64^{defgh}	0.81 ± 0.14^{abcde}	17.56 ± 2.54^{abc}
	E	37.70 ± 1.27^{ab}	47.82 ± 0.99^{mno}	62.29 ± 1.27^{kl}	1.27 ± 0.06^{lm}	19.16 ± 1.34^{def}	15.16 ± 1.23^{defgh}	0.47 ± 0.02^{a}	0.40 ± 0.04^{ij}	13.59 ± 1.22^{def}	0.89 ± 0.10^{cde}	15.42 ± 2.33^{ab}
	F	39.71 ± 2.82^{ab}	46.30 ± 2.71^{lmn}	60.29 ± 2.82^{kl}	1.17 ± 0.14^{kl}	18.34 ± 2.01^{cdef}	14.70 ± 1.67^{def}	0.49 ± 0.03^{a}	0.37 ± 0.06^{ghij}	13.15 ± 1.63^{de}	0.84 ± 0.13^{bcde}	15.89 ± 2.55^{abc}
	G	37.98 ± 1.25^{ab}	50.19 ± 1.33^{o}	62.02 ± 1.25^{kl}	1.32 ± 0.07^{m}	15.44 ± 2.20^{abc}	12.61 ± 1.33^{abc}	0.46 ± 0.02^{a}	0.33 ± 0.04^{cdefg}	11.21 ± 1.21^{abc}	0.62 ± 0.19^{abc}	19.60 ± 6.31^{abc}
0.25% CLA	A	44.67 ± 0.71^{cdef}	40.25 ± 1.45^{fgh}	55.33 ± 0.71^{ghij}	0.90 ± 0.04^{defg}	19.22 ± 1.25^{def}	15.67 ± 1.29^{defghi}	0.61 ± 0.03^{b}	0.35 ± 0.03^{defghi}	14.17 ± 1.29^{defgh}	0.91 ± 0.06^{de}	15.68 ± 1.64^{ab}
(n = 12)	B	47.20 ± 1.99^{fghi}	37.19 ± 1.92^{de}	52.80 ± 1.98^{defg}	0.79 ± 0.07^{cd}	19.09 ± 1.19^{def}	16.11 ± 0.63^{defghi}	0.66 ± 0.06^{bcd}	0.34 ± 0.02^{defgh}	14.77 ± 0.64^{efgh}	0.84 ± 0.18^{bcde}	18.59 ± 5.67^{abc}
	C	42.85 ± 2.17^{c}	43.26 ± 1.91^{ijk}	57.15 ± 2.17^{j}	1.01 ± 0.09^{ghi}	18.15 ± 1.42^{cdef}	14.53 ± 0.65^{cde}	0.60 ± .04^{b}	0.34 ± 0.03^{defgh}	13.00 ± 0.66^{cde}	0.89 ± 0.18^{cde}	15.42 ± 4.39^{ab}
	D	46.60 ± 1.42^{efghi}	38.81 ± 1.41^{efg}	53.40 ± 1.42^{defgh}	0.83 ± 0.05^{cde}	17.96 ± 2.28^{cdef}	15.18 ± 1.26^{defgh}	0.64 ± 0.04^{bcd}	0.33 ± 0.03^{cdefg}	13.81 ± 1.13^{defg}	0.78 ± 0.21^{abcde}	18.95 ± 5.39^{abc}
	E	43.93 ± 1.82^{cde}	41.79 ± 2.35^{hij}	56.07 ± 1.81^{hij}	0.95 ± 0.09^{fgh}	17.86 ± 1.72^{cdef}	14.82 ± 1.23^{defg}	0.61 ± 0.06^{b}	0.34 ± 0.03^{defgh}	13.49 ± 1.22^{def}	0.79 ± 0.14^{abcde}	17.58 ± 2.90^{abc}
	F	44.98 ± 1.65^{cdef}	40.73 ± 2.26^{ghi}	55.02 ± 1.65^{ghij}	0.91 ± 0.08^{efg}	18.70 ± 1.17^{def}	14.87 ± 0.94^{defg}	0.62 ± 0.04^{bc}	0.33 ± 0.02^{cdefg}	13.35 ± 0.96^{def}	0.93 ± 0.09^{e}	14.39 ± 1.41^{ab}
	G	43.73 ± 1.71^{cd}	44.51 ± 2.77^{jkl}	56.27 ± 1.71^{ij}	1.02 ± 0.11^{hij}	14.51 ± 1.85^{ab}	12.37 ± 1.16^{ab}	0.60 ± 0.05^{b}	0.28 ± 0.02^{abc}	11.18 ± 1.14^{abc}	0.57 ± 0.19^{ab}	21.61 ± 6.95^{bc}
0.5% CLA	A	45.80 ± 1.15^{defgh}	37.71 ± 1.52^{def}	54.20 ± 1.15^{efghi}	0.82 ± 0.04^{cde}	20.59 ± 2.77^{f}	17.02 ± 1.81^{hi}	0.64 ± 0.03^{bcd}	0.37 ± 0.04^{fghij}	15.54 ± 1.70^{gh}	0.95 ± 0.25^{e}	17.49 ± 5.61^{abc}
(n = 12)	B	48.62 ± 2.41^{ijk}	35.34 ± 2.02^{bcd}	51.38 ± 2.41^{bcd}	0.73 ± 0.07^{abc}	19.80 ± 2.90^{ef}	16.53 ± 2.07^{fghi}	0.70 ± 0.06^{de}	0.34 ± 0.05^{defgh}	15.18 ± 1.98^{fgh}	0.87 ± 0.21^{cde}	18.13 ± 3.62^{abc}
	C	45.03 ± 1.48^{cdefg}	40.01 ± 1.47^{fgh}	54.97 ± 1.48^{fghij}	0.89 ± 0.06^{def}	19.33 ± 1.31^{def}	15.55 ± 0.66^{defghi}	0.66 ± 0.04^{bcd}	0.35 ± 0.02^{defgh}	14.03 ± 0.75^{defgh}	0.93 ± 0.20^{de}	16.28 ± 6.02^{abc}
	D	47.71 ± 2.09^{ghi}	37.68 ± 1.18^{def}	52.29 ± 2.09^{def}	0.79 ± 0.05^{cd}	17.35 ± 2.02^{bcde}	15.15 ± 1.63^{defgh}	0.69 ± 0.06^{cde}	0.32 ± 0.05^{cdef}	13.96 ± 1.65^{defg}	0.65 ± 0.18^{abcd}	23.08 ± 6.79^{c}
	E	45.73 ± 1.76^{defgh}	40.57 ± 1.56^{ghi}	54.27 ± 1.76^{efghi}	0.89 ± 0.06^{def}	17.27 ± 2.10^{bcde}	14.25 ± 1.32^{bcd}	0.65 ± 0.05^{bcd}	0.31 ± 0.04^{cde}	12.94 ± 1.35^{cde}	0.76 ± 0.19^{abcde}	18.02 ± 5.34^{abc}
	F	47.00 ± 1.85^{fghi}	39.32 ± 1.86^{efgh}	53.00 ± 1.85^{defg}	0.84 ± 0.07^{cde}	17.50 ± 1.36^{cde}	14.22 ± 0.93^{bcd}	0.69 ± 0.05^{cde}	0.30 ± 0.03^{bcd}	12.88 ± 0.93^{bcd}	0.81 ± 0.13^{abcde}	16.33 ± 3.08^{abc}
	G	45.52 ± 2.19^{cdefgh}	43.32 ± 2.55^{ijk}	54.48 ± 2.19^{efghij}	0.96 ± 0.10^{fgh}	14.03 ± 0.90^{a}	11.76 ± 0.66^{a}	0.66 ± 0.07^{bcd}	0.26 ± 0.02^{ab}	10.61 ± 0.75^{a}	0.55 ± 0.11^{a}	20.08 ± 4.99^{abc}
1% CLA	A	47.99 ± 1.80^{hij}	35.34 ± 1.61^{bcd}	52.02 ± 1.80^{cde}	0.74 ± 0.06^{abc}	19.88 ± 2.13^{ef}	17.18 ± 1.14^{i}	0.71 ± 0.04^{de}	0.36 ± 0.03^{efghij}	15.86 ± 0.98^{h}	0.82 ± 0.20^{abcde}	20.35 ± 4.48^{abc}
(n = 12)	B	51.26 ± 2.13^{kl}	32.44 ± 1.67^{a}	48.74 ± 2.13^{ab}	0.63 ± 0.06^{a}	19.05 ± 1.46^{def}	16.75 ± 0.96^{ghi}	0.79 ± 0.08^{f}	0.33 ± 0.03^{cdefg}	15.54 ± 0.91^{gh}	0.76 ± 0.16^{abcde}	21.06 ± 3.72^{abc}
	C	48.60 ± 2.63^{ijk}	36.80 ± 2.18^{cde}	51.39 ± 2.63^{bcd}	0.76 ± 0.08^{bc}	19.18 ± 1.81^{def}	15.21 ± 1.00^{defgh}	0.74 ± 0.09^{ef}	0.31 ± 0.03^{cde}	13.61 ± 0.84^{def}	0.99 ± 0.18^{e}	14.09 ± 2.18^{a}
	D	51.55 ± 1.82^{l}	33.39 ± 1.01^{ab}	48.45 ± 1.82^{a}	0.65 ± 0.04^{ab}	17.95 ± 1.51^{cdef}	15.56 ± 1.17^{defghi}	0.79 ± 0.08^{f}	0.30 ± 0.03^{bcd}	14.32 ± 1.14^{defgh}	0.74 ± 0.10^{abcde}	19.53 ± 2.15^{abc}
	E	49.06 ± 1.55^{ijkl}	36.74 ± 1.27^{cde}	50.94 ± 1.55^{abcd}	0.75 ± 0.04^{bc}	17.19 ± 2.22^{bcde}	14.70 ± 1.32^{def}	0.74 ± 0.05^{ef}	0.30 ± 0.03^{bcd}	13.48 ± 1.19^{def}	0.73 ± 0.23^{abcde}	20.53 ± 6.92^{abc}
	F	51.73 ± 1.67^{l}	34.37 ± 0.78^{abc}	48.27 ± 1.67^{a}	0.67 ± 0.03^{ab}	16.84 ± 2.49^{abcd}	14.41 ± 1.52^{cde}	0.81 ± 0.07^{f}	0.28 ± 0.04^{abc}	13.18 ± 1.40^{de}	0.72 ± 0.23^{abcde}	19.72 ± 4.99^{abc}
	G	50.71 ± 1.71^{jkl}	37.70 ± 1.75^{def}	49.30 ± 1.71^{abc}	0.75 ± 0.06^{abc}	14.02 ± 2.10^{a}	12.14 ± 1.30^{a}	0.79 ± 0.06^{f}	0.24 ± 0.03^{a}	11.04 ± 1.18^{ab}	0.55 ± 0.18^{a}	21.54 ± 5.83^{bc}
Significance level		p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

Means with different superscripts in the same column differ significantly

For MUFA, all sampling positions from the control treatment had significantly ($p<0.001$) higher MUFA content than the corresponding sampling positions of all the CLA treatments (Table 4.13). Within the CLA treatments, all the sampling positions from the 1% CLA treatment had significantly ($p<0.001$) lower MUFA content than the corresponding sampling positions from the 0.25% CLA treatment. Sampling positions, B, C, D, E, F and G from the 1% CLA treatment had significantly ($p<0.001$) lower MUFA content than the same sampling positions from the 0.5% CLA treatment.

For MUFA, sampling position C had significantly ($p<0.001$) lower levels than sampling positions A, B and D, within the control group. Sampling positions E, F and G from the control had significantly ($p<0.001$) higher MUFA content than positions A and B. For the 0.25% CLA treatment, sampling position B had a significantly ($p<0.001$) lower MUFA content than sampling positions A, C, E, F and G. Sampling position G from the 0.25% CLA treatment had significantly ($p<0.001$) higher MUFA content than positions A, B and D. For the 0.5% CLA treatment sampling positions C, E, F and G had significantly ($p<0.001$) higher MUFA content than positions A, B and D. Position G from the 0.5% CLA treatment had significantly ($p<0.001$) higher MUFA content than positions A, B, C, D and F. For the 1% CLA treatment sampling positions B had significantly ($p<0.001$) lower MUFA content than sampling positions A, C, E and G. Sampling position D from the 1 % CLA treatment had significantly ($p<0.001$) lower MUFA content than sampling positions C, E and G.

As mentioned before, the recommendation is to increase the MUFA content of meat from a health point of view (Cordain, Eaton, Sebastian, Mann, Lindeberg, Watkins, *et al*., 2005). From a fat quality perspective, Lizardo *et al*. (2002) proposed a MUFA content < 57 % for good quality fat. Subcutaneous fat from all sampling positions, from all the CLA dietary treatments, conformed to this requirement. Although subcutaneous fat from the control group conformed to the < 57 % requirement, the MUFA content of the subcutaneous fat samples from the CLA treatment groups, was significantly ($p<0.001$) lower than that of the control. This means that CLA supplementation results in subcutaneous fat of better quality, from a MUFA content perspective. The significant differences in MUFA content, between sampling positions, must also be taken into consideration when assessing the suitability of fat for its use in various processed meat products.

For UFA, all sampling positions from the control treatment had significantly ($p<0.001$) higher UFA content than the corresponding sampling positions of all the CLA treatments. Within the CLA treatments, sampling positions A, B, D, E, F and G from the 1% CLA treatment had significantly ($p<0.001$) lower UFA content than the corresponding sampling positions from the 0.25% CLA treatment. Sampling positions C, D, E, F and G from the 1% CLA treatment had

significantly ($p<0.001$) lower UFA content than the same sampling positions from the 0.5% CLA treatment.

For UFA, the control group's sampling position C had a significantly ($p<0.001$) higher UFA content than sampling positions B and D. Sampling positions C and G from the 0.25% CLA treatment had significantly ($p<0.001$) higher UFA contents than sampling positions B and D. For the 0.5% CLA treatment, sampling position B had significantly ($p<0.001$) lower UFA content than sampling positions A, C, E and G. For the 1% CLA treatment, sampling positions D and F had significantly ($p<0.001$) lower UFA content than sampling positions A and C.

Consumers are also advised to increase the intake of UFAs for improved health (Zock, 2006). The opposite is true from a meat technology point of view. The UFA content for good quality fat has been proposed as < 59 % (Prabucki, 1991). All the samples from the three CLA containing treatment groups had UFA contents lower than this value, some even as low as 48.27% (position F; 1% CLA group) (Table 4.13). The linear increase of CLA concentration in the subcutaneous fat implies that increased dietary CLA level produced subcutaneous fat with improved health properties (Table 4.13). Significant sampling position differences also had important fat quality implications. A good example is the control group, where six of the seven sampling positions had UFA values > 60 % (poor fat quality), with position D having an UFA content of 59.95%, approaching the proposed cut-off point of 59 % for good fat quality (Table 4.13).

For MUFA/SFA ratio, all sampling positions from the control had significantly ($p<0.001$) higher MUFA/SFA ratios than the corresponding sampling positions of all the CLA treatments. Within the CLA treatments, all sampling positions from the 1% CLA treatment had significantly ($p<0.001$) lower MUFA/SFA ratios than the corresponding sampling positions from the 0.25% CLA treatment. Sampling positions C, D, E, F and G from the 1% CLA treatment also had significantly ($p<0.001$) lower MUFA/SFA ratios than the same sampling positions from the 0.5% CLA treatment.

For the control, sampling positions C and G had a significantly ($p<0.001$) higher MUFA/SFA ratio than sampling positions A, B, D and F. For the 0.25% CLA treatment, sampling position G had a significantly ($p<0.001$) higher MUFA/SFA ratio than sampling positions A, B, D and F. Also for the 0.25% CLA treatment, sampling position C had significantly ($p<0.001$) higher MUFA/SFA ratio than positions B and D. Sampling position G from the 0.5% CLA treatment had significantly ($p<0.001$) higher MUFA/SFA ratio than positions A, B, D and F. Also for the 0.5% CLA treatment, sampling positions C and E had significantly ($p<0.001$) higher MUFA/SFA ratios than position B. For the 1% CLA treatment, sampling positions C and E had significantly ($p<0.001$) higher MUFA/SFA ratio than sampling position B.

A higher MUFA/SFA ratio for subcutaneous fat would be beneficial from a human health and nutritional point of view (Cordain *et al*., 2005; Zock, 2006, Muchenje *et al*., 2009), whereas a lower MUFA/SFA ratio would be better from a fat quality point of view (Lea *et al*., (1970). The lower MUFA/SFA ratio of subcutaneous fat, observed with increased dietary CLA content (Table 4.13), confirmed the conclusions made from the previously discussed ratios, that dietary CLA supplementation improve subcutaneous fat quality. Lea *et al*. (1970) reported that decreased MUFA/SFA ratio is associated with increased fat firmness, which can be considered as beneficial from a fat quality point of view. The significant ($p<0.001$) difference in MUFA/SFA ratios between sampling positions, within dietary treatments, is also important from a fat quality point of view.

Although a significant ($p<0.001$) decrease was observed for PI and *n*-3 content, along with a significant ($p<0.001$) increase for *n*-6/*n*-3 ratio, with increased dietary CLA content, no significant differences were found for the same sampling position, between dietary treatments. Polyunsaturated fatty acid and *n*-6 contents were not significantly ($p<0.001$) influenced by dietary treatment (Table 4.5). For the 0.25, 0.5 and 1% CLA treatments, PI from sampling position G was significantly ($p<0.001$) lower than sampling positions A, B, C, D, E and F. For the control, sampling position G had a significantly ($p<0.001$) lower PI than sampling positions A, B, C, D and E. For the 0.5% CLA treatment, sampling position A had a PI significantly ($p<0.001$) higher than sampling positions D, E, F and G. For the 1% CLA treatment, sampling position A had significantly ($p<0.001$) higher PI values than sampling positions C, E, F and G.

Sampling position G had significantly ($p<0.001$) lower PUFA content than all the other sampling positions, for all dietary treatments. For the 0.5% CLA treatment, sampling positions E and F had significantly ($p<0.001$) lower PUFA content than sampling positions A and B. For the 1% CLA treatment, sampling positions C had significantly ($p<0.001$) lower PUFA content than sampling position A.

For the control, 0.5 and 1% CLA treatment groups, sampling position G had significantly ($p<0.001$) lower *n*-6 content than all other sampling positions. For the 0.25 % CLA treatment, sampling position G had significantly ($p<0.001$) lower *n*-6 content than sampling positions A, B, D, E and F.

For the control and 0.25% CLA treatment, sampling position G had significantly ($p<0.001$) lower *n*-3 content than sampling positions A, B, C and F. For the 0.5% CLA treatment, sampling position G had significantly ($p<0.001$) lower *n*-3 content than sampling positions A, B and C, whereas sampling position A had significantly higher ($p<0.001$) *n*-3 content than sampling positions D and G. For the 1% CLA treatment, sampling position G had significantly ($p<0.001$) lower *n*-3 content than sampling position C.

No significant differences were observed for the *n*-6/*n*-3 ratio, within the control and 0.25% CLA treatment, between sampling positions. For the 0.5% CLA treatment, sampling position E had significantly ($p<0.001$) higher *n*-6/*n*-3 ratio than all other sampling groups. For the 1% CLA treatment, sampling position G had significantly ($p<0.001$) higher *n*-6/*n*-3 ratio than sampling position C.

Although no specific standard is available for PI, the statistically significant ($p<0.001$) decrease in the PI of subcutaneous fat, with increased dietary CLA content (Table 4.13), implies that meat products, manufactured from meat with increased levels of CLA, will be more resistant to oxidative breakdown than those manufactured from meat with no CLA or lower CLA levels. The same argument is valid for the significant ($p<0.001$) differences between sampling positions, within dietary treatments. The lower PI of sampling position G implies that meat products, manufactured from subcutaneous fat from that area, will be more stable than products manufactured with fat from other areas.

From a human health perspective, the general recommendation is to reduce SFA intake and to increase PUFA intake (Levnedmiddelstyrelsen, as cited by Madsen, Jakobsen, & Mortensen, 1992; Honkavaara, 1989, Muchenje *et al*., 2009). From a fat quality point of view, the opposite is true, with the recommendation to reduce the PUFA content of the subcutaneous fat. As noted earlier, various levels of PUFA have been proposed for good quality fat, ranging from < 12% (Prabucki, as cited by Houben & Krol, 1983), to < 13% (Wenk *et al*., as cited by Warnants, *et al*., 1996) to < 15% (Houben & Krol, 1983). For this study, values ranging from 11.76% (position G, 0.5% CLA) to as high as 17.18% (position A, 1% CLA) were measured (Table 4.13). The trend towards an increase in subcutaneous PUFA content with increased dietary CLA content, observed in Table 4.13, means that from a PUFA point of view, the subcutaneous fat quality improved with increased levels of dietary CLA. The statistically significant ($p<0.001$) differences in PUFA content, between sampling positions, are also of importance. Again, sampling position G had lower PUFA content and therefore better subcutaneous fat quality than the other sampling positions.

In the past, the general recommendation was to increase all PUFAs in food to improve its health properties (Kaizer, Boyd, Kriukos, & Fritchter, 1989). The modern trend is to increase the *n*-3 PUFA and reduce the *n*-6 PUFA content of food, thus reducing the *n*-6/*n*-3 ratio of fats for human consumption (Okuyama & Ikemoto, 1999, Muchenje *et al*., 2009). An *n*-6/*n*-3 ratio of 1:1 is in a safe range, because hunters and gatherers (primitive man) were estimated to consume foods with *n*-6/*n*-3 ratios of roughly 1:1 (Leaf & Weber, 1987). Currently available meats have *n*-6/*n*-3 ratios of above 10. Therefore, the feed materials for animals must be changed in order to lower the *n*-6/*n*-3 ratios of human foods as much as possible (Okuyama & Ikemoto, 1999). A *n*-6/*n*-3 ratio of 1:1

may not be a realistic goal, therefore Okuyama (1997) consequently proposed a *n*-6/*n*-3 ratio of 2:1, while Verbeke *et al*. (1999) proposed an even more conservative goal of 6:1.

Although the *n*-6 content of the subcutaneous fat from the present study was not significantly influenced by dietary treatment (Table 4.5 and Table 4.13), a statistically significant ($p<0.001$) decrease was observed for *n*-3 content, with increased dietary CLA level (Table 4.5 and Table 4.13). That resulted in a significant ($p<0.001$) increase of the *n*-6/*n*-3 ratio, with increased dietary CLA content (Table 4.5 and Table 4.13). Dietary CLA supplementation, therefore, result in the deterioration of the health properties of subcutaneous fat, from a purely FA profile point of view. This negative effect on FA profile must be weighed against the positive health implications of elevated CLA levels. Although dietary CLA supplementation resulted in a deterioration of *n*-6/*n*-3 ratio of subcutaneous fat, the values for all treatments were generally poor. The *n*-6/*n*-3 ratios ranged between 14.09:1 and 23.08:1. That is very far from the recommended value of 6:1, proposed by Verbeke *et al*. (1999). The only method to improve this value is by including *n*-3 rich feed sources like linseed (Nürnberg *et al*., 2005) and fishmeal (Wood *et al*., 1999) in pig diets. The variation in *n*-6/*n*-3 ratio between sampling positions are also important. The *n*-6/*n*-3 ratio of sampling position C was consistently lower than that of other sampling positions.

For the atherogenicity index (AI), all sampling positions from the control had significantly ($p<0.001$) lower AI's than the corresponding sampling positions of all the CLA treatments. Within the CLA treatments, all sampling positions from the 1% CLA treatment had significantly ($p<0.001$) higher AI's than the corresponding sampling positions from the 0.25% CLA treatment. Sampling positions B, C, D, E, F and G from the 1% CLA treatment had significantly higher AI's than the same sampling positions from the 0.5% CLA treatment. The only sampling position difference, for AI, was observed in the 1% CLA treatment, where sampling positions B, D, F and G had significantly ($p<0.001$) higher AI's than sampling position A. With an increase in the CLA concentration in the diets, there was a linear increase in the AI contents for all sampling positions, between dietary treatments (Table 4.13). This can be ascribed to the significant ($p<0.001$) increase in C12:0, C14:0 and C16:0 of the subcutaneous fat (Table 4.8), with increased dietary CLA content. This confirms the findings of Ostrowska *et al*. (2003), who also observed a negative effect on this calculated index, due to CLA supplementation. An increase in AI implies that dietary CLA supplementation had a negative effect on the health properties of subcutaneous fat, from a FA profile point of view. This must be weighed against the other positive effects of elevated CLA levels in meat.

For the PUFA/SFA ratio, sampling positions B, C, D, E, F and G from the control had significantly ($p<0.001$) lower PUFA/SFA ratios than the corresponding sampling positions of all the CLA treatments. No significant differences were found for the same sampling position, between the

different CLA dietary treatments. For all the dietary treatments, sampling position G had a significantly ($p<0.001$) lower PUFA/SFA ratio than sampling positions A, B, C, D and E. For the 0.5% CLA treatment, sampling position F had a significantly ($p<0.001$) lower PUFA/SFA ratio than sampling positions A, B and C. Consumers are advised to increase the PUFA/SFA ratio of their diet (Enser *et al*., 1996). Polyunsaturated fatty acid/SFA ratios from 0.23-0.45 (Phelps, 1991), to 0.45-0.50 (Honkavaara, 1989; Levnedmiddelstyrelsen, as cited by Madsen *et al*., 1992, Wood *et al*., 2008; Alfaia, Alves, Martins, Costa, Fontes, Lemos, *et al*., 2009; Muchenje *et al*., 2009), to even as high as 0.6-0.7 (Verbeke *et al*., 1999) were proposed as the minimum value for acceptable health properties of animal fat. The significant ($p<0.001$) increase in the PUFA/SFA ratio of the subcutaneous fat, with increased dietary CLA level, implies that health properties of subcutaneous fat also deteriorate from a PUFA/SFA ratio point of view. The ratios in the present study ranged from 0.24 (position G, 1% CLA group) to 0.41 (positions B and C, control group).

The only FA ratios with nutritional, health and fat quality implications that exhibited significant ($p<0.05$) interactions between dietary treatment and sampling position, were SFA, UFA and MUFA/SFA (Table 4.5).

Fatty acid ratios with technological implications

Table 4.14 is a summary of the FA ratios of the subcutaneous fat with technological implications. Dietary treatment had a significant (at least $p<0.05$) effect on all ratios, except pentaenoic FAs (Table 4.5). Increased dietary CLA level resulted in a significant ($p<0.001$) decrease in dienoic, tetraenoic, pentaenoic hexaenoic, penta + hexaenoic and C16:1+C18:1/C16:0+C18:0 ratios (Table 4.14). The trienoic FAs, C16:0+C18:0, C18:0/C18:2, and C16:0/C18:2 ratios, and DBI's showed an increase, with increased dietary CLA level. According to Table 4.5, sampling position had a statistically significant ($p<0.001$) effect on all the ratios of subcutaneous fat, with technological implications. Statistically significant ($p<0.001$) sampling position differences, within dietary treatments, were found for all FA ratios with technological implications, with the exception of tetraenoic and hexaenoic FAs (Table 4.14).

Although a statistically significant ($p<0.001$) decrease in dienoic acid content was observed with increased dietary CLA content, no significant differences were observed for the same sampling position between dietary treatments. Within dietary treatment groups, sampling position G had significantly ($p<0.001$) lower dienoic acid content than all other sampling positions, for all four dietary treatments. Within the 0.5% CLA treatment, subcutaneous fat from sampling positions E and F had significantly ($p<0.001$) lower dienoic acid content than sampling positions A and B. Within the 1% CLA treatment, sampling positions C, E and F had significantly ($p<0.001$) lower

Table 4.14: Fatty acid ratios with technological implications of subcutaneous fat from gilts fed four diets containing different levels of supplemented CLA.

Dietary Treatment	Position	Dienoic	Trienoic	Tetraenoic	Pentaenoic	Hexaenoic	Penta + Hexaenoic	C16:0+C18:0	C16:1 + C18:1*c*9 /C16:0+C18:0	C18:0/C18:2	C16:0/C18:2
Control (n = 12)	A	14.21 ± 0.67^{defgh}	0.51 ± 0.08^{def}	0.13 ± 0.04^{ef}	0.16 ± 0.08^{abcd}	0.24 ± 0.11^{bcde}	0.40 ± 0.18^{bcdef}	37.92 ± 2.22^{abc}	1.17 ± 0.13^{ij}	0.98 ± 0.17^{abc}	1.80 ± 0.08^{ab}
	B	14.60 ± 1.13^{defgh}	0.53 ± 0.08^{def}	0.13 ± 0.04^{f}	0.14 ± 0.08^{abcd}	0.23 ± 0.12^{bcde}	0.37 ± 0.18^{abcdef}	38.11 ± 0.72^{bc}	1.15 ± 0.05^{ij}	0.95 ± 0.12^{ab}	1.78 ± 0.14^{a}
	C	13.38 ± 1.43^{def}	0.48 ± 0.08^{bcde}	0.13 ± 0.04^{def}	0.19 ± 0.08^{bcd}	0.30 ± 0.12^{e}	0.49 ± 0.18^{f}	35.35 ± 1.35^{a}	1.35 ± 0.09^{l}	0.92 ± 0.14^{a}	1.87 ± 0.21^{abcd}
	D	13.92 ± 1.62^{defg}	0.46 ± 0.06^{bcd}	0.12 ± 0.02^{cdef}	0.14 ± 0.04^{abcd}	0.22 ± 0.08^{abcde}	0.35 ± 0.10^{abcdef}	38.26 ± 1.03^{bc}	1.16 ± 0.08^{ij}	1.01 ± 0.14^{abcd}	1.89 ± 0.20^{abcd}
	E	13.45 ± 1.20^{def}	0.47 ± 0.05^{bcde}	0.13 ± 0.02^{f}	0.16 ± 0.04^{abcd}	0.26 ± 0.04^{de}	0.42 ± 0.08^{def}	35.93 ± 1.28^{ab}	1.31 ± 0.06^{kl}	0.94 ± 0.18^{a}	1.87 ± 0.18^{abcd}
	F	13.02 ± 1.62^{de}	0.46 ± 0.08^{bcd}	0.13 ± 0.02^{f}	0.15 ± 0.05^{abcd}	0.23 ± 0.07^{abcde}	0.38 ± 0.10^{abcdef}	37.88 ± 2.87^{abc}	1.21 ± 0.15^{jk}	1.08 ± 0.28^{abcde}	2.01 ± 0.33^{abcdef}
	G	11.12 ± 1.19^{abc}	0.35 ± 0.04^{a}	0.09 ± 0.05^{abcdef}	0.09 ± 0.06^{ab}	0.18 ± 0.12^{abcde}	0.27 ± 0.16^{abcdef}	36.28 ± 1.21^{ab}	1.36 ± 0.07^{l}	1.18 ± 0.21^{abcdef}	2.28 ± 0.26^{efg}
0.25% CLA (n = 12)	A	14.07 ± 1.27^{defgh}	0.54 ± 0.04^{def}	0.11 ± 0.02^{bcdef}	0.15 ± 0.04^{abcd}	0.22 ± 0.05^{abcde}	0.37 ± 0.06^{abcdef}	42.33 ± 0.69^{defg}	0.94 ± 0.04^{efg}	1.15 ± 0.12^{abcdef}	1.99 ± 0.22^{abcde}
	B	14.70 ± 0.66^{efgh}	0.55 ± 0.06^{def}	0.07 ± 0.06^{abcd}	0.11 ± 0.07^{abcd}	0.18 ± 0.09^{abcde}	0.29 ± 0.15^{abcdef}	44.89 ± 1.86^{ghij}	0.82 ± 0.08^{de}	1.20 ± 0.08^{abcdef}	1.96 ± 0.15^{abcde}
	C	12.90 ± 0.64^{cde}	0.50 ± 0.03^{def}	0.10 ± 0.05^{bcdef}	0.15 ± 0.08^{abcd}	0.23 ± 0.10^{abcde}	0.38 ± 0.16^{abcdef}	40.36 ± 2.15^{cd}	1.06 ± 0.10^{hi}	1.11 ± 0.18^{1abcdef}	2.14 ± 0.14^{bcdef}
	D	13.73 ± 1.09^{defg}	0.52 ± 0.07^{def}	0.08 ± 0.05^{abcdef}	0.10 ± 0.08^{abc}	0.16 ± 0.10^{abcde}	0.26 ± 0.16^{abcde}	44.29 ± 1.40^{fghij}	0.86 ± 0.05^{def}	1.27 ± 0.17^{cdefgh}	2.09 ± 0.19^{abcdef}
	E	13.39 ± 1.19^{def}	0.50 ± 0.06^{def}	0.10 ± 0.04^{bcdef}	0.13 ± 0.06^{abcd}	0.16 ± 0.07^{abcde}	0.28 ± 0.12^{abcdef}	41.56 ± 1.64^{de}	1.00 ± 0.09^{gh}	1.14 ± 0.13^{abcdef}	2.10 ± 0.20^{abcdef}
	F	13.23 ± 0.96^{de}	0.53 ± 0.05^{def}	0.12 ± 0.03^{cdef}	0.17 ± 0.04^{abcd}	0.24 ± 0.04^{cde}	0.41 ± 0.07^{cdef}	42.57 ± 1.65^{defgh}	0.95 ± 0.09^{fgh}	1.21 ± 0.12^{abcdefg}	2.13 ± 0.16^{bcdef}
	G	11.12 ± 1.13^{abc}	0.40 ± 0.07^{abc}	0.06 ± 0.05^{abc}	0.07 ± 0.07^{a}	0.10 ± 0.10^{abc}	0.17 ± 0.16^{a}	41.46 ± 1.60^{de}	1.06 ± 0.11^{hi}	1.38 ± 0.18^{efgh}	2.52 ± 0.17^{gh}
0.5% CLA (n = 12)	A	15.44 ± 1.67^{gh}	0.57 ± 0.09^{f}	0.10 ± 0.05^{bcdef}	0.18 ± 0.09^{bcd}	0.20 ± 0.13^{abcde}	0.38 ± 0.20^{abcdef}	43.24 ± 1.27^{efghi}	0.86 ± 0.04^{def}	1.11 ± 0.22^{abcde}	1.82 ± 0.18^{abc}
	B	15.07 ± 1.95^{fgh}	0.55 ± 0.09^{def}	0.11 ± 0.05^{bcdef}	0.13 ± 0.07^{abcd}	0.20 ± 0.11^{abcde}	0.32 ± 0.15^{abcdef}	46.01 ± 2.38^{jkl}	0.76 ± 0.07^{abcd}	1.24 ± 0.37^{abcdefgh}	1.98 ± 0.34^{abcde}
	C	13.92 ± 0.75^{defg}	0.51 ± 0.03^{def}	0.11 ± 0.04^{bcdef}	0.20 ± 0.08^{cd}	0.22 ± 0.13^{abcde}	0.42 ± 0.18^{def}	42.16 ± 1.50^{def}	0.94 ± 0.06^{efg}	1.10 ± 0.14^{abcde}	2.05 ± 0.10^{abcdef}
	D	13.89 ± 1.65^{defg}	0.47 ± 0.05^{bcd}	0.07 ± 0.05^{abcde}	0.08 ± 0.07^{a}	0.10 ± 0.10^{abc}	0.18 ± 0.15^{ab}	45.15 ± 2.09^{hijk}	0.82 ± 0.05^{de}	1.27 ± 0.29^{cdefgh}	2.14 ± 0.28^{bcdef}
	E	12.85 ± 1.33^{cd}	0.46 ± 0.05^{bcd}	0.09 ± 0.05^{abcdef}	0.13 ± 0.06^{abcd}	0.17 ± 0.11^{abcde}	0.30 ± 0.16^{abcdef}	43.24 ± 1.65^{efghi}	0.93 ± 0.06^{efg}	1.28 ± 0.28^{defgh}	2.25 ± 0.28^{efg}
	F	12.78 ± 0.92^{bcd}	0.46 ± 0.04^{bcd}	0.09 ± 0.03^{abcdef}	0.17 ± 0.02^{abcd}	0.18 ± 0.10^{abcde}	0.35 ± 0.12^{abcdef}	44.31 ± 1.78^{fghij}	0.88 ± 0.07^{def}	1.30 ± 0.18^{defgh}	2.30 ± 0.18^{efg}
	G	10.55 ± 0.74^{a}	0.35 ± 0.04^{a}	0.06 ± 0.03^{abc}	0.09 ± 0.06^{ab}	0.11 ± 0.06^{abcd}	0.20 ± 0.11^{abcd}	42.97 ± 1.99^{defghi}	1.00 ± 0.10^{gh}	1.50 ± 0.22^{ghi}	2.74 ± 0.17^{h}
1% CLA (n=12)	A	15.78 ± 0.98^{h}	0.56 ± 0.05^{ef}	0.08 ± 0.04^{abcdef}	0.14 ± 0.08^{abcd}	0.12 ± 0.12^{abcd}	0.26 ± 0.18^{abcde}	45.17 ± 1.93^{hijk}	0.77 ± 0.07^{abcd}	1.12 ± 0.15^{abcdef}	1.83 ± 0.16^{abc}
	B	15.47 ± 0.91^{gh}	0.56 ± 0.05^{ef}	0.07 ± 0.04^{abcd}	0.11 ± 0.05^{abcd}	0.09 ± 0.08^{ab}	0.20 ± 0.12^{abc}	48.28 ± 2.05^{l}	0.66 ± 0.06^{a}	1.26 ± 0.13^{cdefgh}	1.96 ± 0.19^{abcde}
	C	13.51 ± 0.83^{def}	0.53 ± 0.07^{def}	0.10 ± 0.02^{bcdef}	0.20 ± 0.06^{d}	0.26 ± 0.09^{de}	0.46 ± 0.12^{ef}	45.42 ± 2.52^{ijk}	0.80 ± 0.09^{cd}	1.33 ± 0.17^{efgh}	2.17 ± 0.22^{cdefg}
	D	14.26 ± 1.13^{defgh}	0.51 ± 0.05^{def}	0.06 ± 0.03^{abc}	0.12 ± 0.05^{abcd}	0.11 ± 0.05^{abc}	0.23 ± 0.09^{abcd}	48.65 ± 1.68^{l}	0.68 ± 0.04^{ab}	1.41 ± 0.18^{fgh}	2.13 ± 0.27^{bcdef}
	E	13.42 ± 1.16^{def}	0.49 ± 0.08^{cdef}	0.06 ± 0.04^{ab}	0.14 ± 0.07^{abcd}	0.11 ± 0.10^{abc}	0.24 ± 0.16^{abcd}	46.11 ± 1.54^{jkl}	0.79 ± 0.05^{bcd}	1.37 ± 0.19^{efgh}	2.21 ± 0.27^{defg}
	F	13.12 ± 1.37^{de}	0.48 ± 0.07^{cdef}	0.06 ± 0.04^{ab}	0.12 ± 0.08^{abcd}	0.12 ± 0.15^{abcd}	0.24 ± 0.17^{abcd}	48.67 ± 1.60^{l}	0.70 ± 0.03^{abc}	1.53 ± 0.26^{hi}	2.36 ± 0.38^{fg}
	G	11.01 ± 1.16^{ab}	0.38 ± 0.06^{ab}	0.03 ± 0.03^{a}	0.09 ± 0.07^{ab}	0.08 ± 0.09^{a}	0.17 ± 0.14^{a}	47.70 ± 1.75^{kl}	0.78 ± 0.06^{abcd}	1.76 ± 0.24^{i}	2.78 ± 0.41^{h}
Significance level		p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

Means with different superscripts in the same column differ significantly.

dienoic acid content than sampling positions A and B. According to Haüser and Prabucki (1990), subcutaneous fat must contain < 10 % dienoic FAs to qualify as subcutaneous fat of good quality. Subcutaneous fat from none of the dietary treatments or sampling positions could conform to this quality requirement. These relatively high dienoic acid levels measured can be attributed to dietary CLA inclusion, which resulted in increased CLA content in the subcutaneous fat (Table 4.11). Since increased CLA content resulted in fimer BF (Table 4.4), the relevance of dienoic acid content as afat quality parameter may be questioned. The significantly lower dienoic acid content of sampling position G, compared to other sampling positions, implies that subcutaneous fat from the leg area had better fat quality than fat from other sampling positions.

A significant ($p<0.001$) decrease in trienoic FA content was observed with increased dietary CLA content (Table 4.14). No significant differences were observed for the same sampling position, between dietary treatments. Within dietary treatment groups, sampling position G had significantly ($p<0.001$) lower trienoic acid content than all other sampling positions, for all four dietary treatments. Within the 0.5% CLA treatment, subcutaneous fat from sampling positions D, E and F had significantly ($p<0.001$) lower trienoic acid content than sampling position A.

According to Haüser and Prabucki (1990), subcutaneous fat must contain < 1 % trienoic FAs to qualify as subcutaneous fat of good quality. Subcutaneous fat from all dietary treatments and sampling positions conformed to this quality standard. The fact that subcutaneous trienoic acid content decreased with increased dietary CLA level, confirmed that dietary CLA supplementation improved subcutaneous fat quality of pigs. It also demonstrated that sampling position G had significantly ($p<0.001$) better fat quality than all other sampling positions, for all dietary treatments. A significant ($p<0.001$) decrease in tetraenoic FA content was also observed with increased dietary CLA content. No significant differences were observed for the same sampling position, between dietary treatments. Within dietary treatment groups, sampling position G had significantly ($p<0.001$) lower tetraenoic acid content than all other sampling positions, for all four dietary treatments. According to Haüser and Prabucki (1990), subcutaneous fat must contain < 0.5 % tetraenoic acid to qualify as subcutaneous fat of good quality. Subcutaneous fat from all dietary treatments and sampling positions conformed to this quality requirement. The fact that subcutaneous tetraenoic acid content decreased with increased dietary CLA level, again confirmed that dietary CLA supplementation improved subcutaneous fat quality of pigs.

Total pentaenoic acid was significantly ($p<0.001$) influenced by sampling position, but not by dietary treatment, whereas total hexaenoic acid was significantly ($p<0.001$) influenced by dietary treatment, as well as sampling position. No fat quality parameters are available for pentaenoic and hexaenoic acids, as individual FAs. Haüser and Prabucki (1990) proposed a pentaenoic +

hexaenoic FA content of 1% of the total FAs, as the maximum for good fat quality. These two FAs will therefore be considered together.

A significant ($p<0.001$) decrease in penta + hexaenoic FA content was observed, with increased dietary CLA content. No significant differences were observed for the same sampling position between dietary treatments. Within the 0.25% CLA treatment, subcutaneous fat from sampling position G had significantly ($p<0.001$) lower penta + hexaenoic acid content than sampling position F. Within the 0.5% CLA treatment, subcutaneous fat from sampling position F had significantly ($p<0.001$) lower penta + hexaenoic acid content than sampling position C. Within the 1% CLA treatment, subcutaneous fat from sampling position G had significantly ($p<0.001$) lower penta + hexaenoic acid content than sampling position C, while sampling position C had significantly higher penta + hexaenoic acid content than sampling positions B, D, F and G. Subcutaneous fat from all dietary treatments and sampling positions conformed to Haüser and Prabucki's (1990) fat quality requirement of < 1 % penta + hexaenoic acid. The penta + hexaenoic acid content confirmed the findings for the previously mentioned ratios, that dietary CLA supplementation improve subcutaneous fat quality of pigs. It also demonstrates that some sampling positions have significantly better fat quality than other sampling positions.

Although no specific value is attached to C16:0+C18:0 as a fat quality parameter, an increase in this ratio has been associated with fat firmness (Enser, 1984). A statistically significant ($p<0.001$) increase in C16:0+C18:0 content was observed, with increased dietary CLA content. For C16:0+C18:0 content, all sampling positions from the control had significantly ($p<0.001$) lower values than the corresponding sampling positions from all the CLA treatments. All sampling positions from the 025 % CLA treatment had significantly ($p<0.001$) lower C16:0+C18:0 content than the corresponding sampling positions from the 1% CLA treatment. No significant differences were observed for the same sampling position, between the 0.5 and 1% CLA treatments. Within all dietary treatments, sampling position C had significantly ($p<0.001$) lower C16:0+C18:0 content than sampling positions B and D. The increase in C16:0+C18:0 contents, with increase in dietary CLA level, imply that CLA supplementation will result in firmer subcutaneous fat, which is advantageous from a meat technology point of view (Enser, 1984).

Another FA ratio that has been associated with fat firmness is C16:1+C18:1*c*9/C16:0+C18:0 (Lea *et al*., 1970). A significant ($p<0.001$) increase in C16:1+C18:1*c*9/C16:0+C18:0 ratio was observed with increased dietary CLA content. For the C16:1+C18:1*c*9/C16:0+C18:0 ratio, all sampling positions from the control had a significantly ($p<0.001$) higher ratio than the corresponding sampling positions of all the CLA treatments. All sampling positions from the 0.25% CLA treatment had significantly ($p<0.001$) lower C16:1+C18:1*c*9/C16:0+C18:0 content than the corresponding sampling positions from the 1% CLA treatment. No significant differences were

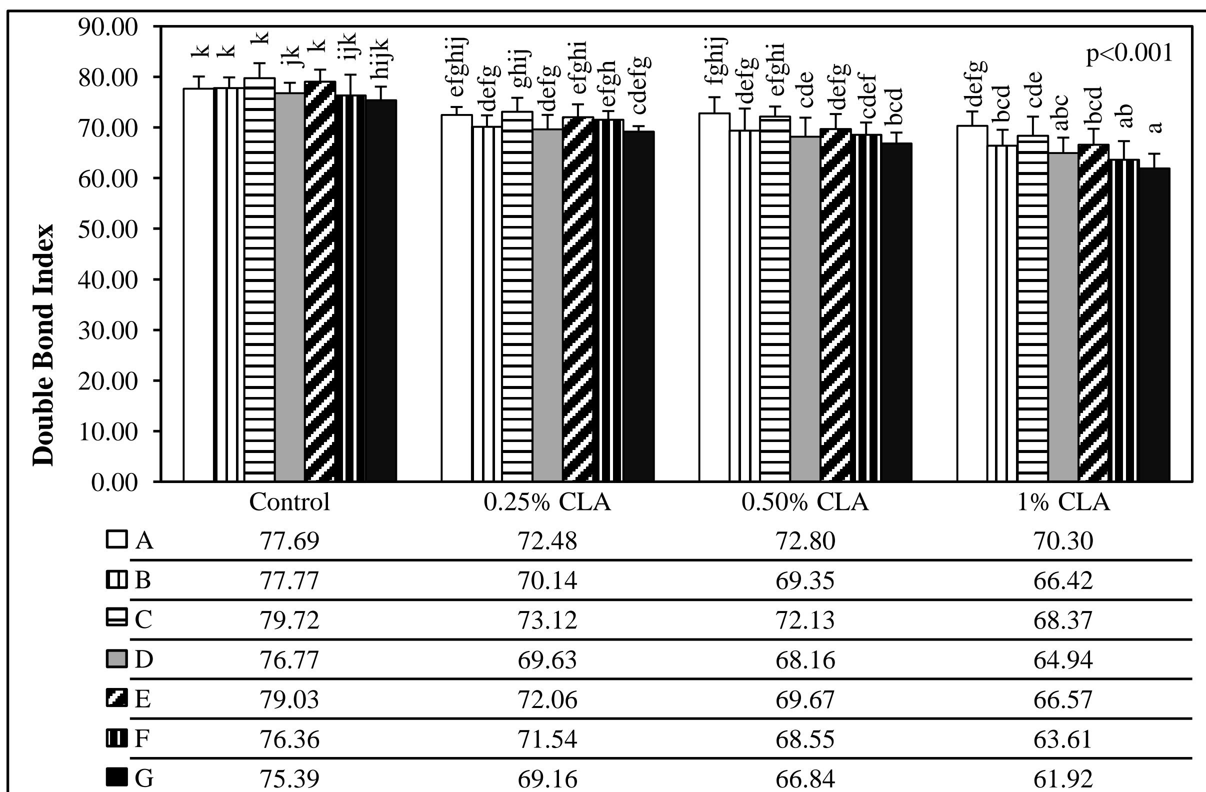
observed for the same sampling position between the 0.25 and 0.5% CLA treatments. Sampling positions C, D and G from the 0.5% CLA treatment had a significantly ($p<0.001$) higher C16:1+C18:1*c*9/C16:0+C18:0 ratio than the corresponding sampling positions for the 1% CLA treatment. Statistically significant ($p<0.001$) sampling position differences were also observed within dietary treatment groups. Sampling position C and G generally had higher C16:1+C18:1*c*9/C16:0+C18:0 ratios than the other sampling positions. The lower C16:1+C18:1*c*9/ C16:0+C18:0 ratios of the CLA supplemented pigs also indicate improved subcutaneous fat quality.

The C16:0/C18:2 ratio has also been associated with fat firmness (Enser, 1984). A significant ($p<0.001$) increase in C16:0/C18:2 ratio was observed with increased dietary CLA content. This means that subcutaneous fat firmness increase with increased dietary CLA content. Sampling positions E and G from the control had a significantly ($p<0.001$) lower C16:0/C18:2 ratio than sampling position E and G from the 0.5 and 1% CLA treatments. Within the control, sampling positions F and G had significantly ($p<0.001$) higher C16:0/C18:2 content than sampling positions A, B, C, D and E. Within every CLA treatment, sampling position G had significantly ($p<0.001$) higher C16:0/C18:2 ratios than the other sampling positions. This sampling position effect implies that subcutaneous fat from sampling positions A and G had better fat quality than that from the other sampling positions.

Enser, Dransfield, Jolley, Jones and Leedham (1984), and Honkavaara (1989) concluded that the C18:0/C:18:2 ratio is the best measure of fat quality. With C18:0/C18:2 ratios of > 1.2 and < 1.2, the sensory consistency of fresh subcutaneous fat was firm and soft, respectively (Honkavaara, 1989). Enser *et al*. (1984) proposed a C18:0/C18:2 ratio of > 1.47 for good subcutaneous fat quality. A significant ($p<0.001$) increase in C18:0/C18:2 ratio was observed in the subcutaneous fat from the pigs that received CLA. For the C18:0/C18:2 ratio, sampling positions B, C, D, E, F and G from the control had significantly ($p<0.001$) lower C18:0/C18:2 ratios than the corresponding sampling positions from the 1% CLA treatment. Sampling positions E and G from the control had significantly ($p<0.001$) lower C18:0/C18:2 ratios than the corresponding sampling positions from the 0.5% CLA treatment. No significant differences in the C18:0/C18:2 ratio were observed between sampling positions, within the control and 0.25% CLA treatment. Within the 0.5% CLA treatment, sampling position G had a significantly ($p<0.001$) higher C18:0/C18:2 ratio than sampling positions A and C. Within the 1% CLA treatment, sampling position G had a significantly ($p<0.001$) higher C18:0/C18:2 ratio than sampling positions A-F. Within the control, no sampling positions conformed to Honkavaara's (1989) minimum proposed C18:0/C18:2 ratio of 1.2, or Enser *et al*.'s (1984) minimum proposed value of 1.47. In the 0.25% CLA treatment, sampling positions B, D, F and G conformed to the minimum value of 1.2, whereas no group's subcutaneous fat could conform to the minimum value of 1.47. In the 0.5% CLA treatment,

sampling positions B, D, E, F and G also conformed to the minimum value of 1.2, while sampling position G also conformed to the minimum value of 1.47 for C18:0/C18:2. In the 1% CLA treatment, sampling positions B-G conformed to the minimum value of 1.2 and sampling positions F and G conformed to the minimum value of 1.47. From these findings it is clear how dietary CLA supplementation improved subcutaneous fat quality.

Despite IV, the DBI is probably the most commonly used fat quality parameter. For good quality and consistency, subcutaneous fat should have a DBI of < 80 (Haüser & Prabucki, 1990; Prabucki, 1991). Figure 4.2 is a representation of the DBI contents of the subcutaneous fat, of the gilts from the four dietary treatments.



	Control	0.25% CLA	0.50% CLA	1% CLA
A	77.69	72.48	72.80	70.30
B	77.77	70.14	69.35	66.42
C	79.72	73.12	72.13	68.37
D	76.77	69.63	68.16	64.94
E	79.03	72.06	69.67	66.57
F	76.36	71.54	68.55	63.61
G	75.39	69.16	66.84	61.92

Means with different superscripts differ significantly

Figure 4.2: Double bond index values of the subcutaneous fat from the gilts fed the four dietary treatments.

A significant (p<0.001) decrease in DBI was observed, with increased dietary CLA content. This means that subcutaneous fat quality increased with increased dietary CLA content. All sampling positions from the control had significantly (p<0.001) higher DBI's than the corresponding sampling positions in all the CLA treatments. No significant differences in DBI were observed between corresponding sampling positions for the 0.25 and 0.5% CLA treatments. Sampling positions C, D, E, F and G from the 1% CLA treatment had significantly (p<0.001) lower DBI's than the corresponding positions from the 0.25% CLA treatment. Sampling positions F and

G from the 1% CLA treatment had significantly ($p<0.001$) lower DBI's than the same positions from the 0.5% CLA treatment. No significant sampling position differences were observed for DBI, within the control and 0.25% CLA treatment. The best example of a sampling position difference, within a dietary treatment, is position G within the 1 % CLA treatment group, with a DBI value of 61.92, which differs significantly ($p<0.001$) from sampling positions A (70.30), B (66.42), C (68.37), D (64.94) and E (66.57).

Although all dietary treatments and sampling positions conformed to the DBI maximum of 80 proposed by Prabucki (1991), it is clear that all CLA treatment groups had better DBI values than the control group and that there is a linear improvement in DBI value, with increased dietary CLA level.

Significant ($p<0.05$) interactions for dietary treatment X sampling position were reported for C16:0+C18:0, C16:1+C18:1/C16:0+C18:0, C18:0/C18:2 and C16:0/C18:2 (Table 4.5). A significant ($p<0.05$) interaction between dietary treatment X sampling position was also observed for C18:0 (Table 4.5), which probably contributed to the significant ($p<0.05$) interactions for all the above mentioned C18:0 containing FA ratios. A significant (at least $p<0.01$) dietary treatment X sampling position interaction was observed for both CLA isomers, which probably contributed to the significant ($p<0.05$) dietary treatment X sampling position interaction for the C18:0/C18:2 and C16:0/C18:2 ratios.

Differential scanning calorimetry of subcutaneous fat

In general, triacylglycerides do not only differ in FA composition, but also in their stereospecific arrangement on the three s*n*-positions of the glycerol (Christie, 1983). The distribution in animal fats is also different from that of vegetable fats. Due to the complexity of the chemical methods used to determine this arrangement, fats of only a few animals have been studied. While the overall FA composition amongst mammalian fats may differ, the stereospecific arrangement shows common features. For pig fat, most of the UFAs are located in the s*n*-1 and s*n*-3 positions (Table 4.15). While the stereospecific distribution sets some limits to the amount of different possible molecular triaglyceride species that can be formed, a large number is still possible.

Simple fats with single or limited types of FAs regularly form β crystals, which is the most stable form, or they may be transformed to the less stable β' and least stable α crystals (Mortensen, 1983; Hagemann, 1988). In complex fats, such as animal fats, the combination of different triglyceride molecule species lead to complicated crystallization patterns. Polymorphism is observed during crystallization, i.e. more than one crystal type is formed due to different patterns of molecular packaging in the crystal.

Table 4.15: Positional distribution of major fatty acids in pig fat (mole %) (Breckenridge, 1978).

***sn*-position**	**C14:0**	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2**
1	0.90	9.50	2.40	29.50	51.30	6.40
2	4.10	72.30	4.80	2.10	13.40	3.30
3	0.00	0.40	1.50	7.40	72.70	18.20

The crystallization and melting of fat from pig lard has been studied to some extent (Svenstrup *et al*., 2005; Sasaki *et al*., 2006). Due to the large number of triglyceride molecule species present, the crystallization is very complex, resulting in the formation of compound crystals. It is not possible to relate specific molecules to the formation of these crystals, however, six major crystal types have been identified in pig fats. The melting points observed by DSC were found to be in agreement with results from a study of particle size distribution in lard fat, which was prepared by fractional crystallization at different temperatures (Wang & Lin, 1995).

As mentioned earlier, pig fat composition may be altered with nutritional intake, which may affect the textural propeties (D'Souza & Mullan, 2002; Wiegand *et al*., 2002; Corino *et al*., 2003). Differences in FA composition of pig fat also affect the crystallization properties of the fat, as was shown for fat from lard and leaf fat (Svenstrup *et al*., 2005). The effect of nutritional intake on crystallization properties, irrespective of the location in the pig carcass, has not yet been determined.

The FA compositions of the subcutaneous fat, from the different sampling positions, are given in Table 4.8-4.14. From Table 4.11 it can be seen that an increase of CLA (*cis* 9,*trans* 11 and *trans* 10,*cis* 2) in the diet, resulted in a significant ($p<0.001$), at least four fold, increase of these FAs in both sampling positions B (BF) and F (belly fat), with increased dietary CLA level. The *cis* 9,*trans* 11 content typically increased from 0.25% in the 0.25% CLA treatment group, to 1.25% in the 1% CLA treatment group. The *trans* 10,*cis* 2 content typically increased from 0.10% in the 0.25% CLA treatment group, to 0.6% in the 1% CLA treatment group.

The C14:0 content increased from ± 1.30% in the control group, to ± 2.35% in the 1% CLA treatment group (Table 4.8). Similarly, the C16:0 content increased from ± 24% in the control group to ± 30% in the 1% CLA treatment group, whereas the C18:0 content increased from ± 12% in the control group to ± 19% in the 1% CLA treatment group (Table 4.8). This resulted in a netto increase of ± 12% in SFAs. Some UFA contents also increased. The C16:1 content increased from ± 2% in the control group to ± 2.4% in the 1% CLA treatment, while the C18:1t9 content increased from ± 0.03% in the control group to ± 0.15% in the 1% CLA treatment group, for both sampling positions B and F (Table 4.9). In contrast, the content of C18:1*c*7 decreased from ± 2.6% in the control group to ± 2% in the 1% CLA treatment group, for both sampling position B and F (Table 4.9). Also, the content of C18:1*c*9 decreased from ± 40% in the control group to ± 30% in the 1%

CLA treatment group (Table 4.9). This resulted in an approximate 12% netto decrease in UFAs, similar to the increase in the SFAs mentioned earlier. Similarly, the C18:0/C18:2 ratio typically increased from 1 in the control group, to 1.2 in the 0.25% CLA treatment group, to 1.4 in the 1% CLA treatment group, for both sampling position B and F (Table 4.14).

The setting and melting thermograms of the pigs' subcutaneous fat, of sampling positions B (BF) and F (bellyfat), are shown in Figures 4.3 and 4.4. The setting isotherms of all the fats displayed an exotherm between -20 and -30°C, with a peak at ca. -22°C. This enthalpy (dH) was large for the controls, (dH ca. 30 W/g), smaller for the fats of the 0.25 and 0.5% CLA fed pigs (dH ca. 20-27 W/g), and very small for the 1% CLA fed pigs (dH ca. 15 W/g). In general, the difference in dH of this - 22°C endotherm was only significant between the fat from the 1% CLA fed pigs and the other three treatments (Table 4.16). In specific, the enthalpy of the BF from the control pigs (dH ca. 33 W/g) was significantly higher than that of the fat from the CLA fed pigs (Table 4.16). The setting isotherms of fats from the control pigs showed an exotherm at ca. 10°C, with an onset of ca. 13°C and a dH of ca. 3W/g (Figures 4.3 and 4.4). In specific, upon further cooling of the control fat, this was followed by a large exotherm at ca. 5°C, between 9 and 4°C, of ca. 32 W/g (Figures 4.3 and 4.4). The setting of the fats from pigs fed 0.25 and 0.5% CLA commenced at ca. 20°C, with a peak at ca. 17°C and dH of ca. 7 W/g (Figures 4.3 and 4.4). A shift was therefore noted from a 10°C exotherm to a 17°C exotherm. Upon cooling, this was followed by a large exotherm between 14 and -8°C, with a peak at ca. 8°C and dH ca. 13 W/g. Setting of the fats from pigs fed 1% CLA commenced at ca. 26°C, with a peak at ca. 20°C and dH ca. 11W/g. A shift was therefore noted, from a 17°C exotherm to a 20°C exotherm. Upon cooling, this was followed by a large exotherm between 16 and -9°C, with a peak at ca. 10°C and dH ca. 36W/g. Again another shift was noted, from an 8°C exotherm to a 10°C exotherm.

For statistical calculations, the onset and final setting temperatures, and the enthalpy of all the peaks, were taken into account (Table 4.16). However, all exotherms with peaks between 8 and 12°C were regarded as one exotherm and no distinction was made between exotherms that showed a small shift in temperature. Melting of fat from the control pigs commenced at ca. -18°C to 11°C, with an endotherm peak at ca. -2°C. The onset of melting of some fats from CLA fed pigs commenced at temperatures up to -12°C, with peaks at between 0 and -2°C. The dH of the 0°C endotherm was large for the fat from the control and 0.25% CLA fed pigs (dH ca. 13-21 W/g), and smaller for the fats from the 0.5 and 1% CLA fed pigs (dH 8-12 W/g). It was only significant ($p<0.001$) between the fat from the 1% CLA fed pigs and the controls, as well as the BF of the 0.25% CLA pigs (Table 4.16). Upon heating, a large endotherm followed between 11 and 38°C, which contained small peaks between 11 and 25°C, and a large peak at ca. 30°C. Also, upon heating the fats from the pigs fed 0.25 and 0.5% CLA, similar endotherms were shown, however,

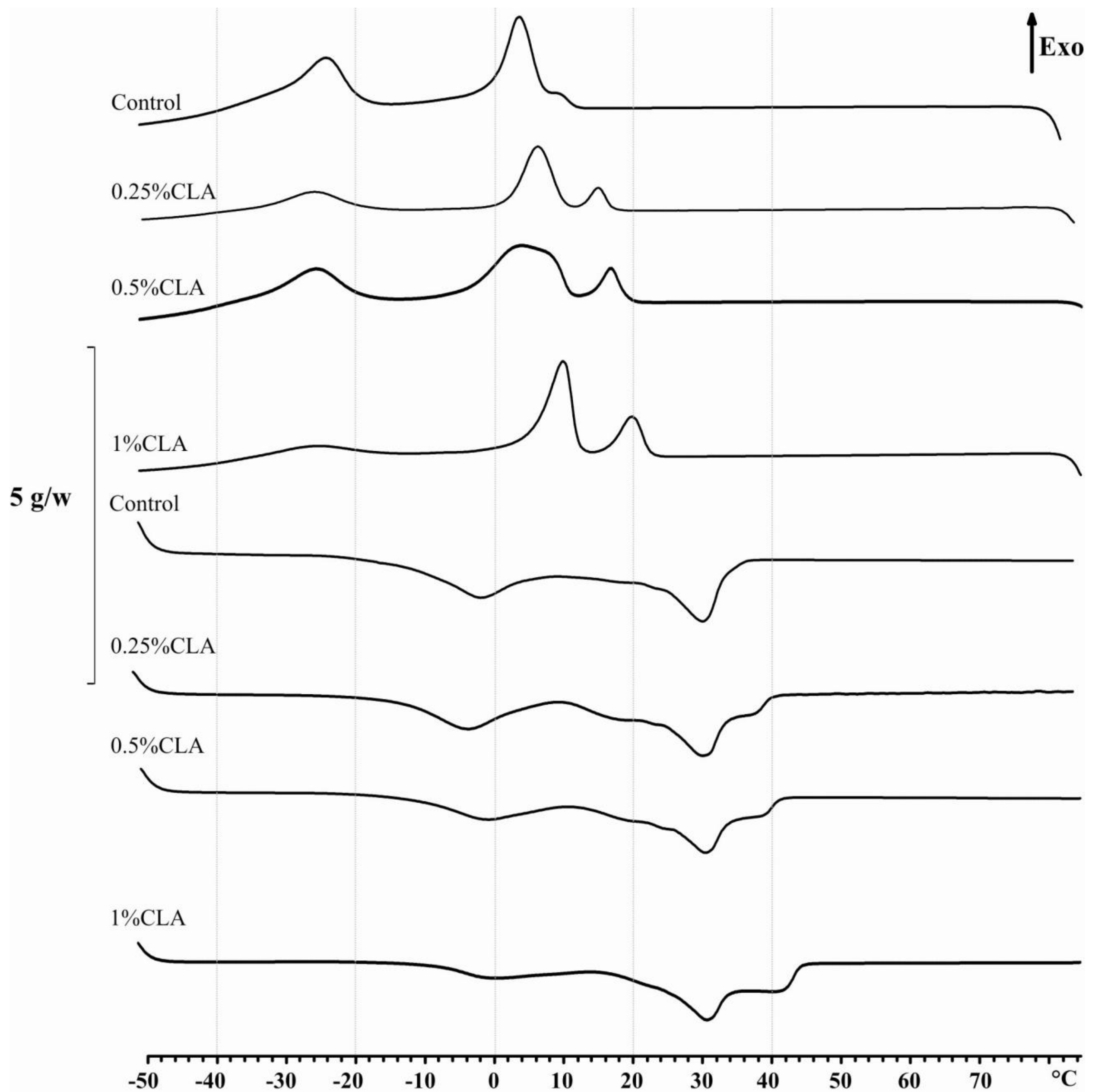


Figure 4.3: The setting (top) and melting (bottom) thermograms of the BF from gilts fed four different concentrations of CLA.

with higher enthalpies between 11 and 25°C, and the large peak at ca. 30°C. Additional endotherms were seen between ca. 36 and 42°C, with a peak at ca. 38°C. The same was observed for the fat from the 1% CLA fed pigs, but the high temperature endotherm was extended to a final melting temperature of 45°, and peaked at 40°C. Because the enthalpies between ca. 11 and 25°C, and the 30° C peak were not clearly separated, they were regarded as one peak for statistical calculations. The dH of this enthalpy was ca. -28 W/g for the control fats, significantly ($p<0.001$) differing from the ca. -36 W/g of the fat from all CLA fed pigs. For the statistical analysis, endotherms

commencing above 35°C were regarded as similar, irrespective of the fact that the endotherms of the 1% CLA fed pigs extended to higher melting temperatures.

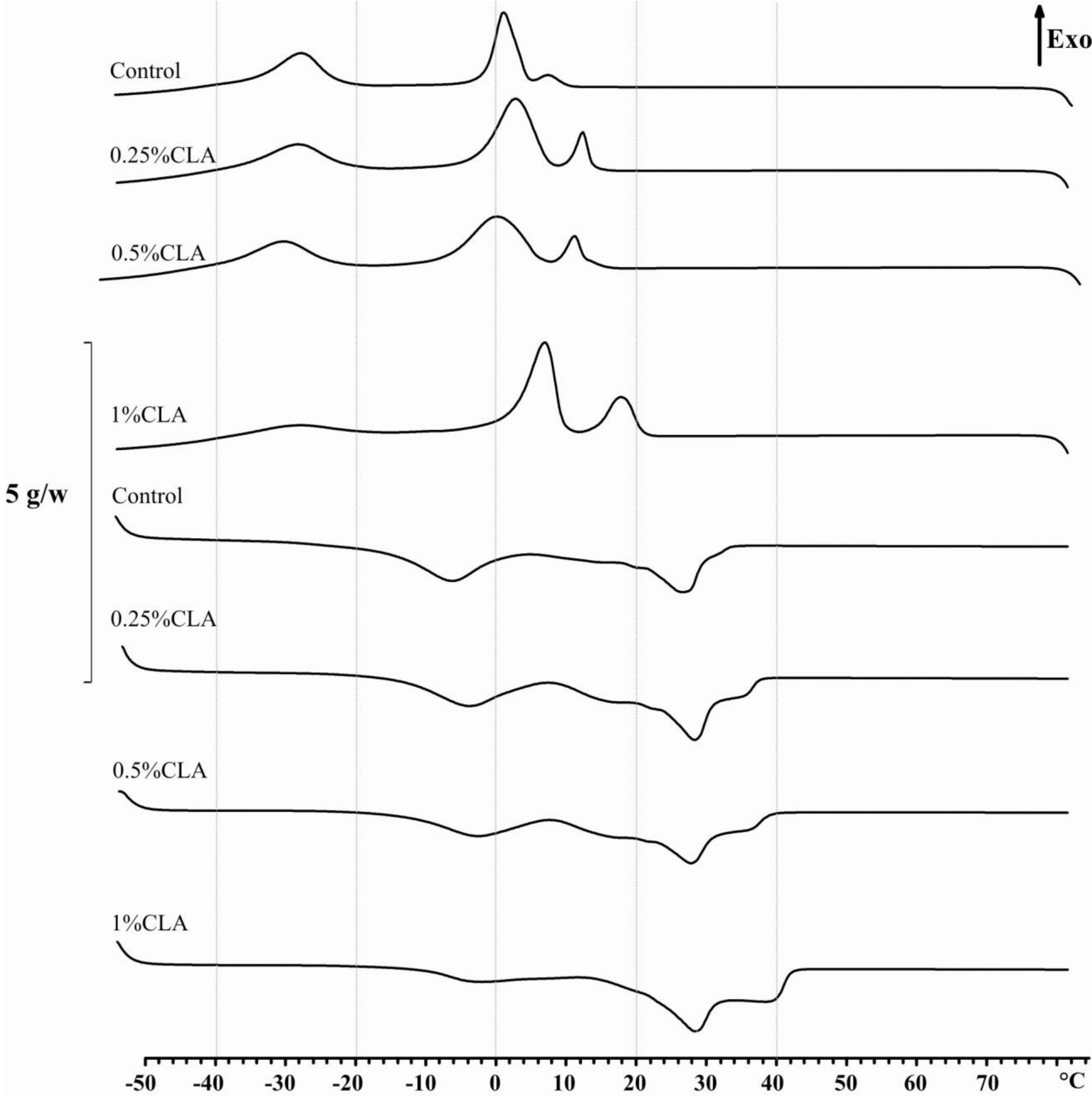


Figure 4.4: The setting (top) and melting (bottom) thermograms of the belly fat from gilts fed four different concentrations of CLA.

Small, but significant ($p<0.05$) differences were noted for the endotherms of the fats extracted from the BF and the belly fat. Specifically, there was a high dH of the -22°C setting exotherm of the BF from the control and a low -22°C setting exotherm of the belly fat from the 1% CLA fed pigs (Table 4.16). The same was found for the melting isotherm at 0°C for the same fat samples. In turn, the belly fat of the 1% CLA fed pigs showed the highest dH of the 40°C melting

Table 4.16: Setting and melting properties of backfat and belly fat of gilts from the four dietary treatments.

Treatment	**Control**	**Control**	**0.25% CLA**	**0.25% CLA**	**0.5% CLA**	**0.5% CLA**	**1% CLA**	**1% CLA**	**Sign Level**
Position	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	
	Setting								
T max	13.33 ± 0.88[a]	13.96 ± 1.64[ab]	18.34 ± 3.20[c]	16.46 ± 0.31[abc]	19.16 ± 3.85[c]	17.27 ± 0.62[bc]	23.60 ± 1.19[d]	26.23 ± 1.14[d]	p<0.001
ca 20°C dH	ND	ND	2.46 ± 3.87[a]	ND	3.23 ± 4.99[a]	ND	10.22 ± 1.79[b]	11.06 ± 1.44[b]	p<0.001
ca 17°C dH	ND	ND	4.41 ± 3.57	7.17 ± 1.46	4.42 ± 3.44	7.31 ± 1.44	ND	ND	NS
ca 10°C dH	2.80 ± 0.72[a]	3.99 ± 0.88[a]	35.50 ± 2.55[b]	36.49 ± 3.25[b]	34.56 ± 2.13[b]	33.53 ± 2.53[b]	36.59 ± 1.98[b]	36.72 ± 3.26[b]	p<0.001
ca 5°C dH	30.85 ± 2.35	34.01 ± 5.47	ND	ND	ND	ND	ND	ND	NS
ca -22°C dH	33.07 ± 1.74[e]	27.48 ± 2.64[cde]	27.92 ± 6.81[de]	22.10 ± 4.91[bcd]	21.82 ± 6.00[bcd]	19.80 ± 2.14[abc]	18.50 ± 2.83[ab]	12.95 ± 3.61[a]	p<0.001
T min	-38.90 ± 0.66[ab]	-37.69 ± 2.26[b]	-42.33 ± 1.60[a]	-37.71 ± 3.16[b]	-41.58 ± 2.31[a]	-37.10 ± 0.80[b]	-40.42 ± 1.56[ab]	-37.88 ± 2.25[b]	p<0.001
	Melting								
ca 0°C dH	-19.96 ± 2.32[a]	-17.84 ± 2.14[ab]	-20.71 ± 8.19[a]	-17.70 ± 2.91[ab]	-13.06 ± 6.11[abc]	-15.67 ± 1.40[abc]	-11.62 ± 2.60[bc]	-8.26 ± 3.99[c]	p<0.001
T min	-18.80 ± 2.10[a]	-16.17 ± 3.74[ab]	-16.95 ± 2.46[ab]	-13.47 ± 4.02[ab]	-16.19 ± 3.44[ab]	-14.33 ± 1.47[ab]	-14.25 ± 2.14[ab]	-12.26 ± 3.45[b]	p<0.05
ca 30°C dH	-28.34 ± 3.82[b]	-30.09 ± 3.96[b]	-42.21 ± 5.27[a]	-46.03 ± 2.40[a]	-43.08 ± 3.12[a]	-43.24 ± 4.05[a]	-43.14 ± 4.55[a]	-39.63 ± 1.83[a]	p<0.001
ca 40°C dH	ND	ND	-7.06 ± 2.10[c]	-6.97 ± 1.00[c]	-8.30 ± 2.36[bc]	-7.66 ± 0.99[bc]	-11.07 ± 3.53[ab]	-12.76 ± 1.85[a]	p<0.001
T max	37.00 ± 1.38[a]	37.83 ± 1.28[a]	42.75 ± 0.88[b]	42.25 ± 0.82[b]	43.67 ± 1.47[bc]	42.80 ± 0.75[b]	45.00 ± 1.38[c]	45.13 ± 0.75[c]	p<0.001

B = BF; F = Bellyfat
Means with different superscripts in the same row differ significantly
ND = Not Detected; NS = Not Significant;

isotherm. It should furthermore be noted that two samples of BF, from each of the 0.25 and 0.5% CLA fed pigs, displayed setting and melting isotherms, characteristic of the fats from the 1% CLA-fed pigs: high temperature setting, with exotherm at 20°C; low enthalpy for the -22°C exotherm; high final melting temperature, with endotherm at 40°C; and low enthalpy for the 0°C endotherm.

According to this stereospecific distribution of the FAs (Table 4.15), it could be mathematically derived that the most prominent molecular species, found in pig fat, would be C18-C16-C18:1 (SPO), followed by C18:1-C16-C18:1 (OPO), with some C18:1-C18:1-C18:1 (OOO) molecules. The melting temperature, for SPO α-crystals, has been determined as either 10 or 24°C, for the β'-crystals, as ranging from 37-40°C and for the β-crystals, as 40-41°C (Hagemann, 1988). For OPO, the respective melting temperatures are -32, -12 and 5°C. Since pig fat does not consist of pure triacylglycerides, but rather a mixture, it could be possible that the mentioned crystal types might not be formed. These fat molecules could rather form complex crystals, with molecules of other FA acid compositions. Nevertheless, the melting isotherms observed for the fats under study, showed that the low melting temperature, at ca. 0°C, may contain the β' and β-type crystals of the OPO. The high isotherms between 20 and 35°C, may indicate the presence of the α and β' crystals of SPO, and the isotherm at 40°C, the β-crystals. The presence of a high proportion of SPO in lard was confirmed by chromatography and DSC studies, by Al-Rashood, Abou-Shoaban, Abdel-Moety and Rauf (1996). It was in the high melting isotherm between 35 and 45°C, of the fat from the 1% CLA fed pigs, that the β', as well as the β-crystals of the latter, seemed to be obvious. This was in agreement with this fat containing the highest amounts of C16:0 and C18:0, and the lowest amounts of UFAs (Tables 4.8 and 4.13). In the fat from the control pigs, the OPO could be in higher abundance, favouring crystals of a lower melting point, hence the larger enthalpy of the 0°C peak. The same molecules could also be responsible for the higher onset of the setting temperature. The SPO could be responsible for the exotherm at 20°C, while at a lower content of C16:0 and C18:0, these triacylglycerides could engage in crystal formation with other molecules, with an onset setting peak at 17°C.

In general, there was hardly any significant ($p<0.001$) difference between the melting isotherms, between the fat from the 0.25 and 0.5% CLA fed pigs, although the FA composition was different. The reason might be that a certain threshold of FA composition has to be passed for the triacylglycerides to clump together in a different crystal structure. The FA composition for the control, the 0.25 and 0.5% CLA fed pigs' fat, and the 1% CLA fed pigs' fat would indicate three threshold levels for such changes in crystal structure.

The two BF samples of each of the 0.25 and 0.5% CLA fed pigs, that displayed setting and melting isotherms similar to the fats from the 1% CLA fed pigs (Table 4.16), deserve further discussion. While, in general, the shift to the higher setting and melting of the fats could be

ascribed to an increase in the SFAs C16:0 and C18:0, and a decrease in the UFAs, the isotherms of these four fats could not be explained by the FA composition. The FA composition of neither was comparable with that of the fat from the 1% CLA fed pigs. The only explanation is that a large enough content of SPO triacylglycerides were present to favour separate crystallization into β-crystals. This would mean that the triacylglyceride synthesis in the BF differed from that in the belly fat, so that distinct amounts of triacylglycerides were synthesized. This phenomenon could not be verified with the data currently available and would merit a separate investigation.

These results, therefore, showed that the physical hardness, observed for the different fats (Table 4.4), might also be ascribed to the melting properties of the fats. It might be argued that the crystallization of the extracted fat would be different from that in the fat tissue. However, the DSC carried out on the fat tissue of different origin in a carcass, showed isotherms with similar peaks as the ones under study, with differences in the high temperature melting above 40°C, concurrent with physical hardness. Harder adipose fat showed a high melting peak, compared to the softer subcutaneous fat (Svenstrup *et al*., 2005; Sasaki *et al*., 2006).

The C18:0/C18:2 ratio, FAs that demonstrated a significant change with increased dietary CLA levels, and the data from Table 4.16 from position B (BF) from the subcutaneous fat and bellyfat, were subjected to PCA, the results of which are shown in Figure 4.5 and 4.6. For the BF, dimension 1, explaining 79.62% of the variables, divides the biplot into two sections, with the control treatment situated to the right of the plot, and the 0.5 and 1%CLA treatment groups to the left. Incidentally, the 0.25% CLA treatment group lies exactly on the axis dividing the biplot. Dimension 2, explaining 18.22% of the variable, divides the biplot into a lower and bottom half, assigning setting and melting properties, FAs and C18:0/C18:2 ratios, to the different treatments. According to this division, the 0.25% CLA group is clustered with the 0.5% CLA treatment group. These results confirmed that the control group had the highest C18:1*c*9 and C18:1*c*7 contents, the highest enthalpy values for melting isotherms at ca. 30ºC dH and setting isotherms at ca. -22ºC dH. The 0.25 and 0.5% CLA treatment groups were clustered together, showing higher contents for C18:0, C16:0 and C18:0/C18:2, as well as significantly higher (than the control group) enthalpy values for setting isotherms at ca. 10ºC dH and melting T max, and an increase in fat hardness. For the 1% CLA treatment group, the C14:0, C18:1*t*9, *cis* 9,*trans* 11 and *trans* 10,*cis* 2 contents were the highest, and setting T max, melting T min, enthalpy values of melting isotherms at ca. 0ºC dH and setting isotherms at ca. 20ºC dH values were the highest. The PCA shows that the fat properties of the BF from the control group, differ substantially from the BF of the CLA treatment groups. Furthermore, the properties of the 0.25% CLA treatment group were not as far removed from the 0.5 and 1% CLA treatment groups, as from the control group.

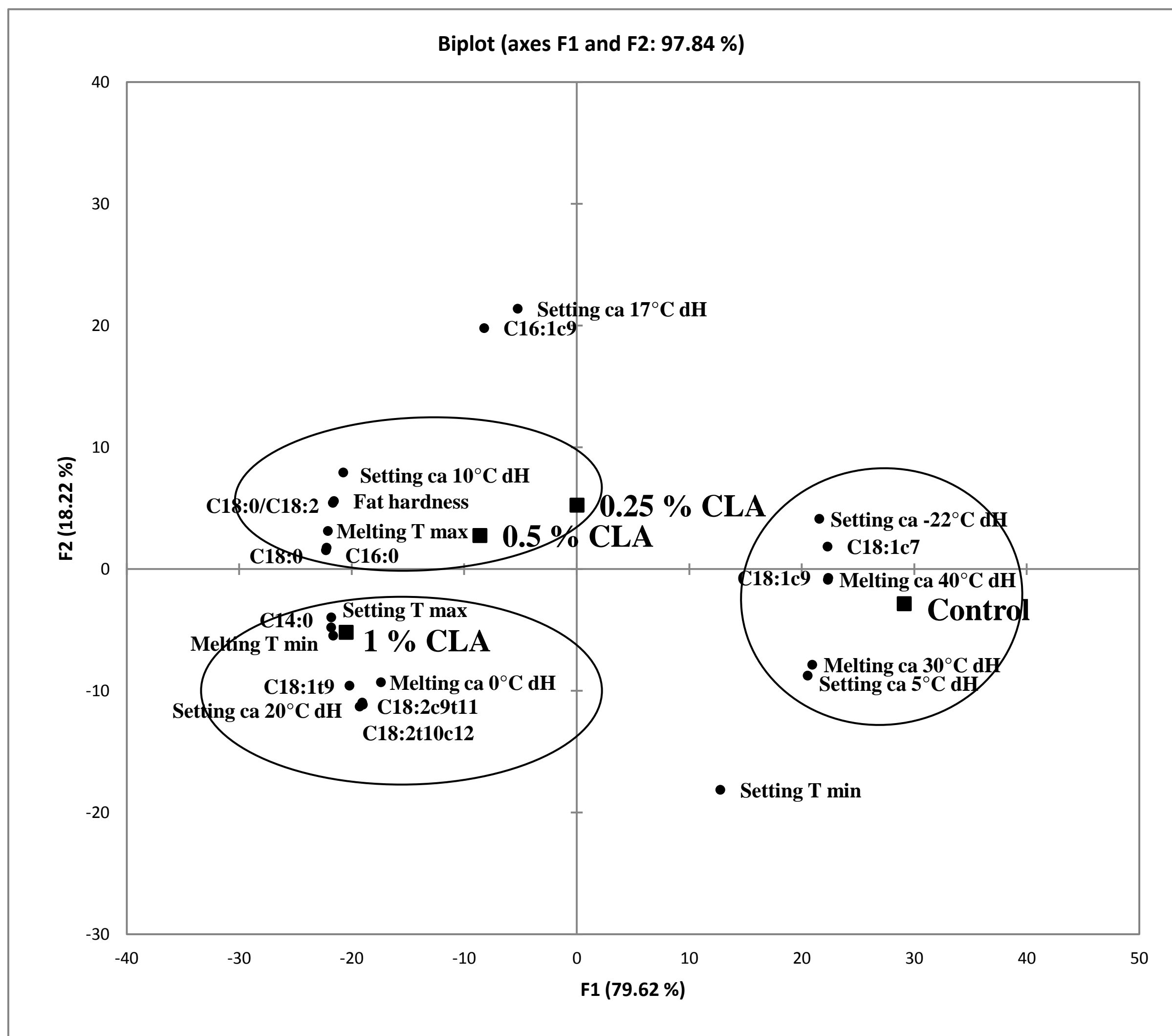


Figure 4.5: Principal Component Analysis of the characteristics of backfat (position B) of gilts from the four dietary treatments.

The BF from the four treatments may be distinguished by PCA, since specific patterns can be recognized, based on the lipid composition, and setting and melting properties. Figure 4.6 is the PCA for the belly fat (position F) of gilts, from the four dietary treatments. Dimension 1, explaining 75.15 % of the variables, again divides the biplot into two sections, with the control and 0.25% CLA treatments situated to the right of the plot, and the 0.5 and 1% CLA treatment groups to the left. For the belly fat, the characteristics of the 0.25% CLA treatment, moved away from the vertical axis, where it was situated for the BF (Figure 4.5). However, this move has placed it in an area without specific characteristics (Figure 4.6). Dimension 2, explaining 20.40% of the variables, divides the biplot into a lower and bottom half, assigning setting and melting properties, FA's and C18:0/C18:2 ratios, to the different treatments. For the control group, C18:1*c*9 content was the highest, and enthalpy values for melting isotherms at ca. 30ºC dH, melting

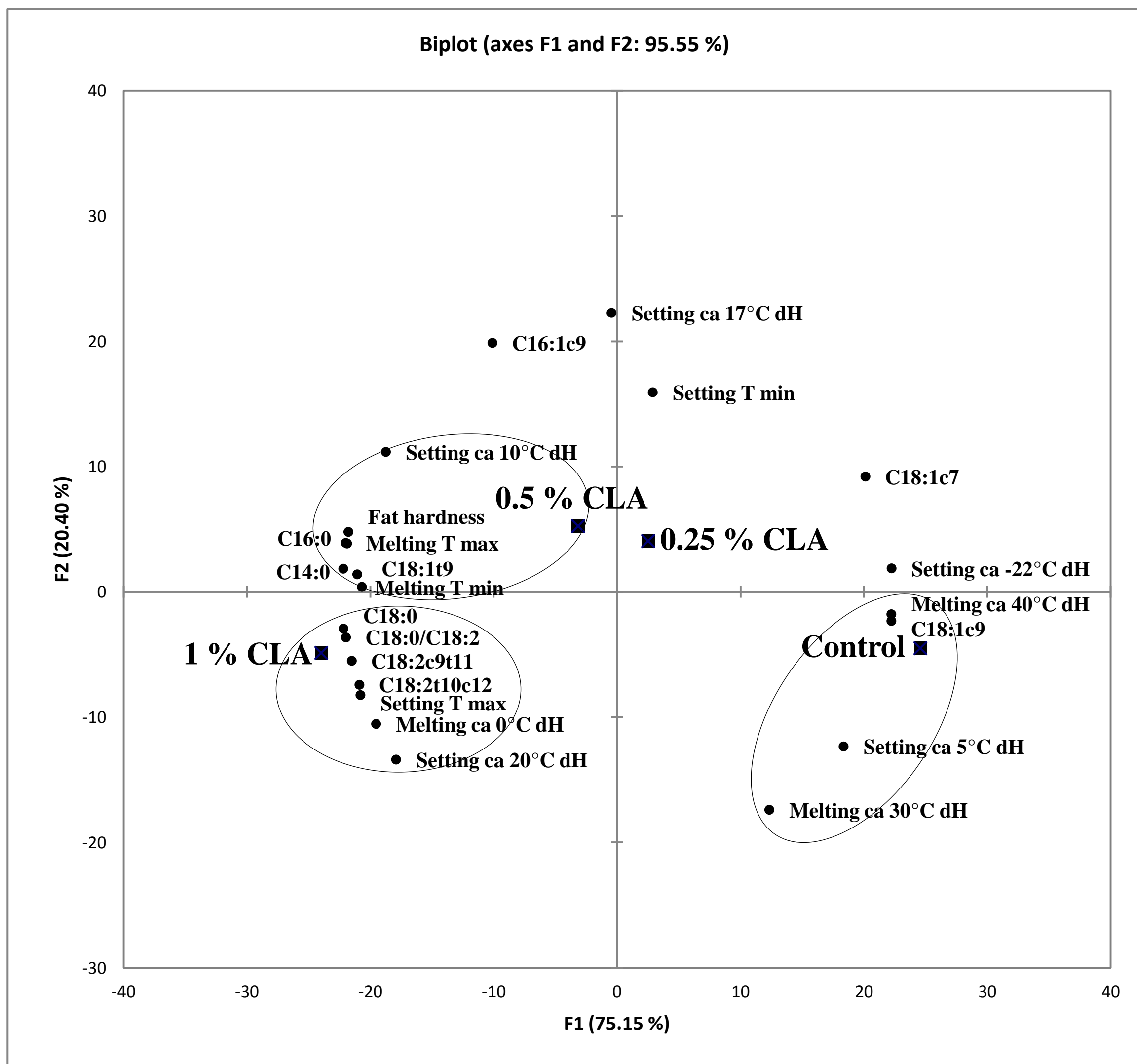


Figure 4.6: Principal Component Analysis of the characteristics of bellyfat (position F) of gilts from the four dietary treatments.

isotherms at ca. 40ºC dH and setting isotherms at ca. 5ºC dH were also high. The 0.5% CLA treatment group showed higher contents for C14:0, C16:0 and C18:1*t*9, as well as significantly higher (than the control group) enthalpy values for setting isotherms at ca. 10ºC dH, melting T max and melting T min, as well as increase in fat hardness. The 1% CLA group had the highest C18:0, C18:0/C18:2, *cis* 9,*trans* 11and *trans* 10,*cis* 2 contents, as well as the highest values for setting T max, enthalpy of melting isotherms at ca. 0ºC dH and setting isotherms at ca. 20ºC dH. The PCA shows that the fat properties of the bellyfat from the control group differ substantially from the bellyfat of the CLA treatment groups. Furthermore, the properties of the 0.5% CLA treatment group were not as far removed from the 1% CLA treatment groups as from the control group. No specific characteristics could be assigned to the 0.25% CLA treatment group.

Clear distinctions could be made from the two biplots (Figures 4.5 and 4.6). The BF control and belly fat control showed more or less the same results for the same characteristics, namely high C18:1*c*9 content and high enthalpy values for melting isotherms at ca. 30ºC dH, melting isotherms at ca. 40ºC dH and setting isotherms at ca. 5ºC dH. There were however, no clear results for the 0.25% CLA treatment, for both the BF and belly fat samples. For the BF, the 0.25% CLA group's characteristics were clustered together with those of the 0.5% CLA treatment. For the belly fat, no clear characteristics could be identified from the PCA. At the higher CLA concentrations, very specific characteristics were associated with specific positions (belly fat versus BF). For the 0.5% CLA treatment, the FAs composition of the BF sample was not the same as that of the belly fat sample. The only characteristics that were shared by both the BF and belly fat samples, were setting isotherms at ca. 10ºC dH, melting T max and increase in fat hardness. Looking at the FA composition for the 1% CLA treatment, only the high *cis* 9,*trans* 11 and *trans* 10,*cis* 2 contents were the same for both fat samples. Again, the high setting T max, melting isotherms at ca. 0ºC dH and setting isotherms at ca. 20ºC dH values were characteristics similar to both fat samples.

Physical and chemical properties of intramuscular fat

Intramuscular fat samples were taken from five muscles in the pig: *M. semimembranosus* (inside) (SM); *M. longissimus thoracis* (LT); *M. biceps femoris* (BF); *M. triceps brachi* (TB); and *M. supra spinatus* (SS) (Figure 3.2). These muscles were chosen in order to cover a broad range of skeletal muscle types and oxidative patterns (Laborde, Talmant, & Monin, 1985; Flores, Alasnier, Aristoy, Navarro, Gandemer, & Toldra, 1996) including: SM (Aristoy & Toldrá, 1998) and LT as glycolytic; TB and SS (Hambrecht, Eissen, Newman, & Smits, 2005) as oxidative; and the BF as intermediate. Oxidative muscles, such as TB and SS, use FAs to supply energy, while the glycolytic muscles, such as SS and LT, use carbohydrates as their source of energy (Hernández et al., 1998).

The present study did not include fiber typing and classical histochemistry of the five muscles. It is known that conventional muscle fiber typing and counting by histochemical methods are very laborious and time consuming, and a task that has a series of technical problems (Rehfeldt, Tuchscherer, Hartung, & Kuhn, 2008).

Dietary treatment, and the interaction between dietary treatment and sampling position, had no statistically significant effect on extractable fat content, FFDM, moisture content and calculated IV (Table 4.17). Sampling position had a statistically significant ($p<0.001$) effect on all the physical and chemical properties of the IMF (Table 4.17).

Bee (2001), Corino *et al*. (2003), and Weber, Ruchert, Belury, Gu, Enright and Schinckel (2006) also reported that CLA supplementation to pigs did not affect the IMF content in pork. The

Table 4.17: Analysis of variance (ANOVA) for main effects and their interactions of the intramuscular fat samples.

	Dietary Treatment	Sampling Position	Dietary Treatment X Sampling Position
Physical and Chemical Properties			
Fat (%)	NS	***	NS
FFDM (%)	NS	***	NS
Moisture (%)	NS	***	NS
IV (Calculated)	NS	***	NS
Fatty Acid Composition (%)			
C10:0	NS	NS	NS
C12:0	**	NS	NS
C14:0	***	***	NS
C15:0	NS	NS	NS
C16:0	***	***	NS
C16:1*c*9	***	***	NS
C17:0	***	***	NS
C18:0	***	***	NS
C18:1*t*9	***	**	NS
C18:1*c*9	***	***	NS
C18:1*c*7	**	**	NS
C18:2*c*9,12 (*n*-6)	**	***	NS
C18:2*c*9*t*11 (*n*-6)	***	***	*
C18:2*t*10*c*12 (*n*-6)	***	***	*
C18:3*c*9,12,15 (*n*-3)	*	***	NS
C18:3*c*6,9,12 (*n*-6)	*	**	NS
C20:0	***	***	NS
C20:1*c*11	***	**	NS
C20:2*c*11,14 (*n*-6)	***	***	NS
C20:3*c*11,14,17(*n*-3)	***	***	NS
C20:4*c*5,8,11,14 (*n*-6)	NS	***	NS
C20:5*c*5,8,11,14,17 (*n*-3)	NS	***	NS
C22:0	NS	***	NS
C22:5*c*7,10,13,16,19 (*n*-3)	**	***	NS
C22:6*c*4,7,10,13,16,19 (*n*-3)	***	***	NS
Fatty Acid Ratios			
SFA (%)	***	***	NS
MUFA (%)	***	***	NS
Dienoic (%)	**	***	NS
Trienoic (%)	**	***	NS
Tetraenoic (%)	NS	***	NS
Pentaenoic (%)	*	***	NS
Hexaenoic (%)	***	***	NS
Penta/Hexaenoic	***	***	NS
PUFA (%)	*	***	NS
UFA (%)	***	***	NS
C16:0+C18:0 (%)	***	***	NS
C16:1+C18:1/C16:0+C18:0	***	***	NS
C18:0/C18:2	NS	***	NS
C16:0/C18:2	NS	***	NS

C18:2/C18:1	NS	***	NS
MUFA/SFA	***	***	NS
DBI	NS	***	NS
PI	*	***	NS
PUFA/SFA	NS	***	NS
Atherogenicity Index	***	***	NS
n-6 (%)	*	***	NS
n-3 (%)	*	***	NS
n-6/*n*-3	*	***	NS
C18:1*c*9/C18:0	***	***	NS

NS = Not Significant; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

findings of the present study is in contrast to the findings of Han *et al*. (2011), who found a significant ($p<0.05$) increase in FFDM content in the LT, after feeding CLA at concentrations of 0.5, 1 and 1.5% CLA. However, Han *et al*. (2011) also found that the IMF content increased, with the increase in CLA in the diet. Intarapichet *et al*. (2008) reported that feeding pigs diets containing 0.5 and 1% CLA, led to higher moisture content and less IMF.

M. supra spinatus from the control had the highest IMF content of 3.96%, while the lowest IMF content of 1.98%, was found for both LT (0.5% CLA group) and SM (1% CLA group) (Table 4.18). *M. supra spinatus*, with the highest fat content, is an oxidative muscle, followed by another oxidative muscle, TB, with 2.54% fat. They were followed by the intermediate muscle, BF (2.34%) and the two glycolytic muscles, the SM (2.20%) whilst the LT (2.19%) had the lowest fat content. Within all four dietary treatments, the extractable fat contents were significantly ($p < 0.001$) higher for the SS than for the other muscles (Table 4.18). Although not statistically significant, a decreasing trend was observed in IMF content (3.96 % - 3.42 %) for SS, with increased dietary CLA content.

It is generally believed that glycolytic muscles contain less fat than oxidative muscles, as glycolytic muscles use carbohydrate glycogen as energy source rather than fat. However, there is some evidence to the contrary. For example, glycolytic muscles such as LT have been shown to have higher total lipid content than more oxidative muscles such as *M. psoas major*, *M. rectus femoris* (Beecher, Cassens, Hoekstra, & Briskey, 1965) and M. m*asseter* (Leseigneur-Meynier & Gandemer 1991). In other cases, white (glycolytic) and red (more oxidative) parts of the same muscle have been found to have similar lipid contents (Beecher, Kastenschmidt, Cassens, & Hoekstra, 1968). Thus, IMF content is not strictly related to the muscle metabolic type (Leseigneur-Meynier & Gandemer 1991).

Although researchers do not always agree on the ideal IMF content of pork (Kauffman, Carpenter, Bray, & Hoekstra, 1964.; Bejerholm & Barton-Gade, 1986; De Vol, McKeith, Bechtel, Novakofski, Shanks, & Carr, 1988; Touraille, Monin, & Legault, 1989; Ellis, Webb, Avery, Brown, & Smithard, 1990; Fernandez, Monin, Talmant, Mourot, & Lebret, 1999; Fortin *et al*., 2005), it is

Table 4.18: Physical properties of intramuscular fat from gilts fed four dietary treatments containing varying levels of CLA.

Dietary Treatment	Position	Intramuscular Fat %	Fat Free Dry Matter%	Moisture %	Iodine Value
Control (n=12)	*M. semimembranosus*	2.20 ± 0.30^{a}	25.26 ± 1.17^{fg}	72.54 ± 1.25^{a}	75.30± 2.42^{def}
	M. longissimus thoracis	2.19 ± 0.28^{a}	25.25 ± 1.04^{fg}	72.56 ± 1.17^{a}	69.28 ± 3.27^{abcde}
	M. biceps femoris	2.34 ± 0.44^{a}	23.77 ± 1.01^{bcdef}	73.81 ± 1.22^{abc}	75.13 ± 4.40^{cdef}
	M. triceps brachi	2.54 ± 0.40^{a}	23.39 ± 1.41^{abcde}	74.0 ± 1.51^{abc}	77.70 ± 3.36^{f}
	M. supra spinatus	3.96 ± 0.43^{d}	21.89 ± 1.06^{a}	73.83 ± 1.04^{abc}	66.07 ± 3.43^{a}
0.25% CLA (n =12)	*M. semimembranosus*	2.16 ± 0.49^{a}	25.20 ± 1.04^{fg}	72.65 ± 0.88^{ab}	74.15 ± 4.64^{cdef}
	M. longissimus thoracis	2.05 ± 0.58^{a}	25.53 ± 1.10^{g}	72.72 ± 0.86^{abc}	70.15 ± 8.45^{abcde}
	M. biceps femoris	2.58 ± 0.74^{ab}	23.81 ± 0.83^{cdefg}	73.61 ± 1.13^{abc}	72.08 ± 6.52^{abcdef}
	M. triceps brachi	2.48 ± 0.37^{a}	23.37 ± 1.75^{abcde}	74.11 ± 1.67^{abc}	74.22 ± 5.42^{cdef}
	M. supra spinatus	3.85 ± 0.42^{d}	22.05 ± 1.00^{ab}	74.10 ± 1.19^{abc}	65.04 ± 3.22^{a}
0.5% CLA (n=12)	*M. semimembranosus*	2.48 ± 0.56^{a}	24.86 ± 1.10^{efg}	72.65 ± 1.13^{ab}	72.20 ± 4.19^{abcdef}
	M. longissimus thoracis	1.98 ± 0.57^{a}	24.52 ± 0.81^{defg}	73.51 ± 0.95^{abc}	68.70 ± 6.08^{abcde}
	M. biceps femoris	2.38 ± 0.73^{a}	23.21 ± 1.32^{abcde}	73.82 ± 0.70^{abc}	71.16 ± 6.30^{abcdef}
	M. triceps brachi	2.73 ± 0.55^{abc}	23.06 ± 1.70^{abcd}	74.22 ± 1.76^{abc}	73.80 ± 4.43^{bcdef}
	M. supra spinatus	3.37 ± 0.72^{bcd}	22.55 ± 1.23^{abc}	74.37 ± 1.50^{bc}	66.53 ± 4.25^{ab}
1% CLA (n=12)	*M. semimembranosus*	1.98 ± 0.55^{a}	24.48 ± 1.04^{defg}	73.55 ± 1.13^{abc}	75.68 ± 4.47^{ef}
	M. longissimus thoracis	2.15 ± 0.46^{a}	24.43 ± 1.33^{defg}	73.42 ± 1.10^{abc}	68.09 ± 6.08^{abcd}
	M. biceps femoris	2.68 ± 0.57^{abc}	23.82 ± 0.99^{cdefg}	73.50 ± 1.13^{abc}	69.40 ± 4.14^{abcde}
	M. triceps brachi	2.60 ± 0.65^{ab}	23.82 ± 1.20^{cdefg}	73.58 ± 1.45^{abc}	75.42 ± 5.58^{def}
	M. supra spinatus	3.42 ± 0.72^{cd}	22.10 ± 0.99^{abc}	74.48 ± 0.87^{c}	67.83 ± 5.41^{abc}
Significance level		p<0.001	p<0.001	p<0.001	p<0.001

Means with different superscripts in the same column differ significantly.

accepted that lower IMF values are usually associated with lower tenderness and juiciness of meat (Wood, 2001). A level of 1.5% IMF was found to be the minimum level necessary to ensure a pleasing eating experience for fresh meat in Canada (Fortin *et al.*, 2005). According to Prabucki (1991), lean meat must contain at least 2.5 % total fat for optimal use in processed meat products. All muscle types from all dietary treatments conformed to the minimum IMF content of 1.5 %, proposed by Fortin *et al*. (2005) for good eating quality. No SS or LT, from any dietary treatment, could conform to the 2.5 % minimum IMF content proposed by Prabucki (1991), for use in processed meat products. With the exception of TB for the 0.25 % CLA treatment, TB and SS from all dietary treatments conformed to the 2.5 % minimum IMF content, proposed by Prabucki (1991). All four dietary treatments also showed significant (p<0.001) differences between the various muscles for FFDM content (%). *M.* l*ongissimus thoracis* (control and 0.25% CLA groups) and SM (control and 0.25% CLA groups) had the highest FFDM contents of 25.20 - 25.53%. The lowest FFDM content, 21.89%, was found for SS from the control treatment group. For the control and

0.25% CLA treatments, SS was significantly (p<0.001) lower than BF, LM and SM, while TB was lower than LT and SM. The two higher concentration CLA treatments showed significantly (p<0.001) lower FFDM % for SS, versus LT and SM. *M. triceps brachi* had lower FFDM % than SM for the 0.5% CLA treatment (Table 4.18). From these results it is clear that two muscles, SS and TB, stood out as being lower in FFDM content, compared to the other muscles (Table 4.18). These two muscles are oxidative muscles, also containing the highest numerical extractable fat content. *M.* l*ongissimus thoracis* and SM are both glycolytic muscles and contained the lowest numerical IMF, which is a confirmation of the inverse relationship between the extractable fat and FFDM contents. *M. biceps femoris* is classified as an intermediate muscle, with IMF and FFDM contents intermediate to the oxidative SS and TB, and glycolytic LT and SM.

The oxidative muscles, TB (control, 0.25 and 0.5% CLA groups) and SS (0.25, 0.5 and 1% CLA groups), had the highest moisture content, ranging from 74.0 to 74.48%. Similarly, the lowest moisture contents, 72.54 – 72.72%, were found for the glycolytic muscles, SM (control, 0.25 and 0.5% CLA groups) and LT (control and 0.25% CLA groups) (Table 4.18). The moisture content of the intermediate BF was also intermediate to the glycolytic (SM and LT) and oxidative (TB and SS) muscles. Acid and neutral lipase activities are correlated and linked with moisture content, thus connecting higher moisture content in meat with greater proteolytic enzyme activity (Hernandez *et al.*, 1998).

As mentioned for the subcutaneous fat, IV is an important fat quality parameter, which evaluates FA unsaturation (Davenel *et al.*, 1999). It has been used to monitor fat firmness (Lea *et al.*, 1970), and the higher the IV (the more unsaturated the fat), the softer the fat tissue (Fischer *et* al., 1989a). As mentioned before, the limits for good quality fat range from < 70 (Barton-Gade, 1983; Girard *et al.*, 1988) to < 66 (Hart, as cited by Houben & Kroll, 1983; Ten Cate, as cited by Fischer, 1989b) and < 65 (Mortensen *et al.,* 1983; Warnants *et al*., 1996), and even < 60, for, e.g. salami (Fischer, 1989b).

No effect of diet was observed in the present study for calculated IVs of IMF (Table 4.17 and Table 4.18), indicating that the shift towards a more saturated IMF FA profile was so subtle, IV value measurements failed to pick it up.

Statistically significant sampling position differences were observed for IV. In confirmation of the results for moisture content, the oxidative TB for the control treatment group had the highest calculated IV of 77.70. The lowest calculated IV value of 65.04 was reported for the oxidative SS for the 0.25% CLA treatment group. This trend continued as the control, 0.25 and 1% CLA dietary groups also had lower calculated IVs for the oxidative SS than for the oxidative TB and glycolytic SM. For the control group, there was a significantly (p<0.001) lower calculated IV for the oxidative SS, versus the intermediate BF (Table 4.18).

Although not statistically significant, there was also a trend towards improved IMF quality with increased dietary CLA content. In the control and 0.25 % CLA treatment only one muscle (LT and SS, respectively) had an IV of < 70. In the 0.5 % CLA treatment, two muscles (LT and SS) had IVs < 70. In the 1 % CLA treatment, three of the muscles (LT, BF and SS) had IVs < 70. Only one sample, the SS from the 0.25% CLA treatment, had an IV of < 66, which was also the lowest IV for IMF (65.04).

Fatty acid composition of intramuscular fat

The FA composition, of the four experimental diets (Table 4.1), was reflected in the FA profiles of the IMF (Tables 4.19 – 4.25). Table 4.17 indicates that nearly all the individual FAs and FA ratios were significantly (at least p<0.05) influenced by dietary treatment and sampling position. The interaction between dietary treatment and sampling position had a significant (p<0.05) effect only for *cis-9, trans-11* and *trans-10, cis-12* contents.

Saturated fatty acids

According to Table 4.19, only trace amounts of C10:0, C12:0, C15:0 and C22:0 were detected in the IMF from all dietary treatments (Table 4.19). Therefore, neither dietary treatment, nor sampling position had a statistically significant effect on C10:0, C12:0 and C15:0 for the IMF tissue (Tables 4.17). In the case of C22:0, a significant sampling position effect was observed for the 0.5 % CLA treatment group, where LT (0.10 %) had a significantly (p<0,001) higher C22:0 content than SS (0.02 %).

Dietary treatment had a statistically significant (at least p<0.01) effect on all other SFAs. Myristic acid and C16:0 contents increased significantly (p<0.001) for specific muscles, with an increase in CLA concentration in the pigs' diets. There were significantly (p<0.001) lower C14:0 content for the BF, TB and SS in the control than for the same muscles for the 1% CLA treatment (Table 4.19). For C16:0, the control was significantly (p<0.001) lower for all the sampling positions, in comparison to the 0.5 and 1% CLA treatments. Also for the BF, the control's C16:0 content was lower than that of the 0.25% CLA treatment (Table 4.19). The 0.25% CLA treatment was also lower for the LT, BF and TB than the 1% CLA treatment, for C16:0 contents (Table 4.19). Margaric acid and C18:0 contents showed no significant differences for the same sampling position, within dietary treatment (Table 4.19). C20:0 content showed one significant sampling position difference between dietary treatments (Table 4.17). The control group had significantly (p<0.001) higher C20:0 content for the LT than the 0.5% CLA treatment (Table 4.19).

Ramsay *et al*. (2001), Thiel-Cooper *et al*. (2001) and Szymczyk (2005) found that CLA supplementation at 0.12, 0.25, 0.5, and 1.0%, exerted a significant increase in the level of mainly

Table 4.19: Saturated fatty acid content (%) of intramuscular fat from gilts from the four diets.

Diet	Position	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C22:0
Control (n=12)	*M. semimembranosus*	0.05 ± 0.04	0.03 ± 0.03	1.19 ± 0.09^{abcd}	0.01 ± 0.02	23.81 ± 0.65^{abc}	0.24 ± 0.03^{cde}	11.84 ± 0.87^{ab}	0.13 ± 0.01^{abcdef}	0.05 ± 0.04^{abc}
	M. longissimus thoracis	0.03 ± 0.04	0.02 ± 0.03	1.22 ± 0.12^{abcde}	0.01 ± 0.01	25.13 ± 0.69^{cdef}	0.20 ± 0.04^{abc}	12.64 ± 0.78^{abc}	0.16 ± 0.01^{g}	0.06 ± 0.04^{abc}
	M. biceps femoris	0.02 ± 0.03	0.01 ± 0.02	1.08 ± 0.08^{ab}	0.02 ± 0.02	23.30 ± 0.56^{a}	0.23 ± 0.06^{bcde}	11.52 ± 0.90^{a}	0.13 ± 0.02^{abcdef}	0.08 ± 0.05^{abc}
	M. triceps brachi	0.02 ± 0.03	0.01 ± 0.02	1.03 ± 0.09^{a}	0.02 ± 0.02	23.44 ± 0.79^{ab}	0.27 ± 0.05^{e}	12.61 ± 1.13^{abc}	0.12 ± 0.02^{abcde}	0.04 ± 0.03^{abc}
	M. supra spinatus	0.05 ± 0.03	0.03 ± 0.02	1.20 ± 0.07^{abcde}	0.01 ± 0.01	24.63 ± 0.66^{abcd}	0.22 ± 0.04^{abcde}	12.78 ± 1.12^{abc}	0.15 ± 0.02^{efg}	0.01 ± 0.02^{a}
0.25% CLA (n =12)	*M. semimembranosus*	0.05 ± 0.04	0.03 ± 0.03	1.25 ± 0.20^{abcde}	0.01 ± 0.02	25.24 ± 0.89^{cdef}	0.23 ± 0.03^{abcde}	12.65 ± 1.30^{abc}	0.11 ± 0.02^{abcd}	0.06 ± 0.04^{abc}
	M. longissimus thoracis	0.05 ± 0.03	0.02 ± 0.03	1.23 ± 0.18^{abcde}	0.01 ± 0.01	25.92 ± 1.50^{defgh}	0.18 ± 0.03^{ab}	12.99 ± 0.84^{bc}	0.15 ± 0.03^{fg}	0.06 ± 0.04^{abc}
	M. biceps femoris	0.03 ± 0.04	0.03 ± 0.03	1.23 ± 0.25^{abcde}	0.01 ± 0.02	24.83 ± 1.59^{bcdef}	0.21 ± 0.03^{abcd}	12.34 ± 0.80^{abc}	0.14 ± 0.02^{cdefg}	0.06 ± 0.04^{abc}
	M. triceps brachi	0.03 ± 0.04	0.02 ± 0.03	1.16 ± 0.15^{abc}	0.01 ± 0.02	24.70 ± 1.06^{abcde}	0.24 ± 0.03^{bcde}	12.86 ± 1.20^{abc}	0.11 ± 0.02^{ab}	0.05 ± 0.05^{abc}
	M. supra spinatus	0.04 ± 0.03	0.05 ± 0.03	1.34 ± 0.12^{cdef}	0.01 ± 0.01	25.94 ± 1.49^{defgh}	0.22 ± 0.03^{abcde}	12.43 ± 0.61^{abc}	0.14 ± 0.03^{cdefg}	0.02 ± 0.03^{ab}
0.5% CLA (n=12)	*M. semimembranosus*	0.05 ± 0.03	0.02 ± 0.03	1.37 ± 0.13^{cdef}	0.02 ± 0.02	26.02 ± 0.55^{defgh}	0.26 ± 0.04^{de}	12.25 ± 0.93^{abc}	0.11 ± 0.02^{abc}	0.08 ± 0.04^{bc}
	M. longissimus thoracis	0.06 ± 0.04	0.04 ± 0.04	1.30 ± 0.14^{bcdef}	0.02 ± 0.02	26.75 ± 0.65^{ghi}	0.23 ± 0.04^{abcde}	12.78 ± 0.91^{abc}	0.13 ± 0.03^{abcdef}	0.10 ± 0.04^{c}
	M. biceps femoris	0.05 ± 0.04	0.04 ± 0.03	1.29 ± 0.17^{bcdef}	0.02 ± 0.02	25.56 ± 0.85^{defg}	0.26 ± 0.04^{cde}	12.00 ± 0.84^{abc}	0.11 ± 0.02^{abc}	0.07 ± 0.06^{abc}
	M. triceps brachi	0.03 ± 0.03	0.02 ± 0.02	1.22 ± 0.13^{abcde}	0.01 ± 0.02	25.33 ± 0.64^{defg}	0.27 ± 0.04^{e}	12.61 ± 0.87^{abc}	0.11 ± 0.02^{a}	0.05 ± 0.05^{abc}
	M. supra spinatus	0.04 ± 0.04	0.03 ± 0.03	1.36 ± 0.17^{cdef}	0.01 ± 0.01	26.18 ± 0.83^{fgh}	0.25 ± 0.04^{cde}	12.30 ± 0.85^{abc}	0.12 ± 0.02^{abcdef}	0.02 ± 0.04^{ab}
1% CLA (n=12)	*M. semimembranosus*	0.03 ± 0.03	0.03 ± 0.04	1.36 ± 0.26^{cdef}	0.01 ± 0.01	26.10 ± 1.18^{efgh}	0.22 ± 0.04^{abcde}	12.71 ± 0.79^{abc}	0.12 ± 0.01^{abcde}	0.07 ± 0.05^{abc}
	M. longissimus thoracis	0.04 ± 0.03	0.04 ± 0.03	1.42 ± 0.21^{def}	0.01 ± 0.01	27.64 ± 1.16^{i}	0.17 ± 0.03^{a}	13.34 ± 0.80^{c}	0.14 ± 0.01^{defg}	0.08 ± 0.05^{bc}
	M. biceps femoris	0.04 ± 0.03	0.05 ± 0.04	1.44 ± 0.19^{ef}	0.01 ± 0.01	26.77 ± 1.09^{ghi}	0.20 ± 0.04^{abc}	12.79 ± 0.91^{abc}	0.14 ± 0.01^{bcdefg}	0.04 ± 0.05^{abc}
	M. triceps brachi	0.04 ± 0.03	0.03 ± 0.03	1.28 ± 0.20^{bcdef}	0.01 ± 0.02	26.16 ± 1.11^{fgh}	0.24 ± 0.05^{bcde}	13.09 ± 0.86^{bc}	0.11 ± 0.02^{abc}	0.04 ± 0.05^{abc}
	M. supra spinatus	0.05 ± 0.03	0.03 ± 0.03	1.51 ± 0.19^{f}	0.01 ± 0.01	27.28 ± 0.86^{hi}	0.21 ± 0.06^{abcd}	12.86 ± 0.79^{abc}	0.12 ± 0.01^{abcdef}	0.05 ± 0.05^{abc}
Significance level		NS	NS	p<0.001	NS	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

Means with different superscripts in the same column differ significantly
NS = Not Significant

C16:0 and C18:0 FAs in the LT. A steady elevation in the feeding of CLA from 0.25, 0.5, 1 to 2% of the diet increased the percentage of C18:0 in the *Lattisimus* muscle (Ramsay *et al*., 2001). Conjugated linoleic acid supplementation at 0.25% in pigs' feed increased C14:0 and C18:0 in the *M. biceps femoris* (Lo Fiego *et al*., 2005). King *et al*. (2004) found, in affirmation of the present study's results, a significant ($p<0.05$) increase in the concentration of C16:0, with increased dietary CLA level. Meat from pigs supplemented with 2% (Migdal *et al*., 2004; Martin *et al*., 2008a) and 4% CLA (Sun *et al*., 2004) had statistically significant higher contents of C14:0, C16:0 and C18:0. Han *et al*. (2011) reported a linear increase in C16:0, with the increment (0.5, 1.0. and 1.5%) of dietary CLA, while concentrations of C14:0, C18:0 and C20:0 were unaffected. In the present study the observed increase in SFA (C14:0 and C16:0) content of the IMF, with increased dietary CLA content, could (as in the case of BF) not be ascribed to an increase in these FAs in the diets of the animals. As mentioned earlier, there was a statistically significant ($p<0.001$) decrease in these FAs in the animal feed, with increased dietary CLA content (Table 4.1). As noted earlier, this phenomenon could be attributed to the inhibition of Δ^9 desaturase activity and mRNA expression by CLA (Han *et al*., 2011).

According to Table 4.17, sampling position had no effect on C10:0, C12:0 and C15:0, probably due to the trace amounts in which they occurred in the muscles (Table 4.19). Myristic acid, C18:0 and C22:0 also showed no change in contents for different muscles, within dietary treatments (Table 4.19). For C16:0, the control group had significantly ($p<0.001$) higher C16:0 contents for the glycolytic LT than the intermediate BF and oxidative TB. The glycolytic LT for the 1% CLA treatment also had a significantly ($p<0.001$) higher C16:0 content than the glycolytic SM and oxidative TB (Table 4.19). For C17:0, both the control and 1% CLA treatment had significantly ($p<0.001$) lower contents for the glycolytic LT than the oxidative TB (Table 4.19). Arachidic acid contents showed for the control treatment group significantly ($p<0.001$) higher values for the glycolytic LT than for the glycolytic SM, intermediate BF and oxidative TB. For the 0.25% CLA treatment, C20:0 contents were significantly higher for the glycolytic LT than the glycolytic SM and oxidative TB. Also for the 0.25% treatment, the oxidative TB had lower C20:0 content than the intermediate BF and oxidative SS (Table 4.19). For the 1% CLA treatment group, the C20:0 content was significantly higher in the glycolytic LT than in the oxidative TB.

It is clear that the glycolytic LT predominantly had higher concentration of SFAs, which is in accordance with the findings of Hernández *et al*. (1998). As noted earlier, a C18:0 content $>$ 12% is an indicator of good fat quality (Häuser & Prabucki 1990). In the present study, IMF from all muscles, from all the CLA treatment groups, as well as IMF from the LT, TB and SS from the control treatment, conformed to this quality requirement (Table 4.19). The exceptions were the IMF samples from the SM and BF from the control group, which had a C18:0 content $<$ 12 % (Table

4.19). As mentioned earlier, no dietary effect was observed for C18:0 and IMF quality was therefore not improved.

Mono-unsaturated fatty acids

Table 4.20 summarizes the MUFA composition of the IMF from the gilts fed the four dietary treatments. No significant differences were found for C18:1*t*9 and C18:1*c*7 contents for the same sampling position, within dietary treatment (Table 4.20). Four of the five muscles, SM, BF, TB and SS, showed significantly ($p<0.001$) lower C16:1 content for the control than for the 0.5 and 1% CLA treatment. The control group also had a significantly ($p<0.001$) lower value for LT, only compared to the 1% CLA treatment (Table 4.20). The 0.25% CLA treatment had lower C16:1 content than the 1% CLA treatment, for the LT and SS. The increase in dietary CLA thus led to a linearly increase in C16:1 content.

Table 4.20: Mono-unsaturated fatty acid content (%) of intramuscular fat from gilts from the four diets.

Diet	Position	C16:1*c*9	C18:1*t*9	C18:1*c*9	C18:1*c*7	C20:1*c*11
Control (n=12)	*M. semimembranosus*	2.80 ± 0.34^{ab}	0.06 ± 0.01^{abc}	38.58 ± 2.05^{def}	3.90 ± 0.26^{ab}	0.52 ± 0.07^{defg}
	M. longissimus thoracis	2.96 ± 0.52^{abc}	0.05 ± 0.03^{abc}	39.80 ± 1.70^{efg}	3.89 ± 0.36^{ab}	0.53 ± 0.07^{fg}
	M. biceps femoris	2.72 ± 0.33^{ab}	0.04 ± 0.04^{abc}	40.30 ± 3.14^{fg}	3.93 ± 0.39^{ab}	0.53 ± 0.08^{fg}
	M. triceps brachi	2.29 ± 0.36^{a}	0.04 ± 0.04^{ab}	36.60 ± 2.69^{bcde}	3.65 ± 0.28^{ab}	0.53 ± 0.05^{efg}
	M. supra spinatus	2.63 ± 0.42^{ab}	0.06 ± 0.02^{abc}	42.38 ± 2.19^{g}	3.73 ± 0.36^{ab}	0.57 ± 0.04^{g}
0.25% CLA (n =12)	*M. semimembranosus*	3.12 ± 0.60^{abcd}	0.04 ± 0.03^{ab}	35.44 ± 1.88^{bcd}	3.93 ± 0.45^{ab}	0.47 ± 0.04^{bcdef}
	M. longissimus thoracis	3.15 ± 0.35^{abcd}	0.04 ± 0.03^{a}	37.53 ± 2.84^{bcdef}	3.85 ± 0.25^{ab}	0.47 ± 0.05^{cdef}
	M. biceps femoris	3.18 ± 0.45^{abcde}	0.04 ± 0.03^{abc}	38.02 ± 1.63^{cdef}	3.91 ± 0.42^{ab}	0.48 ± 0.05^{cdef}
	M. triceps brachi	2.85 ± 0.56^{ab}	0.04 ± 0.02^{ab}	35.92 ± 2.20^{bcd}	3.72 ± 0.35^{ab}	0.47 ± 0.06^{cdef}
	M. supra spinatus	3.25 ± 0.39^{bcde}	0.06 ± 0.03^{abc}	40.37 ± 1.65^{fg}	3.86 ± 0.44^{ab}	0.53 ± 0.07^{fg}
0.5% CLA (n=12)	*M. semimembranosus*	3.81 ± 0.69^{cdef}	0.04 ± 0.03^{abc}	35.48 ± 2.65^{bcd}	3.96 ± 0.21^{ab}	0.45 ± 0.07^{abcdef}
	M. longissimus thoracis	3.82 ± 0.64^{cdef}	0.04 ± 0.03^{ab}	36.28 ± 2.88^{bcd}	4.02 ± 0.30^{ab}	0.44 ± 0.06^{abcd}
	M. biceps femoris	3.85 ± 0.80^{cdef}	0.05 ± 0.04^{abc}	37.15 ± 2.74^{bcdef}	4.05 ± 0.29^{b}	0.44 ± 0.05^{abcde}
	M. triceps brachi	3.35 ± 0.73^{bcdef}	0.04 ± 0.03^{abc}	35.11 ± 2.34^{abc}	3.75 ± 0.40^{ab}	0.44 ± 0.07^{abcd}
	M. supra spinatus	3.92 ± 0.76^{def}	0.06 ± 0.04^{abc}	38.62 ± 2.27^{def}	4.02 ± 0.33^{ab}	0.47 ± 0.04^{cdef}
1% CLA (n=12)	*M. semimembranosus*	3.79 ± 0.90^{cdef}	0.08 ± 0.02^{bc}	32.12 ± 1.16^{a}	3.72 ± 0.46^{ab}	0.38 ± 0.04^{a}
	M. longissimus thoracis	4.18 ± 0.76^{f}	0.05 ± 0.03^{abc}	34.33 ± 1.93^{ab}	3.89 ± 0.47^{ab}	0.38 ± 0.06^{ab}
	M. biceps femoris	4.06 ± 0.50^{ef}	0.08 ± 0.04^{abc}	34.85 ± 1.66^{abc}	3.82 ± 0.34^{ab}	0.40 ± 0.04^{abc}
	M. triceps brachi	3.31 ± 0.71^{bcdef}	0.08 ± 0.04^{abc}	31.95 ± 1.63^{a}	3.50 ± 0.43^{a}	0.40 ± 0.06^{abc}
	M. supra spinatus	4.16 ± 0.90^{f}	0.09 ± 0.02^{c}	34.98 ± 2.21^{abc}	3.68 ± 0.50^{ab}	0.41 ± 0.05^{abc}
Significance level		$p<0.001$	$p<0.001$	$p<0.001$	$p<0.05$	$p<0.001$

Means with different superscripts in the same column differ significantly.

Contrary to the increase in C16:1 content, an increase in dietary CLA led to decreases in C18:1*c*9 and C20:1*c*11 contents (Table 4.20). For the control group, all five muscles were significantly (p<0.001) higher in C18:1*c*9 content than the 1% CLA treatment. The control's LT and SS were also significantly (p<0.001) higher in C18:1*c*9 content than the 0.5% CLA treatment (Table 4.20). The C18:1*c*9 content for the 1% CLA group was lower for the SM, TB and SS, compared to the 0.25% CLA treatment. The 1% CLA group also had significantly (p<0.001) lower C18:1*c*9 content than the 0.5% CLA treatment, for the SM and SS. For C20:1, all the control muscles were significantly (p<0.001) higher in C20:1 content than the 1% CLA treatment. All the muscles, except SM for the control, were significantly (p<0.001) lower in C20:1 content than the corresponding muscles for the 0.5% CLA treatment. For the 0.25% CLA treatment, the SM, LT and SS contained significantly (p<0.001) higher C20:1 content than the 1% CLA group (Table 4.20).

A steady elevation in the dietary inclusion of CLA from 0.25, 0.5, 1 to 2% (Ramsay *et al*., 2001; Migdal *et al*., 2004; Martin *et al*., 2008a), and even 4% of the diet (Sun *et al*., 2004), reduced the C18:1*c*9 concentration in the *M. lattisimus* (Ramsay *et al*., 2001) and loin muscles (Migdal *et al*., 2004; Sun *et al*., 2004; Martin *et al*., 2008a). CLA supplementation at 0.25% increased C16:1, and decreased C18:1c9 and C20:1 contents in the BF muscle (Lo Fiego *et al*., 2005), which are in accordance with the results of the present study. King *et al*. (2004) also found that feeding CLA at a concentration of 1.5% decreased C18:1*c*-9 significantly (p<0.05).

For sampling position, C16:1, C18:1*t*9, C18:1*c*7 and C20:1*c*11 showed no change in contents according to Table 4.20. C18:1*c*9 showed a number of significant (p<0.001) effects for sampling position (Table 4.20). For the control group, the oxidative SS had significantly (p<0.001) higher C18:1*c*9 contents than the glycolytic SM and oxidative TB. The oxidative TB, in turn, had a lower C18:1*c*9 content than the intermediate BF. The 0.25% CLA treatment had a higher C18:1*c*9 content for the oxidative SS than the glycolytic SM and intermediate TB. Only the intermediate TB from the 0.5% CLA treatment was significantly (p<0.001) lower in C18:1*c*9 content, compared to the oxidative SS (Table 4.20). This can be explained by the fact that SS had significantly higher fat content than the other muscles (Table 4.18). Also, TB had a high C18:2 (Table 4.22), which led to a reduction in C18:1content (Hernandez *et al*. 1998). This could be explained by the fact that C18:2 is a potent inhibitor of Δ^9-desaturase, which is responsible for C18:1 FA synthesis (Jeffcoat & James, 1984).

Δ^9 Desaturase index

The Δ^9 desaturase index (C18:1*c*9/C18:0) showed a statistically significant (p<0.001) decrease in the IMF with increased dietary CLA level (Table 4.21). It decreased from ± 3.20 in the control, to

± 2.60 in the 1% CLA treatment (Table 4.21). For the C18:1*c*9/C18:0 ratio, all muscles from the control group had significantly ($p<0.001$) higher values than the corresponding muscles from the 1% CLA treatment. With the exception of TB and SS, SM, LT and BF from the 0.25% CLA treatment had significantly ($p<0.001$) higher C18:1*c*9/C18:0 ratios than the corresponding muscles from the 1% CLA treatment. This explains the increase in SFA content (C14:0 and C16:0) observed in Table 4.19 and the decrease in MUFA content (C18:1*c*9 and C20:1*c*11), observed in Table 4.20. Other researchers also found that dietary CLA supplementation resulted in increased SFAs and decreased MUFAs in the IMF (Ramsay *et al*., 2001; Averette Gatlin *et al.*, 2002b; King *et al*., 2004; Migdal *et al*., 2004; Sun *et al.*, 2004; Lo Fiego *et al*., 2005; Martin *et al*., 2008a) and attributed it to a reduction in Δ^9 desaturase activity (Cordero *et al.*, 2010). The decrease in the Δ^9 desaturase index, for the IMF samples, was not as high as the decrease for the same index for the subcutaneous fat samples. As noted earlier, the Δ^9 desaturase index decreased from ± 3 in the

Table 4.21: The Δ^9 desaturase index of intramuscular fat from gilts from the four dietary treatments.

Diet	Position	C18:1*c*9/C18:0
Control (n=12)	*M. semimembranosus*	3.27 ± 0.25^{efg}
	M. longissimus thoracis	3.16 ± 0.30^{cdefg}
	M. biceps femoris	3.52 ± 0.44^{g}
	M. triceps brachi	2.93 ± 0.36^{bcdef}
	M. supra spinatus	3.34 ± 0.36^{fg}
0.25% CLA (n =12)	*M. semimembranosus*	2.83 ± 0.38^{abcde}
	M. longissimus thoracis	2.90 ± 0.28^{bcdef}
	M. biceps femoris	3.10 ± 0.26^{cdefg}
	M. triceps brachi	2.82 ± 0.36^{abcd}
	M. supra spinatus	3.26 ± 0.24^{defg}
0.5% CLA (n=12)	*M. semimembranosus*	2.91 ± 0.31^{bcdef}
	M. longissimus thoracis	2.85 ± 0.30^{abcde}
	M. biceps femoris	3.11 ± 0.27^{cdefg}
	M. triceps brachi	2.79 ± 0.25^{abc}
	M. supra spinatus	3.16 ± 0.34^{cdefg}
1% CLA (n=12)	*M. semimembranosus*	2.54 ± 0.22^{ab}
	M. longissimus thoracis	2.58 ± 0.25^{ab}
	M. biceps femoris	2.74 ± 0.29^{abc}
	M. triceps brachi	2.45 ± 0.24^{a}
	M. supra spinatus	2.74 ± 0.31^{abc}
Significance level		$p<0.001$

Means with different superscripts in the same column differ significantly.

control, to ± 1.6 in the 1% CLA treatment for the subcutaneous fat samples (Table 4.10). The reduction of the Δ^9 desaturase index activity is therefore not as prominent in the IMF, as in the subcutaneous fat.

Polyunsaturated fatty acids

Table 4.22 is a summary of the dienoic and trienoic PUFA composition of the IMF for the gilts, from the four dietary treatments. Although the C18:2, C18:3*n*-3, C18:3*n*-6 and C20:3*n*-3 contents were all significantly (at least $p < 0.05$) influenced by an increase in dietary CLA level (Table 4.18), no significant differences were observed between corresponding muscles, between different dietary treatment groups. The general trend was, however, for decreased C18:2, decreased C18:3*n*-6 and decreased C20:3*n*-3 contents, with increased dietary CLA content. The C18:3*n*-3 level remained relatively constant for all treatments, although LT from the control had significantly ($p<0.001$) lower C18:3*n*-3 than all the other muscles from all dietary treatment groups (Table 4.22). In a study using a diet containing 0, 1, 2.5, or 5% CLA, the concentration of C18:2 in the IMF of the loins (Joo *et al*., 2002) decreased. It was found by Sun *et al.* (2004) that C18:3*n*-3 contents decreased after feeding pigs CLA at a concentration of 4%.

Both the *cis-9, trans-11* and trans-10 ,cis-12 CLA isomers in the IMF were significantly ($p<0.001$) influenced by dietary treatment (Table 4.17). Since the control group was not fed any CLA, these isomers were not detected (Table 4.22) in the IMF of the control carcasses. All five muscles demonstrated a statistically significant ($p<0.001$) increase in both isomers, with an increase in dietary CLA up to to 1% (Table 4.22). The first effects was noticed for the SS from the 0.5% CLA treatment, which was significantly ($p<0.001$) higher in *cis*-9, *trans*-11 content, compared to the 0.25% CLA treatment (Table 4.22). All the muscles for the 1% CLA treatment had significantly higher *cis-9, trans-11* and trans-10, cis-12 contents than the 0.25 and 0.5% CLA treatments (Table 4.22). This linear increase of CLA isomers in the total FAs (Szymczyk, 2005), as well as in the lean tissue (Thiel-Cooper *et al*., 2001) as a result of dietary CLA supplementation, was reported previously (Joo *et al*., 2002; Wiegand *et al*., 2002; Migdal *et al*., 2004; Sun *et al*., 2004; Intarapichet *et al*., 2008; Martin *et al*., 2008a; Han *et al*., 2011). The present study, as well as previous studies, found that the *cis-9, trans-11* content was higher than the trans-10, cis-12 content (Joo *et al*., 2002; Wiegand *et al*., 2002; Migdal *et al*., 2004; Sun *et al*., 2004; Intarapichet *et al*., 2008; Martin *et al*., 2008a; Han *et al*., 2011). The *cis-9, trans-11* contents were roughly about double that of the t*rans*-10, *cis*-12 contents (Table 4.22). This is in accordance with the results obtained for the subcutaneous fat samples (Table 4.11). As noted previously, the reason for this phenomenon might be due to the variations in absorption, transportation and catabolism (β-

Table 4.22: Dienoic + trinoic polyunsaturated fatty acid content (%) of intramuscular fat from gilts from the four diets.

Diet	Position	C18:2*c*9,12 (*n*-6)	C18:2*c*9*t*11 (*n*-6) (CLA)	C18:2*t*10*c*12 (*n*-6) (CLA)	C18:3*c*9,12,15 (*n*-3)	C18:3*c*6,9,12 (*n*-6)	C20:2*c*11,14 (*n*-6)	C20:3*c*11,14,17 (*n*-3)
Control (n=12)	*M. semimembranosus*	12.54 ± 1.00^{cdefg}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.20 ± 0.03^{bcdef}	0.04 ± 0.03^{ab}	0.30 ± 0.04^{ef}	0.35 ± 0.07^{ef}
	M. longissimus thoracis	9.85 ± 1.58^{abcde}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.15 ± 0.01^{a}	0.02 ± 0.03^{ab}	0.23 ± 0.04^{bcd}	0.31 ± 0.05^{abcdef}
	M. biceps femoris	12.11 ± 1.86^{bcdefg}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.20 ± 0.03^{abcde}	0.03 ± 0.04^{ab}	0.32 ± 0.03^{fg}	0.33 ± 0.08^{def}
	M. triceps brachi	14.71 ± 1.50^{g}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.28 ± 0.03^{hi}	0.05 ± 0.03^{b}	0.37 ± 0.03^{g}	0.40 ± 0.09^{f}
	M. supra spinatus	9.18 ± 1.72^{abc}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.22 ± 0.03^{defg}	0.01 ± 0.02^{ab}	0.31 ± 0.05^{f}	0.20 ± 0.06^{abc}
0.25% CLA (n =12)	*M. semimembranosus*	12.79 ± 2.33^{efg}	0.19 ± 0.22^{ab}	0.00 ± 0.00^{a}	0.22 ± 0.03^{defg}	0.03 ± 0.03^{ab}	0.31 ± 0.07^{f}	0.36 ± 0.04^{ef}
	M. longissimus thoracis	10.31 ± 3.11^{abcde}	0.16 ± 0.13^{ab}	0.02 ± 0.02^{a}	0.17 ± 0.03^{abcd}	0.04 ± 0.03^{ab}	0.23 ± 0.06^{bcd}	0.32 ± 0.12^{cdef}
	M. biceps femoris	11.39 ± 2.02^{abcdef}	0.36 ± 0.20^{bc}	0.05 ± 0.12^{ab}	0.22 ± 0.03^{defgh}	0.03 ± 0.04^{ab}	0.29 ± 0.05^{def}	0.31 ± 0.12^{abcdef}
	M. triceps brachi	13.56 ± 2.57^{fg}	0.33 ± 0.17^{abc}	0.05 ± 0.10^{ab}	0.27 ± 0.04^{hi}	0.02 ± 0.03^{ab}	0.31 ± 0.05^{f}	0.33 ± 0.07^{def}
	M. supra spinatus	9.16 ± 1.19^{abc}	0.28 ± 0.18^{abc}	0.09 ± 0.12^{ab}	0.25 ± 0.03^{fghi}	0.01 ± 0.02^{ab}	0.30 ± 0.03^{ef}	0.19 ± 0.05^{a}
0.5% CLA (n=12)	*M. semimembranosus*	11.16 ± 2.08^{abcdef}	0.73 ± 0.34^{de}	0.21 ± 0.37^{ab}	0.21 ± 0.05^{cdef}	0.03 ± 0.03^{ab}	0.24 ± 0.04^{bcde}	0.32 ± 0.08^{cdef}
	M. longissimus thoracis	9.94 ± 3.11^{abcde}	0.58 ± 0.25^{cd}	0.04 ± 0.13^{ab}	0.15 ± 0.04^{abc}	0.02 ± 0.03^{ab}	0.20 ± 0.05^{ab}	0.29 ± 0.11^{abcdef}
	M. biceps femoris	10.58 ± 2.84^{abcdef}	0.79 ± 0.18^{de}	0.22 ± 0.26^{ab}	0.21 ± 0.03^{def}	0.03 ± 0.03^{ab}	0.24 ± 0.03^{bcde}	0.29 ± 0.12^{abcdef}
	M. triceps brachi	12.48 ± 2.11^{defg}	0.92 ± 0.30^{ef}	0.28 ± 0.36^{ab}	0.27 ± 0.04^{ghi}	0.03 ± 0.03^{ab}	0.29 ± 0.04^{def}	0.32 ± 0.07^{cdef}
	M. supra spinatus	8.84 ± 2.01^{a}	0.83 ± 0.23^{def}	0.34 ± 0.29^{b}	0.24 ± 0.04^{efghi}	0.02 ± 0.02^{ab}	0.23 ± 0.03^{bcd}	0.21 ± 0.10^{abcd}
1% CLA (n=12)	*M. semimembranosus*	12.23 ± 2.18^{cdefg}	1.46 ± 0.13^{gh}	1.08 ± 0.07^{d}	0.22 ± 0.04^{defg}	0.02 ± 0.02^{ab}	0.23 ± 0.03^{abc}	0.32 ± 0.10^{bcdef}
	M. longissimus thoracis	8.87 ± 2.33^{a}	1.15 ± 0.21^{fg}	0.69 ± 0.19^{c}	0.15 ± 0.04^{ab}	0.02 ± 0.02^{ab}	0.17 ± 0.03^{a}	0.24 ± 0.09^{abcde}
	M. biceps femoris	9.40 ± 1.45^{abcd}	1.59 ± 0.21^{h}	1.09 ± 0.20^{d}	0.21 ± 0.04^{def}	0.01 ± 0.02^{ab}	0.22 ± 0.03^{abc}	0.21 ± 0.07^{abcd}
	M. triceps brachi	12.69 ± 2.53^{efg}	1.67 ± 0.57^{h}	1.01 ± 0.48^{d}	0.28 ± 0.04^{i}	0.02 ± 0.04^{ab}	0.27 ± 0.04^{cdef}	0.30 ± 0.10^{abcdef}
	M. supra spinatus	8.97 ± 2.34^{ab}	1.64 ± 0.28^{h}	0.99 ± 0.29^{cd}	0.23 ± 0.05^{defgh}	0.00 ± 0.01^{a}	0.20 ± 0.02^{ab}	0.19 ± 0.07^{ab}
Significance level		$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.05$	$p<0.001$	$p<0.001$

Means with different superscripts in the same column differ significantly.

oxidation, etc.) or the competitive inhibition by the CLA *cis-9, trans-11* isomer of the CLA *trans-10, cis-12* isomer incorporation (Han *et al.*, 2011).

For C20:2, a number of significant ($p<0.001$) effects were reported for dietary treatment (Table 4.22). Generally, the C20:2 contents decreased with an increase in dietary CLA level. For all five muscles the control group had significantly (p<0.001) higher C20:2 content than the 1% CLA treatment. Although not statistically significant, BF, TB and SS also had higher C20:2 contents for the control than for the 0.5% CLA treatment. Only TB for the control had a higher C20:2 content than the 0.25% CLA treatment (Table 4.22). For SM, LT, BF and SS, the 0.25% CLA treatment was higher in C20:2 contents than the 1% CLA treatment. *M. semimembranosus* and SS were also higher in C20:2 contents for the 0.25% CLA treatment than the 0.5% CLA treatment (Table 4.22).

In a study with CLA levels of 0, 1, 2.5, or 5%, the concentration of C18:2 in the IMF of the loins (Joo *et al.*, 2002) decreased. It was found by Sun *et al.* (2004) that C18:3*n*-3 contents decreased after feeding pigs CLA at a concentration of 4%. The increasing level of CLA in the diets caused a linear increase of CLA isomers in the total FAs (Szymczyk, 2005), as well as in the lean tissue (Thiel-Cooper *et al.*, 2001), e.g. the loin. The *cis*-9, *trans*-11 isomer was the highest in concentration (Joo *et al.*, 2002; Wiegand *et al.*, 2002; Migdal *et al.*, 2004; Sun *et al.*, 2004; Intarapichet *et al.*, 2008; Martin *et al.*, 2008a; Han *et al.*, 2011).

No significant differences were observed for C18:3*n*-6 between sampling position, within dietary treatments (Table 4.22). A number of effects were noted for C18:2 contents (Table 4.22). For all four treatments, the oxidative TB showed significantly (p<0.001) higher C18:2 contents than the oxidative SS. The control, 0.25 and 1% CLA treatments had significantly (p<0.001) higher C18:2 contents for the glycolytic SM than the oxidative SS, while the glycolytic LT had significantly (p<0.001) lower C18:2 contents than the oxidative TB. The 1% CLA treatment also had significantly (p<0.001) higher C18:2 contents for the glycolytic SM, compared to the glycolytic LT and a significantly (p<0.001) lower C18:2 content for the intermediate BF, compared to the oxidative TB (Table 4.22). Leseigneur and Gandemer (1991) also noted that LT contained less C18:2 content than oxidative and intermediate muscles.

For both the *cis*-9*, trans*-11 and *trans*-10*, cis*-12 isomers, only the 1% CLA dietary group showed significant (p<0.001) effects. The glycolytic LT showed lower *cis*-9*, trans*-11contents than the intermediate BF, oxidative TB and oxidative SS. For the *trans*-10*, cis*-12 isomer, the glycolytic LT, again, showed lower contents than the glycolytic SM, intermediate BF and oxidative TB (Table 4.22).

α-Linolenic acid had a large number of significant (p<0.001) effects for sampling position (Table 4.22). All four dietary treatments showed significantly (p<0.001) lower values for C18:3*n*-3

for the glycolytic SM versus oxidative TB, glycolytic LT versus oxidative TB and glycolytic LT versus oxidative SS. The control, 0.5 and 1% CLA treatments showed significantly ($p<0.001$) lower C18:3*n*-3 content for the intermediate BF, compared to the oxidative TB. The C18:3*n*-3 contents for the control and 1% CLA treatment were also significantly ($p<0.001$) higher for the glycolytic SM versus glycolytic LT, as well as the oxidative TB versus oxidative SS. The 0.5 and 1% CLA treatments showed significantly ($p<0.001$) lower C18:3*n*-3 contents for the glycolytic LT, compared to the intermediate BF. All dietary treatment groups had significantly ($p<0.001$) lower C18:3*n*-3 contents for the glycolytic SM than the oxidative TB (Table 4.22).

Eicosadienoic acid also showed a number of significant ($p<0.001$) effects for sampling position (Table 4.22). Again, the glycolytic LT muscle showed a significantly ($p<0.001$) lower content than the oxidative TB muscle (all treatments), glycolytic SM (control, 0.25%), oxidative SS (control, 0.25% CLA) and intermediate BF (control). Higher contents for the oxidative TB than the oxidative SS (control and 1% CLA) and glycolytic SM (control) were also noted. For C20:3*n*-3, the oxidative SS showed lower contents for a number of sampling positions, for different dietary treatments (Table 4.22).

Two important observations were made based on the results for the sampling position. Firstly, the glycolytic LT showed lower contents for C18:2, both the *cis*-9*,* *trans*-11 and the *trans*-10*,* *cis*-12 isomers of CLA, C18:3*n*-3 and C20:2. It has been noted earlier that the glycolytic LT predominantly had higher concentration of individual SFAs (Table 4.19), which could explain the lower contents of the individual PUFAs (Table 4.22). Secondly, the oxidative TB featured prominently as having higher contents for PUFAs. That is contrary to the findings of Hernandez *et al*. (1998), who found that the glycolytic LT muscle is associated with having higher values of PUFAs.

Table 4.23 is a summary of the tetraenoic, pentaenoic and hexaenoic PUFA compositions of the IMF from the gilts fed the four dietary treatments. Although the C20:4, C20:5 and C22:5 contents were all significantly ($p<0.001$) influenced by an increase in dietary CLA level (Table 4.23), no significant differences were observed for these FAs between corresponding muscles, for the different dietary treatment groups. The only significant effect was noted for C22:6, which showed lower contents for the oxidative SS from the 0.25% CLA treatment than the 1% CLA treatment (Table 4.23). The general trend was for decreased C20:4 and C20:5 contents and increased C22:5 and C22:6 contents, with increased dietary CLA level (Table 4.23).

As far as sampling position was concerned, a few significant differences were also observed (Table 4.23). The oxidative SS had significantly ($p<0.001$) lower C20:4 contents than the glycolytic SM (control, 0.25 and 1% CLA), intermediate BF and oxidative TB (control and 0.25% CLA), and glycolytic LT (0.25% CLA) (Table 4.23). Similarly, for C20:5, the oxidative SS had significantly

Table 4.23: Tetraenoic, Pentaenoic and Hexaenoic polyunsaturated fatty acid content (%) of intramuscular fat from gilts from the four diets.

Diet	Position	C20:4*c*5,8,11,14 (n-6)	C20:5*c*5,8,11,14,17 (*n*-3)	C22:5*c*7,10,13,16,19 (*n*-3)	C22:6*c*4,7,10,13,16,19 (*n*-3)
Control (n=12)	*M. semimembranosus*	2.00 ± 0.30^{f}	0.31 ± 0.12^{abc}	0.38 ± 0.10^{abcd}	0.65 ± 0.11^{bcde}
	M. longissimus thoracis	1.60 ± 0.45^{bcdef}	0.24 ± 0.08^{abc}	0.35 ± 0.07^{abcd}	0.56 ± 0.12^{abcd}
	M. biceps femoris	1.72 ± 0.72^{cdef}	0.30 ± 0.13^{abc}	0.39 ± 0.16^{abcd}	0.68 ± 0.20^{cde}
	M. triceps brachi	2.06 ± 0.47^{f}	0.32 ± 0.11^{bc}	0.50 ± 0.13^{d}	0.64 ± 0.15^{bcde}
	M. supra spinatus	0.87 ± 0.40^{ab}	0.14 ± 0.08^{a}	0.25 ± 0.09^{a}	0.37 ± 0.11^{ab}
0.25% CLA (n =12)	*M. semimembranosus*	1.95 ± 0.40^{ef}	0.34 ± 0.12^{bc}	0.43 ± 0.17^{abcd}	0.74 ± 0.32^{cde}
	M. longissimus thoracis	1.64 ± 1.00^{bcdef}	0.33 ± 0.16^{bc}	0.44 ± 0.18^{abcd}	0.70 ± 0.31^{cde}
	M. biceps femoris	1.57 ± 0.69^{bcdef}	0.30 ± 0.13^{abc}	0.40 ± 0.15^{abcd}	0.58 ± 0.29^{abcde}
	M. triceps brachi	1.69 ± 0.43^{cdef}	0.27 ± 0.08^{abc}	0.46 ± 0.12^{bcd}	0.59 ± 0.17^{abcde}
	M. supra spinatus	0.74 ± 0.37^{a}	0.14 ± 0.07^{a}	0.27 ± 0.09^{ab}	0.33 ± 0.15^{a}
0.5% CLA (n=12)	*M. semimembranosus*	1.75 ± 0.50^{cdef}	0.35 ± 0.11^{bc}	0.39 ± 0.10^{abcd}	0.69 ± 0.14^{cde}
	M. longissimus thoracis	1.27 ± 0.65^{abcdef}	0.33 ± 0.17^{bc}	0.45 ± 0.19^{bcd}	0.72 ± 0.24^{cde}
	M. biceps femoris	1.36 ± 0.59^{abcdef}	0.30 ± 0.18^{abc}	0.41 ± 0.18^{abcd}	0.64 ± 0.25^{bcde}
	M. triceps brachi	1.66 ± 0.51^{bcdef}	0.30 ± 0.12^{abc}	0.44 ± 0.09^{abcd}	0.67 ± 0.16^{bcde}
	M. supra spinatus	0.98 ± 0.51^{abc}	0.17 ± 0.11^{ab}	0.29 ± 0.09^{abc}	0.44 ± 0.19^{abc}
1% CLA (n=12)	*M. semimembranosus*	1.96 ± 0.52^{ef}	0.38 ± 0.15^{c}	0.50 ± 0.10^{d}	0.88 ± 0.17^{e}
	M. longissimus thoracis	1.40 ± 0.54^{abcdef}	0.34 ± 0.15^{bc}	0.47 ± 0.17^{cd}	0.78 ± 0.25^{de}
	M. biceps femoris	1.20 ± 0.39^{abcde}	0.22 ± 0.15^{abc}	0.41 ± 0.10^{abcd}	0.74 ± 0.21^{cde}
	M. triceps brachi	1.83 ± 0.60^{def}	0.35 ± 0.08^{bc}	0.54 ± 0.11^{d}	0.81 ± 0.22^{de}
	M. supra spinatus	1.07 ± 0.45^{abcd}	0.24 ± 0.08^{abc}	0.38 ± 0.13^{abcd}	0.67 ± 0.25^{bcde}
Significance level		$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

Means with different superscripts in the same column differ significantly.

(p<0.001) lower contents than the oxidative TB (control), and the glycolytic SM and oxidative LT (0.25% CLA). Also for C22:5 the oxidative SS had significantly (p<0.001) lower content than the oxidative TB (control). The same was observed for C22:6, where lower contents were observed for the oxidative SS than the oxidative BF (control), glycolytic SM (0.25% CLA) and glycolytic LT (0.25% CLA) (Table 4.23).

Results clearly indicate that the oxidative SS had a statistically significant (p<0.001) increase in C22:6 content, with increased dietary CLA content from 0.25 to 1% (Table 4.23). As far as sampling position is concerned, SS had significantly lower C20:4, C20:5, C22:5 and C22:6 contents than other muscles within the control treatment. The SS sample always had numerically lower contents of C20:4, C20:5, C22:5 and C22:6, compared to all other muscles within all the other dietary treatments (Table 4.23). This low value, observed for an individual PUFA from an oxidative muscle, was in contrast with the findings of Hernandez *et al*. (1998), who reported an elevated PUFA content in the oxidative muscles. The muscle incorporating the highest content of CLA (combination of both isomers) was the oxidative TB, while the lowest content was found in the glycolytic LT. This was partly in contrast and partly in agreement with the results for the subcutaneous fat, where a tendency was observed for sampling positions on the dorsal (A, B, C) and lateral (D) sides of the carcass to have higher CLA contents. From the results of the subcutaneous fat and the IMF, it appeared as if the most CLA was deposited on the lateral sides of the carcass, namely position D and the TB.

The only PUFAs that exhibited statistically significant (at least p<0.05) interactions between dietary treatment and sampling position, were the two CLA isomers (Table 4.17). This can be ascribed to the different expressions of the two CLA isomers in the different sampling positions, due to different dietary treatments. At 0.25% CLA no significant differences were observed between sampling position for both CLA isomers, whereas at higher inclusion levels, significantly (p<0.001) different sampling position differences were observed.

As mentioned in the section on subcutaneous fat, only C18:2 has a fat quality requirement, ranging from < 15 % (Ellis & Isbell, 1926b; Houben & Krol, 1980; Enser, 1983; Wood, 1983; Whittington *et al*., 1986), to between 12 – 15% (Girard *et al.*, as cited by Fischer, 1989b). All IMF samples from the present study had C18:2 contents < 15%, however, some were as low as 8.84% (SS from the 0.5% CLA treatment) (Table 4.22). The C18:2 content of muscles from pigs receiving CLA supplemented diets were always lower than those of control pigs (Table 4.22). This implies that CLA supplemented pigs also produced IMF more suitable for use in processed meat products.

Fatty acid ratios with nutritional and health implications

Table 4.24 is a summary of the FA ratios with nutritional and health implications. Dietary treatment had a significant (at least p<0.05) effect on all ratios, except the PUFA/SFA ratio (Table 4.17). Increased dietary CLA level resulted in significant (p<0.001) increases in SFAs and AI's, and significant (p<0.001) decreases in MUFA, UFA and MUFA/SFA contents. According to Table 4.17, sampling position had a significant (p<0.001) effect on all the ratios. No statistically significant differences were found for the interaction between dietary treatments and sampling position, for any of the FA ratios with nutritional, health and fat quality implications (Table 4.17).

According to Table 4.24, the control group showed significantly (p<0.001) lower SFA content for all five muscles than the 1% CLA treatment. The control group also had significantly (p<0.001) less SFA's than the 0.5% CLA treatment, for SM, BF and TB. M. *semimembranosus* and BF also showed significantly (p<0.001) lower SFA content for the control, compared to the 0.25% CLA treatment. Both LT and BF had significantly (p<0.001) lower SFA contents for the 0.25% CLA treatment than for the 1% CLA treatment. Only BF had significantly (p<0.001) less SFAs for the 0.5% CLA treatment than the 1% treatment (Table 4.24).

The increase in SFA content of the IMF with increased dietary CLA content in the diet, confirmed the findings of other researchers. A gradual increase in the level of CLA (0.12 0.25, 0.5, to 1.0%) in pigs' feed resulted in increased levels of SFAs in LT (Ramsay *et al*., 2001; Thiel-Cooper *et al*., 2001; Szymczyk, 2005). Supplementation at 0.25% CLA (Lo Fiego *et al*., 2005), 0.5% CLA (Corino *et al*., 2003), 1.5% CLA (King *et al*., 2004) and 2% CLA (Martín *et al.*, 2008b; Cordero *et al*., 2010; Martin *et al*., 2011) also increased total SFAs in LT (Han *et al*., 2011). Dietary CLA at 1 % (Eggert *et al*., 1998; Eggert *et al*., 2001; Averette Gatlin *et al.*, 2002b) resulted in loins containing more SFAs. With feeding 1.25% CLA, it was also found that SFAs increased linearly in the loin tissue (Wiegand *et al*., 2002). From a health point of view, it is recommended to reduce SFA content in meat (Siri-Tarino *et al*., 2010). However, from a fat quality point of view, a SFA content > 41 % (Hauser & Prabucki, 1990) has been proposed for good quality fat. In the present study, LT (0.5 and 1% CLA), BF (1% CLA), TB (1% CLA) and SS (1% CLA) conformed to this standard (Table 4.24). No muscle from the control pigs had IMF > 40%. This, again, is an important finding and implies that dietary CLA supplementation results in IMF of better quality, from a meat technology point of view.

The fact that IMF at some positions on the carcass had significantly (p<0.001) higher SFA content than other, is also important (Tables 4.19 and 4.24). For the control and 1% CLA treatment, the SFA contents were significantly (p<0.001) higher for the glycolytic LT than the glycolytic SM.

Table 4.24: Fatty acid ratios with nutritional, health and fat quality implications of intramuscular fat from gilts from the four diets.

Diet	Position	SFA	MUFA	UFA	MUFA/SFA	PI	PUFA	Athero-genicity Index	PUFA/SFA	*n*-6	*n*-3	*n*-6/*n*-3
Control (n=12)	*M. semimembranosus*	37.35 ± 0.79^{ab}	45.87 ± 1.77^{cdefgh}	62.65 ± 0.79^{hi}	1.23 ± 0.06^{gh}	32.55 ± 3.86^{cde}	16.78± 1.53^{bcdef}	0.46 ± 0.02^{abc}	0.45 ± 0.04^{bcdef}	14.88± 1.21^{bcdefgh}	1.90 ± 0.35^{abcd}	8.00 ± 1.04^{abcd}
	M. longissimus thoracis	39.48 ± 0.62^{cdefg}	47.23 ± 2.28^{efgh}	60.53 ± 0.62^{cdiefg}	1.20 ± 0.06^{fgh}	26.57 ± 4.80^{abc}	13.30 ± 2.29^{abc}	0.50 ± 0.02^{cde}	0.34 ± 0.06^{abc}	11.70 ± 2.06^{abc}	1.60 ± 0.27^{abcd}	7.35 ± 0.66^{abc}
	M. biceps femoris	36.40 ± 1.07^{a}	47.53 ± 3.34^{fgh}	63.60 ± 1.07^{i}	1.31 ± 0.12^{h}	31.23 ± 7.21^{bcde}	16.07 ± 2.95^{abcdef}	0.43 ± 0.02^{a}	0.44 ± 0.08^{bcdef}	14.18 ± 2.51^{abcdefgh}	1.90 ± 0.49^{abcd}	7.66 ± 1.09^{abcd}
	M. triceps brachi	37.57 ± 1.18^{abc}	43.10 ± 2.66^{abcd}	62.43 ± 1.18^{ghi}	1.15 ± 0.10^{defg}	35.91 ± 5.55^{cde}	19.33 ± 2.26^{ef}	0.44 ± 0.02^{ab}	0.52 ± 0.06^{f}	17.20 ± 1.88^{gh}	2.14 ± 0.46^{d}	8.25 ± 1.12^{bcd}
	M. supra spinatus	39.06 ± 1.19^{bcde}	49.38 ± 2.51^{h}	60.94 ± 1.19^{efgh}	1.27 ± 0.09^{gh}	20.41 ± 4.81^{a}	11.56 ± 2.36^{a}	0.48 ± 0.02^{abcd}	0.30 ± 0.06^{a}	10.37 ± 2.08^{a}	1.18 ± 0.30^{ab}	8.88 ± 1.04^{cd}
0.25% CLA (n=12)	*M. semimembranosus*	39.66 ± 1.68^{defg}	43.00 ± 2.64^{abc}	60.34 ± 1.67^{cdef}	1.09 ± 0.09^{bcdef}	33.84 ± 6.30^{cde}	17.34 ± 2.84^{cdef}	0.50 ± 0.04^{cde}	0.44 ± 0.08^{bcdef}	15.27 ± 2.48^{cdefgh}	2.08 ± 0.55^{cd}	7.63 ± 1.58^{abcd}
	M. longissimus thoracis	40.61 ± 2.04^{defgh}	45.03 ± 3.18^{cdefg}	59.39 ± 2.04^{bcdef}	1.11 ± 0.06^{cdef}	29.70 ± 11.04^{abcde}	14.36 ± 4.70^{abcd}	0.52 ± 0.06^{def}	0.36 ± 0.13^{abcd}	12.40 ± 3.94^{abcde}	1.97 ± 0.78^{cd}	6.63 ± 1.06^{ab}
	M. biceps femoris	38.88 ± 2.40^{bcd}	45.63 ± 2.12^{cdefgh}	61.13 ± 2.40^{fgh}	1.18 ± 0.09^{efg}	29.47 ± 7.87^{abcde}	15.49 ± 3.08^{abcdef}	0.49 ± 0.06^{bcd}	0.40 ± 0.10^{abcdef}	13.68 ± 2.56^{abcdefgh}	1.82 ± 0.60^{abcd}	7.92 ± 1.67^{abcd}
	M. triceps brachi	39.18 ± 1.74^{bcde}	43.00 ± 2.77^{abc}	60.88 ± 1.81^{efgh}	1.10 ± 0.08^{bcdef}	32.43 ± 6.56^{bcde}	17.89 ± 3.38^{def}	0.48 ± 0.04^{abcd}	0.46 ± 0.10^{cdef}	15.96 ± 2.96^{efgh}	1.92 ± 0.45^{bcd}	8.40 ± 0.72^{bcd}
	M. supra spinatus	40.19 ± 1.84^{defgh}	48.07 ± 2.21^{gh}	59.81 ± 1.84^{bcdef}	1.20 ± 0.10^{fgh}	19.91 ± 3.71^{a}	11.74 ± 1.72^{a}	0.53 ± 0.05^{defg}	0.29 ± 0.05^{a}	10.57 ± 1.45^{a}	1.17 ± 0.30^{a}	9.36 ± 1.56^{d}
0.5% CLA (n =12)	*M. semimembranosus*	40.17 ± 0.93^{defgh}	43.74 ± 3.03^{bcdef}	59.83 ± 0.93^{bcdef}	1.09 ± 0.08^{bcdef}	31.54 ± 6.17^{bcde}	16.08 ± 3.09^{abcdef}	0.53 ± 0.02^{defg}	0.40 ± 0.08^{abcdef}	14.12 ± 2.75^{abcdefgh}	1.96 ± 0.42^{cd}	7.28 ± 0.97^{abc}
	M. longissimus thoracis	41.40 ± 1.10^{fghi}	44.61 ± 3.45^{cdefg}	58.61 ± 1.10^{abcd}	1.08 ± 0.08^{bcde}	28.31 ± 8.33^{abcde}	14.00 ± 4.09^{abcd}	0.55 ± 0.03^{efgh}	0.34 ± 0.10^{abc}	12.05 ± 3.46^{abcd}	1.95 ± 0.66^{cd}	6.36 ± 0.91^{a}
	M. biceps femoris	39.40 ± 1.27^{cdef}	45.54 ± 3.28^{cdefgh}	60.60 ± 1.27^{defg}	1.16 ± 0.08^{defg}	28.83 ± 8.78^{abcde}	15.06 ± 3.95^{abcde}	0.51 ± 0.03^{cdef}	0.38 ± 0.11^{abcde}	13.21 ± 3.30^{abcdef}	1.85 ± 0.70^{abcd}	7.52 ± 1.24^{abc}
	M. triceps brachi	39.65 ± 0.91^{defg}	42.69 ± 2.81^{abc}	60.35 ± 0.91^{cdef}	1.08 ± 0.07^{bcde}	32.69 ± 6.42^{cde}	17.66 ± 3.04^{cdef}	0.50 ± 0.02^{cde}	0.45 ± 0.08^{bcdef}	15.66 ± 2.67^{defgh}	1.99 ± 0.44^{cd}	8.00 ± 1.17^{abcd}
	M. supra spinatus	40.33 ± 0.96^{defgh}	47.09 ± 2.98^{defgh}	59.68 ± 0.96^{bcdef}	1.17 ± 0.08^{efg}	22.52 ± 6.35^{ab}	12.59 ± 2.92^{ab}	0.53 ± 0.03^{defg}	0.31 ± 0.07^{a}	11.25 ± 2.51^{ab}	1.34 ± 0.46^{abc}	8.86 ± 1.92^{cd}
1% CLA (n=12)	*M. semimembranosus*	40.65 ± 1.21^{defgh}	40.09 ± 2.25^{ab}	59.35 ± 1.21^{bcdef}	0.99 ± 0.05^{ab}	37.29 ± 5.67^{e}	19.27 ± 2.91^{ef}	0.53 ± 0.04^{defgh}	0.48 ± 0.08^{def}	16.97 ± 2.68^{fgh}	2.30 ± 0.42^{d}	7.53 ± 1.41^{abc}
	M. longissimus thoracis	42.88 ± 1.26^{i}	42.84 ± 2.85^{abc}	57.12 ± 1.26^{a}	1.00 ± 0.06^{abc}	29.46 ± 8.15^{abcde}	14.29 ± 3.68^{abcd}	0.59 ± 0.05^{h}	0.34 ± 0.09^{ab}	12.31 ± 3.11^{abcde}	1.98 ± 0.62^{cd}	6.40 ± 0.82^{a}
	M. biceps femoris	41.48 ± 1.28^{ghi}	43.21 ± 2.12^{abcde}	58.52 ± 1.28^{abc}	1.04 ± 0.07^{abcd}	28.73 ± 5.82^{abcde}	15.31 ± 2.25^{abcdef}	0.56 ± 0.04^{fgh}	0.37 ± 0.06^{abcde}	13.52 ± 1.81^{abcdefg}	1.79 ± 0.52^{abcd}	7.87 ± 1.38^{abcd}
	M. triceps brachi	41.00 ± 1.59^{efghi}	39.24 ± 2.57^{a}	59.00 ± 1.59^{abcde}	0.96 ± 0.06^{a}	36.94 ± 7.05^{de}	19.77 ± 3.39^{f}	0.53 ± 0.04^{defg}	0.49 ± 0.09^{ef}	17.49 ± 2.97^{h}	2.28 ± 0.49^{d}	7.79 ± 0.91^{abcd}
	M. supra spinatus	42.11 ± 1.43^{hi}	43.31 ± 3.53^{bcde}	57.89 ± 1.43^{ab}	1.03 ± 0.09^{abc}	27.07 ± 7.67^{abcd}	14.58 ± 3.65^{abcd}	0.58 ± 0.04^{gh}	0.35 ± 0.09^{abc}	12.87 ± 3.13^{abcde}	1.71 ± 0.56^{abcd}	7.73 ± 1.05^{abcd}
Significance level		$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

Means with different superscripts in the same column differ significantly.

For the control, the glycolytic LT also had significantly higher SFAs than the intermediate BF, while the intermediate BF was significantly lower in SFA content than the oxidative SS (Table 4.24). Comparing these muscles with the set limit of > 41% SFA (Hauser & Prabucki, 1990), the glycolytic LT (0.5 and 1% CLA), intermediate BF (1% CLA), oxidative TB (1% CLA) and SS (1% CLA) conformed to this standard (Table 4.24). The only muscle form the 1% CLA treatment that did not meet this limit was SM, at a SFA content of 40.65%. This implies than IMF from the other four muscles may, e.g. be more suitable for the manufacture of products like salami and bacon, which is more sensitive to fat quality.

The MUFA content of IMF decreased significantly ($p<0.001$) with increased concentration of CLA in the diet (Table 4.24). Four of the five muscles, SM, LT, BF and SS, from the control treatment, had significantly ($p<0.001$) higher MUFA content than the corresponding muscles from the 1% CLA treatment (Table 4.24). The oxidative SS showed a significantly ($p<0.001$) higher MUFA content for the 0.25% CLA treatment than the 1% CLA treatment (Table 4.24).

Sampling position demonstrated a statistically significant ($p<0.001$) effect on MUFA content (Table 4.17 and Table 4.24). For all four dietary treatments, the oxidative SS had significantly ($p<0.001$) higher MUFA content than the oxidative TB. The control dietary treatment group also showed a significantly ($p<0.001$) lower MUFA value for the oxidative TB than both the glycolytic LT and intermediate BF. The oxidative SS for the 0.25% CLA treatment also had a significantly ($p<0.001$) higher content than the glycolytic SM and the oxidative TB (Table 4.24).

Other researchers also demonstrated that a gradual increase in the level of CLA (0.12, 0.25, 0.5 to 1.0%) in pigs' feed, also resulted in decreased levels of MUFAs in the LT (Ramsay *et al*., 2001; Thiel-Cooper *et al*., 2001; Szymczyk, 2005). Furthermore, supplementation at 0.25% CLA (Lo Fiego *et al*., 2005), 0.5% CLA (Corino *et al*., 2003), 1.5% CLA (King *et al*., 2004) and 2% CLA (Martín *et al*., 2008b; Cordero *et al*., 2010; Martin *et al*., 2011) also lowered MUFAs in the LT (Han *et al*., 2011).

From a health perspective consumers are advised to increase the MUFA content of meat, as mentioned earlier (Cordain *et al.,* 2005). For good technological fat quality, a MUFA content < 57% has been proposed (Lizardo *et al*., 2002). Intramuscular fat from all sampling positions, from all the CLA dietary treatments, conformed to this requirement. Similarly to the results for the subcutaneous fat, the IMF from the control group also conformed to this limit. However, the MUFA content from the IMF samples from all the CLA treatment groups, was still significantly ($p<0.001$) lower than that of the control. Thus, CLA supplementation also results in IMF of better quality from a MUFA content point of view. The significant differences in MUFA content between different sampling positions must also be taken in consideration, when assessing the suitability of fat for its use in various processed meat products.

For UFAs, all muscles from the control treatment had significantly (p<0.001) higher UFA content than the corresponding muscles from the 1% CLA treatment. For the SM, BF and TB, the control also had significantly (p<0.001) higher UFA contents than the 0.5% CLA treatment. The SM and BF also showed significantly (p<0.001) higher UFA contents for the control than the 0.25% CLA treatment. For the LT and BF, the UFA contents for the 0.25% CLA treatment were significantly (p<0.001) higher than the 1% CLA treatment. Only the BF's UFA content was significantly (p<0.001) higher for the 0.5% CLA treatment than the 1% CLA treatment (Table 4.24).

For both the control and 1% CLA treatment, the glycolytic LT had significantly (p<0.001) lower UFA contents than the glycolytic SM. The glycolytic LT from the control also had a significantly (p<0.001) lower UFA content than the intermediate BF (Table 4.24). These results are in agreement with other studies, where dietary CLA at 1 % (Eggert *et al*., 1998; Eggert *et al*., 2001; Averette Gatlin *et al.*, 2002b) resulted in loins containing less UFAs.

To improve their health, consumers are advised to increase the intake of UFAs (Zock, 2006). Again, from a meat technology perspective, the opposite is true. As mentioned earlier, the UFA content for good quality fat has to be < 59 % (Prabucki, 1991). Only four muscles, the LT (0.5 and 1% CLA), BF (1% CLA) and SS (1% CLA) adhered to this standard (Table 4.24). The linear increase in the CLA concentration of the IMF with increased dietary CLA content caused a decrease in UFA content, which can be considered a negative from a health point of view (Table 4.24). This negative effect health effect is however counteracted by the increase in CLA, with positive health implications. Significant sampling position differences also had important fat quality implications. A good example is the glycolytic LT from the 0.25% CLA treatment, where four of the five muscles had UFA values > 59 % (poor fat quality), with LT having an UFA content of 59.39%, approaching the proposed cut-off point of 59 % for good fat quality (Table 4.24).

For MUFA/SFA ratio, all muscles from the control had significantly (p<0.001) higher MUFA/SFA ratios than the corresponding muscles from the 1% CLA treatment. Furthermore, the SM and BF also had significantly (p<0.001) higher MUFA/SFA contents for the control than the 0.25 and 0.5% CLA treatments. The LT showed a significantly (p<0.001) higher ratio for the control than the 0.5 and 1 % CLA treatments. The 0.25% CLA treatment had significantly (p<0.001) higher MUFA/SFA ratios for the BF, TB and SS, compared to the 1% CLA treatment. For the 0.5% CLA treatment, only the TB and SS showed significantly (p<0.001) higher MUFA/SFA ratios than the 1% CLA treatment (Table 4.24).

Only one significant (p<0.001) effect was observed for sampling position, within dietary treatment. The control had a significantly (p<0.001) lower MUFA/SFA ratio for the intermediate

BF than the oxidative TB (Table 4.24). Intarapichet *et al*. (2008) found a lower MUFA/SFA ratio in the LT, after feeding pigs 0.5 and 1% CLA supplements.

As mentioned earlier, a higher MUFA/SFA ratio would be beneficial for IMF, from a human health and nutritional point of view (Cordain *et al*., 2005; Zock, 2006), whereas a lower MUFA/SFA ratio would be better from a fat quality point of view (Lea *et al*., 1970). The lower MUFA/SFA ratio of the IMF, observed with increased dietary CLA content (Table 4.24), confirmed the conclusions made from the previously discussed ratios, that dietary CLA supplementation also improve IMF quality. It was previously also noted that decreased MUFA/SFA ratios are associated with increased fat firmness (Lea *et al*., 1970), which is considered as beneficial for fat quality. The statistically significant ($p<0.001$) difference of MUFA/SFA ratio between sampling positions, within dietary treatments, is also important from a fat quality viewpoint.

The PI, *n*-3, *n*-6/*n*-3, PUFAs and *n*-6 contents were all significantly ($p<0.05$) influenced by dietary treatment (Table 4.17). Significance levels were generally low, but the general trend was for increased PI, *n*-3, *n*-6/*n*-3, PUFAs and *n*-6 content, with increased dietary CLA content.

For the control, 0.25 and 1% CLA treatments, the PI of the oxidative SS had significantly ($p<0.001$) lower values than the glycolytic SM. For the control, 0.25 and 0.5% CLA treatments, the oxidative SS also showed significantly ($p<0.001$) lower PIs than the oxidative TB. The oxidative SS's PI for the control was also lower than the oxidative TB, intermediate BF and glycolytic SM (Table 4.24).

As mentioned in the discussion on the subcutaneous fat, no specific standard is available for PI. Most of the muscles showed a tendency of lowering in PIs at the CLA introduction level of 0.25%. At 0.5% CLA, PIs increased, decreased or remained the same, depending on the type of muscle. At the concentration of 1% CLA, PIs increased dramatically, to values even higher than that of IMF, with no CLA. This implies that meat products manufactured from meat with increased levels of CLA, will not necessarily be more resistant to oxidative breakdown than those manufactured from meat with no CLA or lower CLA levels. The same argument is valid for the significant ($p<0.001$) differences between sampling positions, within dietary treatments. The lower PI of the SS implies that meat products manufactured from IMF from that muscle, will be more stable than products manufactured with fat from other muscle areas.

Investigators have shown that CLA functioned more as a pro-oxidant (Chen, Chan, Kwan, & Zhang, 1997). Van den Berg *et al*. (1995) showed that CLA did not act as an efficient radical scavenger in any way comparable to vitamin E or BHT. Dietary CLA shifted the whole FA composition to the more saturated side, thus making meats from animals fed CLA, less susceptible to lipid oxidation. Banni, Day, Evans, Corongiu and Lombardi (1995) indicated that there was no antioxidant effect for CLA. In later years, Flintoff-Dye and Omaye (2005) suggested that CLA

isomers did not act as efficient antioxidants *in vitro*. They found that CLA acted as a pro-oxidant, antioxidant, and subsequently reverted to a pro-oxidant again as enrichment levels increased.

For all four dietary treatments, there were significantly ($p<0.001$) higher PUFA contents for the oxidative TB, versus the oxidative SS (Table 4.24). The PUFA contents of the glycolytic SM from the control, 0.25 and 1% CLA treatments, were significantly ($p<0.001$) higher than the oxidative SS. Furthermore, the glycolytic LT's PUFA contents for the control and 1% CLA treatment were significantly ($p<0.001$) lower than the oxidative TB. The PUFA content of the glycolytic SM from the 1% CLA treatment, was significantly ($p<0.001$) higher than the glycolytic LT and oxidative SS (Table 4.24). Ramsay *et al*. (2001), Thiel-Cooper *et al*. (2001) and Szymczyk (2005) found no change in PUFAs from muscle, following a gradual increase in the level of CLA (0.12, 0.25, 0.5, to 1.0%) in pigs' feed.

For good health, consumers are advised to increase PUFA intake (Honkavaara, 1989; Levnedmiddelstyrelsen, as cited by Madsen *et al*., 1992). For good fat quality, PUFA content of IMF must be reduced. The maximum proposed levels of PUFA for good fat quality, range from < 12% (Prabucki, as cited by Houben & Krol, 1983), to < 13% (Wenk *et al*., as cited by Warnants *et al*., 1996) to < 15% (Houben & Krol, 1983). For this study, values ranging from 11.56% (SS, control) to as high as 19.77% (TB, 1% CLA) were measured (Table 4.24). Although dietary treatment had a low statistically significant ($p<0.05$) effect on the PUFA content of IMF, the direction of this effect, with increased dietary CLA content, was not always very clear. Depending on the muscle type, increase in CLA concentration led to no change, decreased or increased PUFA contents (Table 4.24). Except the BF, all the muscles from the 1% CLA treatment showed higher PUFAs than the control. From a PUFA point of view, the IMF quality did not improved with increased levels of dietary CLA. The statistically significant ($p<0.001$) differences in PUFA content between sampling positions, are also of importance. Again, the oxidative SS, this time from the control group, had lower PUFA content and therefore better IMF quality than the other sampling positions.

For AI, all five muscles from the control had significantly ($p<0.001$) lower AI's than the muscles from the 1% CLA treatment. The control also had significantly ($p<0.001$) lower AI's for the SM, BF and TB, compared to the corresponding muscles from the 0.5% CLA treatment. For the BF, the AI from the control group was also significant ($p<0.001$) lower than the 0.25% CLA treatment. The 0.25% CLA treatment had significantly ($p<0.001$) lower AI's for the LT and BF, in comparison to the 1% CLA treatment (Table 4.24).

For AI, the glycolytic LT from the control and 1% CLA treatment was higher than the oxidative TB. Also, the glycolytic LT had a significantly ($p<0.001$) higher AI for the control group than the intermediate BF (Table 4.24).

With an increase in the CLA concentration in the diets, there was a numerical linear increase in the AI contents, for all sampling positions, between dietary treatments (Table 4.24). This can be ascribed to the statistically significant ($p<0.001$) increase in C16:0 of the IMF (Table 4.19), with increased dietary CLA content. An increase in AI implies that dietary CLA supplementation had a negative effect on the health properties of IMF, because of the increase in SFAs. This must be weighed against the other positive effects of elevated CLA levels in meat.

For PUFA/SFA ratio, dietary treatment showed no significant effects (Table 4.17). All four treatments had a significantly ($p<0.001$) lower ratio for the SS, compared to the: glycolytic SM (control, 0.25 and 1% CLA); intermediate BF (control); and oxidative TB (control, 0.25, 0.5 and 1% CLA) (Table 4.24). The glycolytic LT also had a significantly ($p<0.001$) lower PUFA/SFA ratio than the oxidative TB from the control and 1% CLA treatment. For the 1% CLA treatment, the glycolytic LT was also significantly ($p<0.001$) lower for PUFA/SFA ratio than the glycolytic SM (Table 4.24). These results are in agreement with the results of Intarapichet *et al*. (2008), who also found a lower PUFA:SFA ratio in the loin, after feeding pigs 0.5 and 1% CLA supplements.

Consumers are generally advised to increase the PUFA/SFA ratio of their diets (Enser *et al*., 1996). The lower limit for the PUFA/SFA ratio ranges from 0.23-0.45 (Phelps, 1991), to 0.45-0.50 (Honkavaara, 1989; Levnedmiddelstyrelsen, as cited by Madsen *et al*., 1992), to even as high as 0.6-0.7 (Verbeke *et al*., 1999), as the minimum value for acceptable health properties for animal fat. The fact that diet had no effect on the PUFA/SFA ratio of the IMF, implied that the health properties of IMF did not deteriorate or improve. The ratio's in the present study ranged from 0.29 (SS, 0.25% CLA) to 0.52 (TB, control). Also, the PUFA/SFA ratios observed in the present study were generally on the low side (mostly in the 0.23-0.45 category), meaning that IMF obtained from the present study could be considered as rather unhealthy, from a PUFSA/SFA point of view.

For the control dietary treatment group, the *n*-6 value from the oxidative SS was significantly ($p<0.001$) lower than the glycolytic SM and oxidative TB (Table 4.24). The oxidative TB's *n*-6 content was also significantly ($p<0.001$) higher than the glycolytic LT, for the same treatment. The 0.25% CLA treatment showed significantly ($p<0.001$) lower *n*-6 contents for the oxidative SS than the glycolytic SM and intermediate TB. Also, the 0.5% CLA treatment showed a significantly ($p<0.001$) lower content for the oxidative SS than the oxidative TB (Table 4.24). For the 1% CLA treatment, *n*-6 content was significantly ($p<0.001$) lower for the oxidative SS than the glycolytic SM and oxidative TB. Also for the 1% CLA treatment, the *n*-6 content was significantly ($p<0.001$) higher for the oxidative TB than the glycolytic LT and intermediate BF. The *n*-6 content was also significantly ($p<0.001$) higher for the glycolytic SM than the glycolytic LT, from the 1% CLA treatment (Table 4.24).

For the control and 0.25% CLA treatment, the oxidative SS had significantly ($p<0.001$) lower *n*-3 content than the oxidative TB. For the 0.25% CLA treatment, the oxidative SS also had a significantly ($p<0.001$) lower *n*-3 content than the glycolytic SM and glycolytic LT (Table 4.24). Intarapichet *et al*. (2008) also fed pigs 0.5 and 1% CLA supplements and found lower *n-6/n-3* ratios, in the loin.

No significant differences were found in *n*-6/*n*-3 ratio, for the same sampling position between different dietary treatments (Table 4.24). No significant differences were observed for *n*-6/*n*-3 ratio within the control and 1% CLA treatment, between sampling positions. Only the glycolytic LT had a significantly ($p<0.001$) lower *n*-6/*n*-3 ratio than the oxidative SS for the 0.25 and 0.5% CLA treatments.

In the past, as was mentioned in the discussion on the subcutaneous fat, the general recommendation was to increase PUFA content of food, to improve its health properties (Kaizer *et al*., 1989). Nowadays, the recommendation is to increase the *n*-3 PUFA and decrease the *n*-6 PUFA content of food, thereby reducing the *n*-6/*n*-3 ratio of fats for human consumption (Okuyama & Ikemoto, 1999). It was also noted that the safe *n*-6/*n*-3 ratio of 1:1 (Leaf & Weber, 1987) is not easily attainable. This ratio was changed by Okuyama (1997) to 2:1, while Verbeke *et al*. (1999) even lessened it to 6:1.

Because the *n*-6 and *n*-3 content of IMF from the present study was only influenced at a very low significance level ($p<0.05$) by dietary treatment, it is very difficult to establish the direction of the effect (Tables 4.17 and 4.24). Dietary CLA supplementation, therefore, did not result in the deterioration of the health properties of IMF, purely from a FA profile point of view. The *n*-6/*n*-3 ratios for the IMF, ranging from 6.40:1 to 9.36:1, were much lower than that of the subcutaneous fat (between 14.09:1 and 23.08:1). The lowest value for IMF (6.36:1) is very close to the recommended value of 6:1, proposed by Verbeke *et al.* (1999). The variation in *n*-6/*n*-3 ratio between sampling positions are also important. The *n*-6/*n*-3 ratio of LT was consistently lower than that of the other muscles, within the same dietary treatment group (Table 4.24). For the control, 0.25 and 0.5% CLA treatments, the LT had a significantly ($p<0.001$) lower *n*-6/*n*-3 ratio than the SS.

Two very distinct trends were noted from the results for the FA ratios with nutritional and health implications. Firstly, the glycolytic LT showed higher values for the SFA contents, which are in accordance with earlier results for individual SFAs (Table 4.19). As a result of this, LT also showed higher AI's, lower UFAs and lower PUFA/SFA ratios, compared to other muscles. *M. longissimus thoracis* also had lower contents for the tetraenoic, pentaenoic and hexaenoic acids (Table 4.23), thus resulting in the lower *n*-6/*n*-3 ratio. The second notable trend was the data for the oxidative SS. *M. supra spinatus* in the present study showed an atypical FA composition, i.e. lower

PUFAs, *n*-6 and *n*-3 contents, which are supported by the results shown in Table 4.23. It also had lower values for the tetraenoic, pentaenoic and haxaenoic FAs, thus contributing to the total lower PUFA content, *n*-6 and *n*-3 values for this muscle. From Table 4.20, it is also clear that SS had higher individual MUFAs, thus contributing to the higher total MUFA content. Leseigneur and Gandemer (1991) emphasised that variations in FA composition were not related to metabolic type. The more saturated FA profile of the SS muscle can probably be attributed to to its very high IMF content. The IMF content of the SS for all dietary treatments was higher than all the other muscles for all dietary treatments (Table 4.18). It is known that muscles with higher IMF content will have a more saturated FA profile (Sun *et al*., 2004).

Having lower PUFA contents would also explain the lower PUFA/SFA ratios shown by SS. The PUFA/SFA ratio was 0.45 for SM (glycolytic), 0.52 for TB (oxidative), 0.44 for BF (intermediate), 0.34 for LT (glycolytic) and 0.30 for SS (oxidative) for the control group. A PUFA/SFA ratio of 0.45 or slightly higher is preferred and considered an important nutritional benefit to the consumer (Enser *et al*., 1996). Taking this into consideration, the PUFA/SFA ratios for LT, TB and SS, from all four dietary groups, are all well below the required value of 0.45.

Fatty acid ratios with technological implications

Table 4.25 is a summary of the FA ratios of the IMF with technological implications. Dietary treatment had a statistically significant (at least $p<0.05$) effect on all ratios with technological implications, except for the tetraeonic, C18:0/C18:2, C16:0/C18:2, C18:2/C18:1 and DBI contents (Table 4.17). Sampling position had a statistically significant ($p<0.001$) effect on all FA acid ratios with technological implications (Table 4.17). No FA ratio showed any effect for the interaction of dietary treatment X sampling position (Table 4.17).

Although no significant differences in dienoic acid were observed for specific muscles between dietary treatments, the general trend for dienoic acid was an increase with increased dietary CLA content (Table 4.25). The oxidative SS was significantly ($p<0.001$) lower in dienoic acid content than the glycolytic SM (control and 0.25% CLA) and the oxidative TB (control, 0.25, 0.5 and 1% CLA groups). In turn, the oxidative TB was significantly ($p<0.001$) higher in dienoic acid than the glycolytic LT (control, 0.25 and 1% CLA). For the 1% CLA treatment, the oxidative TB was significantly ($p<0.001$) higher than the intermediate BF. The dienoic acid content of the 1% CLA treatment was also significantly ($p<0.001$) higher for the glycolytic SM than the glycolytic LT. According to Haüser and Prabucki (1990), fat must contain < 10 % dienoic FAs to qualify as fat of good quality. Intramuscular fat from only two muscles, SS from the control and 0.25% CLA treatment, could conform to this quality requirement (Table 4.25). All the other relatively high dienoic acid levels can be attributed to dietary CLA inclusion, which resulted in increased CLA

Table 4.25: Fatty acid ratios with technological implications of intramuscular fat from gilts from the four dietary treatments.

Diet	Position	Dienoic	Trienoic	Tetraenoic	Pentaenoic	Hexaenoic	Penta/Hexaenoic	C16:0+C18:0	C16:1+C18:1*c*9/ C16:0+C18:0	C18:0/C18:2	C16:0/C18:2	C18:2/C18:1	DBI
Control (n=12)	*M. semimembranosus*	12.84 ± 1.01^{bcdefg}	0.60 ± 0.07^{bcdef}	2.00 ± 0.30^{f}	0.69 ± 0.19^{abc}	0.65 ± 0.11^{bcde}	1.34 ± 0.30^{abcde}	35.64 ± 0.75^{ab}	1.27 ± 0.07^{ghi}	0.95 ± 0.13^{ab}	1.91 ± 0.13^{abcde}	0.33 ± 0.04^{abcdef}	88.71 ± 3.02^{def}
	M. longissimus thoracis	10.08 ± 1.60^{abc}	0.48 ± 0.08^{abcde}	1.60 ± 0.45^{bcdef}	0.59 ± 0.13^{abc}	0.56 ± 0.12^{abcd}	1.14 ± 0.23^{abcd}	37.77 ± 0.51^{cdef}	1.24 ± 0.07^{efghi}	1.31 ± 0.18^{bcd}	2.63 ± 0.52^{cdef}	0.25 ± 0.05^{ab}	81.48 ± 4.08^{abcde}
	M. biceps femoris	12.42 ± 1.85^{abcdefg}	0.55 ± 0.11^{abcdef}	1.72 ± 0.72^{cdef}	0.69 ± 0.26^{abc}	0.68 ± 0.20^{cde}	1.38 ± 0.41^{bcde}	34.82 ± 0.99^{a}	1.35 ± 0.12^{i}	0.97 ± 0.14^{ab}	1.97 ± 0.30^{abcdef}	0.31 ± 0.07^{abcdef}	88.48 ± 5.54^{cdef}
	M. triceps brachi	15.09 ± 1.50^{fg}	0.72 ± 0.12^{f}	2.06 ± 0.47^{f}	0.82 ± 0.23^{c}	0.64 ± 0.15^{bcde}	1.46 ± 0.36^{cde}	36.05 ± 1.16^{abc}	1.18 ± 0.10^{cdefg}	0.87 ± 0.12^{a}	1.61 ± 0.17^{a}	0.41 ± 0.07^{f}	91.64 ± 4.20^{f}
	M. supra spinatus	9.49 ± 1.74^{a}	0.43 ± 0.07^{ab}	0.87 ± 0.40^{ab}	0.39 ± 0.15^{a}	0.37 ± 0.11^{ab}	0.77 ± 0.25^{ab}	37.40 ± 1.17^{bcde}	1.31 ± 0.09^{hi}	1.44 ± 0.29^{d}	2.77 ± 0.52^{f}	0.22 ± 0.05^{a}	77.33 ± 4.21^{a}
0.25% CLA (n=12)	*M. semimembranosus*	13.29 ± 2.27^{cdefg}	0.60 ± 0.05^{cdef}	1.95 ± 0.40^{ef}	0.76 ± 0.24^{bc}	0.74 ± 0.32^{cde}	1.50 ± 0.50^{cde}	37.90 ± 1.63^{cdef}	1.12 ± 0.10^{bcdef}	1.00 ± 0.18^{abc}	2.00 ± 0.36^{abcdef}	0.36 ± 0.09^{cdef}	87.42 ± 5.76^{cdef}
	M. longissimus thoracis`	10.72 ± 3.14^{abcd}	0.53 ± 0.17^{abcde}	1.64 ± 1.00^{bcdef}	0.77 ± 0.34^{bc}	0.70 ± 0.31^{cde}	1.47 ± 0.65^{cde}	38.91 ± 1.88^{defg}	1.15 ± 0.07^{bcdef}	1.38 ± 0.59^{cd}	2.80 ± 1.31^{f}	0.28 ± 0.10^{abcd}	82.68 ± 10.48^{abcdef}
	M. biceps femoris	12.08 ± 1.97^{abcdef}	0.56 ± 0.13^{abcdef}	1.57 ± 0.69^{bcdef}	0.70 ± 0.27^{abc}	0.58 ± 0.29^{abcde}	1.28 ± 0.51^{abcde}	37.16 ± 2.13^{bcd}	1.22 ± 0.09^{efgh}	1.07 ± 0.20^{abcd}	2.17 ± 0.46^{abcdef}	0.30 ± 0.06^{abcdef}	84.75 ± 8.11^{abcdef}
	M. triceps brachi	14.25 ± 2.56^{efg}	0.63 ± 0.12^{ef}	1.69 ± 0.43^{cdef}	0.73 ± 0.20^{abc}	0.59 ± 0.17^{abcde}	1.32 ± 0.37^{abcde}	37.57 ± 1.65^{cde}	1.13 ± 0.09^{bcdef}	0.95 ± 0.19^{ab}	1.84 ± 0.43^{abc}	0.38 ± 0.09^{def}	87.33 ± 6.63^{cdef}
	M. supra spinatus	9.82 ± 1.33^{ab}	0.45 ± 0.07^{abcd}	0.74 ± 0.37^{a}	0.40 ± 0.14^{a}	0.33 ± 0.15^{a}	0.73 ± 0.26^{a}	38.37 ± 1.76^{defg}	1.24 ± 0.10^{fghi}	1.33 ± 0.19^{bcd}	2.77 ± 0.41^{f}	0.23 ± 0.04^{a}	75.99 ± 3.91^{a}
0.5% CLA (n =12)	*M. semimembranosus*	12.34 ± 2.39^{abcdef}	0.56 ± 0.12^{abcdef}	1.75 ± 0.50^{cdef}	0.74 ± 0.21^{abc}	0.69 ± 0.14^{cde}	1.43 ± 0.34^{cde}	38.27 ± 1.00^{defg}	1.13 ± 0.09^{bcdef}	1.05 ± 0.25^{abcd}	2.24 ± 0.49^{abcdef}	0.32 ± 0.08^{abcdef}	84.97 ± 5.17^{abcdef}
	M. longissimus thoracis	10.76 ± 3.17^{abcd}	0.47 ± 0.15^{abcde}	1.27 ± 0.65^{abcdef}	0.78 ± 0.35^{bc}	0.72 ± 0.24^{cde}	1.50 ± 0.54^{cde}	39.53 ± 1.10^{fgh}	1.12 ± 0.08^{bcde}	1.34 ± 0.52^{bcd}	2.80 ± 1.01^{f}	0.28 ± 0.11^{abcd}	80.82 ± 7.52^{abcde}
	M. biceps femoris	11.83 ± 2.83^{abcdef}	0.52 ± 0.15^{abcdef}	1.36 ± 0.59^{abcdef}	0.71 ± 0.37^{abc}	0.64 ± 0.25^{bcde}	1.35 ± 0.57^{bcde}	37.57 ± 1.23^{cde}	1.20 ± 0.08^{defgh}	1.11 ± 0.34^{abcd}	2.35 ± 0.67^{abcdef}	0.29 ± 0.10^{abcde}	83.59 ± 7.85^{abcdef}
	M. triceps brachi	13.97 ± 2.25^{defg}	0.62 ± 0.11^{def}	1.66 ± 0.51^{bcdef}	0.74 ± 0.19^{abc}	0.67 ± 0.16^{bcde}	1.41 ± 0.35^{cde}	37.94 ± 0.94^{cdef}	1.11 ± 0.08^{bcde}	0.95 ± 0.20^{ab}	1.90 ± 0.36^{abcd}	0.36 ± 0.08^{bcdef}	86.83 ± 5.49^{bcdef}
	M. supra spinatus	10.25 ± 2.06^{abc}	0.46 ± 0.12^{abcde}	0.98 ± 0.51^{abc}	0.46 ± 0.19^{ab}	0.44 ± 0.19^{abc}	0.89 ± 0.36^{abc}	38.48 ± 0.83^{defg}	1.21 ± 0.09^{efgh}	1.27 ± 0.24^{bcd}	2.72 ± 0.59^{def}	0.23 ± 0.07^{a}	77.81 ± 5.34^{ab}
1% CLA (n=12)	*M. semimembranosus*	14.99 ± 2.23^{efg}	0.55 ± 0.13^{abcdef}	1.96 ± 0.52^{ef}	0.88 ± 0.19^{c}	0.88 ± 0.17^{e}	1.76 ± 0.33^{e}	38.81 ± 1.06^{defg}	1.02 ± 0.06^{ab}	0.87 ± 0.10^{a}	1.82 ± 0.35^{abc}	0.38 ± 0.08^{def}	89.27 ± 5.54^{ef}
	M. longissimus thoracis	10.89 ± 2.57^{abcd}	0.41 ± 0.13^{a}	1.40 ± 0.54^{abcdef}	0.81 ± 0.31^{bc}	0.78 ± 0.25^{de}	1.58 ± 0.52^{de}	40.98 ± 1.11^{h}	1.04 ± 0.06^{ab}	1.31 ± 0.34^{bcd}	2.76 ± 0.84^{ef}	0.26 ± 0.08^{abc}	80.16 ± 7.56^{abcd}
	M. biceps femoris	12.31 ± 1.49^{abcdef}	0.44 ± 0.10^{abc}	1.20 ± 0.39^{abcde}	0.63 ± 0.24^{abc}	0.74 ± 0.21^{cde}	1.37 ± 0.43^{bcde}	39.56 ± 1.18^{fgh}	1.08 ± 0.07^{abcd}	1.07 ± 0.13^{abcd}	2.26 ± 0.39^{abcdef}	0.27 ± 0.05^{abcd}	81.51 ± 5.22^{abcde}
	M. triceps brachi	15.63 ± 2.57^{g}	0.61 ± 0.16^{cdef}	1.83 ± 0.60^{def}	0.89 ± 0.18^{c}	0.81 ± 0.22^{de}	1.70 ± 0.38^{de}	39.25 ± 1.49^{efgh}	0.99 ± 0.07^{a}	0.88 ± 0.17^{a}	1.76 ± 0.41^{ab}	0.40 ± 0.10^{ef}	88.93 ± 6.91^{def}
	M. supra spinatus	11.80 ± 2.71^{abcde}	0.42 ± 0.11^{a}	1.07 ± 0.45^{abcd}	0.62 ± 0.21^{abc}	0.67 ± 0.25^{bcde}	1.29 ± 0.46^{abcde}	40.14 ± 1.33^{gh}	1.07 ± 0.10^{abc}	1.15 ± 0.22^{abcd}	2.47 ± 0.57^{bcdef}	0.26 ± 0.09^{abc}	79.56 ± 6.78^{abc}
Signifance level		$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

Means with different superscripts in the same column differ significantly

content in the subcutaneous fat (Table 4.22). The significantly lower dienoic acid content of LT and SS, compared to other muscles, implies that IMF from the dorsal and lateral area had better fat quality than fat from the other muscles.

No significant differences in trienoic acid were observed for specific muscles between dietary treatments. The general trend was, however, for trienoic acid content to decrease with increased dietary CLA level (Table 4.25). The trienoic acids had significant ($p<0.001$) differences for sampling positions (Tables 4.17 and 4.25). For the control, 0.25 and 1% CLA treatments, the oxidative TB was significantly ($p<0.001$) higher in trienoic acid contents than the oxidative SS. For the control and 1% CLA treatment, the oxidative TB was also significantly ($p<0.001$) higher than the glycolytic LT. According to Haüser and Prabucki (1990), IMF must contain < 1 % trienoic FAs to qualify as IMF of good quality. Intramuscular fat from all dietary treatments and muscle types conformed to this quality requirement. The lower trienoic acid content of LT and SS means that this muscles had significantly ($p<0.001$) better fat quality, than some of the other muscles for three of the dietary treatments.

Although dietary treatment had no significant effect on tetraenoic acid content, there was a general trend for tetraenoic acid content to decrease with increased dietary CLA level (Tables 4.17 and 4.25). The tetraeonic acids showed significant ($p<0.001$) differences for sampling positions (Table 4.25). It was mainly the oxidative SS that showed significantly ($p<0.001$) lower levels than the glycolytic SM (control, 0.25 and 1% CLA), intermediate BF (control and 0.25% CLA), oxidative TB (control and 0.25%) and glycolytic LT (0.25% CLA) (Table 4.25). According to Haüser and Prabucki (1990), fat must contain < 0.5 % tetraenoic acid to qualify as fat of good technological quality. No muscle from any of the dietary treatments conformed to this quality requirement. Dietary treatment, therefore, had no effect on the IMF quality from a tetraenoic acid content point of view.

With the exception of SS, no significant differences in pentaenoic, hexaenoic and penta + hexaenoic acid contents were observed for specific muscles between dietary treatments. The general trend was, however, for the pentaenoic, hexaenoic and penta + hexaenoic contents to increase with increased dietary CLA level (Table 4.25). Pentaenoic acid demonstrated significant ($p<0.001$) differences for sampling positions. Only the oxidative SS (control and 0.25% CLA) was lower in pentaenoic acid contents than the oxidative TB (control group), glycolytic SM (0.25% CLA) and glycolytic LT (0.25% CLA).

The hexaenoic acids had significant ($p<0.001$) differences for both the sampling positions and dietary treatments ($p<0.001$) (Table 4.17). The oxidative SS (control and 0.25% CLA), again, was significantly ($p<0.001$) lower in hexaenoic acid contents than the intermediate BF (control), glycolytic SM (0.25% CLA) and glycolytic LT (0.25% CLA). The oxidative SS showed

significantly ($p<0.001$) lower hexaenoic values for the 0.25% CLA treatment, compared to the 1% CLA treatment (Table 4.25).

No fat quality parameters are available for pentaenoic and hexaenoic acids, individually. Haüser and Prabucki (1990) proposed a pentaenoic + hexaenoic FA content of 1% of the total FAs, as the maximum for good fat quality. These two FAs will therefore be considered together, similarly to the subcutaneous fat. The general trend for the penta- + hexaenoic acids was to increase with increased dietary CLA content. The oxidative SS was significantly ($p<0.001$) lower for this ratio than the oxidative TB, for the control group. For the 0.25% CLA treatment, the oxidative SS was also significantly ($p<0.001$) lower in penta- + hexaenoic FA content than the glycolytic SM and glycolytic LT (Table 4.25). Only IMF from the oxidative SS, from the control, 0.25 and 0.5% CLA treatments, conformed to the requirement of < 1 % penta- + hexaenoic acid (Haüser & Prabucki, 1990) (Table 4.25). The penta + hexaenoic acid content confirmed the findings for the previous ratios, that dietary CLA supplementation did not have a major positive effect on the IMF quality of pigs. It also demonstrates that some sampling positions have significantly better fat quality than other sampling positions, in this case, again the oxidative SS.

Although no specific value is attached to C16:0+C18:0 as a fat quality parameter, an increase in this ratio has been associated with fat firmness (Enser, 1984). The C16:0+C18:0 ratio showed significant ($p<0.001$) differences for dietary treatment and sampling position ($p<0.001$) (Tables 4.17 and 4.25). A statistically significant ($p<0.001$) increase in C16:0+C18:0 content was observed with increased dietary CLA content. All five muscles demonstrated a significantly ($p<0.001$) lower C16:0+C18:0 ratio for the control than the 1% CLA treatment. Additionally, the glycolytic SM and intermediate BF had lower C16:0+C18:0 ratios for the control than the 0.25 and 0.5% CLA treatments. For the glycolytic LT and intermediate BF, the 0.25% CLA treatment showed significantly lower C16:0+C18:0 ratios than the 1% CLA treatment. For the intermediate BF, the 0.5% CLA treatment was significantly ($p<0.001$) lower in C16:0+C18:0 content than the 1% CLA treatment (Table 4.25).

Significant ($p<0.001$) differences for C16:0+C18:0 ratios were also observed between muscles, within the same dietary treatment. For the control and 1% CLA treatment, the glycolytic SM was significantly ($p<0.001$) lower than the glycolytic LT for this ratio. The glycolytic LT from the control and 0.5% CLA treatment had significantly higher C16:0+C18:0 ratios than the intermediate BF. The control also had a higher C16:0+C18:0 ratio for the oxidative SS than the intermediate BF.

The increase in C16:0+C18:0 content with increase in dietary CLA level, imply that CLA supplementation will result in firmer IMF, which is advantageous from a meat technology point of view (Enser, 1984).

Another FA ratio that has been associated with fat firmness is C16:1+C18:1c9/C16:0+C18:0 (Lea *et al*., 1970). The C16:1+C18:1*c*9/C16:0+C18:0 was also significantly ($p<0.001$) influenced by dietary treatment and sampling position ($p<0.001$) (Tables 4.17 and 4.25). All five muscles had significantly ($p<0.001$) higher C16:1+C18:1*c*9/C16:0+C18:0 ratios for the control, compared to the 1% CLA treatment. The glycolytic SM and intermediate BF also had higher C16:1+C18:1*c*9/C16:0+C18:0 ratios for the control than the 0.25 and 0.5% CLA treatments. For the intermediate BF, oxidative TB and oxidative SS, the 0.25% CLA treatment showed significantly higher C16:1+C18:1*c*9/C16:0+C18:0 ratios than the 1% treatment. The oxidative TB and oxidative SS also showed significantly ($p<0.001$) higher C16:1+C18:1*c*9/C16:0+C18:0 ratios for the 0.5% CLA treatment, than the 1% CLA treatment (Table 4.25). Only one significant difference was observed for muscle type, within a specific dietary treatment. The oxidative TB showed a significantly lower C16:1+C18:1*c*9/C16:0+C18:0 ratio than the intermediate BF and oxidative SS, for the control dietary group (Table 4.25). The lower C16:1+C18:1c9/C16:0+C18:0 ratio of the CLA supplemented pigs also indicate improved IMF quality.

The C16:0/C18:2 ratio has also been associated with fat firmness (Enser, 1984). Dietary treatment had no effect on the intramuscular C16:0/C18:2 ratio, implying that C16:0/C18:2 ratio was not a good indicator of IMF firmness. Tables 4.17 and 4.25 do, however, show significant ($p<0.001$) differences in sampling position, within dietary treatment for the C16:0/C18:2 ratio. The oxidative TBs for all the treatments were significantly lower for this ratio, than the glycolytic LT, for all four dietary treatments. Furthermore, the oxidative TB's ratio was also significantly lower than the oxidative SS for the control and 0.25 % CLA treatment. The C16:0/C18:2 ratio was also lower for the glycolytic SM (control and 1% CLA), than the oxidative SS (control) and LT (1% CLA group) (Table 4.25). This sampling position effect implies that IMF from this sampling position had a lower fat quality than the other muscles.

According to Honkavaara (1989) and Enser *et al*. (1984), C18:0/C:18:2 ratio is the best measure of fat quality. With C18:0/C18:2 ratios of > 1.2 and < 1.2, the sensory consistency of fresh fat was firm and soft, respectively (Honkavaara, 1989). Enser *et al*. (1984) proposed a C18:0/C18:2 ratio > 1.47 for good fat quality. Although C18:0/C18:2 was not significantly influenced by dietary treatment, significant ($p<0.001$) differences were observed for sampling positions within specific dietary treatment (Tables 4.17 and 4.25). The glycolytic LT had significantly higher C18:0/C18:2 ratios for the control, 0.25 and 1% CLA treatments than the oxidative TB. The oxidative SS was higher in C18:0/C18:2 ratio, than the glycolytic SM, intermediate BF and oxidative TB for the control. For the 1% CLA treatment, the glycolytic SM had a lower C18:0/C18:2 ratio than the glycolytic LT. Within the control, 0.25 and 0.5% CLA treatments, LT and SS conformed to the minimum proposed ratio of 1.2, but not to the value of 1.47. In the 1 % CLA treatment, only LT

conformed to the 1.2 minimum value, but no IMF sample from this treatment could conform to the 1.47 minimum value. From these findings it is clear that from a C18:0/C18:2 ratio point of view, dietary CLA supplementation had no effect on IMF quality. Also, from this point of view, LT and SS had better IMF quality than the other muscles.

For good quality and consistency, IMF should also have a DBI < 80 (Haüser & Prabucki, 1990; Prabucki, 1991). The DBI was not significantly influenced by dietary treatment, but sampling position, within dietary treatment, had a statistically significant ($p<0.001$) effect (Table 4.25). The oxidative SS had statistically ($p<0.001$) lower DBI's than the glycolytic SM and oxidative TB, for the control, 0.25 and 1% CLA treatments. For the control group, the glycolytic LT had a significantly lower DBI than the oxidative TB, while the intermediate BF's DBI was significantly ($p<0.001$) higher than the oxidative SS. For the 1% CLA treatment, the DBI for the glycolytic SM was significantly ($p<0.001$) higher than that of the glycolytic LT (Table 4.25).

For all the treatments, only SS had DBI's < 80, implying that this muscle had a better quality fat than the other muscles. The atypical FA composition for SS also resulted in FA ratios with positive technological implications. An explanation for this was presented earlier.

Sensory and chemical stability

Sensory analysis of pork meat and backfat samples

The results for the pork meat and BF samples, as obtained from the sensory panel, are summarized in Table 4.26 (also see Table 3.4 and Annexure 1). From Table 4.26 it is clear that no significant differences were observed in meat aroma attributes, juiciness of meat, sustained impression of juiciness of meat, flavour of meat and aftertaste for the meat from the different treatment groups. Chemical aroma of fat, which is regarded as a negative attribute, differed significantly ($p<0.05$) between groups. Fat from the higher dietary CLA inclusion levels showed higher scores for chemical aroma than fat from the lower CLA inclusion level. The panel defined the chemical aroma of fat as aromatics generally associated with many different types of compounds, such as solvents, cleaning compounds and hydrocarbons (Table 3.4). Fresh pork fat aroma, associated with cooked fresh pork fat (Table 3.4) was significantly ($p<0.05$) lower for the 0.5% CLA treatment than the control and 0.25% CLA treatments. It should also be noted that aspects such as the specific cooking method can also influence the sensory profiles. A more intense sweet odour in pan fried pork, compared to oven prepared pork, could be related to the formation of Maillard reaction-derived compounds, while the more intense piggy flavour and sourish taste in oven roasted pork, could be related to lipid oxidation-derived compounds (Mottram, 1985).

D'Souza and Mullan (2002) also found inferior eating quality regarding flavour, as a result of CLA feeding at a concentration of 5%. Migdal *et al.* (2004) found that C18:1*c*9, which is

Table 4.26: Sensory properties of the pork *M.longissimus* muscle and fat samples from pigs receiving different dietary levels of CLA.

Attributes	p-value	SEM	LSD	Control	0.25% CLA	0.5% CLA	1% CLA
Aroma – fat							
Fresh pork fat	0.03	0.07	0.19	4.74 ± 1.17[b]	4.78 ± 1.29[b]	4.49 ± 1.17[a]	4.70 ± 1.23[ab]
Roast pork fat (caramel)	0.91	0.08	0.23	5.54 ± 1.08	5.60 ± 0.95	5.51 ± 1.03	5..59 ± 1.05
Chemical	0.03	0.08	0.21	1.55 ± 0.85[ab]	1.37 ± 0.62[a]	1.69 ± 0.83[b]	1.58 ± 0.76[b]
Sour	0.09	0.05	0.13	1.20 ± 0.47	1.12 ± 0.32	1.29 ± 0.52	1.25 ± 0.43
Piggy (Old Musty)	0.24	0.07	0.20	1.88 ± 0.95	1.78 ± 0.83	1.96 ± 0.91	1.93 ± 0.86
Aroma – meat							
Roast pork meat	0.85	0.09	0.25	3.70 ± 1.26	3.71 ± 1.28	3.65 ± 1.30	3.65 ± 1.29
Cooked pork meat (fresh)	0.85	0.08	0.22	5.35 ± 0.84	5.23 ± 0.83	5.33 ± 0.89	5.31 ±0 .89
Musty	0.18	0.06	0.16	1.45 ± 0.68	1.45 ± 0.68	1.57 ± 0.85	1.60 ± 0.78
Livery (metallic/bloody)	0.17	0.07	0.20	1.64 ± 0.71	1.51 ± 0.68	1.71 ± 0.81	1.71 ± 0.71
Juiciness of meat							
Initial impression of juiciness	0.56	0.12	0.34	3.81 ±1 .20	4.04 ± 1.21	4.09 ± 1.15	3.96 ± 1.33
Texture – meat							
First bite	<.001	0.13	0.35	3.72 ± 1.32[a]	4.25 ± 1.21[b]	4.51 ± 1.11[b]	4.25 ± 1.25[b]
Tough	<.001	0.15	0.42	3.33 ± 1.29[a]	4.00 ± 1.25[b]	4.38 ± 1.26[c]	4.30 ± 1.22[bc]
Tender	<.001	0.15	0.41	4.49 ± 1.45[c]	3.91 ± 1.29[b]	3.57 ± 1.28[a]	3.45 ± 1.40[a]
Sustained impression of juiciness- meat							
Dry	0.34	0.13	0.35	4.22 ± 1.21	4.48 ± 1.12	4.30 ± 0.99	4.49 ± 1.13
Juicy	0.19	0.12	0.34	3.59 ± 1.17	3.33 ± 1.26	3.61 ± 1.22	3.36 ± 1.25
Flavour – meat							
Meaty	0.47	0.10	0.27	3.46 ± 1.39	3.38 ± 1.31	3.51 ± 1.28	3.57 ± 1.40
Metallic (tin/aluminium)	0.71	0.07	0.20	1.88 ± 0.87	1.75 ± 0.81	1.86 ± 0.84	1.77 ± 0.88
Cooked pork	0.70	0.10	0.27	5.26 ± 1.22	5.12 ± 1.12	5.12 ± 1.19	5.17 ± 1.27
Sour	0.19	0.07	0.19	1.59 ± 0.67	1.43 ± 0.65	1.59 ± 0.67	1.61 ± 0.67
After-taste (Off-flavour of meat)							
Metallic	0.49	0.06	0.17	1.75 ± 0.90	1.71 ± 0.84	1.64 ± 0.79	1.74 ± 0.92
Sour	0.16	0.06	0.15	1.51 ± 0.58	1.35 ± 0.54	1.43 ± 0.53	1.49 ± 0.59

[ab] = Values in the same row with different superscripts differed significantly ($p < 0.05$)
Score 1 – extremely bland for aroma and flavour intensity; extremely tough for first bite and overall tenderness, extremely abundant for residual connective tissue and extremely dry for juiciness
Score 8 - extremely intense for aroma and flavour intensity; extremely tender for first bite and overall tenderness, practically devoid of residual connective tissue and extremely juicy for juiciness

positively correlated to pork flavour (Cameron & Enser, 1991), decreased as result of CLA being fed to pigs. On the other hand, C18:2, which is negatively correlated to pork flavour (Cameron & Enser, 1991), increased when CLA was added to pigs' feed (Migdal *et al.,* 2004). Also, a decrease in C20:4, together with an increase in C16:0, contributed to a difference in flavour (Migdal *et al.,* 2004). For the current study, the C18:1*c*9 values decreased with an increase in CLA concentration to 1%, while the C18:2 and C20:4 contents remained unchanged, and the C16:0 contents increased

(Tables 4.8, 4.9, 4.11, 4.12, 4.19, 4.20, 4.22 and 4.23). Similar results were obtained by Cameron, Enser, Nute, Whittington, Penman, Fisken, *et al*. (2000), who also reported that C18:2, C18:3*n*-6, C20:3*n*-3, C20:4 and C22:5 were all negatively correlated to pork flavour, while C16:1*c*9 was positively correlated. Campo *et al*. (2003) found that C18:1*c*9 was characterized by an oily odour, C18:2 by a cooking oil odour and C18:3*n*-6 by fishy and linseed odours.

In contrast, Tikk, Tikk, Aaslyng, Karlsson, Lindahl and Andersen (2007) found that C18:2, C18:3*n*-6 and C20:3*n*-3 were significantly ($p<0.001$) and positively correlated with the sensory attributes 'fried meat odour' and 'sweet odour', but not with 'piggy flavour'. Also, in contrast C17:0, C18:0 and C18:1*c*9 were significantly ($p<0.001$) and positively correlated with 'piggy flavour', and inversely associated with 'sweet odour' and 'fried meat odour' (Tikk *et al*., 2007).

The lower content of PUFAs in the experimental groups from the present study (Tables 4.11, 4.12, 4.22 and 4.23), was consistent with previous studies by Enser (1984), Cameron and Enser (1991), and Migdal *et al*. (2004). They reported that the FA composition of pork meat was related to eating quality, with the SFAs and MUFAs being positively correlated to pork flavor, and the PUFAs negatively correlated. Increased PUFA levels have often been reported to give rise to off-flavour problems after reheating, because of their greater susceptibility to oxidative breakdown and the formation of unwanted volatile compounds during cooking (Schackelford, Reagan, Haydon & Miller, 1990; Larick, Turner, Scheonherr, Coffey, & Pilkington, 1992). Analysis of the FA composition from the LD muscle in the current study (Table 4.24), showed an increase in the saturation of the fat from the gilts receiving CLA in their feed. The PUFA and *n-6/n-3* contents remained unchanged (Table 4.24). The other fat aroma attributes (roast pork fat, sour and piggy) did not differ significantly between treatments, which is in agreement with the results of Dugan *et al*. (1999) at 2% CLA, Wiegand *et al.* (2001) at 1.25% CLA and Tischendorf *et al.* (2002) at 2% CLA.

The other descriptive sensory attributes that differed significantly included three textural descriptors, i.e. first bite, toughness and tenderness (Table 4.26). First bite was defined as the impression formed on the first bite. Toughness and tenderness shared the same definition, namely the impression of toughness/tenderness of the meat; depending on whether the break down was easy or tough (Table 3.4). The control treatment was rated to be significantly tenderer by sensory, as well as physical texture (shear force) analysis (Tables 4.26 and 4.27). For first bite and toughness the samples from this treatment scored the lowest value of 3.72 and 3.33, respectively, and the highest value of 4.49 for tenderness (Table 4.27). Shear force measurement for the control, 0.25 and 1% CLA did not differ significantly at 4 kg, but the control sample differed significantly ($p<0.1$) from the 4.8 kg shear value for the 0.5 % CLA treatment group (Table 4.27).

Previous findings by D'Souza and Mullan (2002), and Intarapichet *et al*. (2008) also confirmed that CLA supplementation had a negative effect on meat tenderness. Migdal *et al.* (2004) reported an increase in the number and diameter of white muscle fibres and a decrease in the number and diameter of red muscle fibres, as a result of dietary CLA supplementation. Such muscle fibre hypertrophy is associated with increased meat toughness (Maltin, Warkup, Matthews, Grant, Porter & Delday, 1997).

Table 4.27: Cooking data of the *M. longissimus* muscle according to treatments.

Attributes	p-value	SEM	Control	0.25% CLA	0.5% CLA	1% CLA
Shear force (kg)	0.09	0.24	3.92 ± 0.57[a]	4.25 ± 0.53[ab]	4.80 ± 0.38[b]	4.42 ± 0.77[ab]
Total cooking loss (%)	0.09	0.66	28.23 ± 1.75	27.18 ± 1.84	27.58 ± 1.59	25.79 ± 1.20
Thawing loss (%)	0.97	0.35	1.66 ± 0.99	1.70 ± 0.73	1.94 ± 1.39	1.75 ± 1.06
Drip loss (%)	0.02	0.77	13.85 ± 2.30[b]	12.83 ± 1.74[b]	12.36 ± 2.04[ab]	10.21 ± 1.37[a]
Evaporation loss (%)	0.28	0.53	14.38 ± 1.34	14.35 ± 1.35	15.22 ± 1.46	15.5 9± 1.01

[ab] =Values in the same row with different superscripts differed significantly ($p<0.05$) and ($p<0.1$) for shear force

There were no statistically significant differences in thawing loss and evaporation loss between treatment groups (Table 4.27). Drip loss differed significantly ($p<0.05$) among the treatment groups, mainly due to a similar trend in cooking losses. Drip loss is measured as the sum of fat drip and "stock" drip (meat juices). Meat from the control, 0.25 and 0.5% CLA treatments showed the highest drip loss, with losses of 13.85%, 12.83% and 12.36%, respectively. The 1% CLA treatment differed significantly ($p<0.05$) from the control and 0.25% CLA treatments, with the lowest drip loss of 10.21%. Joo, Kaufman, Borggaard, Stevenson-Barry, Park and Kim (2000) proposed a positive relationship between WHC and IMF content. In 2002, Joo *et al*. reported that the WHC of pork might be improved due to increased IMF resulting from dietary CLA at a concentration of 5%, which led to the stability of cell membrane lipids. Szymczyk (2005) also found that the technologically most beneficial WHC was found in the meat from 0.1 and 0.2% CLA fed pigs. The 0.4 and 0.6% CLA supplemented pig meat had a better WHC than the control group. For the present study, no differences were found for either the % IMF, % FFDM or % moisture of the LD between the four treatments (Table 4.18).

Although not significant, the results obtained in this study did point to a relationship between the presence of CLA in a carcass and better WHC (Table 4.4). This was confirmed by Hayek, Han, Wu, Watkins, Meydani, Dorsey, *et al.* (1999), who suggested that the incorporation of CLA isomers into the fraction of phospholipids and the effect thereof on the composition of FAs, might reduce the permeability of cell membranes, thus limiting drip loss. This was ascribed to decreased susceptibility of phospholipids to oxidation, by reduction of the content of C18:2 and

C20:4, and the concurrent increase in the level of SFAs (Belury, 2002). For this study, C18:2 and C20:4 contents did not change (Tables 4.22 and 4.23), while the SFAs did increase (Table 4.19). The WHC of meat is also highly correlated to its pH value (Szymczyk, 2005). It was shown that the WHC rapidly decreased at a pH of 5 (iso-electric point), because the electric charge of the protein then approached neutral (Toldra, 2003). The pH_{24} of the pork meat ranged from 5.47-5.55, in the present study (Table 4.4) and thus could not explain the better WHC for the 1% CLA treatment, compared to the control treatment.

In an attempt to simplify the interpretation of descriptive sensory evaluation, the multivariate statistical procedure PCA was used (Meilgaard, Civille, & Carr, 1999). Figure 4.7 is the PCA biplot of the significant ($p<0.05$) sensory and physical attributes, for meat samples from pigs fed CLA at four different concentrations. The first dimension explained 71.21% and the second dimension 19.79% of the variance in the data. The two dimensions accounted for 91.00% of the total variation in the data. The first dimension divided the four treatments into two groups, with the control and 0.25% CLA-treatments on the left side of the plot, and 0.5 and 1% CLA-treatments on the right. This dimension explains the differences in meat tenderness. The control treatment had the tenderest texture. The highest shear force was found in the meat samples from the 0.5% CLA treatment. The 1% CLA treatment produced the toughest first bite and meat texture.

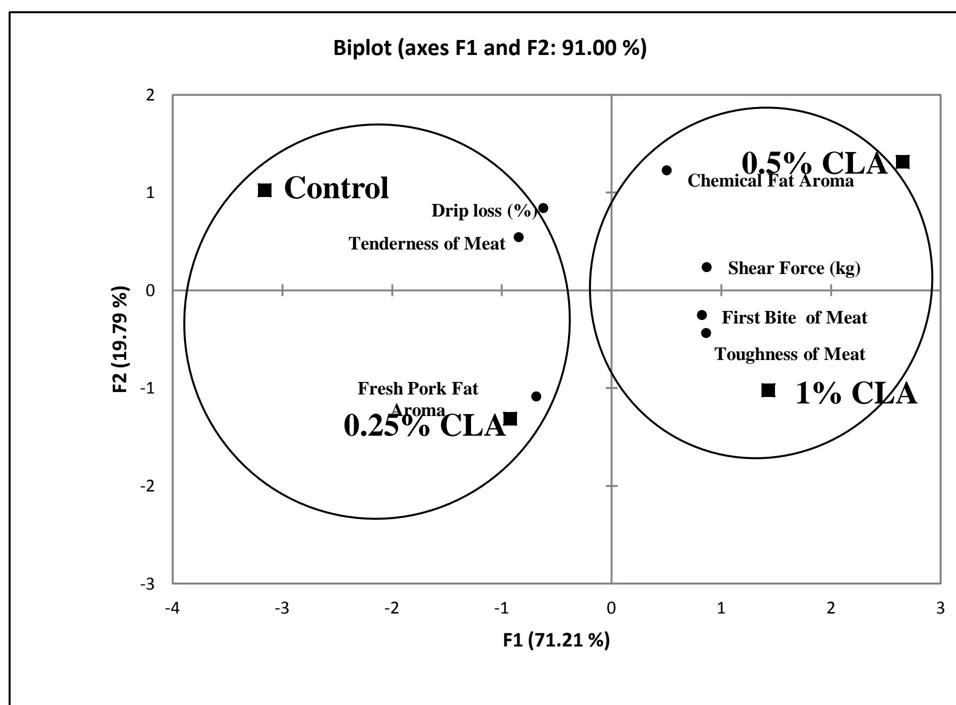


Figure 4.7: Principle component analysis biplot of significant sensory and physical attributes for meat from pigs fed CLA at four different concentrations.

The second dimension, which adds to the explanation of differences among treatments, indicates differences in the quality of aroma of the pork fat in the treatment. In addition, it also indicates the differences in the drip loss percentages, among treatments. The control treatment had the highest drip loss. Fresh pork fat aroma was associated with the 0.25% CLA samples, while the 0.5% CLA fat samples had a chemical fat aroma. These results clearly demonstrate the negative influence of higher CLA concentrations on the sensory quality characteristics of pork. It should be noted that the scalar differences in tenderness of meat is far greater than the differences in fat aroma. Consumers may possibly tolerate greater variation in tenderness, as opposed to variation in aroma or slight off- or undesirable odours.

Chemical Stability Studies

Accelerated oxidation test (Schaal oven test)

Figure 4.8 illustrates the pattern and general trend of changes that took place in the PV, during 13 days of storage of the extracted BF at 63 ± 0.5 ^{o}C, with free access to air. The results from the accelerated oxidation test (Table 4.28) indicated that the BF from the control did not become rancid faster than BF from the three CLA treatments. The lack of anti-oxidant effect of CLA observed in

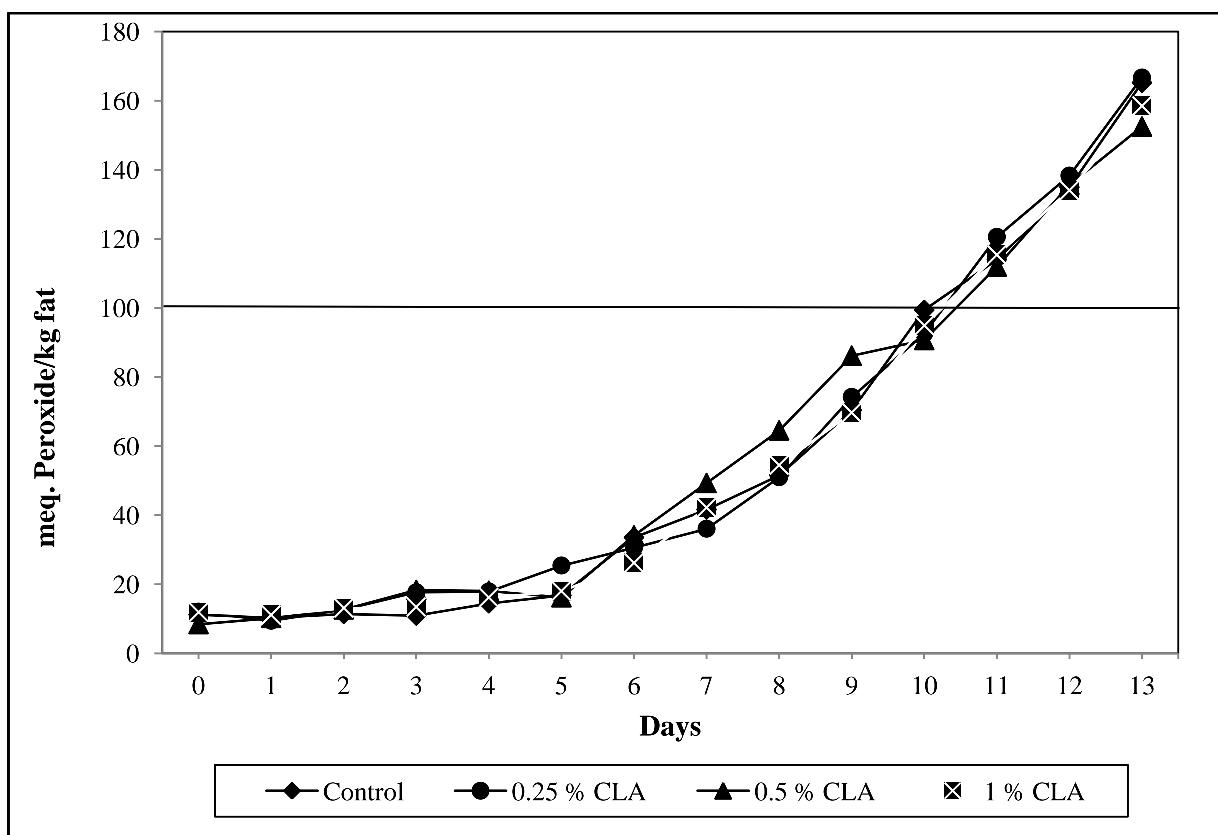


Figure 4.8: Pattern and general trend of changes that took place in peroxide values of backfat of different treatments, during 13 days storage of extracted backfat at 63 ± 0.5 ^{o}C, with free access to air.

this accelerated oxidation test, confirmed the conflicting reports in literature regarding the anti-oxidant properties of CLA. Although anti-oxidant properties have been ascribed to CLA by Hur, Park and Joo (2007), Van den Berg *et al*. (1995) showed that CLA did not act as an efficient radical scavenger, in any way comparable to antioxidants such as vitamin E or BHT. Pro-oxidant properties have even been ascribed to CLA (Chen, Chan, Kwan, & Zhang, 1997). The anti-oxidant effect of CLA can most probably be ascribed to the fact that dietary CLA shifted the whole FA composition of fat tissue to be more saturated, thus making meats from animals fed CLA less susceptible to lipid oxidation (Eggert *et al*., 1998; Eggert *et al*., 2001). The present study also failed to demonstrate that effect. It is however possible that the severe oxidative conditions, created in this experiment, overwhelmed the subtle anti-oxidative effect created by the increase in SFA content (Tables 4.8 and 4.13) of fat tissue, due to dietary CLA supplementation.

Table 4.28: Results of accelerated oxidation test for backfat (Schaal oven test).

	Control n = 12	**0.25% CLA** n = 12	**0.5% CLA** n = 12	**1% CLA** n = 12	**Sign. level**
Days to reach peroxide value of 100	10.67 ± 1.21	10.50 ± 1.38	10.33 ± 1.63	11.00 ± 0.89	NS

NS = Not significant

Stability of fresh and frozen pork chops

It had been established that atmospheric oxygen (Lanari *et al*., 1995) and fluorescent light (Andersen & Skibsted, 1991) accelerate oxidation. Oxygen permeable packaging material for fresh and frozen pork chops, combined with displaying under fluorescent lights as used in this experiment with the fresh and frozen chops, should result in lipid oxidation. Furthermore, lipolytic enzymes, such as lipases, esterases and phospholipases play an important role in the quality of meat during storage (Martin, Muriel, Antequera, Andres, & Ruiz, 2009). These enzymes are responsible for the lipolysis of triacylglycerols and phospholipids. The released FFAs are considered more prone to lipid oxidation and, in turn, contribute to the generation of flavour compounds (Flores *et al*., 1996; Alasnier, David-Braind, & Gandemer, 2000). Several studies have found an effect of CLA on lipolytic processes (Park, Albright, Liu, Storkson, Cook, & Pariza, 1997), such as CLA-induced increase in basal lipolysis (Park *et al*., 1997). Martin, Ruiz, Flores and Toldra (2006) detected a significant effect of dietary CLA on neutral lipase and acid esterase activities of pork. They also reported that the combination of CLA and MUFA levels in pig diets also influenced the acid lipase, acid esterase and neutral esterase activities of meat.

The display of meat under fluorescent light at 4°C for 8 days, resulted in significant ($p<0.01$) differences in certain aspects of muscle colour between the four treatments, but had no effect on lipid stability (Table 4.29). At day 0, the L* values from the 0.5 and 1% CLA treatments were significantly ($p<0.01$) lower than from the control. This implied that at packaging, the muscle from the control was whiter in colour than the higher CLA containing treatments. After two days of display at 4°C, the chops from the 0.25% CLA treatment also differed significantly ($p<0.01$), along

Table 4.29: Chemical stability of fresh and frozen pork chops.

Dietary Groups		Control n=12	0.25% CLA n=12	0.5% CLA n=12	1% CLA n=12	Sign. level
FRESH PORK CHOPS STORED AT 4°C						
Muscle Colour Stability:						
L* value:	Day 0	64.01 ± 3.92^{b}	60.37 ± 4.91^{ab}	54.71 ± 4.72^{a}	55.89 ± 4.86^{a}	p<0.01
	Day 2	64.48 ± 1.44^{b}	58.23 ± 3.27^{a}	56.75 ± 4.73^{a}	55.92 ± 3.48^{a}	**
	Day 4	65.26 ± 1.37^{b}	60.08 ± 2.78^{ab}	57.62 ± 4.43^{a}	57.25 ± 3.85^{a}	p<0.01
	Day 6	64.83 ± 2.99^{b}	61.36 ± 2.35^{ab}	59.63 ± 4.85^{ab}	57.74 ± 2.25^{a}	p<0.01
	Day 8	65.05 ± 3.66	63.06 ± 4.48	60.99 ± 4.22	59.31 ± 2.34	NS
a* value:	Day 0	7.20 ± 0.95	6.05 ± 1.38	6.36 ± 1.37	5.71 ± 1.29	NS
	Day 2	6.48 ± 0.48	6.12 ± 1.08	6.29 ± 1.58	5.74 ± 1.00	NS
	Day 4	5.36 ± 0.61	5.11 ± 1.20	5.37 ± 1.37	4.83 ± 0.80	NS
	Day 6	5.00 ± 1.13	4.66 ± 0.97	4.94 ± 1.28	4.49 ± 0.90	NS
	Day 8	4.72 ± 1.11	4.21 ± 0.97	4.72 ± 1.32	4.42 ± 1.17	NS
b* value:	Day 0	12.33 ± 1.05^{b}	10.37 ± 1.59^{ab}	9.17 ± 2.32^{a}	9.26 ± 1.33^{a}	p<0.05
	Day 2	12.64 ± 0.77^{b}	10.70 ± 1.34^{ab}	10.33 ± 1.86^{a}	9.91 ± 1.00^{a}	p<0.01
	Day 4	12.55 ± 0.48^{b}	10.62 ± 1.17^{ab}	10.30 ± 2.08^{a}	10.06 ± 1.12^{a}	p<0.05
	Day 6	12.67 ± 0.63^{b}	11.14 ± 0.66^{ab}	10.88 ± 1.68^{a}	10.21 ± 0.88^{a}	p<0.01
	Day 8	12.94 ± 0.95^{b}	11.37 ± 1.00^{ab}	11.27 ± 1.51^{ab}	10.71 ± 0.49^{a}	p<0.01
Chroma:	Day 0	14.32 ± 1.07^{b}	12.06 ± 1.96^{ab}	11.22 ± 2.41^{a}	10.93 ± 1.52^{a}	p<0.05
	Day 2	14.22 ± 0.84^{b}	12.36 ± 1.47^{ab}	12.17 ± 2.01^{ab}	11.50 ± 1.10^{a}	p<0.05
	Day 4	13.67 ± 0.61^{b}	11.82 ± 1.36^{ab}	11.66 ± 2.26^{ab}	11.21 ± 0.97^{a}	p<0.05
	Day 6	13.69 ± 0.62^{b}	12.12 ± 0.73^{ab}	12.03 ± 1.60^{ab}	11.21 ± 0.90^{a}	p<0.01
	Day 8	13.84 ± 0.85^{b}	12.19 ± 0.83^{ab}	12.32 ± 1.44^{ab}	11.66 ± 0.49^{a}	p<0.01
Hue Angle	Day 0	59.72 ± 3.70	60.02 ± 4.03	54.99 ± 6.42	58.42 ± 5.39	NS
	Day 2	62.86 ± 1.38	60.27 ± 4.24	58.80 ± 7.03	59.94 ± 4.35	NS
	Day 4	66.91 ± 1.91	64.49 ± 5.06	62.53 ± 5.30	64.22 ± 5.00	NS
	Day 6	68.52 ± 4.77	67.40 ± 4.44	65.49 ± 6.91	66.29 ± 4.51	NS
	Day 8	69.92 ± 4.94	69.57 ± 5.21	67.28 ± 6.82	67.69 ± 5.79	NS
Muscle Lipid stability:						
TBARS:	Day 0	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	NS
	Day 2	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	NS
	Day 4	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	NS
	Day 6	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.04 ± 0.02	NS
	Day 8	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.03	NS
FRESH PORK CHOPS STORED AT -18°C						
Muscle Lipid Stability:						
TBARS:	Day 0	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	NS
	Month 3	0.08 ± 0.03	0.06 ± 0.03	0.07 ± 0.03	0.05 ± 0.02	NS
	Month 6	0.13 ± 0.02^{b}	0.09 ± 0.02^{a}	0.10 ± 0.02^{a}	0.09 ± 0.03^{a}	p<0.05

Means with different superscripts in the same row differ significantly.
NS = Not significant

with the other two CLA treatments, from the control, which was still the highest in L* value, implicating a whiter muscle colour. Similar to day 0, L* values for the 0.5 and 1% CLA treatments were significantly ($p<0.01$) lower than the control. After six days of display, only the 1% CLA treatment had BF with L* values significantly ($p<0.01$) lower than the control. There was no difference between the treatment groups for the L* value at the end of the experiment (eight days). This confirmed the findings of Martin, Antequera, Muriel, Andres and Ruiz (2008a), that L* values of loin chops decreased with refrigerated storage at 4°C as a result of dietary CLA supplementation. These authors also reported that the highest L* value was measured for the 2% CLA level, with the lowest L* value at the 1% CLA level. Furthermore, they did not detect differences in the L* value due to dietary CLA, beyond two days of storage. Wiegand *et al*. (2001) also found higher L* values in chops from CLA-fed pigs than in the control chops, after seven days of refrigerated storage.

Although colour a* values of muscle demonstrated a decrease with increased dietary CLA level, it was not significant over the storage period of eight days between muscles from the four dietary treatments (Tables 4.29). Martin *et al*. (2008a) reported a decrease in a* values of refrigerated loin chops from CLA supplemented pigs, but concluded that CLA level was not responsible for the change in a* values.

From day 0 ($p<0.05$) to day 2 ($p<0.01$) to day 4 ($p<0.05$) and to day 6 ($p<0.01$) of the present study, b* values for the control were significantly (at least $p<0.05$) higher than the 0.5 and 1% CLA treatments. At the end of the experiment (eight days), only chops from the the 1 % CLA treatment had b* values significantly ($p<0.001$) lower than the control (Table 4.29). Martin *et al.* (2008a) found an increase in b* values from day 0 to day 4, followed by a decrease in the muscle of CLA supplemented chops. These authors also found that CLA level did not influence the changes in b* values, throughout the refrigerated storage period of the chops.

Saturation index (chroma) is related to the quantity of pigments and high values represent a more vivid colour, and denote lack of greyness (Ripoll & Munoz, 2011). The control muscle had higher chroma values than muscle from the CLA treatments, but it was only significant (at least $p<0.05$) between the control and 1% CLA treatment (Table 4.29). This data implies that CLA supplementation had a negative effect on the vividness of pork muscle colour.

Hue angle is the attribute of colour perception denoted by blue, green, yellow, red, purple, etc., and is related to the state of pigments (Ripoll & Munoz, 2011). A clear numerical increase in hue angle of chops with storage time and a clear decrease with increased dietary CLA supplementation was observed. The dietary CLA effect was not statistically significant.

In contrast to the present results, Hur, Ye, Lee, Ha, Park and Joo (2004) reported an improvement in meat colour stability of beef patties, manufactured from meat of animals receiving CLA supplemented diets. Hur *et al.* (2004) attributed this observation to inhibition of lipid and oxymyoglobin oxidation by CLA, although the mechanism for the improvement of colour stability by CLA remains unclear. Martin *et al*. (2008a) reported no improvement on meat colour as a result of dietary supplemented CLA. They also concluded that the MUFA level of the diet did not interact with the dietary CLA in the instrumental measured colour values.

No differences were observed in TBARS values of fresh meat over the display period of eight days, between all four treatments (Table 4.29). This is in agreement with the results of Martin *et al*. (2008a), who also found that CLA supplementation ($\leq$ 1%) in pig diets did not affect lipid oxidation of refrigerated pork loins. They reported that dietary CLA at 2%, however, led to higher TBARS values for loin chops at day 7 of refrigerated storage ($p<0.05$). This can be explained by the fact that CLA acts as pro-oxidant at low levels, then as antioxidant at higher doses and subsequently reverts to a pro-oxidant at the highest levels (Flintoff-Dye & Omaye, 2005).

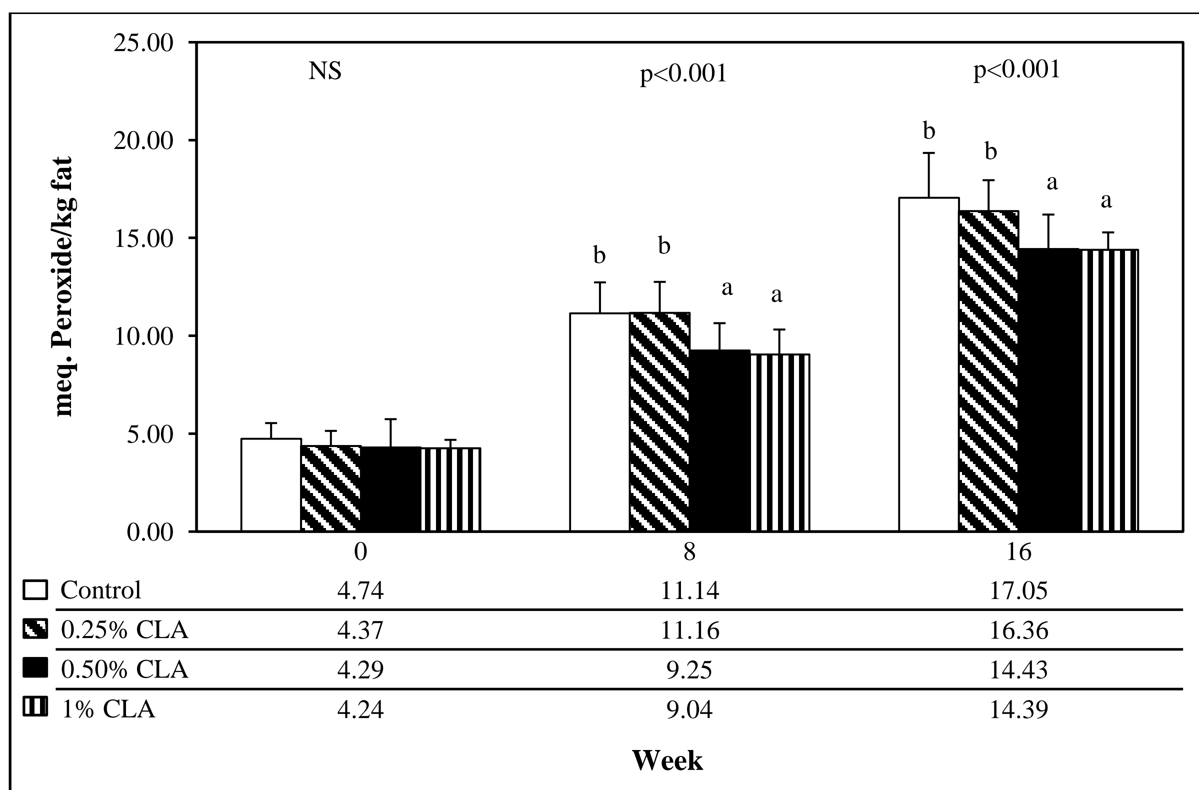
No differences in TBARS values were observed between pork chops from the different dietary treatments, during frozen storage (-18ºC) for up to 3 months. After 6 months of frozen storage, the TBARS values of pork chops from CLA supplemented pigs were significantly ($p<0.05$) lower than the control (Table 4.29). This might be due to the antioxidant effect of the supplemented CLA (Hur *et al*., 2007) or the more SFA profile of meat from CLA supplemented pigs, which makes meats from animals, fed CLA, less susceptible to lipid oxidation (Eggert *et al*., 1998; Eggert *et al*., 2001).

Lipid stability of frozen pork patties

Figure 4.9 is a presentation of the PVs of the pork patties, taking at day 0, week 8 and week 16 of frozen storage at -18ºC. At day 0, there was no difference between the samples from the four different dietary treatments. However, samples taken at week 8 of frozen storage showed significantly ($p<0.001$) lower PVs for the 0.5 and 1% CLA treatments, compared to the control and 0.25% CLA treatment. The same trend was observed at week 16, when the PVs from the 0.5 and 1% CLA treatment again were significantly ($p<0.001$) lower than the control and the 0.25% CLA treatment. The explanation for these lower peroxidation levels could be similar to the explanation above, for the improved stability of the fresh pork chops during frozen storage. Alternatively, PVs may be lower, because peroxides are primary oxidation products, causing levels to decrease because secondary oxidation had progressed.

The TBARS of the frozen pork patties at week 0, 8 and 16, are shown in Figure 4.10. No differences occurred at day 0 for all four dietary treatments. At week 8, the TBARS decreased

significantly ($p<0.01$) for the three CLA treatments, compared to the control. After 16 week of frozen storage, only the 1% CLA treatment showed significantly ($p<0.05$) lower TBARS values than the control. When comparing these results with the TBARS from the chops stored at -18°C, it is evident that no significant differences occurred in TBARS values of chops, between the four treatment groups, until the sixth month of frozen storage. Only then did the control show significantly ($p<0.05$) higher TBARS than the three CLA treatments. Nonetheless, these TBARS values were between 0.09 and 0.13 mg malonaldehyde/kg meat (Table 4.29), compared to the range of 1.04 to 1.18 mg malonaldehyde/kg meat for the frozen patties (Figure 4.10). From these values it was clear that the mincing of the lean and the BF, and the addition of salt, led to an increase in the TBARS values despite the antioxidant effects of CLA (Ha *et al.*, 1990). In a study by Hur *et al.* (2004), beef patties were prepared from lean meat taken from animals receiving 0.5 and 2% CLA in their diets. The patties were only refrigerated, but after 14 days of storage the TBARS for patties from the CLA treatments were significantly ($p<0.05$) lower than the control. Du, Ahn and Sell (1999) and Du, Ahn, Nam and Sell (2000) reported that as dietary CLA concentrations increased, the TBARS values and hexanal content of chicken meat patties decreased during aerobic storage.



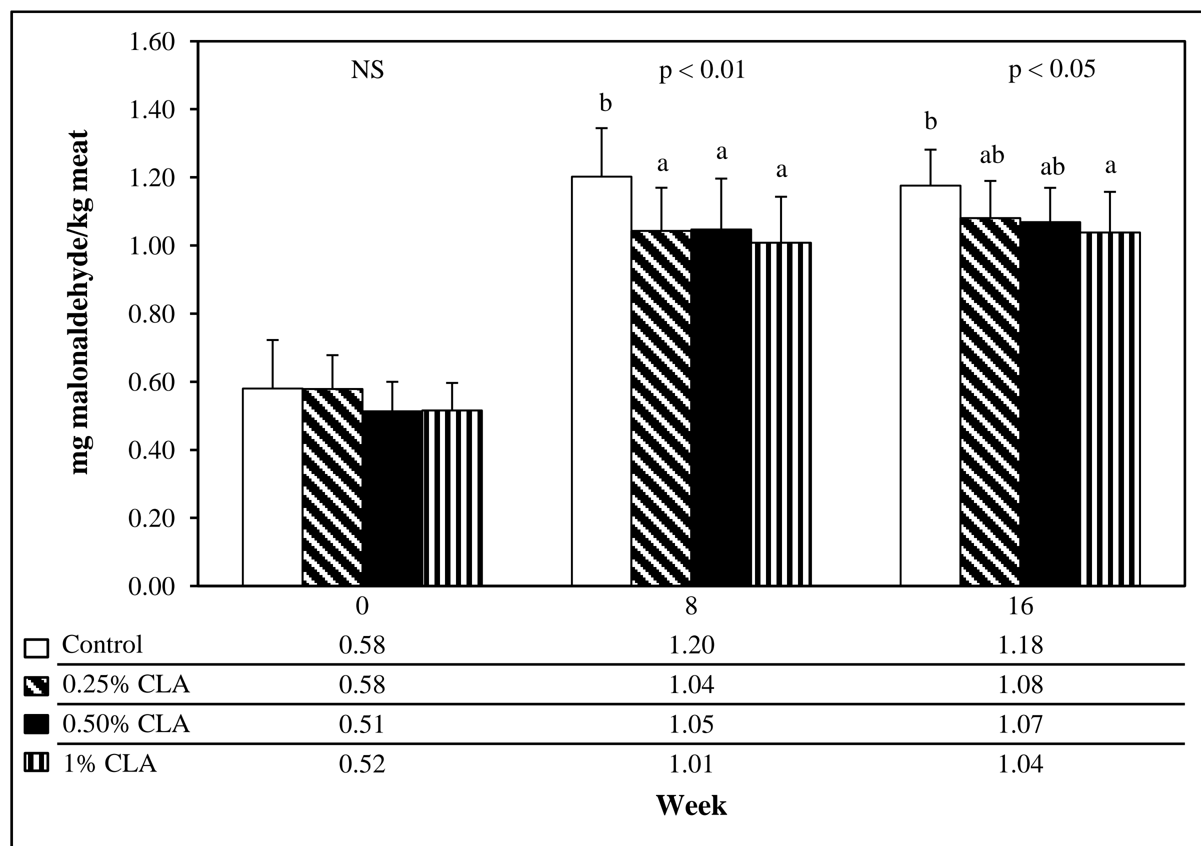
	0	8	16
Control	4.74	11.14	17.05
0.25% CLA	4.37	11.16	16.36
0.50% CLA	4.29	9.25	14.43
1% CLA	4.24	9.04	14.39

Means with different superscripts at the same sampling time differed significantly.

Figure 4.9: Peroxide values of pork patties during frozen storage.

Lipid stability of fermented salami

Salami is probably one the most popular meat products, because of its textural, sensory and nutritional characteristics. Actually, salami is not a specific sausage, but rather a generic term



Means with different superscripts at the same sampling time differed significantly.

Figure 4.10: Thiobarbituric acid reactive substance values of pork patties during frozen storage.

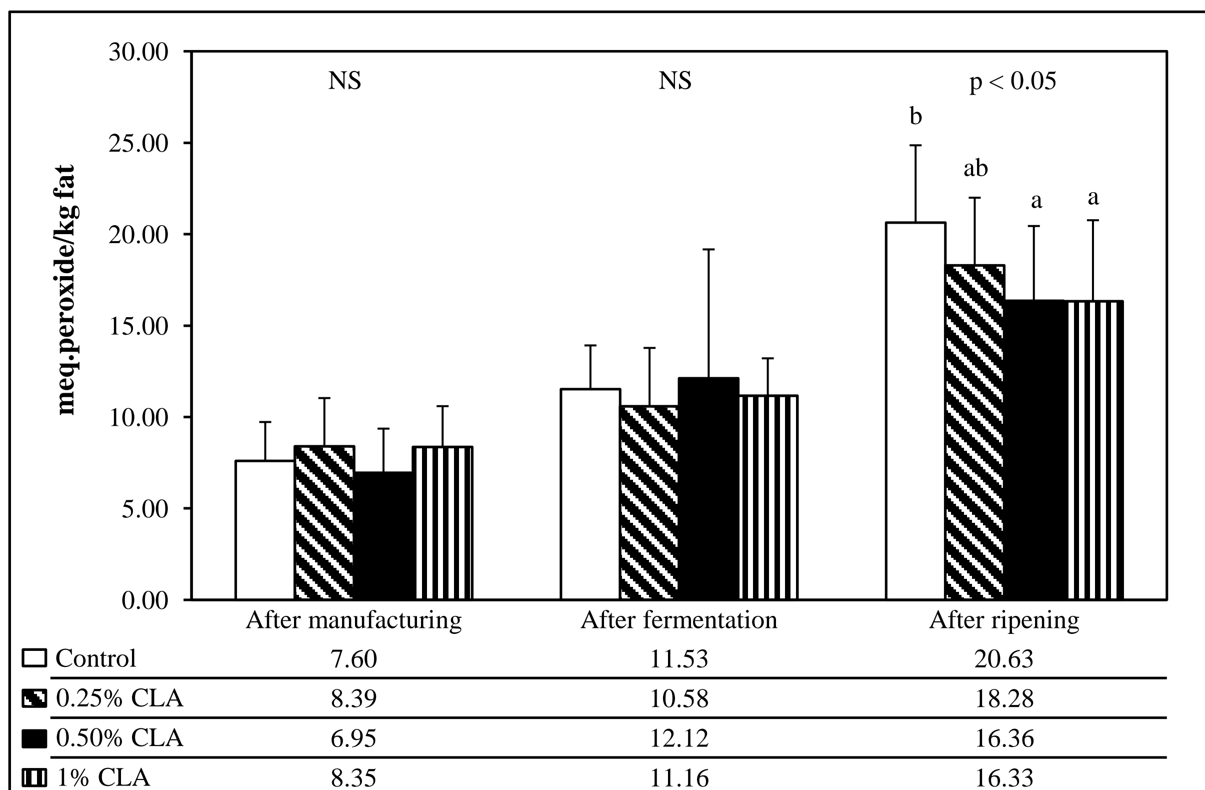
describing any type of encased (*insaccati*) meat product. Salami is differentiated by the fineness of the ground meat and each variety has different meat formulations, consistency and spice profile (Herrnaz, Ordóñez, de la Hoz, Hierro, Soto, & Cambero, 2008).

Although salami contains nitrite, a strong antioxidant (Zanardi, Novelli, Ghiretti, & Chizzolini, 2000), there are factors present during salami manufacturing that will accelerate the oxidative processes. These factors include: exposure to oxygen (Allen & Foegeding, 1981); high fat content of salami; grinding during processing; a relatively high salt content (Zanardi *et al*., 2000); the naturally high PUFA content of pork fatty tissue (Warrants, van Oeckel, & Boucqué, 1998); and the fact that the product is exposed to high temperatures during fermentation and drying (Zanardi *et al*., 2000).

A slight increase was observed in PVs for salamis from all dietary treatments, from manufacturing till after fermentation. However, no significant differences were observed in the PVs of salami from all dietary treatments directly after manufacturing and after 48 h of fermentation at 22 ^{o}C (Figure 4.11). Peroxide values were also quite stable and increased from ± 7

meq.peroxide/kg fat after manufacturing to ± 10 meq.peroxide/kg fat after fermentation. There was, however, a clear increase in the PVs of salamis from all dietary treatments towards the end of the ripening period. At this stage, the salamis from the 0.5 and 1% CLA treatments had significantly ($p<0.001$) lower PVs than from the control and 0.25% CLA treatment.

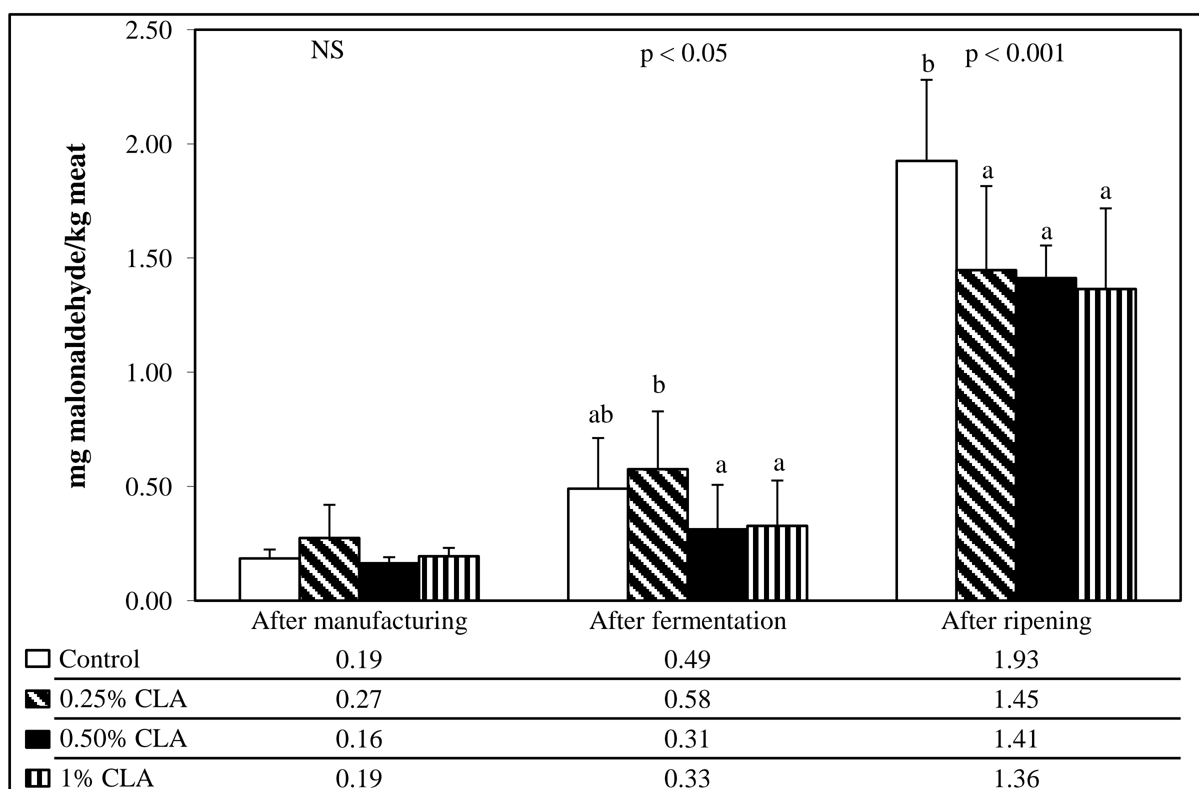
As in the case of PV, no significant differences were observed in TBARS values (mg maldonaldehyde/kg meat) between salamis from different treatments directly after manufacturing (Table 4.12). After fermentation there was already a trend towards lower TBARS values for salamis from the 0.5 and 1 % CLA treatments, compared to salamis from the control and 0.25 %



Means with different superscripts at the same processing stage differed significantly

Figure 4.11: Changes in peroxide values during salami manufacturing.

CLA treatment. The TBARS values for salamis from the 0.5 and 1 % CLA treatments were significantly ($p<0.001$) lower than the 0.25% CLA treatment. Although the TBARS values were still fairly low (± 0.5 mg maldonaldehyde/kg meat) at the end of fermentation, they were already roughly double the value of the raw meat mixtures (±0.1-0.2 mg maldonaldehyde/kg meat). A drastic increase was observed in TBARS values for all treatments towards the end of ripening. At the end of ripening, TBARS values of salamis from all CLA treatments were significantly ($p<0.001$) lower than the control.



	After manufacturing	After fermentation	After ripening
Control	0.19	0.49	1.93
0.25% CLA	0.27	0.58	1.45
0.50% CLA	0.16	0.31	1.41
1% CLA	0.19	0.33	1.36

Means with different superscripts at the same processing stage differed significantly

Figure 4.12: Changes in thiobarbituric acid reactive substance values during salami manufacturing.

This finding that CLA supplementation increased the oxidative stability of salami is quite interesting, considering that contradictory results were reported in literature, regarding the effect of increased levels of CLA on the oxidative stability of processed meat products. Lee, Lee, Kwack, Ha, Jung, Lee, *et al*. (2003) found that TBARS values of an emulsion-type sausage containing CLA, increased significantly as the storage period at 4°C advanced to 28 days. Martin, Ruiz, Kivikari and Puolanne (2008c) found a decrease in TBARS rather than an increase, when they replaced pork fat with conjugated linoleic acid in liver pâtés. Hah, Yang, Hur, Moon, Ha and Park (2006) studied the use of different CLA levels in the formulation of a finely chopped sausage. They found that the increase in TBARS values was lower with a higher CLA content of the product. In the case of supplementing pig feed with CLA, a reduction in lipid oxidation has also been reported in pork loin or pork liver (Joo *et al*., 2002; Martin *et al*., 2011).

These lower PVs and TBARS values observed in salami could only be attributed to the antioxidant effects of CLA or the increase in SFAs of CLA supplemented meat, resulting in increased oxidative stability (Eggert *et al*., 1998; Eggert *et al*., 2001). Because CLA is a stable component (Shantha, Ram, O'Leary, Hicks, & Decker, 1995), it was postulated that relatively high levels of CLA could reduce the formation of FA radicals and subsequent oxidative reactions. It is

also suggested that the conjugated structure makes CLA less susceptible to free radical attacks, without CLA itself acting as an antioxidant (Du *et al*., 2000).

Lipid stability of bacon

Researchers (D'Souza & Mullan, 2002; Wiegand *et al*., 2002; Corino *et al*., 2003) have already determined that pig fat composition may be altered with nutritional intake, which in turn may affect the textural properties. Fat composition can also have a marked effect on the sliceability of bacon (Shackelford *et al*., 1990; Averette Gatlin, See, Odle, & Larick, 2002c; Rentfrow, Sauber, Allee, & Berg, 2003). Differences in the FA composition of pig fat also affect the crystallization properties of the fat (Svenstrup *et al*., 2005). Previous research have shown that CLA supplementation led to increased subjective belly firmness measurements (Eggert *et al*. 1998; Carroll *et al*., 1999; Schinckel *et al*., 2000; Eggert *et al*., 2001; Weber *et al*., 2001), as well as increased belly weights (Averette Gatlin *et al*., 2002b).

The same result was obtained in the current experiment, where the control fresh belly fat scored a significantly ($p<0.001$) lower value for fat firmness, compared to the belly fat from the CLA treatments (Table 4.30). Firmness values increased from 566.27 for the control belly fat, to 873.44 for the belly fat from the 1% CLA treatment. Belly fat from the 1% CLA treatment was also significantly ($p<0.001$) firmer than the belly fat from the control and 0.25% CLA treatment, but not from the 0.5% CLA treatment. The firmness did not differ between the 0.25 and 0.5% CLA bellies, but the 0.5% CLA bellies were significantly ($p<0.001$) firmer than the control bellies. The DSC results, reported on earlier, explained the firmness differences elegantly. Compared to the control, fat from CLA fed pigs contained increasing amounts of CLA, but a 10% overall decrease in UFAs and a 5% overall increase in each of the C16:0 and C18:0 FAs (Tables 4.8 and 4.13). Thus, the higher content of saturated fat resulted in changes in the melting properties. In addition, the presence of β'-crystals of the C18:0-C16:0-C18:1 triacylglycerides in the fat from the 0.25 and 0.5% CLA fed pigs, and β-crystals, in the fat from the 1% CLA fed pigs were observed, thus creating a more firmer fat.

Table 4.30: Fresh belly fat firmness of gilts in the four dietary groups.

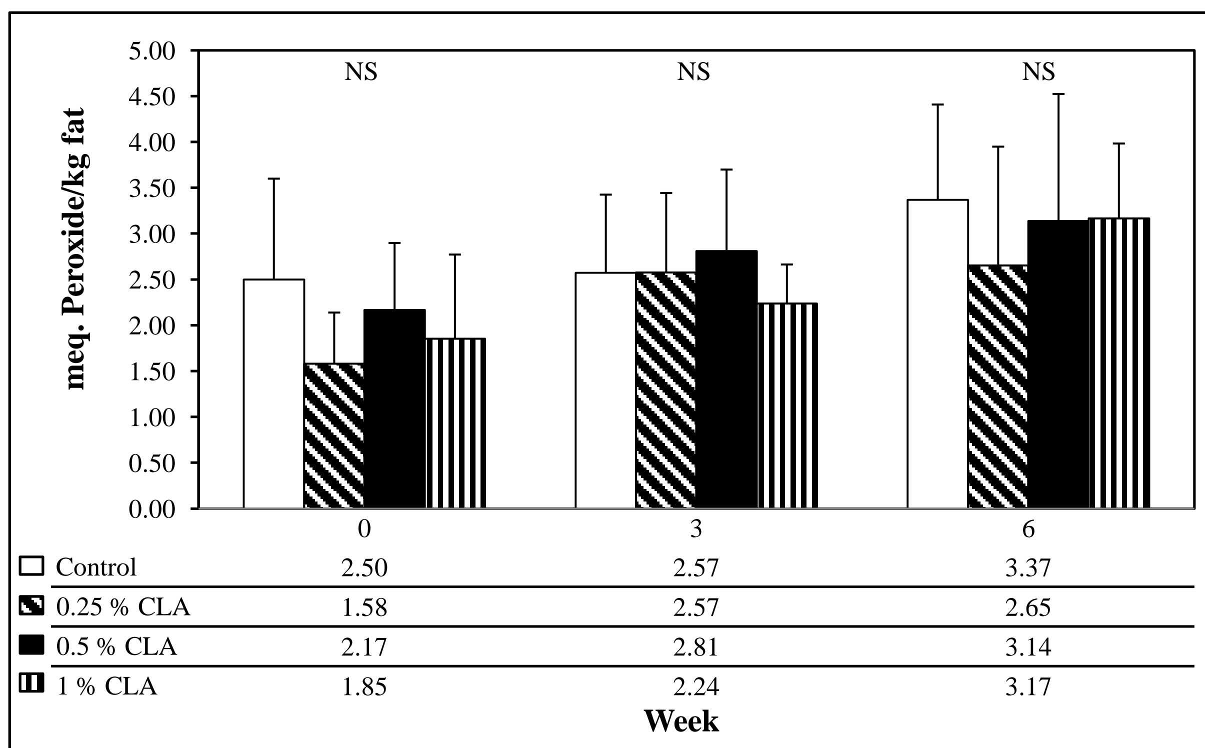
Dietary Groups	Control n = 12	0.25% CLA n = 12	0.5% CLA n = 12	1% CLA n = 12	SL
Fat Hardness	566.27 ± 116.47^{a}	736.88 ± 158.41^{b}	805.09 ± 129.58^{bc}	873.44 ± 143.14^{c}	$p<0.001$

SL = significance level Means with different superscripts in the same row differed significantly

Furthermore, a C18:0/C18:2 ratio > 1.47 for bacon fat has been proposed by Enser (1983) and Enser *et al*. (1984). From Table 4.14, this ratio increased significantly from 0.95 (control) to

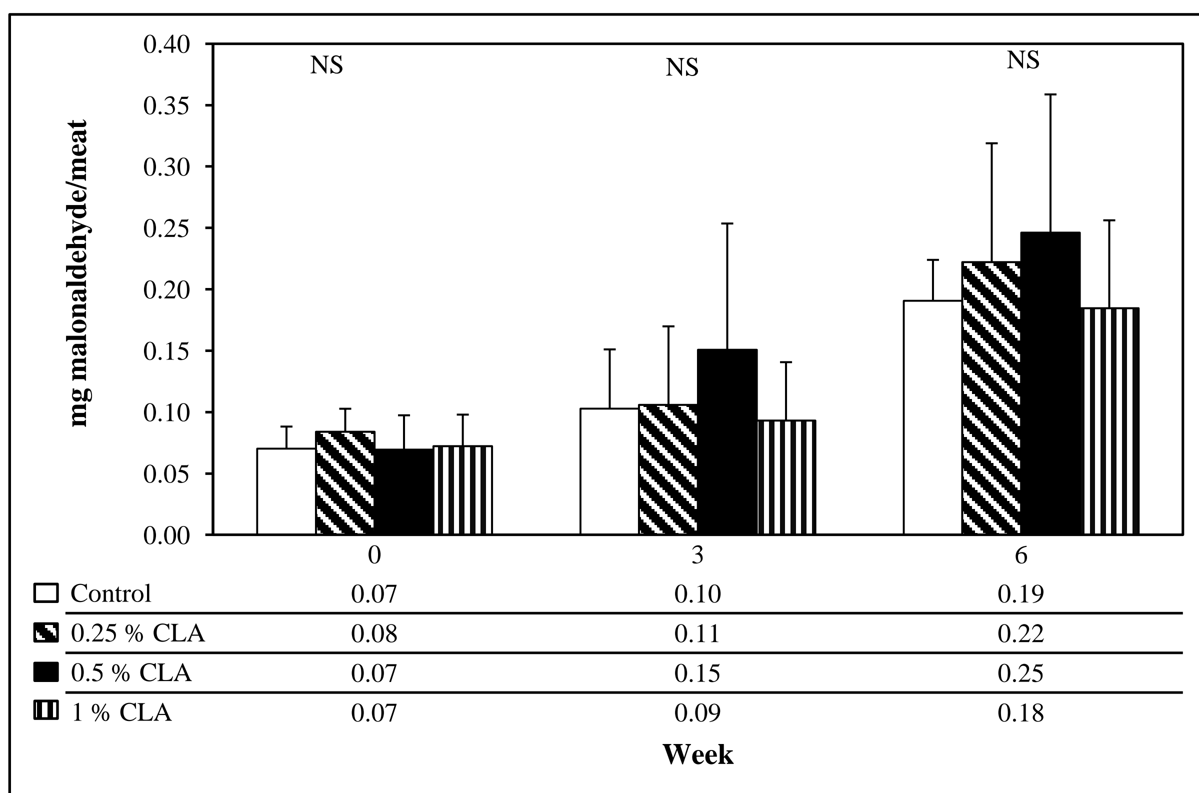
1.20 (0.25% CLA), 1.24 (0.5% CLA), and 1.26 (1% CLA) for the belly sampling position of subcutaneous fat. These values from the CLA-containing bellies were in accordance with the proposed C18:0/C18:2 ratio of 1.2 for good fat quality (Honkavaara, 1989). In the present study there were also significant ($p<0.001$) increases in the amount of SFAs and significant ($p<0.001$) decreases in the MUFA fraction of the belly sampling position (Table 4.13). A higher saturation ratio would mean firmer bellies and fewer slicing problems, but would be less desirable from the human health perspective (Schmid, Collomb, Sieber, & Bee, 2006). A C18:2 content < 15 % was set by Enser (1983) for good fat quality in bacon. All belly samples from the present study, even the control, had C18:2 contents < 15% (Table 4.11), conforming to the standard of <15 for good fat quality (Ellis & Isbell, 1926ab; Houben & Krol, 1980; Enser, 1983; Wood, 1983; Whittington *et al.*, 1986).

No significant differences were observed between the four bacon treatments for either PV or TBARS values, over the course of the six week refrigerated storage (Figures 4.13 and 4.14). The PVs recorded for the bacons were fairly low, the highest being <3.5 meq.peroxide/kg fat (control; after 6 weeks of refrigerated storage) (Figure 4.13), in comparison to the values of >20 meq.peroxide/kg fat observed for the control salami after ripening (Figure 4.11) and >15 meq.peroxide/kg fat for the control frozen patties, after 16 weeks of storage (Figure 4.9). This



NS = Not Significant

Figure 4.13: Peroxide value of bacons during refrigerated storage.



NS = Not Significant.

Figure 4.14: Thiobarbituric acid reactive substance values of bacon during refrigerated storage.

stability of the bacons against oxidation was propably due to the injection of the brine, which contained sodium tripolyphosphate, sodium nitrite and ascorbic acid, all being considered antioxidants (Van den Berg *et al*., 1995).

The TBARS values were also relatively low (<0.25 mg malonaldehyde/kg meat for the 0.5% CLA bacons at 6 week of refrigerated storage) (Figure 4.14), in comparison to the values reported for the frozen pork patties, which were <1.2 mg malonaldehyde/kg meat, for the control patties at week 8 of storage (Figure 4.10). Again the presence of three antioxidants, in the case of the control bacons, and four antioxidants, in the case of the CLA containing bacons, probably inhibited the oxidation process.

A consumer panel compared the acceptability of bacon containing different levels of CLA, with bacon manufactured from control bellies. Table 4.31 shows the demographic profile of the

Table 4.31: Demographic Profile of Consumer Panel for Bacon

Gender:	% of Total	Age:	% of Total
Female	92	**< 20**	20
Male	8	**20-29**	69
		30-39	1
		40-49	4
		50-59	5
		>60	1

consumer panel. Sixty-nine females and six males took part, while the age ranged between 20 and 60. Most participants were between 20-29 years of age and were mostly under- or postgraduate students.

From Table 4.32 it is evident that liking for the four treatment bacons differed significantly ($p>0.05$) for the control and 0.25% CLA treatment. The control bacon was more acceptable to consumers. However, liking for the 0.5 and 1% CLA bacon did not differ from both the control or 0.25% CLA bacon. The control sample had the highest mean which corresponds to between "like moderately" and "like slightly" on the hedonic scale. The lowest score was for the bacon sample from the 0.25% treatment (6.03), representing "like slightly" on the hedonic scale. In a study by Larsen *et al*. (2009), sensory scores for bacon from pigs fed 1.25% CLA showed no differences with control bacons.

Table 4.32: Mean values for the preference of bacon samples from 4 treatments. (n=75)

Bacon samples from different treatments	**Liking (Mean value out of nine)**	**p-value <0.05**
Control	6.87 ± 1.61^{b}	
0.25% CLA	6.03 ± 1.89^{a}	
0.5% CLA	6.59 ± 1.90^{ab}	
1% CLA	6.41 ± 1.78^{ab}	

Means with different superscripts (ab) differed significantly 1= Dislike extremely; 9 = Like extremely

Figure 4.16 shows the frequency of the hedonic scale ratings per treatment and can be

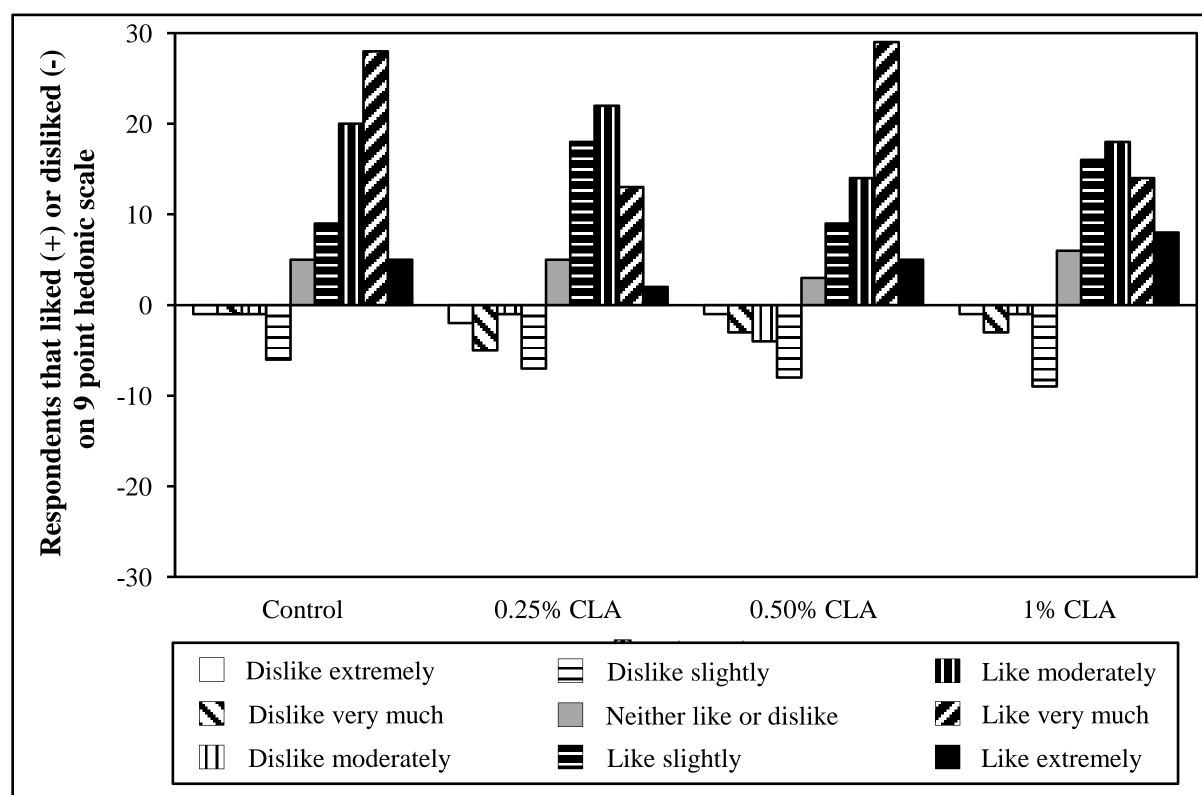


Figure 4.15: Frequency of consumers' ratings per treatment on the nine-point hedonic scale.

divided into two parts: i) bars pointing upwards, indicating the positive or "like" side of the hedonic scale, ranging from "like extremely" to "neither like nor dislike"; and ii) bars pointing downwards, indicating the negative or "dislike" side of the hedonic scale, categorized as between "dislike slightly" to "dislike extremely". It is clear that the positive indicators were in general used more frequently than the negative ones.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Pork meat was often controversial in the past because it was considered to contain an excess of fat, SFAs and cholesterol (Hernández *et al.*, 1998). The global meat industry responded by producing leaner pigs through modern pig breeding and feeding techniques (Blanchard, 1995). In pigs, the FA composition of the fat tissue triglycerides can be changed by altering the FA composition of dietary fat, which are absorbed intact from the small intestine and incorporated directly into the fat tissue (Rhee *et al.*, 1990). One way of designing functional pork products, by means of dietary manipulation, is by supplementing pig diets with CLA. The *cis-9, trans-11* isomer of CLA is apparently the biologically active isomer, which can be incorporated into phospholipids in the body (Pariza *et al.*, 2001). Experiments on laboratory animals indicate that CLA has beneficial effects on improving the immune function, preventing cancer, reducing the incidence of heart disease, improving blood sugar level, decrease blood cholesterol and reduce body weight (Migdal *et al.*, 2004).

In pigs, CLA has also shown to improve performance and reduce fat deposition, and increase lean meat content. Pigs fed CLA also had less BF, more carcass lean, bigger loin muscle area and better feed conversion (Swan *et al.*, 2001; Wiegand *et al.*, 2001; D'Souza, & Mullan, 2002). With respect to meat quality, CLA increased the SFA/UFA ratio in adipose tissue and IMF, and improved belly firmness (Migdal *et al.*, 2004). Most researchers found no detrimental effect of CLA supplementation on the eating and sensory quality of pork (Dugan *et al.*, 1999; Wiegand *et al.*, 2002; Corino *et al.*, 2003). D'Souza and Mullan (2002), however, found inferior eating quality, i.e. flavour, tenderness, juiciness and overall acceptability, as a result of CLA feeding. Except for the observation of increased belly firmness, little information is available regarding the effect of CLA supplementation on the technological properties of the fatty tissue. Furthermore, little information is available on the oxidative stability of processed meat products, manufactured from CLA supplemented pork.

In Chapter 2 of this dissertation an attempt was made to provide an adequate and orderly overview of the literature available, explaining consumption patterns of pork and pork quality in general. The effect of CLA in human medical trials and the controversy surrounding it was discussed. Conjugated linoleic acid has a pronounced effect on animal performance and meat quality. Research into the effects of CLA on the carcass quality, FA composition and sensory data vary amongst researchers. The author believes that this literature review has made a contribution in that it has highlighted the positive effects of CLA supplementation on animal performance and identified areas in need of clarification.

The practical work of this dissertation focused on the influence of different levels of CLA supplementation on pork. Worldwide there has been interest in the supplementation of pig feed with CLA (Dugan *et al.*, 1997; Eggert *et al.,* 1998; Stahl *et al.*,1999; Heckart *et al.*,2000; Weber *et al.*, 2000; Thiel-Cooper *et al.*, 2001; D'Souza & Mullan, 2002; Corino *et al.*, 2003; Migdal *et al.*, 2004; Szymczyk, 2005; Martin *et al.*, 2008a; Martin, Muriel, Gonzalez, Viguera, & Ruiz, 2008b; Cordero, *et al.*, 2010; Han *et al.*, 2011) and the subsequent manufacturing of processed products from this meat (D'Souza & Mullan, 2002; Wiegand *et al.*, 2002; Corino *et al.*, 2003). In the first part of this dissertation the optimum level of dietary CLA supplementation was determined, which would deliver the required positive effects in pork, without having a negative effect on eating quality.

All pig performance, growth traits and slaughter characteristics remained unchanged. The lack of significance in ADFI implies that the increase in CLA concentration did not have a negative effect on the acceptability of the food to the animals. It was found that pigs on the 1% CLA diet needed 0.17 kg less feed to increase 1 kg in weight, representing a 5.36 % increase in feed efficiency.

Lean classification of pigs improves due to CLA supplementation. For this study, 66.7% of the pigs in the control and 0.25 % CLA group obtained P and O classifications, compared to 83.3% in the 0.5 and 100% in the 1% CLA treatments. This finding is of practical significance for the South African pig producer, since more pigs being classified as P and O means more profitable pig production.

This study demonstrated that after feeding dietary CLA, differences exist in the BF and *M. longissimus thoracis* from a technological and quality point of view. Although not statistically significant, there was a gradual numerical increase in WHC of *M.longissimus thoracis* with increased exposure to dietary CLA. Backfat became firmer and more stable with elevated CLA levels, resulting in subcutaneous fat with improved technological properties. This finding is of special importance for manufacturers of processed products, like bacon and salami. A firmer BF will result in less cutting losses during bacon packaging, as well as salami of better quality and higher stability. The yellowness of the BF increased with increase in CLA levels, possibly because CLA acts as a pro-oxidant at higher inclusion levels.

Descriptive sensory analysis confirmed the findings of D'Souza & Mullan (2002) that higher inclusion levels of CLA may cause inferior eating quality. Increase in CLA levels led to increase in toughness, resistance, shear force and WHC for the pork meat, while chemical aroma increased in the pork fat. The hypothesis for the first aim of the study is therefore accepted.

In the second part of the study, the effect of CLA was studied on the physical and chemical properties, as well as FA composition of subcutaneous fat. This is the most far reaching study to

date, involving seven subcutaneous sampling positions. Subcutaneous fat samples were taken from the neck, BF, chump, rib area, shoulder, belly and leg. Data from this part of the study clearly demonstrate that CLA supplementation resulted in the improved technological quality of subcutaneous fat. Conjugated linoleic acid supplementation reduced IVs in the subcutaneous fat to < 60. According to Fischer (1989b), subcutaneous fat with an IV < 60 conforms to the very strict fat quality requirements for fat tissue used in firm cutting sausages, such as salami. Fat free dry matter content and IV demonstrated an interaction between dietary treatment and sampling position, implying that feeding different concentrations of CLA resulted in different expression of FFDM and IV for different sampling positions, e.g. for the 1% CLA treatment the belly had more FFDM than the neck.

Data showed that nearly all the individual FAs and FA ratios were influenced by CLA levels and sampling position. The Δ^9 desaturase index (C18:1*c*9/C18:0) decreased with increased CLA levels, confirming the increase in SFAs and decrease in MUFAs of the subcutaneous fat. The two CLA isomers also increased and the *cis*-9, *trans*-11 CLA isomer was about double the amount of the *trans*-10, *cis*-12 isomer at all sampling positions in the subcutaneous fat. Although not always statistically significant, there was a tendency for sampling positions on the dorsal (A, B, C) and lateral (D) sides of the carcass to have higher CLA content than sampling positions on the ventral (E, F and G) side of the carcass. An interesting effect observed, was the significantly different sampling position effects for both isomers at higher CLA inclusion levels.

From a health point of view, SFAs, *n*-6 and *n*-6/*n*-3 must decrease, while UFAs, MUFAs, MUFA/SFA, PUFA, *n*-3 and PUFA/SFA have to increase (Levnedmiddelstyrelsen, as cited by Madsen *et al*., 1992; Honkavaara, 1989, Muchenje *et al*., 2009). With increased dietary CLA in the present study, SFA, UFA, *n*-6/*n*-3 and PUFA/SFA contents of the subcutaneous fat increased and MUFA, PUFA, MUFA/SFA and *n*-3 contents decreased, while *n*-6 remained unchanged. Furthermore, all the CLA-containing dietary groups showed SFAs, MUFAs, UFAs, MUFA/SFA, PUFAs, PUFA/SFA values conforming to the standards set for each ratio. These important findings imply that dietary CLA supplementation results in subcutaneous fat of better quality from a meat technology point of view. The negative effect on FA profile must be weighed against the positive health implications of elevated CLA levels. It should also be noted that a SFA such as C16:0 which increased, is regarded as a healthy SFA, indicating that the FA profile of subcutaneous fat from the CLA treatments still had a healthy balance of FAs.

All FA ratios concerned with fat firmness increased with increase in dietary CLA. Whenever quality standards for good fat quality applied, subcutaneous fat from the CLA treatments conformed to it, confirming that dietary CLA supplementation improved subcutaneous fat quality of pigs. Increased dietary CLA also decreased the PI of subcutaneous fat, implying that meat products

with increased levels of CLA will be more resistant to oxidative breakdown. Increased CLA resulted in higher SFA contents in the BF and rib area, making these positions more suitable for the manufacturing of products like salami and bacon, which is more sensitive to fat quality. The leg had lower PUFAs, dienoic acids, trienoic acids and PIs and therefore better subcutaneous fat quality. The *n*-6/*n*-3 ratio of the chuck was consistently lower than other sampling positions.

For the first time research has been done to reveal the effect of CLA dietary supplementation on the formation of and change in fat crystals, subsequently explaining the observed increase in fat hardness. Differential scanning calorimetry of BF and belly fat showed that fat from CLA fed pigs contained increasing amounts of CLA, but a 10% overall decrease in UFAs and a 5% overall increase in each of C16:0 and C18:0. The higher content of saturated fat resulted in changes of melting properties. The onset setting temperature increased from ca. 14°C to 18°C, for fat from the 0.25 and 0.5% CLA treatments and to 26°C, for fat from the 1% CLA treatment. Respectively, the final melting temperatures increased from 37°C to 43°C and 45°C. The presence of β'-crystals of 18:0-16:0-18:1 triacylglycerides, in fat from 0.25 and 0.5% CLA fed pigs and β-crystals, in fat from 1% CLA fed pigs were observed. Small significant differences were noted in the setting and melting properties between BF and belly fat.

Another part of this extensive study was the analysis of IMF taken from five muscles, representing different oxidative patterns. The effect of CLA on the physical and chemical properties, as well as FA composition, were determined, making it the most extensive CLA related study to date, involving a broad range of skeletal muscle types. Dietary treatment did not affect extractable fat content, FFDM, moisture content and calculated IVs. The physical and chemical properties were different for the IMF from different muscles. It is important to note that IMF content is not strictly related to the metabolic type (Leseigneur-Meynier & Gandemer 1991). Only TB and SS from all dietary treatments conformed to the 2.5 % minimum IMF content (Prabucki 1991), and had the lowest FFDM and highest moisture content (74.0 - 74.48%). Iodine values remained unchanged, indicating that dietary CLA did not shift the FA composition to the more saturated side in the IMF. *M. Triceps brachi* from the control treatment had the highest calculated IV and SS (0.25% CLA treatment) the lowest calculated IV. There was a trend towards improved IMF quality with increased dietary CLA content.

Nearly all the individual FAs and FA ratios were influenced by dietary treatment and sampling position. The Δ^9 desaturase index (C18:1*c*9/C18:0) decreased with increased CLA levels, although not as high as for the subcutaneous fat samples. The reduction of the Δ^9 desaturase index activity is therefore not as prominent in the IMF, resulting in lower increase in SFAs and lower decrease in MUFAs. The two CLA isomers increased and the *cis*-9, *trans*-11 CLA isomer was, as with subcutaneous fat, double the amount of the *trans*-10, *cis*-12 isomer in all the muscles. There

was again a tendency for muscles on the lateral (TB) sides of the carcass to have higher CLA content.

From a health point of view, SFAs, *n*-6 and *n*-6/*n*-3 must decrease, while UFAs, MUFAs, MUFA/SFA, PUFA, *n*-3 and PUFA/SFA have to increase (Levnedmiddelstyrelsen, as cited by Madsen *et al*., 1992; Honkavaara, 1989, Muchenje *et al*., 2009). Increased dietary CLA level resulted in increases in PI, *n*-3, *n*-6, *n*-6/*n*-3, PUFAs, SFAs, AI, dienoic acids, trienoic acids and penta- + hexaenoic acids, while MUFA, UFA and MUFA/SFA contents decreased and tetraenoic acids remained unchanged. Intramuscular fat from CLA treatments conformed to all requirements for good quality fat, wherever applicable, implying that dietary CLA supplementation results in IMF of better quality from a meat technology point of view. Increased dietary CLA content caused a decrease in UFA content, which can be considered a negative from a health point of view. This negative health effect is however counteracted by the increase in CLA, which have positive health implications. The fact that diet had no effect on the PUFA/SFA ratio of the IMF, implied that the health properties of IMF did not deteriorate.

The increase in C16:0+C18:0 and C16:1+C18:1*c*9/C16:0+C18:0 contents, with increase in dietary CLA level, imply that CLA supplementation results in firmer and more stable IMF, which is advantageous from a meat technology point of view. However, dietary CLA did not affect C16:0/C18:2, C18:0/C18:2 contents and DBI. The glycolytic LD showed higher values for the SFA and AI, and lower C18:2, *cis*-9*, trans*-11, *trans*-10*, cis*-12, C18:3*n*-3, C20:2, UFA, PUFA/SFA, tetraenoic, pentaenoic, hexaenoic *n*-6/*n*-3 contents, compared to other muscles. *M. supra spinatus* had an atypical FA composition for an oxidative muscle, i.e. lower PUFA, *n*-6, *n*-3, tetraenoic, pentaenoic, haxaenoic, PUFA, *n*-6, *n*-3 and PI contents. *M. supra spinatus* also had higher individual MUFAs, thus contributing to the higher total MUFA content, implying better fat quality and more stable fat. Furthermore, it also had higher C22:6 content, and lower C20:4, C20:5, C22:5 and C22:6 contents. It should be noted, again, that variations in FA composition are not related to metabolic type (Leseigneur-Meynier & Gandemer 1991). This atypical behaviour of the *M. supra spinatus* was attributed to its very high IMF content. It is known that muscles with a high IMF contend tend to have a more SFA profile (Sun *et al*., 2004). The second hypothesis of this study is therefore also accepted.

The accelerated oxidation test indicated that CLA levels did not demonstrate an antioxidative affect for BF. Lightness, yellowness and SI for CLA pork chops increased after being on display under fluorescent light at 4°C for 8 days, while TBARS values remained unchanged. No differences in TBARS values were also observed after 3 months of frozen storage. After 6 months of frozen storage, the TBARS values of the CLA pigs were lower than the control, clearly showing the antioxidative effect of the dietary CLA. For frozen patties after eight and 16weeks of frozen

storage, the antioxidative effect of CLA was already clear in lower PVs for the 0.5 and 1% CLA treatments. Differences in TBARS values for the different treatments became evident after eight weeks for the frozen patties. For the frozen pork chops, differences in TBARS values became apparent only after sixth months of storage. The TBARS values for the frozen chops were substantially lower than that of the frozen patties. The mincing of the lean and the BF, and the addition of salt, led to an increase in the TBARS values despite the antioxidant effects of CLA.

The effect of the antioxidative properties of CLA was not clear in bacon, because of the presence of other antioxidants. Belly fat from the control was not as firm as belly fat from the CLA treatments. According to the consumer panel's results, the control bacon was preferred to the 0.25% CLA bacon. The third hypothesis of this study is not fully accepted, because the antioxidative effect of CLA in the diet at 0.5% was not always clear. Conjugated linoleic acid demonstrated no antioxidant properties during refrigerated display. In fresh meat the antioxidant properties of CLA become only evident after 6 months of frozen storage. In processed products such as patties and salami, the antioxidant properties of CLA were clearer. In bacon CLA demonstrated no antioxidant properties. The antioxidative property of CLA is possibly masked by the presence of other antioxidants in the formulation of bacon.

Future research may include a group feeding study with CLA fed pigs. Pigs were individually penned in this experiment. It is possible to make conclusions and recommendations regarding animal production efficiency, more applicable to industry, by using a group feeding system. A group feeding system may also be preferred, because pigs, due tot their social order, perform better in groups than when individually housed.

A further experiment with more animals in a group feeding system will help to clarify some of the questions regarding the effect of CLA supplementation on animal production efficiency. The experimental design of such an experiment will also include animals of the different gender groups, as well as different slaughter weights. That will enable us to investigate the interaction between CLA supplementation, gender of animals and slaughter weight. The observation in this study that the meat from CLA supplemented animals were less preferred by a sensory panel and were also less tender as demonstrated by sensory analysis and shear measurement also deserve further investigation with advanced sensory, physical and chemical techniques. Another research project for the future is to evaluate the effect of synthetic fortification of CLA on the quality and stability of processed meat products.

CHAPTER 6
REFERENCES

AASLYNG, M.D., & STØIER, S. (2004). The effect of intramuscular fat on eating quality of pork depending on end point temperature. In: *Proceedings for the 50th International Congress of Food Scientists and Technologists* (pp. 548-550). Finland: Helsinki.

AGRICULTURAL STATISTICS. (2009). Pig numbers, numbers slaughtered and average prices, production and consumption, p.62. Department of Agriculture, Republic of South Africa.

ALAM, S.Q., & ALAM, B.S. (1986). Effect of essential fatty acid deficiency on acyl group composition, membrane fluidity and adenylate cyclase activity in plasma membranes of rat submandibular salivary glands. *Journal of Nutrition,* 116, 1620-1630.

ALASNIER, C., DAVID-BRAIND, E., & GANDEMER, G. (2000). Lipolysis in muscles during refrigerated storage as related to the metabolic type of the fibres in the rabbit. *Meat Science*, 54, 127–134.

ALFAIA, C.P.M., ALVES, S.P., MARTINS, S.I.V., COSTA, FONTES, C.M.G.A., LEMOS, J.P.C., *et al*. (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discrimatory ability. *Food Chemistry*, 114, 939-946.

ALLEN, C.E., & FOEGEDING, E.A. (1981). Some lipid characteristics and interactions in muscle foods – a review. *Food Technology*, 35, 253-257.

AL-RASHOOD, K.A., ABOU-SHAABAN, R.R.A., ABDEL-MOETY, E.M., & RAUF, A. (1996). Compositional and thermal characterization of genuine and randomized lard: a comparative study. *Journal of American Oil Chemists Society*, 73, 303-309.

AMERICAN SOCIETY FOR TESTING AND MATERIALS (ASTM). (1996). Standard definitions of terms relating to sensory evaluation of materials and products. In: *Annual Book of ASTM Standards*. Philadedphia: American Society for Testing and Materials.

AMINOT-GILCHRIST, D.V., & ANDERSON, H.D.I. (2004). Insulin resistance-associated cardiovascular disease: potential benefits of conjugated linoleic acid. *American Journal of Clinical Nutrition*, 79, 1159S-1163S.

ANDERSEN, H.J. (2000). What is pork quality? In C. Wenk, J.A. Fernandez & M. Dupuis (Eds.), *Quality of meat and fat in pigs as affected by genetics and nutrition:* In: *Proceedings of the joint session of the EAAP commissions on pig production, animal genetics and animal nutrition.* (pp. 15-16). Zurich, Switzerland.

ANDERSEN, H.J., & SKIBSTED, L.H. (1991). Oxidative stability of frozen pork patties. Effect of light and salt. *Journal of Food Science*, 56(5), 1182-1184.

ANDERSON, B.W. (1988). The living world of the Old Testament. London: Langman.

ANGEL, A. (2004). Preface. *American Journal of Clinical Nutritionists*, 79, 1131S.

ANONMOUS. (2005). Pigs force rethink on human history. University of Oxford Press office. March 11, 2005.

ARISTOY, M-C., & TOLDRA, F. (1998). Concentration of free amino acids and dipeptides in porcine skeletal muscles with different oxidative patterns. *Meat Science*, 50, 327-332.

ASSOCIATION OF ANALYTICAL CHEMISTS. (2000). Official Methods of Analysis (14th edition). AOAC, Washington, DC, USA.

ASTM MANUAL SERIES: MNL 13. (1992). Manual on Descriptive Analysis Testing for Sensory Evaluation (R.C. Hootman, Ed.) Philadelphia: Library in Congress Cataloging-in-Publication Data.

ATKINSON, R.L. (1999). Conjugated linoleic acid for altering body composition and treating obesity. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza & G. Nelson (Eds.), *Advances in conjugated linoleic acid research*. (Vol. 1), (pp. 348-353). Champaign: AOCS Press.

ATTAR-BASHI, N.M., WEISINGER, R.S., BEGG, D.P., LI, D., & SINCLAIR, A.J. (2007). Failure of conjugated linoleic acid supplementation to enhance biosynthesis of docosahexaenoic acid from alpha-linoleic acid in healthy human volunteers. *Prostagladins, Leukotrienes and Essential fatty Acids*, 76, 121-130.

AVERETTE GATLIN, L., SEE, M.T., HANSEN, J.A., SUTTON, D., & ODLE, J. (2002a). The effects of dietary fat sources, levels, and feeding intervals on pork fatty acid composition. *Journal of Animal Science*, 80, 1606-1615.

AVERETTE GATLIN, L., SEE, M.T., LARICK, D.K., LIN, X., & ODLE, J. (2002b). Conjugated linoleic acid in combination with supplemental dietary fat alters pork fat quality. *Journal of Nutrition*, 132, 3105-3112.

AVERETTE GATLIN, L., SEE, M.T., ODLE, J., & LARICK, D.K. (2002c). Improving bacon quality in lean genotype swine: effects of dietary fat and supplemental CLA. Annual Swine Report 2002, Extension Swine Husbandry, Department of Animal Sciences, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, North Carolina.

BABOL, J., & SQUIRES, E.J. (1995). Quality of meat from entire male pigs. *Food Research International, 28*, 201–212.

BANNI, S., DAY, B.W., EVANS, R.W., CORONGIU, F.P., & LOMBARDI, B. (1995). Detection of conjugated diene isomers of linoleic acid in liver lipids of rats fed a choline-devoid diet indicates that the diet does not cause lipoperoxidation. *Journal of Nutritional Biochemistry*, 6, 281-289.

BARBUT, S., SOSNICKI, A.A., LONERGAN, S.M., KNAPP, T., CIOBANU, D.C., GATCLIFFE, L.J., *et al.* (2008). Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. *Meat Science*, 79, 46-63.

BARTON-GADE, P.A. (1983). Some experience on measuring the quality of pork fat. In J.D. Wood (Ed.), *Fat Quality in Lean Pigs*. (Special Report No. 2, EUR 8901 EN, CEC Workshop), (pp.47-52), Brussels, Belgium.

BARTON-GADE, P. A. (1987). Meat quality in boars, castrates and gilts. *Livestock Production Sciences, 16,* 187–196.

BASF. (2003). Technical Information. Johannesburg. South Africa.

BEE, G. (2001). Dietary conjugated linoleic acids affect tissue lipid composition but not *de novo* lipogenesis in finishing pigs. *Animal Research*, 50, 383-399.

BEE, G., GEBERT, S., & MESSIKOMMER, R. (2002). Effect of dietary energy supply and fat source on the fatty acid pattern of adipose and lean tissues and lipogenesis in the pig. *Journal of Animal Science*, 80, 1564-1574.

BEECHER, G.R., CASSENS, R.G., HOEKSTRA, W.G., & BRISKEY, E.J. (1965). Red and white fiber content and associated post mortem properties of seven porcine muscles. *Journal of Food Science,* 30(6), 969-976.

BEECHER, G.R., KASTENSCHMIDT, L.L., CASSENS, R.G., HOEKSTRA, W.G., & BRISKEY, E.J. (1968). A comparison of light and dark portions of a striated muscle. *Journal of Food Science*, 33(1), 84-88.

BEJERHOLM, C., & BARTON-GADE, P.A. (1986). Effect of intramuscular fat level on eating quality of pig meat. 32nd European Congress Meat Research Workshop, (pp. 389-391), 24-29 August, Gent, Belgium.

BELURY, M.A. (2002). Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. *Annual review of Nutrition*, 22, 505-531.

BELURY, M.A., & VAN DEN HEUVEL, J.P. (1999). Modulation of diabetes by conjugated linoleic acid. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza & G. Nelson (Eds.), *Advances in conjugated linoleic acid research.* (Vol. 1), (pp. 404-411). Champaign: AOCS Press.

BFAP. (2010). South African outlook - Meat. The South African Agricultural Baseline. Bureau for Food and Agricultural Policy. (pp. 33-37). [Available on internet:] www.bfap.co.za 20 DECEMBER 2011.

BLANCHARD, P. (1995). Pork Quality: How can the eating quality of pork be influenced by management on the farm? 1. Fatness. *Meat Focus International,* 4(8), 329-335.

BOCHICCHIO, D., FAETI, V., MARCHETTO, G., POLETTI, E., MARANESI, M., MORDENTI, A.L., *et al.* (2005). Effect of feeding partially hydrogenated lard on *trans*-fatty acid content of muscle and backfat of heavy pigs. *Meat Science*, 71, 651-656.

BONNE, K., & VERBEKE, W. (2008). Muslim consumer trust in halal meat status and control in Belgium. *Meat Science*, 79, 113-123.

BORKMAN, M., CHISHOLM, D.J., FURLER, S.M., STORLEIN, L.H., KRAEGEN, E.W., SIMONS, L.A., *et al*. (1989). Effects of fish oil supplementation on glucose and lipid metabolism in NIDDM. *Diabetes*, 38, 1314-1319.

BRECKENRIDGE, W.C. (1978). Stereospecific analysis of triacylglycerols. In A. Kuksis (Ed.), *Handbook of lipid research. Fatty acids and glycerides.* (pp. 197-232). New York: Plenum Press.

BREWER, M.S., ZHU, L.G., BIDNER, B., MEISINGER, D.J., & McKEITH, F.K. (2001). Measuring pork color: effects of bloom time, muscle, pH and relationship to instrumental parameters. *Meat Science*, 57, 169-176.

BROWNBILL, R.A., PETROSIAN, M., & IIICH, J.Z. (2005). Association between dietary conjugated linoleic acid and bone mineral density in postmenopausal women. *Journal of the American College of Nutrition*, 24, 177-181.

BRUWER, G.G. (1992). The evaluation of pig carcasses for the development of a classification system. Ph.D. dissertation, University of Pretoria, Pretoria.

CAMERON, N.D., & ENSER, M.B. (1991). Fatty acid composition of lipid in *Longissimus Dorsi* muscle of Duroc and British Landrace pigs and its relationship with eating quality. *Meat Science,* 29, 295-307.

CAMERON, N.D., ENSER, M., NUTE, G.R., WHITTINGTON, F.M., PENMAN, J.C., FISKEN, A.C., *et al*. (2000). Genotype with nutrition interaction on fatty acid compositin of intramuscular fat and the relationship with flavor of pig meat. *Meat Science*, 55, 187-195.

CAMPO, M.M., NUTE, G.R., WOOD, J.D., ELMORE, S.J., MOTTRAM, D.S., & ENSER, M. (2003). Modelling the effect of fatty acids in odour development of cooked meat in vitro: part I-sensory perception. *Meat Science*, 63, 367-375.

CARROLL, A.L., EGGERT, J.M., SCHINCKEL, A.P., & RICHERT, B.T. (1999). Effects of high oil corn and duration of conjugated linoleic acid (CLA) supplementation on pig growth, pork quality and carcass composition. Purdue University Swine Day Report. (pp 34-40).

CAVE, W.T. (1992). *n*-3 Fatty acid affects on tumorigenesis. In J.C. Frölich & C. Von Schacky (Eds.), *Fish, Fish Oils and Human Health* (pp. 179-189). Münich: W. Zuckschwerdt Verlag.

CHAJES, V., LAVILLONNIERE, F., MAILLARD, V., GIRAUDEAU, B., JOURDAN, M.L., SEBEDIO, J.L., *et al*. (2003). Conjugated linoleic acid content in breast adipose tissue of breast cancer patients and the risk of metastasis. *Nutrition and Cancer*, 45, 17-23.

CHAN, W.K.M., FAUSTMAN, C., & RENERRE, M. (1996). Model system for studying pigment and lipid oxidation relevant to muscle based foods. In Fereidoon Shahidi (Ed.), National Antioxidants, Chemistry, Health Effects and Application. pp. 319-330. Champaign: ACDS Press.

CHANG, K.C., DA COSTA, N., BLACKLEY, R., SOUTHWOOD, O., EVANS, G., & PLASTOW, G. (2003). Relationships of myosin heavy chain fibre types to meat quality traits in traditional and modern pigs. *Meat Science*, 64, 93-103.

CHEN, Z.Y, CHAN, P.T., KWAN, K.Y., & ZHANG, A (1997). Reassessment of the antioxidant activity of conjugated linoleic acid. *Journal of American Oil Chemists Society*, 74, 749-753.

CHILLIARD, Y., FERLAY, A., ROUEL, J., & LAMBERET, G. (2003). A review of nutritional and physiological factors affecting goat milk lipid synthesis and lypolysis. *Journal of Dairy Science*, 86(5), 1751-1770.

CHRISTIE, W.W. (1983). The composition and structure of milk lipids. In P.F. Fox (Ed.), *Developments in Dairy Chemistry.* (Vol 2), (pp.1-36). London: Applied Science Publishers

CHRISTIE, W.W., DOBSON, G., & GUNSTONE, F.D. (1997). Isomers in commercial samples of conjugated linoleic acid. *Journal of American Oil Chemists Society*, 74, 1231.

CHRISTIE, W.W., JENKINSON, D.M., & MOORE, J.H. (1972). Variation in lipid composition through the skin and subcutaneous adipose tissue of pigs. *Journal of the Science of Food and Agriculture*, 23, 1125-1129.

COOK, M.E., DEVONEY, D., DRAKE, B., PARIZA, M.W., WHIGHAM, L., & YANG, M. (1999). Dietary control of immune-induced cachexia: conjugated linoleic acid and immunity. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza & G. Nelson (Eds.), *Advances in conjugated linoleic acid research.* (Vol. 1), (pp. 226-237). Champaign: AOCS Press.

COOPER, K.C., SINCLAIR, L.A., WILKINSON, R.G., HALLETT, K.G., ENSER, M., & WOOD, J.D. (2004). Manipulation of the *n*–3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. *Journal of Animal Science*, 82, 1461-1470.

CORDAIN, L., EATON, S.B., SEBASTIAN, A., MANN, N., LINDEBERG, S., WATKINS, B.A., *et al.* (2005). Origins and evolution of the Western diet: health implications of the 21st century. *The American Journal of Clinical Nutrition*, 81(2), 341-354.

CORDERO, G., ISABEL, B., MENOYO, D., DAZA, A., MORALES, J., PIñEIRO, C., *et al.* (2010). Dietary CLA alters intramuscular fat and fatty acid composition of pig skeletal muscle and subcutaneous adipose tissue. *Meat Science*, 85, 235-239.

CORINO, C., SPARK, S.J.C., PARRISH, F.C., & ZIMMERMAN J. (2003). Effect of conjugated linoleic acid on meat quality, lipid metabolism and sensory characteristics of dry-cured hams from heavy pigs. *Journal of Animal Science*, 81, 2219-2229.

COURTINE, R.J. (1994). Larousse Gastronomique. London: Paul Hamlyn.

CZANDERNA, M., KOWALCZYK, J., NIEDŹWIEDZKA, K.M, WĄSOWSKA, I., PASTUSZEWSKA, B., BULSKA, E., *et al.* (2004). Liver and body mass gain, content of conjugated linoleic acid (CLA) isomers and other fatty acids in the liver of rats red CLA isomers and selenium. *Journal of Animal Feed Science*, 13, 353-369.

DAFF. (2010). A profile of the South African pork market value chain. Department of Agriculture, Forestry and Fisheries, South Africa.

DAVENEL, A., RIAUBLANC, A., MARCHAL, P., & GANDEMER, G. (1999). Quality of pig adipose tissue: relationship between solid fat content and lipid composition. *Meat Science*, 51, 73-79.

DEAN, H.K., & HILDITCH, T.P. (1933). The body fats of the pigs III. The influence of body temperature on the composition of depot fats. *Biochemical Journal,* 27, 1950-1961.

DE KOCK, H.L., HEINZE, P.H., POTGIETER, C.M., DIJKSTERHUIS, G.B., & MINNAAR, A. (2001). Temporal aspects related to the perception of skatole and androstenone, the major boar odour compounds. *Meat Science*, 57, 61-70.

DEMAREE, S.R., GILBERT, C.D., MERSMANN, H.J., & SMITH, S.B. (2002). Conjugated linoleic acid differentially modifies fatty acid composition in subcellular fractions of muscle and adipose tissue but not adiposity of post weanling pigs. *Journal of Nutrition*, 132, 3272-3279.

DE VOL, D.L., MCKEITH, F.K., BECHTEL, P.J., NOVAKOFSKI, J., SHANKS, R.D., & CARR, T.R. (1988). Variation in composition and palatability traits and relationships between muscle characteristics and palatability in a random sample of pork carcasses. *Journal of Animal Science,* 66, 385-395.

DOYLE, L., JEWELL, C., MULLEN, A., NUGENT, A.P., ROCHE, H.M., & CASHMAN, K.D. (2005). Effect of dietary supplementation with conjugated linoleic acid on markers of calcium and bone metabolism in healthy adult men. *European Journal of Clinical Nutrition,* 59, 432-440.

DOYLE, M.P., & ERICKSON, M.C. (2006). Emerging microbiological food safety issues related to meat. *Meat Science*, 74, 98-112.

DRANSFIELD, E. (2008). The taste of fat. *Meat Science*, 80, 37-42.

D'SOUZA, D.N., & MULLAN B.P. (2002). The effect of genotype, sex and management strategy on the eating quality of pork. *Meat Science*, 60, 95-101.

DU, M., AHN, D.U., NAM, K. C., & SELL, J.L. (2000). Influence of dietary conjugated linoleic acid on volatile profiles, colour and lipid oxidation of irradiated raw chicken meat. *Meat Science,* 56, 387-395.

DU, M., AHN, D.U., & SELL, J.L. (1999). Effect of dietary conjugated linoleic acid on the composition of egg yolk lipids. *Poultry Science*, 78, 1639-1645.

DUGAN, M.E.R., AALHUS, J.L., JEREMIAH, L.E., KRAMER, J.K.G., & SCHAEFER, A.L. (1999). The effects of feeding conjugated linoleic acid on subsequent pork quality. *Canadian Journal of Animal Science*, 79, 45-51.

DUGAN, M.E.R., AALHUS, J.L., & KRAMER, J.K.G. (2004). Conjugated linoleic acid pork research. *American Journal of Clinical Nutrition*, 79, 1212S-1216S.

DUGAN, M.E.R., AALHUS, J.L., SCHAEFER, A.L., & KRAMER, J.K.G. (1997). The effect of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. *Canadian Journal of Animal Science*, 77, 723-725.

EDWARDS, J.W., CANNELL, R.C., GARRETT, R.P., SAVELL, J.W., CROSS, H.R., & LONGNECKER, M.T. (1989). Using ultrasound, linear measurements, and live fat thickness estimates to determine the carcass composition of market lambs. *Journal of Animal Science*, 67, 3322-3330.

EGGERT, J.M., BELURY, M.A., KEMPA-STECZKO, A., MILLS, S.E., & SCHINCKEL, A.P. (2001). Effects of conjugated linoleic acid on the belly firmness and fatty acid composition of genetically lean pigs. *Journal of Animal Science*, 79, 2866-2872.

EGGERT, J.M., BELURY, M.A., & SCHINCKEL, A.P. (1998). The effects of conjugated linoleic acid (CLA) and feed intake on lean pig growth and carcass composition. Purdue University Swine Day Report, September, pp. 21-25.

ELLIS, N.R., & ISBEL, H.S. (1926a). Soft pork studies. 2. The influence of the character of the ration upon the composition of the body fat of hogs. *Journal of Biological Chemistry,* 69, 219-238.

ELLIS, N.R., & ISBEL, H.S. (1926b). Soft pork studies. 3. The effect of food fat upon body fat, as shown by the separation of the individual fatty acids of the body fat. *Journal of Biological Chemistry,* 69, 239-248.

ELLIS, M., WEBB, A.J., AVERY, P.J., BROWN, I., & SMITHARD, R. (1990). Evidence for genetic variation in the eating quality of pork. In: *Proceeding 4th World Congress*. General Applied Livestock Products, (pp. 553-556), 23-29 July, Edinburgh, Scotland.

ENSER, M.B. (1983). The relationship between the composition and the consistency of pig backfat. In J.D. Wood (Ed.), *Fat Quality in Lean Pigs* (Special Report No. 2, EUR 8901 EN, CEC Workshop), (pp.53-57), Brussels, Belgium.

ENSER, M. (1984). The chemistry, biochemistry and nutritional importance of animal fats. In J Wiseman (Ed.), *Fats in Animal Nutrition.* (pp. 25-51). London: Butterworths.

ENSER, M., DRANSFIELD, E., JOLLEY, P.D., JONES, R.C.D., & LEEDHAM M. (1984). The composition and consistency of pig backfat as it affects the quality of vacuum-packed rindless bacon rashers. *Journal of Science and Food Agriculture*, 35, 1230-1240.

ENSER, M., HALLET, K., HEWITT, B., FURSEY, G.A.J., & WOOD, J.D. (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science*, 42, 443-456.

ESKORT. (2011). The South African meat Industry. [Available on internet:] www.eskort.co.za 6 December 2011.

EVANS, M., GEIGERMAN, C., COOK, J., CURTIS, L., KUEBLER, B., & MCINTOSH, M. (2000). Conjugated linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3-L1 preadipocytes. *Lipids*, 35, 899-910.

EYNARD, A.R., & LOPEZ, C.B. (2003). Conjugated linoleic acid (CLA) versus saturated fats/cholesterol: their proportion in fatty and lean meats may affect the risk of developing colon cancer. *Lipids in Health and Disease*, 2, 6.

FARNWORTH, E.R., & KRAMER, J.K.G. (1987). Fat metabolism in growing swine: A review. *Canadian journal of Animal Science*, 67(2), 301-318.

FERNANDEZ, X., MONIN, G., TALMANT, A., MOUROT, J., & LEBRET, B. (1999). Influence of intramuscular fat content on the quality of pig meat – 2. Consumer acceptability of *m.longissimus lumborum. Meat Sci*ence, 53, 67-72.

FIELD, C.J., & SCHLEY, P.D. (2004). Evidence for potential mechanisms for the effect of conjugated linoleic acid on tumor metabolism and immune function: lessons from *n*-3 fatty acids. *American Journal of Clinical Nutrition,* 79, 1190S-1198S.

FISCHER, A. (1989a). Requirements asked from and properties found in pig's fatty tissue: on the processing of fats from slaughter animals. I. *Die Fleischherei*, 8, iv-v.

FISCHER, A. (1989b). Requirements asked from and properties found in pig's fatty tissue: on the processing of fats from slaughter animals. II. *Die Fleischherei*, 9, iii-v.

FLINTOFF-DYE, N.L., & OMAYE, S.T. (2005). Antioxidant effects of conjugated linoleic acid isomers in isolated human low-density lipoproteins. *Nutritional Research*, 25, 1-12.

FLORES, M., ALASNIER, C., ARISTOY, M.C., NAVARRO, J.L. GANDEMER, G., & TOLDRA, F. (1996). Activity of aminopeptidase and lipolytic enzymes in five skeletal muscles with various oxidative patterns. *Journal of Science and Agriculture*, 70, 127-130.

FOLCH, J., LEES, M., & SLOANE-STANLEY, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry*, 226, 497-509.

FORTIN, A., ROBERTSON, W.M., & TONG, A.K.W. (2005). The eating quality of Canadian pork and its relationship with intramuscular fat. *Meat Science*, 69, 297-305.

FORTIN, A., WOOD, J.D., & WHELEHAN, O.P. (1985). The partition and distribution of fat in entire male and female Large white, Pietrain, and Iron age pigs. *Animal Production*, 40, 542.

FOSTER, C.B., AMASON, J.T., & BRIGGS, C.J. (2005). Natural health products and drug disposition. *Annual Reviews of Pharmacological Toxicology*, 45, 203-226.

GAULLIER, J.-M., BERVEN, G., BLANKSON, H., & GUDMUNDSEN, O. (2002). Clinical trial results support a preference for using CLA preparations enriched with two isomers rather than four isomers in human studies. *Lipids,* 37, 1019-1025.

GAULLIER, J.-M., HALSE, J., HØYE, K., KRISTIANSEN, K., FAGERTUN, H., VIK, H., *et al*, (2004). Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. *American Journal of Clinical Nutrition*, 79, 118-1125.

GIRARD, J.P., BOUT, J., & SALORT, D. (1988). Lipides et qualité des tissus adipeux et musculaires de porc, facteurs de variation. *Journale Research de Porcine France*, 20, 255-270.

GRAU, R., & HAMM, R. (1953). Eine einfache Methode zur Bestimmung der Wasserbindung im Muskel. *Die Naturwissenschaften,* 40, 29.

GRIINARI, J.M., & BAUMANN, D.E. (1999). Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk ruminants. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza & G. Nelson (Eds.), *Advances in conjugated linoleic acid research*. (Vol. 1), (pp. 180-200). Champaign: AOCS Press.

HA, Y.L., STORKSON, J., & PARIZA, M.W. (1990). Inhibition of benzo(a)pyrene-induced mouse for stomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Research*, 50, 1097-1101.

HADORN, R., EBERHARD, P., GUGGISBERG, D., PICCINALI, P., & SCHLICTHERLE-CERNY, H. (2008). Effect of fat score on the quality of various meat products. *Meat Science*, 80, 765-770.

HAGEMANN, J.W. (1988). Thermal behavior and polymorphism of acylglycerides. In N. Garti & K. Sato (Eds.), *Crystallization and polymorphism of fats and fatty acids.* (pp 9-96). New York: Dekker.

HAH, K.H., YANG, H.S., HUR, J.S., MOON, S.S., HA, Y.L., & PARK, G.B. (2006). Effect of substituted conjugated linoleic acid for fat on meat qualities, lipid oxidation and residual nitrite content in emulsion-type sausage. *Asian-Australasian Journal of Animal Science*, 19, 744-750.

HAM, B., SHELTON, R., BUTLER, B., & THIONVILLE, P. (1998). Calculating the iodine value for marine oils from fatty acid profiles. *Journal of the American Oil Chemists Society,* 75(10), 1445-1446.

HAMBRECHT, E., EISSEN, J.J., NEWMAN, D.J., SMITS, C.H.M., VERSTEGEN, M.W.A., & DEN HARTOG, L.A. (2005). Pre-slaughter handling effects on pork quality and glycolytic potential in two muscles differing in fiber type composition. *Journal of Animal Science*, 83(4), 900-907.

HAN, X.F., FENG, F.J., YU, J.P., TANG, S.X., BAMIKOLE, M.A., TAN, Z.L., *et al.* (2011). Effects of conjugated linoleic acid supplementation on growth, carcass characteristics and fatty acid profiles of muscle and fat in growing-finishing pigs. *Journal of Animal and Feed Sciences*, 20, 171-185.

HAUSER, A., & PRABUCKI, A.L. (1990). Ergebnisse eines "screenings"betreffend die Fettqualität bei Mastschweinen in schweizerischen Schlachthöfen. *Journal of Animal Physiology and Animal Nutrition*, 64, 36.

HAYEK, M.G., HAN, S.N., WU, D., WATKINS, B.A., MEYDANI, M., DORSEY, J.L., *et al.* (1999). Dietary conjugated linoleic acid influences the immune response of young and old C57BL/6NCrlBR mice. *Journal of Nutrition*, 129, 32-38.

HECKART, M.L., EGGERT, J.M., SCHINCKEL, A.P., MILLS, S.E., & DONKIN, S.S. (2000). Feeding conjugated linoleic acids (CLA) decreases lipogenesis and alters expression of lipogenic genes in porcine adipose tissue. Purdue University Swine day Report. (pp. 54-59).

HERNÁNDEZ P., NAVARRO J.L., & TOLDRÁ, F. (1998). Lipid composition and lipolytic enzyme activities in porcine skeletal muscles with different oxidative pattern. *Meat Science,* 49, 1- 10.

HERRANZ, B., ORDÓÑEZ, J.A., DE LA HOZ, L., HIERRO, E., SOTO, E., & CAMBERO, M.I. (2008). Fatty acid composition of salami from different countries and their nutritional implications. *International Journal of Food Sciences and Nutrition*, 59, (7/8), 607-618.

HERRERA, J.A., SHABABUDDIN, A.K., ERSHENG, G., WEI, Y., GARCIA, R.G., & LOPEZ-JARAMILLO, P. (2005). Calcium plus linoleic acid therapy for pregnancy-induced hypertension. *International Journal of Gynaecology and Obstetrics*, 91, 221-227.

HERTZMAN, C., GÖRANSSON, L., & RUDÉRUS, H. (1988). Influence of fishmeal, rape seed, and rape seed meal in feed on the fatty acid composition and storage stability of porcine body fat. *Meat Science,* 23, 37-53.

HONKAVAARA, M. (1989). Influence of porcine stress and breed on the fatty acid profiles of subcutaneous and intramuscular total lipids. *Fleischwissenschaft*, 69(9), 1429-1432.

HOUBEN, J. H., & KROL, B. (1983). Pig fats and the manufacture and storage of meat products. In J.D. Wood (Ed.), *Fat Quality in Lean Pigs*, Meat Research Institute Special Report No. 2, Document number EUR 8901 EN, CEC Workshop. (pp. 15–26). Brussels, Belgium.

HUGHES, P.E., HUNTER, W.J., & TOVE, S.B. (1982). Biohydrogenation of unsaturated fatty acids, purification and properties of *cis*-9, *trans*-11 octadecadienoate reductase. *Journal of Biological Chemistry*, 257, 3643-3649.

HUGO, A., & ROODT, E. (2007). Significance of porcine fat quality in meat technology: a review. *Food reviews International*, 23, 175-198.

HUR, S.J., PARK, G.B., & JOO, S.T. (2007). A comparison of the effects of dietary conjugated linoleic acid contents, cholesterol, lipid oxidation and drip loss in pork loin and chicken breast. *Journal of Muscle Foods*, 18, 264-275.

HUR, S.J., YE, B.W., LEE, J.L., HA, Y.L., PARK, G.B., & JOO, S.T. (2004). Effects of conjugated linoleic acid on colour and lipid oxidation of beef patties during cold storage. *Meat Science*, 66, 771-775.

INGR, I. (1989). Meat quality. Defining the term by modern standards. *Fleischwissenschaft*, 69(8), 1268-1270.

INNIS, S.M., SPRECHER, H., HACHEY, D., EDMOND, J., & ANDERSON, R.E. (1999). Neonatal polyunsaturated fatty acid metabolism. *Lipids*, 34, 139-149.

INTARAPICHET, K.-O., MAIKHUNTHOD, B., & THUNGMANEE, N. (2008). Physicochemal characteristics of pork fed palm oil and conjugated linoleic acid supplements. *Meat Science*, 80, 788-794.

IP, C. (1994). Conjugated linoleic acid in cancer prevention research: a report of current status and issues. Research report no. 100-4. National Livestock and Meat Board, Chicago, IL.

IP, C., SINGH, M., THOMPSON, H.J., & SCIMECA, J.A. (1994). Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Research,* 54, 1212-1215.

IRIE, M., IZUMO, A., & MOHRI, S. (1996). Rapid method for determining water-holding capacity in meat using video image analysis and simple formulae. *Meat Science,* 42, 95–102.

IWATA, T., KAMEGAI, T., YAMAUCHI-SATO, Y., OGAWA, A., KASAI, M., AOYAMA, T., *et al*. (2007). Safety of dietary conjugated linoleic acid (CLA) in a 12-weeks trail in healthy overweight Japanese male volunteers. *Journal of Oleo Science*, 56, 517-525.

JACKSON, J.E., PASCHKE, R.F., TOLBERG, W. BOYD, H.M., & WHEELER, D.H. (1952). Isomers of linoleic acid. Infrared and ultraviolet properties of methyl esters. *Journal of American Oil Chemists Society*, 29, 229-234.

JEFFCOAT, R., & JAMES, A.T. (1984). The control of stearoyl-CoA desaturase by dietary linoleic acid. *FEBS Letters*, 85(1), 114-118.

JEWELL, C., CUSACK, S., & CASHMAN, K.D. (2005). The effect of conjugated linoleic acid on transepithelial calcium transport and mediators of paracellular permeability in human intestinal-like Caco-2 cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 72, 163-171.

JOO, S.T., KAUFMAN, R.G., BORGGAARD, C., STEVENSON-BARRY, J.M., PARK, G.B., & KIM, B.C. (2000). Objectively predicting quality of post-rigor pork musculature: I. Initial comparison of techniques. *Asian-Australian Journal of Animals*, 13, 68-76.

JOO, S.T., KAUFFMAN, R.G., KIM, B.C., & KIM, C.J. (1995). The relationship between colour and water-holding capacity in post rigor porcine *Longissimus* muscle. *Journal of Muscle Foods*, 6(3), 211-226.

JOO, S.T., LEE, J.I., HA, Y.L., & PARK, G.B. (2002). Effects of dietary conjugated linoleid acid on fatty acid composition, lipid oxidation, color and water-holding capacity of pork loin. *Journal of Animal Science*, 80, 108-113.

KAIZER, L., BOYD, N.F., KRIUKOV, V., & TRITCHTER, D. (1989). Fish consumption and breast cancer risk: an ecological study. *Nutrition and Cancer*, 12, 61-68.

KARMALI, R.A. (1996). Historical perspective and potential use of n-3 fatty acids in therapy of cancer cachexia. *Nutrition*, 12 (Suppl 1), S2-S4.

KAUFFMAN, R.G., CARPENTER, Z.L., BRAY, R.W., & HOEKSTRA, W.G. (1964). Biochemical properties of pork and their relationship to quality. II. Intramuscular fat. *Journal of Food Science*, 29, 70-74.

KELLEY, D.S., & ERICKSON, K.L. (2003). Modulation of body composition and immune cell functions by conjugated linoleic acid in humans and animal models: benefits vs. risks. *Lipids*, 38, 377-386.

KEPLER, C.R., HIRONS, K.P., MCNEILL, J.J., & TOVE, S.B. (1966). Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens. Journal of Biological Chemistry,* 241, 1350-1354.

KHAN, M.Z. (1997). The Qur'an: the eternal revelation vouchsafed to Muhammad, the seal of Prophets/Arabic text with a new translation by Muhammad Zafrulla Khan. New York: Olive Branch Press.

KIM, Y.L., LIU, R.H., RYCHLIK, J.L., & RUSSELL, J.B. (2002). The enrichment of a ruminal bacterium (*Megaspheara elsdenii* YJ-4) that produces the *trans*-10, *cis*-12 isomer of conjugated linoleic acid. *Journal of Applied Microbiology*, 92, 976-982.

KING, D.A., BEHRENDS, J.M., JENSCHKE, B.E., RHOADES, R.D., & SMITH, S.B. (2004). Positional distribution of fatty acids in triacylglycerols from subcutaneous adipose tissue of pigs fed diets enriched with conjugated linoleic acid, corn oil, or beef tallow. *Meat Science*, 67, 675-681.

KNAPP, H. R. (1999). Conjugated linoleic acid as a nutraceutical: observations in the context of 15 years of 2-3 polyunsaturated fatty acid research. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza & G. Nelson (Eds.), *Advances in conjugated linoleic acid research*. (Vol. 1), (pp. 412-419). Champaign: AOCS Press.

KNEKT, P., & JÄRVINEN, R. (1999). Intake of dairy products and breast cancer risk. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza & G. Nelson (Eds.), *Advances in conjugated linoleic acid research.* (Vol. 1), (pp. 444-468). Champaign: AOCS Press.

KOLETSKO, B., & SINCLAIR, A. (1999). Long-chain polyunsaturated fatty acids in diets for infants: choices for recommending and regulating bodies and for manufacturers of dietary products. *Lipids*, 34, 215-220.

KOUBA, M., & BONNEAU, M. (2009). Compared development of intermuscular and subcutaneous fat in carcass and primal cuts of growing pigs from 30 to 140 kg body weight. *Meat Science*, 81, 270-274.

KOUBA, M., ENSER, M., WHITTINGTON, F.M., NUTE, G.R., & WOOD, J.D. (2003). Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition and meat quality in the growing pig. *Journal of Animal Science*, 81, 1967-1979.

KOUBA, M., MOUROT, J., & PEINIAU, P. (1997). Stearoyl-CoA-desaturase activity in adipose tissues and liver of growing Large White and Meishan pigs. *Comparative Biochemistry and Physiology*, 118B, 509-514.

KRAMER, J.K.G., BLACKADAR, C.B., & ZHOU, J. (2002). Evaluation of two GC columns (60-m SUPELCOWAX 10 and 100-m CP Sil 88) for analysis of milk fat with emphasis on CLA, 18:1, 18:2 and 18:3 isomers, and short- and long-chain FA. *Lipids*, 37, 823-835.

KRAMER, J.K.G., CRUZ-HERNANADEZ, C. DENG, Z. ZHOU, J. JAHREIS, G., & DUGAN, M.E.R. (2004). Analysis of conjugated linoleic acid and *trans* 18:1 isomers in synthetic and animal products. *American Journal of Clinical Nutrition,* 79(Suppl), 1137S-1145S.

KRAMER, J.K.G., SEHAT, N., FRITSCHE, J., MOSSOBA, M.M., EULITZ, K., YURAWECZ, M.P., *et al*. (1999). Separation of conjugated fatty acid isomers. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza, & G. Nelson (Eds.), *Advances in conjugated linoleic acid research.* (Vol. 1), (pp. 83-109). Champaign: AOCS Press.

KRITCHEVSKY, D. (1999). Conjugated linoleic acid and experimental atherosclerosis in rabbits. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza & G. Nelson (Eds.), *Advances in conjugated linoleic acid research*. (Vol. 1), (pp. 397-403). Champaign: AOCS Press.

LABORDE, D., TALMANT, A., & MONIN, G. (1985). Enayme, metabolic and contractile activities in 30 pig muscles and their relationship with ultimate postmortem pH. *Reproduction, Nutrition, Development*, 25, 619-628.

LAMARCHE, B., & DESROCHES, S. (2004). Metabolic syndrome and effects of conjugated linoleic acid in obesity and lipoprotein disorders: the Quebec experience. *American Journal of Clinical Nutrition*, 79, 1149S-1152S.

LANARI, M.C., SCHAEFER, D.M., & SCHELLER, K.K. (1995). Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. *Meat Science*, 41, 237-250.

LARICK. D.K., TURNER, B.E., SCHOENHERR, W.D., COFFEY, M.T., & PILKINGTON, D.H. (1992). Volatile compound content and fatty acid composition of pork as influenced by linoleic acid content of the diet. *Journal of Animal Science*, 70, 1397-1403.

LARSEN, T.M., TOUBRO, S., & ASTRUP, A. (2003). Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and human studies. *Journal of Lipid Research*, 44, 2234-2241.

LARSEN, S.T., WIEGAND, B.R., PARRISH Jr., F.C., SWAN, J.E., & SPARKS, J.C. (2009). Dietary conjugated linoleic acid changes belly and bacon quality from pigs fed varied lipid sources. *Journal of Animal Science*, 87, 285-295.

LAURIDSEN, C., MU, H., & HENCKEL, P. (2005). Influence of dietary conjugated linoleic acid (CLA) and age at slaughtering on performance, slaughter- and meat quality, lipoproteins, and tissue deposition of CLA in barrows. *Meat Science*, 69, 393-399.

LAURIDSEN, C., NIELSEN, J.H., HENCKEL, P., & SǾRENSEN, M.T. (1999). Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E, and copper. *Journal of Animal Science*, 77, 105-115.

LEA, C.H., SWOBODA, P.A.T., & GATHERUM, D.P. (1970). A chemical study of soft fat in cross bred pigs. *Journal of Agricultural Science*, 74(2), 279-289.

LEAF, A., & WEBER, P. (1987). A new era for science in nutrition. *American Journal of Clinical Nutrition*, 45, 1048-1053.

LEAF, A., JORGENSEN, M.B., JACOBS, A.K., COTE, G., SCHOENFELD, D.A, SCHEER, J., *et al*. (1994). Do fish oils prevent restenosis after coronary angioplasty? *Circulation*, 90, 2248-2257.

LEAF, A., & KANG, J.X. (1998). Omega 3 fatty acids and cardiovascular disease. *World Reviews of Nutrition and Diet*, 83, 24-37.

LEE, J.I., LEE, J.H., KWACK, S.C., HA, Y.J., JUNG, J.D., LEE, J.W., *et al*. (2003). Effects of CLA-vegetable oils and CLA-lard additives on quality characteristics of emulsion-type sausage. *Journal of Animal Science and Technology*, 45(2), 283-296.

LESEIGNEUR-MEYNIER, A., & GANDEMER, G. (1991). Lipid composition of pork muscle in relation to the metabolic type of the fibres. *Meat Science*, 29, 229-241.

LIZARDO, R., VAN MILGEN, J., MOUROT, J., NOBLET, J., & BONNEAU, M. (2002). A nutritional model of fatty acid composition in the growing-finishing pig. *Livestock Production Science*, 75, 167-182.

LO FIEGO, D.P., MACCHIONI, P., SANTORO, P., PASTORELLI, G., & CORINO, C. (2005). Effect of dietary conjugated linoleic acid (CLA) supplementation on CLA isomers content and fatty acid composition of dry-cured Parma ham. *Meat Science*, 70, 285-291.

MacDERMOND, R., & DORSCHEID, D.R. (2011). Conjugated linoleic acid (CLA): is it time to supplement asthma therapy? *Pulmonary Pharmacology & Therapeutics*, 24, 540-548.

MADSEN, A., JAKOBSEN, K., & MORTENSEN, H.P. (1992). Influence of dietary fat on carcass quality in pigs. A review. *Animal Science*, 42(4), 220-225.

MALTIN, C.A., WARKUP, C.C., MATTHEWS, K.R., GRANT, C.M., PORTER, A.D., & DELDAY, M.I. (1997). Pig muscle fibre characteristics as a source of variation in eating quality. *Meat Science* 47, 237-248.

MARTIN, J.C., GREGOIRE, S., SIESS, M.H., GENTY, M., CHARDIGNY, J.M., BERDEAX, O., *et al.* (2000). Effects of conjugated linoleic acid isomers on lipid metabolizing enzymes in male rats. *Lipids*, 35, 91-98.

MARTíN, D., ANTEQUERA, T., GONZALES, E., LóPEZ-BOTE, C.J., & RUIZ, J. (2007). Changes in the fatty acid profile of the subcutaneous fat of swine throughout fattening as affected by dietary conjugated linoleic acid and mono-unsaturated fatty acids. *Journal of Agricultural and Food Chemistry*, 55, 10820-10826.

MARTÍN, D., ANTEQUERA, T., MURIEL, E., ANDRES, A.I., & RUIZ, J. (2008a). Oxidative changes of fresh loin from pig, caused by dietary conjugated linoleic acid and mono-unsaturated fatty acids, during refrigerated storage. *Food Chemistry*, 111, 730-737.

MARTÍN, D., ANTEQUERA, T., MURIEL, E., PEREZ-PALACOIS, T., & RUIZ, J. (2011). Effect of dietary conjugated linoleic acid in combination with mono-unsaturated fatty acids on the composition and quality traits of cooked loin. *Food Chemistry*, 124, 518-526.

MARTÍN, D., MURIEL, E., ANTEQUERA, T., ANDRES, A.I., & RUIZ, J. (2009). Quantitative changes in the fatty acid profile of lipid fractions of fresh loin from pigs as affected by dietary conjugated linoleic acid and mono-unsaturated fatty acids during refrigerated storage. *Journal of Food Composition and Analysis*, 22, 102-111.

MARTÍN, D., MURIEL, E., GONZALEZ, E., VIGUERA, J., & RUIZ, J. (2008b). Effects of dietary conjugated linoleic acid and mono-unsaturated fatty acids on productive, carcass and meat quality traits of pigs. *Livestock Science*, 117(2/3), 155-164.

MARTÍN, D., RUIZ, J., FLORES, M., & TOLDRA, F. (2006). Effect of dietary conjugated linoleic acid and mono-unsaturated fatty acid content on pig muscle and adipose tissue, lipase and esterase activity. *Journal of Agricultural and Food Chemistry*, 54, 9241-9247.

MARTÍN, D., RUIZ, J., KIVIKARI, R., & PUOLANNE, E. (2008c). Partial replacement of pork fat by conjugated linoleic acid and/or olive oil in liver pâtés: effect on physiochemical characteristics and oxidative stability. *Meat Science*, 80, 496-504.

MARTIN, J.-C., GRÉGOIRE, S., SIESS, M.-H., GENTY, M., CHARDIGNY, J.-M., BERDEAUX, O., *et al.* (2000). Effects of conjugated linoleic acid isomers on lipid-metabolizing enzymes in male rats. *Lipids*, 35, 91-98.

MAW, S.J., FOWLER, V.R., HAMILTON, M., & PETCHEY, A.M. (2003). Physical characteristics of pig fat and their relation to fatty acid composition. *Meat Science*, 63, 185-190.

McCANN, S.E., IP, C., IP, M.M, McGUIRE, M.K., MUTI, P., EDGE, S.B., *et al.* (2004). Dietary intake of conjugated linoleic acids and risk of premenopausal and postmenopausal breast cancer, Western New York exposures and breast cancer study (WEB study). *Cancer Epidemiology, Biomarkers & Prevention,* 13, 1480-1484.

McGINLEY, L. (1991). Analysis and quality control for processing and processed fats. In J.B. Rossell & J.L.R. Pritchard (Eds.), *Analysis of Oilseeds, Fats and Fatty Foods.* (pp. 465-466). Elsevier Applied Science: London.

McGUIRE, M.K., MCGUIRE, M.A., RITZENTHALER, K., & SHULTZ, T.D. (1999). Dietary sources and intakes of conjugated linoleic acid intake in humans. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza, & G. Nelson (Eds.), *Advances in conjugated linoleic acid research*. (Vol. 1), (pp. 296-306). Champaign: AOCS Press.

McLEOD, R.S., LEBLANC, A.M., LANGILLE, M.A., MITCHELL, P.L., & CURRIE, D.L. (2004). Conjugated linoleic acids, artherosclerosis, and hepatic very-low-density lipoprotein metabolism. *American Journal of Clinical Nutrition*, 79, 1169S-1174S.

MEILGAARD, M., CIVILLE, G.V., & CARR, B.T. (1999). *Sensory evaluation techniques*. (Third Ed). Boca Raton, Florida: CRC Press.

MIGDAL, W., PAŚCIAK, P., WOJTYSIAK, D., BAROWICZ, T., PIESZKA, M., & PIETRAS, M. (2004). The effect of dietary CLA supplementation on meat and eating quality, and the histochemical profile of the *M. longissimus dorsi* from stress susceptible fatteners slaughtered at heavier weights. *Meat Science*, 66, 863-870.

MIR, P.S., MCALLISTER, T.A., SCOTT, S., AALHUS, J., BARON, V., MCCARTNEY, D., *et al*. (2004). Conjugated linoelic acid-enriched beef production. *American Journal of Clinical Nutrition*, 79, 1207S-1211S.

MITCHAOTHAI, J., YUANGKLANG, C., WITTAYAKUN, S., VASUPEN, K., WONGSUTTHAVAS, S., SRENANUL, P., *et al.* (2007). Effect of dietary fat type on meat quality and fatty acid composition of various tissues in growing-finishing swine. *Meat Science*, 76, 95-101.

MONZIOLS, M., BONNEAU, M., DAVENEL, A., & KOUBA, M. (2007). Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. *Meat Science,* 76, 54-60.

MOONEY, D., McCARTHY, C., & BELTON, O. (2012). Effects of conjugatedlinoleic acid isomers on monocyte, macrophage and foam cell phenotype in stherosclerosis. *Prostaglandins and Other Lipid Mediators*, doi:10.1016/j.prostaglandins.2011.12.006.

MORTENSEN, B.K. (1983). Physical properties and modification of milk fat. In P.F. Fox (Ed.), *Developments in dairy chemistry.* (Vol. 2), (pp 159-194). London: Applied Science Publishers.

MORTENSEN, H.P., MADSEN, A., BEJERHOLM, C., & BARTON-GADE, P. (1983). Report No. 540, National Institute of Animal Scientists. Copenhagen, Denmark.

MOTTRAM, D. S. (1985). The effect of cooking conditions on the formation of volatile heterocyclic compounds in pork. *Journal of the Science of Food and Agriculture*, 36, 377-382.

MOUROT, J., KOUBA, M., & PEINIAU, P. (1995). Comparative study of *in vitro* lipogenesis in various adipose tissues in the growing domestic pig (*Sus domesticus*). *Comparative Biochemistry and Physiology*, 111B, 379-384.

MUCHENJE, V., DZAMA, K., CHIMONYO, M., STRYDOM, P.E., HUGO, A., & RAATS, J.G. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chemistry*, 112, 279-289

MUCHENJE, V., & NDOU, S.P. (2011). How pig pre-slaughter welfare affects pork quality and the pig industry. [Available on internet:] www.sapork.biz 5 May 2012.

MÜLLER, E. (1983). Hormones and fat deposition in pigs. In:J.D. Wood (Ed.), *Fat Quality in lean Pigs*. p. 69-73. Special report No. 2, EUR 8901 EN, CEC Workshop, Brussels, Belgium.

MÜLLER, H.L., KIRCHGESSNER, M. ROTH, F.X., & STANGL, G.I. (2000). Effect of conjugated linoleic acid on energy metabolism in growing-finishing pigs. *Journal of Animal Physiology and Animal Nutrition*, 83, 85-94.

MÜLLER, M., WENK, C., & SCHEEDER, M.R.L. (2008). Determination of fatty acid composition and consistency of raw pig fat with near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, 16, 305-309.

NAWAR, W.N. (1996) Lipids. In O.R. Fennema (Ed.), *Food Chemistry*. Third edition (pp. 226-314). New York: Marcel Dekker Inc.

NCSS. (2007). Statistical System for Windows. Number Cruncher Statistical Systems, Kaysville, Utah, USA.

NGAPO, T.M., MARTIN, J.F., & DRANSFFIELD, E. (2007a). International preferences for pork appearance: I. Consumer choices. *Food Quality and Preference*, 18, 26.

NGAPO, T.M., MARTIN, J.F., & DRANSFFIELD, E. (2007b). International preferences for pork appearance: II. Factors influencing consumer choices. *Food Quality and Preference*, 18, 139.

NÜRNBERG, K., FISCHER, K., NÜRNBERG, G., KUECHENMEISTER, G., KLOSOWSKA, D., ELIMINOWSKA-WENDA, G., *et al.* (2005). Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. *Meat Science*, 70, 63-74.

NÜRNBERG, K., WEGNER, J., & ENDER, K. (1998). Factors influencing fat composition in muscle and adipose tissue of farm animals. *Livestock production Science*, 56, 145-156.

O'CONNOR, R.T. (1960). Spectral properties. In K.S. Markley (Ed.), *Fatty acids: their chemistry, properties, production uses* (Part 1) (pp.410). New York: Interscience.

OKUYAMA, H. (1997). Recommended lipid intake-president's summary from the Japan Society for Lipid Nutrition. *Journal of Lipid Nutrition*, 6, 5-42.

OKUYAMA, H., & IKEMOTO, A. (1999). Needs to modify the fatty acid composition of meats for human health. In *Proceedings of the 45th International Congress of Meat Science Technologists, Yokohama, Japan*. (pp. 638-640).

O'QUINN, P.R., SMITH, J.W.,II, NELSSEN, J.L., TOKACH, M.D., GOODBAND, R.D., & SMITH, J.S. (1998). Effects of modified tall oil versus conjugated linoleic acid on finishing pig growth performance and carcass characteristics. Purdue University Swine Day Report. pp 157-161.

O'SHEA, M, BASSAGANYA-RIERA, J., & MOHEDE, I.C.M. (2004). Immunomodulatory properties of conjugated linoleic acid. *Journal of American Clinical Nutrition*, 79, 1199S-1206S.

OSTHOFF, G., HUGO, A., JOUBERT, C.C., & SWARTS, J.C. (2011). Differential scanning calorimetry of milk fat from dairy and non-dairy animals. *South African Journal of Chemistry*, 64, 241-250.

OSTROWSKA, E., CROSS, R.F., MURALITHARAN, M., BAUMAN, D.E., & DUNSHEA, F.R. (2003). Dietary conjugated linoleic acid differentially alters fatty acid composition and increases conjugated linoleic acid content in porcine adipose tissue. *British Journal of Nutrition,* 90, 915-928.

OSTROWSKA, E., MURALITHARAN, M., CROSS, R.F., BAUMAN, D.E., & DUNSHEA, F.R. (1999). Dietary conjugated linoleic acid increase lean tissue and decrease fat deposition in growing pigs. *Journal of Nutrition*, 129, 2037-2043.

PAMPLONA, R., PORTERO-OTÍN, M., RIBA, D., RUIZ, C., PRAT, J., BELLMUNT, M.J., *et al.* (1998). Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. *Journal of Lipid Research,* 39, 1989-1994.

PARISH, F.C., Jr. WIEGAND, B.R., BEITZ, D.C., AHN, D.U., DU, M., & TRENKLE, A. (1999). Use of dietary CLA to improve composition and quality of animal-derived foods. In J.-L. Sébèdio, W.W. Christie & R. Adlof (Eds.), *Advances in conjugated linoleic acid research*. (Vol. 2), (pp. 189-217). Champaign: AOCS Press.

PARIZA, M.W. (1999). The biological activities of conjugated linoleic acid. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza & G. Nelson (Eds.), *Advances in conjugated linoleic acid research*. (Vol. 1), (pp. 12-20). Champaign: AOCS Press.

PARIZA, M.W., PARK, Y., & COOK, M.E. (2001). The biologically active isomers of conjugated linoleic acid. *Progress in Lipid Research*, 40, 283-298.

PARK, Y., ALBRIGHT, K. J., CAI, Z. Y., & PARIZA, M. W. (2001). Comparison of methylation rocedures for conjugated linoleic acid and artefact formation by commercial (trimethylsilyl) diazomethane. *Journal of Agricultural and Food Chemistry*, 49(3), 1158–1164.

PARK, Y., ALBRIGHT, J., LIU, W., STORKSON, J., COOK, M., & PARIZA, M. (1997). Effect of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Lipids*, 32, 853-858.

PARK, Y., ALBRIGHT, K.J., STORKSON, J.M., LIU, W., & PARIZA, M.W. (1999). Evidence that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids*, 34, 235-241.

PARK, Y., ALBRIGHT, K.J., STORKSON, J.M., LIU, W., & PARIZA, M.W. (2007). Conjugated linoleic acid (CLA) prevents body fat accumulation and weight gain in an animal model. *Journal of Food Science*, 72, S612-S617.

PARK, Y., & PARIZA, M.W. (2007). Mechanisms of body fat modulation by conjugated linoleic acid (CLA). *Food Research International*, 40, 311-323.

PARK, Y., PARIZA, M.W., & PARK, Y. (2008). Co-supplementation of dietary calcium and conjugated linoleic acid (CLA) improves bone mass in mice. *Journal of Food Science*, 73, C556-C560.

PARODI, P.W. (1997). Cows' milk fat as potential anticarcinogenic agents. *Journal of Nutrition*, 127, 1055-1060.

PARODI, P.W. (2003). Conjugated linoleic acid in food. In J.-L. Sebedio, W.W. Christie, & R. Adlof (Eds.), *Advances in conjugated linoleic acid research*. (2), (pp. 101-122). Champaign (IL): AOCS Press.

PEARSON, D. (1968). Application of chemical methods for the assessment of beef quality III – Methods related to fat spoilage. *Journal of the Science of Food and Agriculture,* 19, 553-556.

PHELPS, A. (1991). A time for a rethink on fat. *Farmers Weekly*, May, 18-19.

PIG PROGRESS. (2009). Pork consumption overtakes lamb in South Africa. [Available on internet:] www.pigprogress.net 26 December 2011.

PLOURDE, M., JEW, S., CUNNANE, S.C., & JONES, P.J.H. (2008). Conjugated linoleic acids: why the discrepancy between animal and human studies? *Nutrition Reviews*, 66(7), 415-421.

PORK.ORG. (2005). Porcine mycology. University of Lincoln, Nebraska. [Available on internet:] http://porcine.unl.edu/porcine2005 30 December 2011.

PRABUCKI, A.L. (1991). Qualitätsanforderungen an Schweinefleisch. In *Schweinefleischqualität – Qualitätsschweinefleisch.* Tagunsbericht, p 5-10, 22 May 1991, ETH-Zürich, Institut für Nutztierwissenschaften.

RAHARJO, S., SOFOS, J.N.S., & SCHMIDT, G.R. (1992). Improved speed specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C_{18} method for measuring lipid peroxidation in beef. *Journal of Agricultural and Food Chemistry,* 40, 2182-2185.

RALOFF, J. (2003). Food for thought: Global food trends. Science News Online. May, 31, 2003.

RAMSAY, T.G., EVOCK-CLOVER, C.M., STEELE, N.C., & AZAIN, M.J. (2001). Dietary conjugated linoleic acid alters fatty acid composition of pig skeletal muscle and fat. *Journal of Animal Science*, 79, 2152-2161.

RASTAFARI MOVEMENT. (2009). Diet. [Available on internet:] http://en.wikipedia.org/wiki/Rastafari_movement#Diet 4 May 2009.

RASTMANESH, R. (2011). An urgent need to include risk-benefit analysis in clinical trials investigating conjugated linoleic acid supplements in cancer patients. *Contemporary Clinical Trials*, 32, 69-73.

REALINI, C.E., DURAN-MONTAGÉ, R., LIZARDO, R., GISPERT, M., OLIVER, M.A., & ESTEVE-GARCIA, E. (2010). Effect of source of dietary fat on pig performance, carcass characteristics and carcass fat content, distribution and fatty acid composition. *Meat Science*, 85, 606-612.

REANEY, M.J.Y., LIU, Y.-D., & WESTCOTT, N.D. (1999). Commercial production of conjugated linoleic acid. In M.P. Yurawecz, M.M., Mossoba, J.K.G. Kramer, M.W. Pariza, & G. Nelson (Eds.), *Advances in conjugated linoleic acid research*. (1), (pp. 39-54). Champaign: AOCS Press.

REHFELDT, C., TUCHSCHERER, A., HARTUNG, M., & KUHN, G. (2008). A second look at the influence of birth weight on carcass and meat quality in pigs. *Meat Science*, 78, 170-175.

RENTFROW, G., SAUBER, T.R., ALLEE, G.L., & BERG, E.P. (2003). The influence of diets containing either conventional corn, conventional corn with choice white grease, high oil corn or high oil high oleic corn on belly bacon quality. *Meat Science*, 64, 459-466.

RHEE, K.S., DAVIDSON, T.L., CROSS, H.R., & ZIPRIN, Y.A. (1990). Characteristics of pork products from swine fed a high mono-unsaturated fat diet: Part 1 – Whole muscle products. *Meat Science,* 27, 329-341.

RICHARD, J.W., WIEGAND, B.R., POMPEU, D., HINSON, R.B., GERLEMANN, G.D., DISSELHORST, R., *et al*. (2011). The effect of corn distiller's dried grains with soluble, ractopamine, and conjugated linoleic acid on the carcass performance, meat quality, and shelf-life characteristics of fresh pork following three different storage methods. *Meat Science*, 90, 643-654.

RIPOLL, R., JOY, M., & MUÑOZ, F. (2011). Use of dietary vitamin E and selenium (Se) to increase the shelf life of modified atmosphere packaged light lamb meat. *Meat Science*, 87, 88-93.

RISERUS, U., BASU, S., JOVINGE, S., FREDERIKSON, G.N., ARNLOV, J., & VESSBY, B. (2002). Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. *Circulation,* 106, 1925-1929.

RISSANEN, H., KNEKT, P., JARVINEN, R., SALMINEN, I., & HAKULINEN, T. (2003). Serum fatty acids and breast cancer incidence. *Nutrition and Cancer*, 45, 168-175.

ROSENVOLD, K., & ANDERSEN, H.J. (2003). Factors of significance for pork quality – a review. *Meat Science*, 64, 219-237.

RUBIN, J.S. (2004). The Maker's Diet. Strang Communications: Florida.

SAMIC. (2009). Export statistics for beef, sheep, pork and offal for 2008. [Available on internet:] www.samic.co.za 22 April 2009.

SA PORK. (2011). Latest import figures. [Available on internet:] www.sapork.biz 31 December 2011.

SASAKI, K., MITSUMOTO, M., NISHIOKA, T., & IRIE, M. (2006). Differential scanning calorimetry of porcine adipose tissues. *Meat Science,* 72, 789–792.

SATHER, A.P., JONES, S.D.M., ROBERTSON, W.M., & ZAWADSKI, S. (1995). Sex effects on fat hardness meter readings of market weight pigs. *Canadian Journal of Animal Science*, 75(3), 509-515.

SCHEERDER, M.R.L., GLÄSER, K.R., EICHENBERGER, B., & WENK, C. (2000). Influence of different fats in pig feed on fatty acid composition of phospholipids and physical meat quality characteristics. *European Journal of Lipid Science and Technology*, 102, 391-401.

SCHINCKEL, A.P., EGGERT, J.M., RICHERT, B.T., & CARROLL, A.L. (2000). Effects of conjugated linoleic acid (CLA) supplementation on pig growth, pork quality and carcass composition in two genetic populations of gilt. Purdue University Swine Day Report, pp. 51-53.

SCHMID, A., COLLOMB, M., SIEBER, R., & BEE, G. (2006). Conjugated linoleic acid in meat and meat products: a review. *Meat Science* 73, 29-41.

SEVENTH-DAY ADVENTIST CHURCH. (2009). Health and diet. [Available on Internet:] http://en.wikipedia.org/wiki/Seventh_day_Adventist_Church#health_and_diet 4 May 2009.

SHACKELFORD, S.D., MILLER, M.F., HAYDON, K.D., LOVEGREN, N.V., LYON, C.E., & REAGAN, J.O. (1990). Acceptability of bacon as influenced by the feeding of elevated levels of mono-unsaturated fats to growing-finishing swine. *Journal of Food Science*, 55, 621-624.

SHANTHA, N.C., RAM, L.N., O'LEARY, J., HICKS, C., & DECKER, E.A. (1995). Conjugated Linoleic acid concentrations in dairy products as affected by processing and storage. *Journal of Food Science*, 60, 695-697.

SHAW, P.E., MOSHONAS, M.G., BUSLIG, B.S., BARROS, S.M., & WIDMER, W.W. (1999). Discriminant and principle component analyses to classify commercial orange juice based on relative amounts of volatile juice constituents. *Journal of Science of Food and Agriculture*, 79, 1949-1953.

SHEARD, P.R., NUTE, G.R., RICHARDSON, R.I., & WOOD, J.D. (2005). Effects of breed and marination on the sensory attributes of pork from Large White and Hampshire-sired pigs. *Meat Science*, 70, 699-707.

SINK, J.D., WATKINS, J.L., ZIEGLER, J.H., & MILLER, R.C. (1964). Analysis of fat deposition in swine by gas-liquid chromatography. *Journal of Animal Science*, 23, 121-125.

SIRI-TARINO, P.W., SUN, Q., HU, F.B., & KRAUSS, R.M. (2010). Saturated fat, carbohydrate and cardiovascular disease. *American Journal of Clinical Nutrition*, 91, 502-509.

SMITH, S.R., HIVELY, T.S., CORTESE, G.M., HAN, J.J., CHUNG, K.Y., CASTEÑADA, P., *et al*. (2002). Conjugated linoleic acid decreases the Δ^9 desaturase index and stearoyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue. *Journal of Animal Science*, 80, 2110-2115.

SOLOMON, C. (1996). Encyclopedia of Asian Food. Melbourne: William Heineman.

SONG, H.J., GRANT, I., ROTONDO, D., MOHEDE, I., SATTAR, N., HEYS, S.D., *et al*. (2005). Effect of CLA supplementation on immune function in young healthy volunteers. *European Journal of Clinical Nutrition*, 59, 508-517.

STAHL, C.A., EGGERT, J.M., RICHERT, B.T., GERRARD, D.E., FORREST, J.C., BOWKER, B.C., *et al*. (1999). Effects of corn and conjugated linoleic acid (CLA) on the storage and shelf life of fresh pork. Purdue University Swine Day Report. pp. 41-45.

STANTON, C., MURPHY, J., MCGRATH, E., & DEVERY, R. (1999). Animal feeding strategies for conjugated linoleic acid enrichment of milk. In J.-L. Sébèdio, W.W. Christie, & R. Adlof (Eds.), *Advances in conjugated linoleic acid research*. (Vol. 2), (pp. 123-145). Champaign: AOCS Press.

STORLIEN, L.H., KRAEGEN, E.W., CHISHOLM, D.J., FORD, G.L., BRUCE, D.G., & PASCOE, W. (1987). Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science*, 237, 885-888.

STRONG, R. (2002). Feast: a history of grand eating. London: Jonathan Cape.

SUN, D., ZHU, X., QIAO, S., FAN, S., & LI, D. (2004). Effects of conjugated linoleic acid levels and feeding intervals on performance, carcass traits and fatty acid composition of finishing barrows. *Archives of Animal Nutrition,* 58(4), 277-286.

SVENSTRUP, G., BRÜGGEMANN, D., KRISTENSEN, L., RISBO, J., & SKIBSTED, L.H. (2005). The influence of pretreatment on pork fat crystallization. *European Journal of Lipid Science and Technology,* 107, 607–615.

SWAN, J.E., PARRISH, F.C.J., WIEGAND, B.R., LARSEN, S.T., BAAS, T.J., & BERG, E.P. (2001). Total body electrical conductivity (TOBEC) measurement of compositional differences in hams, loins and bellies from conjugated linoleic acid (CLA)-fed stress-genotype pigs. *Journal of Animal Science*, 79, 1475-1482.

SZYMCZYK, B. (2005). Effects of conjugated linoleic acid (CLA) on fatty acid composition, lipid oxidation and quality of pork meat. *Annals of Animal Science*, 5(1), 145-157.

TAYLOR, C.G., & ZAHRADKA, P. (2004). Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. *American Journal of Clinical Nutrition*, 79, 1164S-1168S.

TERPSTRA, A.H.M. (2004). Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an overview of the literature. *American Journal of Clinical Nutrition*, 79, 352-361.

TEYE, G.A., SHEARD, P.R., WHITTINGTON, F.M., NUTE, G.R., STEWART, A., & WOOD, J.D. (2006a). Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. *Meat Science*, 73, 157-165.

TEYE, G.A., WOOD, J.D., WHITTINGTON, F.M., STEWART, A., & SHEARD, P.R. (2006b). Influence of dietary oils and protein level on pork quality. 1. Effects on properties of fat and processing characteristics of bacon and frankfurter-style sausages. *Meat Science*, 73, 166-177.

THIEL-COOPER, R.L., PARRISH, F.C.Jr., SPARKS, J.C., WIEGAND, B.R., & EWAN, R.C. (2001). Conjugated linoleic acid changes swine performance and carcass composition. *Journal of Animal Science*, 79(7), 1821-1828.

THIEL-COOPER, R.L., WIEGAND, B.R., PARRISH, F.C. JR., & LOVE, J.A. (1999). Effects of CLA supplementation on quality and sensory characteristics of pork. *Journal of Animal Science*, 77(Suppl.1), 23 (Abstract).

THOMPSON, E.H., & ALLEN, E. (1969). Relationship between stearic acid desaturase and fatty acid composition. *Journal of Animal Science*, 29, 127-128.

TIIKKAINEN, M., BERGHOLM, R., RISSANEN, A., ARO, A., SALMINEN, I., TAMMINEN, M., *et al.* (2004). Effects of equal weight loss with orlistat and placebo on body fat and serum fatty acid composition and insulin resistance in obese women. *American Journal of Clinical Nutrition*, 79, 22-30.

TIKK, K., TIKK, M., AASLYNG, M.D., KARLSSON, A.H., LINDAHL, G., & ANDERSEN, H.J. (2007). Significance of fat supplemented diets on pork quality – connections between specific fatty acids and sensory attributes of pork. *Meat Science*, 77, 275-286.

TISCHENDORF, F., SCHÖNE, F., KIRCHHEIM, U., & JAHREIS, G. (2002). Influence of conjugated linoleic acid mixture on growth, organ weights, carcass traits and meat quality in growing pigs. *Journal of Animal Physiology and Animal Nutrition*, 86, 117-128.

TOLDRA, F. (2003). Muscle foods: water, structure and functionality. *Food Science and Technology International*, 9, 3, 173-177.

TOURAILLE, C., MONIN, G., & LEGAULT, C. (1989). Eating quality of meat from European X Chinese crossbred pigs. *Meat Science*, 25, 177-186.

TRICON, S., BURDGE, G.C., KEW, S., BANERJEE, T., RUSSELL, J.J., GRIMBLE, R.F., *et al.* (2004). Effects of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 conjugated linoleic acid on immune cell function in healthy humans. *American Journal of Clinical Nutrition*, 80, 1626-1633.

TRICON, S., & YAQOOB, P. (2006). Conjugated linoleic acid and human health: a critical evaluation of the evidence. *Current Opinion in Clinical Nutrition and Metabolic Care*, 9, 105-110.

TROPP, B. (1982). The Modern Art of Chinese Cooking. New York: Hearst Books.

TURPEINEN, A.M., YLONEN, N., VON WILLEBRAND, E., BASU, S., & ARO, A. (2008). Immunological and metabolic effects of *cis*-9, *trans*-11 –conjugated linoleic acid in subjects with birch pollen allergy. *The British Journal of Nutrition*, 100, 112-119.

USDA. (2006). Livestock and poultry: World markets and trade. Circular Series DL&P 2-06, Foreign Agricultural Service, United States Department of Agriculture, October 2006.

VAN DEN BERG, J.I.M, COOK, N.E., & TRIBBLE, D.L. (1995). Reinvestigation of the antioxidant properties of conjugated linoleic acid. *Lipids*, 30, 599-605.

VERBEKE, W., VAN OECKEL, M.J., WARNANTS, N., VIAENE, J., & BOUCQUÉ, C.H.V. (1999). Consumer perception, facts and possibilities to improve acceptability of health and sensory characteristics of pork. *Meat Science,* 53, 77-99.

VILLEGAS, F.J., HEDRICK, H.B., VEUM, T.L., McFATE, K.L., & BAILEY, M.E. (1973). Effect of diet and breed on fatty acid composition of porcine adipose tissue. *Journal of Animal Science*, 36, 663-668.

WAHLE, K.W.L., HEYS, S.D., & ROTONDO, D. (2004). Conjugated linoleic acids: Are they beneficial or detrimental to health? *Progress in Lipid Research*, 43, 553-587.

WALLINFORD, J.C., & YETTY, E.A. (1991). Development of the health claims regulations: the case of omega-3 fatty acids and heart disease, *Nutritional Reviews*, 49, 323-331.

WAHLE, K.W.J., & JAMES, W.P.T. (1993). Review isomeric fatty acids in human health. *European Journal of Clinical Nutrition*, 47, 828-839.

WANG, F-S., & LIN, C-W. (1995). Contribution of Particle Sizes and Particle Size Distributions in Crystalline Fractionation of Lard. *Journal of Agriculture and Food Chemistry,* 43, 785-790.

WANG, Y., JACOME-SOSA, M.M., & PROCTOR, S.D. (2011). The role of ruminant *trans* fat as a potential nutraceutical in the prevention of cardiovascular disease. *Food Research International*, doi:10.1016/jfoodres.2011.08.019.

WANG, Y., & JONES, P.J.H. (2004). Dietary conjugated linoleic acid and body composition. *American Journal of Clinical Nutrition*, 79, 1153S-1158S.

WARNANTS, N., VAN OECKEL, M.J., & BOUCQUÉ, Ch.V. (1996). Incorporation of dietary polyunsaturated fatty acids in pork tissues and its implications for the quality of the end products. *Meat Science*, 44, 125-144.

WARNANTS, N., VAN OECKEL, M.J., & BOUCQUÉ, Ch.V. (1998). Effect of incorporation of dietary fatty acids in pork on the quality of salami. *Meat Science*, 49, 435-445.

WATKINS, B.A., LI, Y. LIPPMAN, H.E. REINWALD, S., & SEIFERT, M.F. (2004). A test of Ockham's razor: Implications of conjugated linoleic acid in bone biology. *American Journal of Clinical Nutrition*, 79, 1175S-1185S.

WEBER, T.E., DECAMP, S.A., BOWERS, K.A., HERR, C.T., KNOLL, S.L., RICHERT, B.T., *et al.* (2000). Evaluation of conjugated linoleic acid (CLA) and dietary antibiotics as growth promotants in weanling pigs. Purdue University Swine Day report, pp. 43-50.

WEBER, T.E., ENRIGHT, K., RICHERT, B.T., & SCHINCKEL, A.P. (2001). Evaluation of the effects of dietary fat, conjugated linoleic acid and ractopamine on growth performance and carcass quality in genetically lean gilts. Purdue University Swine Day report. pp. 60-64.

WEBER, T.E., RICHERT, B.T., BELURY, M.A., GU, Y., ENRIGHT, K., & SCHINCKEL, A.P. (2006). Evaluation of the effects of dietary fat, conjugated linoleic acid and ractopamine on growth performance, pork quality and fatty acid profiles in genetically lean gilts. *Journal of Animal Science*, 84, 720-732.

WEILER, H., AUSTIN, S., FITZPATRICK-WONG, S., NITSCHMANN, E., BANKOVIC-CALIC, N., MOLLARD, R., *et al*. (2004). Conjugated linoleic acid reduces parathyroid hormone in health and in polycystic kidney disease in rats. *American Journal of Clinical Nutrition*, 79, 1186S-1189S.

WHITTINGTON, F.M., PRESCOTT, N.J., WOOD, J.D., & ENSER, M. (1986). The effect of dietary linoleic acid on the firmness of backfat in pigs of 85 kg live weight. *Journal of Science and Food Agriculture*, 37, 753-761.

WIEGAND, B.R., PARRISH, F.C.JR., & SPARKS, J.C. (1999). Effects of CLA supplementation on pork quality characteristics in cross-bred growing-finishing barrows. *Journal of Animal Science*, 77(Suppl. 1), 47 (Abstract).

WIEGAND, B.R., PARRISH, F.C., SWAN, J.E., LARSEN, S.T., & BAAS, T.J. (2001). Conjugated linoleic acid improves feed efficiency, decreases subcutaneous fat and improves certain aspects of meat quality in Stress-Genotype pigs. *Journal of Animal Science*, 79, 2187-2195.

WIEGAND, B.R., SPARK, S.J.C., PARRISH, F.C., & ZIMMERMAN, D.R. (2002). Duration of feeding conjugated linoleic acid influences growth performance, carcass traits and meat quality of finishing barrows. *Journal of Animal Science*, 80, 637-643.

WOOD, J.D. (1984). Fat deposition and the quality of fat tissue in meat animals. In J. Wiseman (Ed.), *Fats in animal nutrition* (pp.401-435). London: Butterworths.

WOOD, J.D. (2001). Meat quality and the designer pig. In T.P. Lyons & D.J.A. Coles (Eds.), *Concepts of meat science* (pp. 17-31). Nottingham: Nottingham Nutrition International.

WOOD, J.D., ENSER, M., FISHER, A.V., NUTE, G.R., SHEARD, P.R., RICHARDSON, R.I., *et al*. (2008). Fat deposition, fatty acid composition and meat quality: a review. *Meat Science*, 78, 343-358.

WOOD, J.D., ENSER, M., WHITTINGTON, F.M., MONCRIEFF, C.B., & KEMPSTER, A.J. (1989). Backfat composition in pigs: differences between fat thickness groups and sexes. *Livestock Production Science*, 22, 351-362.

WOOD, J.D., NUTE, G.R., RICHARDSON, R.I., WHITTINGTON, F.M., SOUTHWOOD, O., PLASTOW, G., *et al*. (2004). Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science*, 67, 651-667.

WOOD, J.D., SHEARD, P.R., ENSER, M., NUTE, G.R., RICHARDSON, R.I., & GILL, B.P. (1999). Increasing the n-3 polyunsaturated fatty acid content of pigmeat and effects on meat quality. In *Proceedings of the 45th International Congress of Meat Science Technologists, Yokohama, Japan*. (pp. 672-673).

WOOD, J.D., WHELEHAN, O.P., ELLIS, M., SMITH, W.C., & LAIRD, R. (1983). Effects of selection for low backfat thickness in pigs on the sites of tissue deposition in the body. *Animal Production*, 36, 389-397.

XUE, J., DIAL, G. D., & PETTIGREW, J. E. (1997). Performance, carcass, and meat quality advantages of boars over barrows: A literature review. *Journal of Swine Health and Production, 5,* 21–28.

YU, L., DONKIN, S.S., & WATKINS, B.A. (1998). CLA reduced arachidonic acid formation in primary cultures of rat hepatocytes. *FASEB Journal*, 12, A535.

ZOCK, P.L. (2006). Do favourable effects of increasing unsaturated fat intake on cardiovascular disease risk outweigh the potential adverse effect on body weight? *International Journal of Obesity*, 30, S10-S15doi:10.1038/sj.ijo.0803486.

ZANARDI, E., NOVELLI, E., GHIRETTI, G.P., & CHIZZOLINI, R. (2000). Oxidative stability of lipids and cholesterol in salame Milano, coppa and parma ham: dietary supplementation with vitamin E and oleic acid. *Meat Science*, 55, 169-175.

CHAPTER 7
SUMMARY

Forty eight gilts were fed one of four dietary treatments containing 0, 0.25, 0.5 and 1% CLA, until their weight reached 95 kg and were then slaughtered. There were a lack of significant differences in pig performance and growth traits (weight increase, ADG, ADFI, FCR), and slaughter characteristics (SLW, hot carcass weight, cold carcass weight, dressing percentage, BFT, MT and LMC). There were no change in *M.longissimus thoracis* area, drip loss, WHC, pH_{45} and pH_{24}, while L*-and b* values decreased with increased dietary CLA. Colour a*-values and SI also did not differ between the four treatments. For the BF, IVs decreased with increased dietary CLA, while RI, colour a* and SI values remained unchanged, and colour b* values and hardness increased.

Conjugated linoleic acid supplementation resulted in improved technological quality of subcutaneous fat, demonstrated by reduced IVs, unchanged RI and extractable fat content, and increased FFDM. With increase in CLA supplementation, C18:0, c*is* 9, *trans* 11, *trans* 10, *cis* 12, UFA, SFA, *n*-6/*n*-3, PUFA/SFA, dienoic acid, C16:0+C18:0, C16:0/C18:2, C16:1+C18:1*c*9/C16:0+C18:0 and C18:0/C:18:2 increased, C18:1*c*9/C18:0, MUFA, PUFA, MUFA/SFA, *n*-3, PI, trienoic, tetraenoic, penta + hexaenoic acid and DBI decreased, while *n*-6 remained unchanged. There was a tendency for sampling positions on the dorsal (neck, BF, chuck) and lateral (rib area) sides of the carcass to have higher CLA content. Differential scanning calorimetry of subcutaneous fat showed the presence of β'-crystals in fat from 0.25 and 0.5% CLA-fed pigs and β-crystals in fat from 1% CLA-fed pigs.

For IMF samples, increased dietary CLA led to no change in IV, C18:0, C18:1*t*9, C18:1*c*7, C18:3*n*-3, PUFA/SFA, tetraenoic acid, C16:0/C18:2 and C18:0/C18:2 contents, while C16:1*c*9, *cis*-9, *trans*-11, t*rans*-10, *cis*-12, C22:5, C22:6, SFA, AI, PI, *n*-3, *n*-6/*n*-3, PUFA, *n*-6, dienoic acid, trienoic acid, penta- + hexaenoic acid, C16:0+C18:0 and C16:1+C18:1*c*9/C16:0+C18:0 contents increased and C18:1*c*9, C20:1*c*11, C18:1*c*9/C18:0, C18:2, C18:3*n*-6, C20:3*n*-3, C20:2, C20:4, C20:5, MUFA, UFA, MUFA/SFA and AI contents decreased. Most CLA was deposited on the lateral sides of the carcass, namely the *M.triceps brachi*. *M.supra spinatus* showed an atypical FA composition.

Descriptive sensory analysis was performed on oven-broiled pork chops and fat samples by a trained panel. The control was rated most tender, confirming the results from the physical texture analysis. The control also had least resistance for first bite, with the 0.5% CLA treatment having most resistance. The 0.5% CLA treatment had a chemical aroma for the fat.

The accelerated oxidation test indicated that BF from the control did not become rancid faster than BF from the three CLA treatments. Refrigerated display of pork chops for 8 days resulted in increased L*and b* values for the CLA treatments, unchanged TBARS values, while SI decreased. After frozen storage for 3 months, TBARS values remained unchanged for pork chops from the different dietary treatments. After 6 months of frozen storage, TBARS values decreased for pork chops from CLA supplemented pigs. After eight and 16 weeks of frozen storage, PVs for frozen patties decreased for the 0.5 and 1% CLA treatments. Differences in TBARS values became evident after eight weeks for the frozen patties, compared to sixth months for frozen pork chops. The TBARS values for the frozen chops were lower than the frozen patties. At the end of the ripening period, PVs for salamis from the 0.5 and 1% CLA treatments decreased, along with TBARS values. Belly fat from the CLA treatments was firmer than the control. No significant differences were observed between the four bacon treatments for either PV or TBARS values over the course of the six week refrigerated storage. According to the consumer panel, the control bacon was preferred to the 0.25% CLA bacon.

Keywords: conjugated linoleic acid, subcutaneous, intramuscular, fatty acids, pig, sensory, stability

OPSOMMING

Agt en veertig jong sôe is een van vier diëte wat 0, 0.25, 0.5 en 1% gekonjugeerde linoleïensuur (CLA) bevat het, gevoer, tot 'n gemiddelde gewig van 95 kg bereik is en toe geslag. Daar was nie betekenisvolle verskille ten opsigte van varkprestasie en groei-eienskappe (massatoename, gemiddelde daaglikse massatoename, gemiddelde daaglikse voerinname, voer-omskakelingsverhouding), en slagkenmerke (slagmassa, warm karkasmassa, koue karkasmassa, dresseerpersentasie, rugvetdikte, spierdikte en maervleisinhoud) nie. Daar was ook nie verskille in *M.longissimus thoracis-* oppervlakte, drupverlies, waterhouvermoë, pH_{45} en pH $_{24}$ nie, terwyl kleur L*- en b*-waardes verminder het met toename in CLA in die dieet. Kleur a* -waardes en "chroma" en "hue angle" het ook nie verskil tussen die vier behandelings nie. Jodium- en kleur b*-waardes vir die rugvet het verminder, met gepaardgaande toename in CLA in die dieet, terwyl kleur a*-waarde, "chroma" en "hue angle" nie verander het nie, en vethardheid toegeneem het.

Supplementering met CLA het die tegnologiese kwaliteit van die onderhuidse vet verbeter, soos aangedui deur verminderde jodiumwaardes, onveranderde ekstraheerbare vetinhoud en toename in vetvrye droë materiaal. Met toename in CLA, het 14:0, C16:0, C18:0, C18:1*t*9, cis-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA, SFA, arterogenetiese indeks, *n*-6/*n*-3, trienoïese suur, C16:0+C18:0, C16:0/C18:2, C18:0/C:18:2 en DBI verhoog, terwyl C18:1*c*9, C18:1*c*7, C20:1*c*11, C18:1*c*9/C18:0, C18:2, C18:3, C20:2, C20:3, C20:4, C22;5, C22:6, MUFA, UFA, MUFA/SFA, PI, *n*-3, dienoïese suur, trienoïese suur, tetraenoïese suur, pentaenoïiese suur, heksaenoïese suur, penta+hexaenoïese suur en C16+C18:1/C16:0+C18:0 verlaag het, en C16:1*c*9 en *n*-6 nie verander het nie. Differensiële skanderingskalometrie van die onderhuidse vet het die teenwoordigheid van β'-kristalle getoon in die vet van varke wat 0.25 en 0.5% CLA gevoer is, en β-kristalle in die vet van varke wat 1% CLA gevoer is.

Vir die spiervetmonsters het die verhoging van CLA in die dieet gelei tot geen verandering in ekstraheerbare vetinhoud, vetvrye droë material, voginhoud, jodiumwaarde, C18:0, C20:0, C22:0, C18:1*t*9, C18:1*c*7, C18:3, C16:0/C18:2, C18:0/C18:2 en DBI, terwyl C14:0, C16:0, C16:1*c*9, *cis*-9, *trans*-11 CLA, t*rans*-10, *cis*-12 CLA, C22:5, C22:6, SFA, arterogenetiese indeks, peroksidasieindeks, *n*-3, *n*-6/*n*-3, PUFA, *n*-6, dienoïese suur, pentaenoïese suur, heksaenoïese suur, penta+ heksaenoïese suur en C16:0+C18:0 inhoud verhoog het en C18:1*c*9, C20:1*c*11, C18:1*c*9/C18:0, C18:2, C18:3, C20:3, C20:2, C20:4, C20:5, MUFA, UFA, MUFA/SFA, MUFA/UFA, trienoïese suur, tetraenoïese suur en C16:1+C18:1*c*9/C16:0+C18:0 inhoud verlaag het. Die meeste CLA is neergelê aan die sye van die karkas, naamlik die *M.triceps brachi*. Die *M.supraspinatus* het 'n a-tipiese vetsuursamestelling getoon.

‘n Opgeleide paneel het oondgeroosterde varkkotelette and vetmonsters geëvalueer met behulp van beskrywende analise. Die kontrole was die sagste, wat die resultate van die fisiese tekstuuranalise bevestig het. Die kontrole het ook die minste weerstand gebied teen byt, terwyl die 0.5% CLA behandeling die meeste weerstand gebied het. Die 0.5% CLA behandeling het ook ‘n chemiese vet aroma gehad.

Die versnelde oksiedasietoets het getoon dat die rugvet van die kontrole nie vinniger galsterig geword het, as rugvet van die drie CLA-behandelings nie. Verkoelde uitstalling van varkkotelette vir agt dae het gelei tot verhoogde L*- en b*-waardes vir die CLA-behandelings, terwyl tiobarbituriensuur reaktiewe bestandeelwaardes (TBARS) onveranderd gebly en versadigingsindeks verlaag het. Na bevrore opberging vir drie maande, het die TBARS-waardes steeds nie verander vir varkkotelette van die verskillende dieetbehandelings nie. Na ses maande van bevrore opberging, het die TBARS-waardes van die CLA-gevoerde varke verlaag. Na agt en 16 weke van bevrore opberging het die peroksiedwaardes van die bevrore vleiskoekies van die 0.5 en 1% CLA-behandelings gedaal. Vir die bevrore vleiskoekies het veranderinge in die TBARS-waardes duidelik geword na agt weke, teenoor die ses maande vir die bevrore varkkotelette. Die TBARS-waardes van die bevrore kotelette was laer as díe van die bevrore vleiskoekies. Aan die einde van die rypwordingsperiode het die peroksiedwaardes van die salamis van die 0.5 en 1% CLA-behandelings gedaal, saam met die TBARS-waardes. Rugvet van die CLA-behandelings was fermer as die kontrole. Geen betekenisvolle verskille in peroksied- of TBARS-waardes, tussen die spek van die vier behandelings, het voorgekom tydens die ses week verkoelde opberging nie. Volgens die verbruikerspaneel is die spek van die kontrole verkies bo die spek van die 0.25% CLA-behandeling.

Sleutelwoorde: gekonjugeerde linoleïensuur, onderhuidse, binnespierse, vetsure, stabiliteit, sensories, vark

ANNEXURE 1

Sensory evaluation of CLA PORK and PORK FAT Name:……………………………..
Date…………………….. Session……………… Sensory Code…………
Read instructions carefully

Please complete form i.e. name, date code etc.

1. **AROMA:** Smell the pork fat by lifting the foil closure at least half way off the glass container and inserting your nose into the top part of the glass beaker. Smell the fat.
2. **FLAVOUR, TEXTURE, JUICINESS AND AFTERTASTE.**
   Now bite through the meat, evaluate first bite the chew and swallow. Be cognisant of the mouth-feel and aftertaste while tasting. The aftertaste should be judged as the flavour in the mouth immediately after swallowing the product.

AROMA – FAT	***None***	***Hint***	***Slight***	***Weak***	***Moderate***	***High***	***Very High***	***Extremely***
Fresh pork fat	1	2	3	4	5	6	7	8
Roast pork fat (caramel)	1	2	3	4	5	6	7	8
Chemical	1	2	3	4	5	6	7	8
Sour	1	2	3	4	5	6	7	8
Piggy (Old Musty)	1	2	3	4	5	6	7	8
AROMA – MEAT	***None***	***Hint***	***Slight***	***Weak***	***Moderate***	***High***	***Very High***	***Extremely***
Roast pork meat	1	2	3	4	5	6	7	8
Cooked pork meat (fresh pork)	1	2	3	4	5	6	7	8
Musty	1	2	3	4	5	6	7	8
Livery (metallic/bloody)	1	2	3	4	5	6	7	8
JUICINESS OF MEAT	***None***	***Hint***	***Slight***	***Weak***	***Moderate***	***High***	***Very High***	***Extremely***
Initial impression of juiciness	1	2	3	4	5	6	7	8
TEXTURE – MEAT	***None***	***Hint***	***Slight***	***Weak***	***Moderate***	***High***	***Very High***	***Extremely***
First bite	1	2	3	4	5	6	7	8
Tough	1	2	3	4	5	6	7	8
Tender	1	2	3	4	5	6	7	8
SUSTAINED IMPRESSION OF JUICINESS – MEAT	***None***	***Hint***	***Slight***	***Weak***	***Moderate***	***High***	***Very High***	***Extremely***
Dry	1	2	3	4	5	6	7	8
Juicy								
FLAVOUR – MEAT	***None***	***Hint***	***Slight***	***Weak***	***Moderate***	***High***	***Very High***	***Extremely***
Bland	1	2	3	4	5	6	7	8
Metallic (tin/aluminium)	1	2	3	4	5	6	7	8
Cooked pork	1	2	3	4	5	6	7	8
Sour	1	2	3	4	5	6	7	8
AFTER TASTE (Off-flavour of meat)	***None***	***Hint***	***Slight***	***Weak***	***Moderate***	***High***	***Very High***	***Extremely***
Metallic	1	2	3	4	5	6	7	8