EFFECTS OF DIETARY FISH OIL OR OTHER LIPIDS AND SANOVITE™ ON PIG PERFORMANCE AND PORK QUALITY

A thesis presented in partial fulfilment of the requirements for the Degree of Master of Science in Animal Science at Massey University, Palmerston North, New Zealand

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2010

ABSTRACT

In this experiment, the effects of (1) lipid type (soy bean oil, tallow and fish oil), (2) the period the fish oil was provided and (3) a dietary supplement containing conjugated linoleic acid (CLA), selenium (Se), vitamin E and vitamin C on pig performance and pork quality were studied.

Forty-eight female pigs (PIC hybrids, with a mean live weight of 16.19 kg ± 1.56 SD) were obtained from a single commercial operation in the North Island of New Zealand. The pigs were rank ordered by weight and assigned to one of six dietary treatment groups. The diet base was either a combination of animal and plant feedstuffs (AT and PTS), plant feedstuffs only (PO, POS) or plant feedstuffs combined with fish oil (PFSe and PFSl). The diets also differed depending on the presence or absence of the nutritional supplement Sanovite^{TM} and a vitamin C supplement. Sanovite^{TM} is a trademarked dietary supplement containing CLA (BASF, Auckland, New Zealand), organic Selenium (Alltech Inc., Nicholasville, KY) and vitamin E (Morel et al., 2008). Diets POS, PTS, PFSe, PFSl contained Sanovite^{TM} and a vitamin C supplement. Diets PO and POS were used to establish the effect of the supplementation with Sanovite^{TM} and the vitamin C supplement.
Pigs fed diet PFSe received plant feedstuffs and fish oil with supplement between days 1 and 35 and then diet POS up to day 84. Pigs fed diet PFSl received diet POS between days 1 and 35; plant feedstuffs and fish oil with supplement between days 36 and 56 and then diet POS up to day 84. Pigs in group PFSe and PFSl both received the same total amount of fish oil per pig (2.52 l/ 2.31 kg). Between days 1 and 56 of the experiment grower diets were fed, and finisher diets were fed between days 57 and 84 of the experiment.

The pigs were kept in pens of six, but fed individually twice daily (at approximately 8 am and 3.30 pm) according to a fixed feeding schedule. Water was available at all times. Individual feed intakes were measured daily and live weight recorded weekly.

Faeces were collected once a day during two days in week five of the trail for digestibility determination. Carcass quality characteristics determined at the abattoir included carcass weight and back fat thickness as measured at the end of the slaughter line. Meat quality assessments were performed on the Semimembranosus muscle (SM) from one of the topside cuts of each pig. Measurements of fatty acid profile (loin and backfat) and the Se content (lean meat) were conducted in Singapore by Mrs. J. Leong (MSc).

In this study plant or animal feedstuffs, lipid type, Sanovite^{TM} and vitamin C supplementation had no significant effects on growth performance and carcass quality.

There were no differences in apparent faecal digestibility characteristics for dry matter (DDM) and organic matter (DOM) in the un-supplemented animal (AT) and plant based (PO) diets.

Lipid type had a significant effect on the digestibility of ash (DA), and an increased ratio of unsaturated fatty acid to saturated fatty acid resulted in increases in DDM and DOM. DDM and DOM increased when soybean and linseed oil (POS) were used instead of tallow (PTS) or fish oil (PFS). The main differences in DDM, DOM and DA were observed between diets PO and POS. A positive effect of selenium, vitamin E and CLA supplementation is suggested.

Increased cooking temperatures reduced tenderness (higher mean, peak force, yield force and peak force – yield force) and increased cooking loss. There was a significant negative relationship between ultimate pH and relative lightness (L^*).

There were highly significant positive correlations between all three measurements of expressed juice, and there was a significant positive correlation between cooking loss at 60 and 70^{o}C (P<0.01) but correlations between expressed juice values and cooking loss were not significant.

The P-values for the contrasts for cooking loss at 70 ^{0}C were significant for all contrasts except for AT vs PO and PFSe vs PFSl. For all other contrasts, the P-values for cooking loss at 70 ^{0}C were significantly higher in group POS than for groups PO, PTS and PFSe+PFSl.

The P-value for (Peak force – Yield force) at 70°C was significantly higher in samples from group PFSe than for samples from group PFSl.

The P-value for the myofibrillar fragmentation index (MFI) was significantly higher for group AT in contrast to group PO. Group POS had a significantly higher P-value for MFI in contrast to groups PO, PTS and PFSe and PFSl.

Group POS had a lower P-value for sarcomere length in contrast to groups PFSe and PFSl. Group PFSe had a significantly higher P-value for sarcomere length in contrast to group PFSl.

Group PFSe had a significantly higher P-value for expressed juice percentage loss in weight in contrast to group PFSl.

Supplementing with Sanovite^{TM} increased the Se content (P = 0.002) in lean meat as analysed by J. Leong (2010, personal communication).

In general it was concluded that an increase in the ratio of unsaturated fatty acid to saturated fatty acid (U/S) in the diet resulted in higher levels of unsaturated fatty acids in loin and backfat. The fatty acid profile in the diet reflected the fatty acid profile of pork. Backfat of pigs fed diets including soybean and linseed oil contained higher levels of linoleic and α-linolenic acids.

Diets PO and POS were used to establish the effect of the supplementation of CLA.
The backfat of pigs fed diet POS contained higher levels of CLA (C18:2-*trans*-10, *cis*-12) and α-linolenic acid than pigs fed diet PO. The loin of pigs fed diet POS contained higher levels of palmitoleic and linoleic acid and CLA (C18:2-*cis*-9, *trans*-11) and lower levels of oleic acid than pigs fed diet PO.

The use of fish-oil as a lipid type resulted in the highest levels of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in loin and backfat. The loin and backfat of pigs fed fish-oil in the second part of the grower phase (PFSl) contained higher levels of EPA, DPA and DHA than pigs fed fish-oil in the first part of the grower phase (PFSe).

By enriching the swine diet with long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) it was possible to increase the EPA, DPA and DHA content of pork. Enriching pork with LC n-3 PUFA will contribute to achieving standards for adequate intake (AI), but might not be suitable to reach suggested dietary targets (SDT).

In conclusion, it was possible to change the pork composition by dietary manipulation without compromising pig performance and meat quality. There were a few significant effects from treatments on meat quality characteristics, but differences reported in this study were small and relatively unimportant. A negative influence of the dietary regime on palatability and meat processing was expected, but these issues are beyond the scope of this experiment.

ACKNOWLEDGEMENTS

First of all I would like to thank my primary supervisor Associate Professor Patrick C.H. Morel from the Institute of Food Nutrition and Human Health for his valuable and friendly guidance, advice and support throughout my research. Thanks for your open-door policy and for sharing your knowledge about pigs, nutrition and statistical analysis.

I would also like to thank my secondary supervisors Associate Professor Roger W. Purchas and Dr. Brian H. P. Wilkinson from the Institute of Food Nutrition and Human Health for their valuable advice and friendly guidance throughout my research.
A special thanks to Associate Professor Roger W. Purchas for sharing his knowledge and his help with the meat quality assessments and the statistical analysis.

Special appreciation to Mrs. Jasmine Leong for sharing her results of the FA analysis of the samples and the sensory evaluation.

I am extremely grateful to Mrs Kelly Rutherfurd of the Institute of Food Nutrition and Human Health for the support she offered during the trial. Thank you very much for your advice, your optimistic views, your awesome pep talks and all the other good conversations that were totally not work related. Thanks for giving me the opportunity to learn and become more and more independent during the trial. I wouldn't have been able to grow those pigs without you!

A special thanks to the other technicians from the Institute of Food Nutrition and Human Health. I would like to thank Miss Maggie Honeyfield-Ross for guiding me through the set-up of the trial. I would also like to thank Mr. Ed A. James for his help and advice in the feed mill and his assistance during the trial. I want to thank Miss Margreet Hekman for helping out in times of need and her dedication and enthusiasm on sample days.
Thanks to all of you for your enthusiasm, patience and sharing your knowledge. I've learned a lot from you.

I would like to thank Dr. Eric Neumann from the Institute of Veterinary, Animal and Biomedical Sciences for sharing his incredible knowledge and giving me the opportunity to learn. Thanks for teaching me and providing me with veterinary advice when necessary.

A special thanks to Mr. Gajen Sinnathamby for his help during the weekends. Thanks to Dr. Shay Rutherford-Hill for helping out in times of need.

Special appreciation to John Fitness and staff from Land Meat New Zealand Ltd.,Whanganui. Thanks for giving us the opportunity to take samples in the abattoir.

Special thanks to Mr. Graham Pearson for his help and sharing his knowledge on the slaughter day and during the meat processing. Thanks for your hospitality and getting up early!

Thanks to the staff of the nutrition laboratory of the Institute of Food Nutrition and Human Health for their help with the analysis of the samples.

Thanks to Mr. Garry C. Radford from the Institute of Food Nutrition and Human Health for helping out in the Food Pilot Plant and the Meat Quality lab.

I'm very grateful to Mr. Grant Skilton for providing the strong and healthy pigs that formed the most important base of my study.

Thanks to my flatmates for putting up with the pig smell, the endless pig stories and helping out when needed.

I would also like to thank my friends both in The Netherlands and New Zealand for the good times and the laughter but also for always being there for me when needed.

Last but not least, a very special thanks to my family back in The Netherlands for their unconditional love, support, encouragement and enthusiasm. Thanks for making me believe in myself. It is thanks to you that I had the courage to live my dream and study in New Zealand. I've learned more from you then any University degree could ever teach me. There are no words to describe how much you mean to me. I would like to dedicate this work to my grandmother Oma Nuijten, unfortunately she passed away before she could see the end result.

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LIST OF ABBREVIATIONS

a*	**Relative redness**
ADF	**Acid-detergent fibre**
ADFI	**Average daily feed intake**
ADG	**Average daily gain**
AI	**Adequate intake**
b*	**Relative yellowness**
BW	**Body weight**
CLA	**Conjugated linoleic acid**
CP	**Crude protein**
CVD	**Cardio vascular disease**
DA	**Apparent digestibility ash**
DDM	**Apparent digestibility dry matter**
DHA	**Docosahexaenoic acid**
DM	**Dry matter**
DOM	**Apparent digestibility organic matter**
DPA	**Docosapentaenoic acid**
EE	**Ether extract**
EPA	**Eicosapentaenoic acid**
FA	**Fatty acid**
FAME	**Fatty acid methyl esters**
FCR	**Feed conversion ratio**
FI	**Feed intake**
GE	**Gross energy**
He-Ne	**Helium-neon**
KCL	**Potassium chloride**
L*	**Relative lightness**
LC	**Long-chain**
LM	**Longissimus muscle**
LW	**Live weight**

MCFA	**Medium-chain fatty acid**
MFI	**Myofibrillar fragmentation index**
N	**Newton**
n-3	**Omega-3**
n-6	**Omega-6**
NaCl	**Sodium chloride**
NDF	**Neutral-detergent fibre**
NRC	**National Research Council**
OM	**Organic matter**
pHu	**Ultimate pH**
PM	**Psoas major muscle**
PUFA	**Poly-unsaturated fatty acid**
SCF	**Subcutaneous fat**
SD	**Standard deviation**
SDT	**Suggested dietary target**
Se	**Selenium**
SEM	**Standard error of the mean**
SFA	**Saturated fatty acids**
SL	**Sarcomere length**
SM	**Semimembranosus muscle**
TBA	**Thiobarbituric acid**
TBARS	**Thiobarbituric acid reactive substances**
TiO_2	**Titanium dioxide**
U/S	**Unsaturated fatty acid to saturated fatty acid ratio**
WBSF	**Warner-Bratzler shear force**
WHC	**Water-holding capacity**

CHAPTER I

GENERAL INTRODUCTION

Consumers are becoming more aware and concerned about the composition of food (Verbeke et al., 1999). According to Verbeke et al. (1999), the health conscious consumer prefers pork with an increased level of polyunsaturated fatty acid (PUFA). According to Coates et al. (2009) meat and eggs can be enriched with long chain omega-3 (LC n-3) PUFA by feeding appropriate sources of these fatty acids to monogastric animals creating healthier products.

The long-chain polyunsaturated fatty acids, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) offer a wide range of potential health benefits and are more and more recognised as essential nutrients in the human diet according to Simopoulos (1991) as stated in Sioutis et al. (2008). As described in Coates et al. (2009) LC n-3 PUFA have been implicated in the prevention of cardio vascular disease (Harris, 2007), chronic inflammatory disorders (Calder, 2006) and mental health conditions (Assisi et al., 2006).

The goal of this study was to find out if it is possible to change the composition of pork by dietary manipulation without compromising pig performance and meat quality.
In order to do this, the effects of (1) lipid type (soy bean oil, tallow and fish oil), (2) the period the fish oil was provided and (3) a dietary supplement (Sanovite^{TM}) containing conjugated linoleic acid (CLA), selenium, vitamin E and vitamin C on pig performance and pork quality were studied.

As described in Morel et al. (2008), several studies have shown that diet composition has an effect on the composition of pork in terms of PUFA (Bryhni et al., 2002; Irie and Sakimoto, 1992), CLA (Thiel-Cooper et al., 2001), selenium (Mahan et al., 1999), and vitamin E (Asghar et al., 1991a; Cannon et al., 1996; Hoving-Bolink et al., 1998; and Hasty et al., 2002). In a review of the effects of swine nutrition on pork quality by Pettigrew and Esnaola (2001) it was noted that the dietary fat composition reflects the body fat composition in pigs to some extent. Different dietary fat sources will therefore result in a different fat composition of pork cuts.

The current experiment was based on a previous study by Morel et al. (2008) in which the influence of diets supplemented with Sanovite^{TM} with or without animal protein on the quality of pork from female pigs was studied. In that study it was concluded that changing from a part animal component of the diet to an all plant diet had no effect on pig performance, but affected the fatty acid profile of pork. Morel et al. (2008) found that the nutritional value of pork could be improved by supplementing the diet with CLA, selenium and vitamin E.

The difference between the study by Morel et al. (2008) and the current study is that Morel et al. (2008) did not attempt to increase the amount of long chain n-3 PUFA in pork by feeding appropriate sources of these fatty acids to pigs. In the current study, the aim was to increase the amount of long chain n-3 PUFA in pork by feeding fish oil and to study the effects of varying the period during which fish oil is provided.

The main question of the current study was:

Is it possible to change the composition of pork by dietary manipulation without compromising pig performance and meat quality?

In order to be able to answer the main question, the following sub-questions are discussed in this thesis:

1. What is the effect of feedstuffs, lipid type and Sanovite^{TM} on growth performance and carcass quality?
2. What is the effect of feedstuffs, lipid type and Sanovite^{TM} on the digestibility of DDM, DOM and DA?
3. What is the effect of feedstuffs, lipid type and Sanovite^{TM} on the quality of pork?
4. What is the effect of feedstuffs, lipid type and Sanovite^{TM} on the fatty acid profile of loin muscle and backfat?
5. Is it possible to increase the EPA, DPA and DHA content of pork by diet manipulation sufficient to create pork providing adequate intake levels of these fatty acids for human consumption?
6. What is the effect of feedstuffs, lipid type and Sanovite^{TM} on palatability and meat processing?

Sub-questions 5 and 6 will be addressed with speculation and discussion, but there is no data available to substantiate the statements made in this thesis. The sensory evaluation conducted in Singapore was part of the project of another student, and results will therefore be published separately.

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CHAPTER II

REVIEW OF LITERATURE

This chapter is an overview of the literature relevant for this study. The effects of feedstuffs, lipid types and the supplementation of selenium (Se), vitamin E, vitamin C and conjugated linoleic acid (CLA) on growth performance, carcass quality, nutrient digestibility, meat quality and fatty acid profile of pork found in previous studies are discussed. This literature review also contains sections on adequate human dietary intake of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), meat palatability, and meat processing.

2.1 Growth performance

2.1.1 Feedstuff and lipid type

Animal versus plant

According to Shelton et al. (2001) as described in Morel et al. (2008) only a few studies have been conducted in which direct comparisons were made between the effect of animal versus plant feedstuffs in the diet on the performance of pigs. The previous work by Morel et al. (2008) is described in more detail in Table 2.1.2.1 and in Section 5.1.1 of the Discussion. Morel et al. (2008) found a similar growth performance in pigs fed diets containing either animal and plant components or plant components only. They concluded that changing from a partly animal component of the diet to an all plant diet will not change the growth performance of pigs, as long as the diets are similar in their composition with respect to minerals and proteins.

Fish oil

In a study by Haak et al. (2008), 154 crossbred pigs (barrows and gilts) with a mean body weight (BW) of 36.4 kg (SD = 4.5 kg) were randomly allocated to 7 treatment groups as shown in Table 2.1.1.1. The linseed and fish oil were added at 3% and 6%, respectively, on an as-fed basis. The trial consisted of two phases in which the fat source differed depending on the treatment group. Three treatment groups were fed the same fatty acid source during both phases, the other groups switched between fatty acid sources after the first phase.

Table 2.1.1.1: Dietary treatments in the study by Haak et al. (2008)

Group	Dietary treatment	
	Phase 1[a]	Phase 2[b]
BB	Basal diet	Basal diet
LL	Linseed	Linseed
FF	Fish oil	Fish oil
BL	Basal diet	Linseed
BF	Basal diet	Fish oil
LF	Linseed	Fish oil
FL	Fish oil	Linseed

[a] *Phase 1 until approximately 70 kg*
[b] *Phase 2 until an approximate slaughter weight of 100 kg*

In this study, the different diets had no effect on the growth performance of pigs. The average daily gain (ADG) was within the normal range of fattening pigs with an average of 637.3 and 675.8 g/d in phase 1 and phase 2, respectively. According to Van Oeckel et al. (1992) as described in Haak et al. (2008), fish oil influences the growth rate of pigs, they advised a maximum of 5% fish oil in the diet to obtain a normal feed intake (FI) and growth.

2.1.2 Supplement

A summary of the effects of the supplementation of Se, vitamin E, vitamin C and CLA on growth performance of pigs found in previous work comparable to the current study is given in Table 2.1.2.1.

Sanovite™

In the study by Janz et al. (2008) and Morel et al. (2008), supplementation of Sanovite™ had no effect on growth performance.

Selenium

In a study by Mahan et al. (1999) with 351 crossbred pigs with an average BW of 20.4 kg at the start and slaughtered at 105 kg BW, no effect of selenium supplementation on growth performance was found.

Vitamin E

The effect of vitamin E on growth performance is unclear. In studies by Cannon et al. (1996) and Hoving-Bolink et al. (1998), no effect of vitamin E supplementation on growth performance was found. In contrast, Asghar et al. (1991) found that higher levels of vitamin E (100 or 200 mg/kg feed) improved ADG and feed conversion ratio (FCR) in the early growth phase. In a study by Hasty et al. (2002), vitamin E supplementation increased average daily feed intake (ADFI) linearly ($P<0.05$) and tended to have an inconsistent effect on ADG and FCR ($P<0.10$).

Vitamin E and vitamin C

In a study by Eichenberger et al. (2004) on 40 barrows with an average BW of 25 kg to 106 kg vitamin E and/or vitamin C supplementation did not have an effect on growth performance. Details of this study are described in Table 2.1.2.1 and in Section 5.1.2 of the Discussion.

Table 2.1.2.1: A summary of results from studies with pigs into the effects of vitamin E, vitamin C, selenium and CLA on growth performance, and carcass or meat quality characteristics

Reference	Animals	Dietary treatments	Effects on Growth Performance	Effects on carcass, or meat quality (LM = longissimus muscle, SM = semimembranosus muscle, PM= psoas major muscle)
Sanovite™				
Janz et al. (2008)	59 gilts grown from an average of 11.4 kg to 101.9 kg on diets with or without animal products	Contr.: • 38.5 mg/kg Vit. E • 0 mg/kg Vit. C • 0.3 mg/kg Se • 0 % CLA in diet Suppl.: • 164.4 mg/kg Vit. E • 2700 mg/kg Vit. C • 0.7 mg/kg Se • 0.25 % CLA in diet	No effect	• Ultimate pH (pHu) of LM lower for samples from pigs that received an animal-based diet containing the supplement (P<0.04) • Drip loss (SM) and Expr. Juice (LM) lower for samples from pigs that received an animal-based diet containing the supplement (P< 0.01) but not after adjustments for pHu (P<0.07). • Rancid odor was more often detected in samples from pigs that received a plant based diet containing the supplement compared with control plant group (25 vs 12%)
Morel et al. (2008)	60 gilts grown from an average of 11.4 kg to 101.9 kg on diets with or without animal products	Contr.: • 38.5 mg/kg Vit. E • 0 mg/kg Vit. C • 0.3 mg/kg Se • 0 % CLA in diet Suppl.: • 164.4 mg/kg Vit. E • 2700 mg/kg Vit. C • 0.7 mg/kg Se • 0.25 % CLA in diet	No effect	• Inclusion of the supplement increased Se and vitamin E content in LM (P<0.001) • Inclusion of suppl. increased LM intramuscular fat (P<0.05), increased the CLA level in different tissues (P<0.001), lowered contents of oleic acid (P<0.001) and increased contents of stearic acid (P<0.05) • Sensory evaluation as by Janz et al. (2008)
Selenium				
Mahan et al. (1999)	351 pigs grown from an average of 20.4 kg to 105.0 kg on basal diets	Contr.: • 0 mg/kg Se Suppl: • 0.05, 0.1, 0.2, 0.3 mg/kg Se	No effect	• Organic selenium in diet increased Se content in muscle tissue.
Vitamin E				
Cannon et al. (1996)	30 pigs (24 barrows and 6 gilts) grown from an average of 40.3 kg to 110.6 kg on grower/finisher diets	Contr.: • 0 mg/kg Vit. E Suppl.: • 100 mg/kg Vit. E	No effect	• Supplementation of vitamin E resulted in higher α-Tocopherol concentrations (P<0.05) in longissimus muscle and lower TBA values during extended retail display (P<0.05). • Palatability ratings were more desirable when vitamin E was supplemented (P<0.05).
Hoving-Bolink et al. (1998)	72 pigs (32 castrates and 40 gilts) grown from an average of 44.0 kg to 111.0 kg on tapioca based diets	Contr.: • 8 mg/kg Vit. E Suppl.: • 208 mg/kg Vit. E	No effect	• Vitamin E levels were 5 times higher in LM and PM muscles of treated group. • Vitamin E treatment reduced TBA- values in both muscles and improved colour stability in LM after 6 days of storage.
Asghar et al. (1991a)	60 pigs (barrows and gilts) grown from an average of 29.0 kg to 103.7 kg on grower diets	Contr.: • 10 mg/kg Vit. E[a] Suppl.: • 100, 200 mg/kg Vit. E[a]	Higher levels of vitamin E (100 or 200 mg/kg) improved ADG and FCR (P<0.05) in early growth phase.	• Concentrations of α-Tocopherol in blood plasma and different tissues increased (P<0.05) with increasing levels of dietary vitamin E.

[a] *Supplemented with DL-α-tocopheryl acetate, 1 IU of vitamin E is equivalent to 1 mg of with DL-α-tocopheryl acetate*

(continued overleaf)

Table 2.1.2.1 (continued)

Reference	Animals	Dietary treatments	Effects on Growth Performance	Effects on carcass, or meat quality (LM = longissimus muscle, SM = semimembranosus muscle, PM= psoas major muscle)
Vitamin E (continued)				
Hasty et al. (2002)	240 pigs (barrows and gilts) grown from an average of 87.0 kg to 129.5 on basal diets	Contr.: • 0 mg/kg Vit. E Suppl.: • 75, 150, 300, 600 mg/kg Vit. E	Vitamin E increased (P<0.05) ADFI linearly (P<0.05) and tended to affect ADG and FCR (P<0.10)	• Vitamin E tended (P<0.07) to increase b* values linearly (P<0.06). • Vitamin E supplementation increased tissue concentrations of vitamin E linearly (P<0.001).
Vitamin E and C				
Eichenberger et al. (2004)	40 barrows grown from an average of 25.0 kg to 106.0 kg on basal diets	Contr.: • 0 mg/kg Vit. E Suppl.: • 200 mg/kg Vit. E and/or • 300 mg/kg Vit. C	No effect	• Supplementation of vitamin E led to significantly higher concentrations of vitamin E in tissues. • Supplementation of vitamin C tended to enhance vitamin E levels in tissues, except for ham samples. • Differences in vitamin C content in tissues were only significant in LM and spleen • The correlation between vitamin E and vitamin C was positive in LM, ham and kidney, but negative in heart. • In the outer layer of backfat, oxidative stability (TBARS) was positively influenced by dietary vitamin E supplementation. • TBARS were higher in diets supplemented with vitamin C.
CLA				
Thiel-Cooper et al. (2001)	40 barrows grown from an average of 26.3 kg to 114.0 kg on basal diets	Contr.: • 0 % CLA in diet Suppl.: • 0.12, 0.25, 0.5, 1.0 % CLA in diet	ADG and FCR improved linearly when CLA level increased (P<0.05)	• CLA supplementation decreased both 10th rib backfat (P<0.05), fat depth over loin eye at the 10th rib (P<0.05), intramuscular fat (P<0.001) and subcutaneous fat (P<0.05). • Supplementation of CLA increased belly firmness linearly as the concentration of CLA increased (lean side up: P<0.001; lean side down: P<0.05). • CLA was incorporated into pig tissues, CLA concentration increased linearly in both subcutaneous fat and lean tissue (P<0.001)
Dugan et al. (1997)	108 pigs (54 barrows, 54 gilts) grown from an average of 61.5 kg to 106.0 kg on cereal based diets containing either CLA or sun flour oil	Contr.: • 0 % CLA in diet Suppl.: • 2 % CLA in diet	CLA suppl. tended to reduce FI (P=0.07) and improve FCR (P=0.06)	• Pigs fed CLA deposited less subcutaneous fat (P=0.01) and gained more lean (P=0.03)
Dugan et al. (2001)	216 barrows grown from an average of 36.0 to 115.0 kg on diets containing different levels of dietary CLA and total oil	Contr.: • 0 % CLA in diet Suppl.: • 0.25, 0.5 % CLA in diet	No effect	• An increased CLA content increased the lean content of commercial cuts by 2.7% (P = 0.008). • Supplementing CLA reduced the amount of subcutaneous fat (SCF) by 6.6% (P = 0.002)

Vitamin C

In studies by Zhao et al. (2002), vitamin C supplementation in growing pigs had no effect on growth performance. In contrast, Mahan et al. (1994) and de Rodas et al. (1998) did find an effect of vitamin C on growth performance. According to de Rodas et al. (1998) the inconsistent findings may be partly due to the instability of vitamin C.

The study by Zhao et al. (2002) consisted of 2 experiments on pigs with different weaning age. The experimental diets were supplemented with different levels of vitamin C, as shown in Table 2.1.2.2. In both experiments, there was no effect of vitamin C supplementation on ADG, FI or FCR.

The study by Mahan et al. (1994) consisted of 2 experiments as described in Table 2.1.2.2. Weanling pigs were used in Experiment 1, grower-finisher swine were used for Experiment 2. The diets were supplemented with 0, 50, or 500 mg/kg vitamin C in both experiments. The starter pigs grew faster ($P<0.05$) and had an improved FCR ($P<0.05$) when vitamin C was supplemented during the fist two weeks post-weaning. Vitamin C did not affect the growth performance of the grower-finisher pigs.

Table 2.1.2.2: Summary of experiments involving feeding supplements of vitamin C to pigs

	Mahan et al. (1994)		de Rodas et al. (1998)		Zhao et al. (2002)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
No. pigs	288	216	72	120	48	96
Weaning age, days	23 ± 2	-	14 ± 2	20 ± 3	30	20 ± 2
Average BW, kg	6.37	22.3	4.98	7.2	7.7 ± 0.9	7.1 ± 0.5
Vitamin C level, mg/kg	0, 50, 500	0, 50, 500	0, 75, 150	0, 75, 150	0, 300	0, 75, 300

In a study by de Rodas et al. (1998), two experiments were conducted. The two experiments are summarised in Table 2.1.2.2. In Experiment 1, an increased level of vitamin C resulted in a linear improvement of ADG ($P<0.05$) and FCR ($P<0.1$) for the overall 42 day experiment.
In Experiment 2, an increased level of vitamin C resulted in a linear ($P<0.1$) improvement of ADG and FCR for 0 – 17 days after weaning.

Vitamin C supplementation may have a positive effect on growth performance under specific conditions such as in young piglets and/or when animals are stressed (de Rodas et al., 1998).

CLA

Different studies have found variable results when it comes to the effect of CLA on growth performance. Thiel-Cooper et al. (2001) found improvements in ADG and FCR, and improvements in FCR were also found by Dugan et al. (1997). On the other hand, a more recent study by Dugan et al. (2001) found no effect of CLA on either ADG or FCR. A more detailed description and discussion of these studies is given in Table 2.1.2.1 and in Section 5.1.2. In a review by Dugan et al. (2004) it was concluded that feeding CLA to pigs had the potential to improve animal performance, but also that evidence on CLA improving ADG or FCR is limited.

2.2 Digestibility

2.2.1 Feedstuff and lipid type

Cera et al. (1989) found that, in weaned pigs, different fat sources have an effect on nutrient digestibility. They also concluded that these differences decrease with age.

Animal vs Plant

The study by Cera et al. (1989) consisted of 2 experiments; Experiment 1 was a digestibility trial whereas Experiment 2 was a performance trial. In this Section, Experiment 1 is summarised. In Experiment 1, 27 barrows with a weaning age of 21 days (average BW 6.1 kg) were fed a corn-soybean meal-dried whey diet containing 8% coconut oil, corn oil or tallow as a fat source.

The coconut oil contained more than 60% medium-chain fatty acids (MCFA), while the corn oil and tallow contained more than 95% long-chain fatty acids. In Experiment 1, diets with tallow as a fat source had the lowest apparent fat digestibility, and the coconut oil diet was found to have the highest apparent fat digestibility in the initial 3 weeks post-weaning. This, according to Cera et al. (1989) suggests an improved absorption of unsaturated versus saturated long-chain fatty acids in weanling pigs.

Jorgensen and Fernandez (2000) reported an increased digestibility for soybean oil in comparison to tallow. In their study, eight different fat sources (3 animal fat batches and 5 vegetable fat batches) were added to a basal diet to create dietary fat levels of 5, 10, 15, 20, 25 or 30%. In each experiment, three litters of six female pigs, weighing 50-75 kg were used. Jorgensen and Fernandez (2000) found that inclusion of soybean oil, which contains a high level of polyunsaturated fatty acids (PUFA) enhanced protein digestibility.

As described in Jorgensen and Fernandez (2000), the fatty acid pattern of animal fat depends on the origin of the raw materials. Fat from ruminant offal contains a higher level of saturated fatty acids than fat from monogastric animals. They found that both a high level of free fatty acids and a low content of fatty acid relative to the fat content had a negative effect on digestibility.

An increase in the ratio of unsaturated fatty acids to saturated fatty acids (U/S) results in an increased fat digestibility (Wiseman et al. 1990; 1998).

Fish oil

Jorgensen et al. (2000) studied the digestion of fat and fatty acids in diets containing oils with different fatty acid composition. The four barrows (average BW 35 kg) used in this experiment were assigned to a basal, fish oil, rapeseed oil or coconut oil diet and fed according to a 4 x 4 Latin square design. Diets were formulated to contain 150 g oil/kg diet. Jorgensen et al. (2000) found that digestibilities of PUFAs in the fish and rapeseed oil diets were higher ($P<0.05$) than in the coconut oil diet.

2.2.2 Supplement

Sanovite™

The effect of Sanovite™ on DDM, DOM and DA has not been studied in the past.

Selenium

Adkins and Ewan (1984) found a linear increase of the apparent digestibility of dry matter ($P<0.01$) as Se supplementation increased. In contrast, Tian et al. (2006) did not find an effect of Se supplementation on dry matter or ash digestibility.

In the study by Adkins and Ewan (1984), 65 crossbred pigs (barrows and gilts) weaned at 21 days of age were used in the digestibility trial. The different treatments consisted of a basal diet (< 0.02 mg/kg Se) supplemented with 0, 0.025, 0.050, 0.075 or 0.100 mg/kg Se as selenious acid.

Tian et al. (2006) studied the effect of different Se products and levels on nutrient digestibility. In their metabolic study, 21 barrows with an average BW of 50.21 kg ± 0.62 SD were used. A non-Se-fortified basal diet served as the negative control in this experiment. The experimental diets were supplemented with three different Se products (sodium selenite, Se-enriched yeast-organic Se and organic Se) at two dietary levels (0.1 or 0.3 mg/kg).

According to Adkins and Ewan (1984), as described in Tian et al. (2006) improvements of DM digestibility were observed more clearly when pigs were Se deficient.

Vitamin E and Vitamin C

Sahin and Kucuk (2001) concluded that a higher level of dietary vitamin C and vitamin E increased nutrient digestibility ($P<0.02$ and $P = 0.07$, for vitamin C and vitamin E respectively) in Japanese quails reared under chronic heat stress (34^0C). The nutrient digestibility parameters were: dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE). For this study, a total of 180 10-day-old Japanese quails were assigned to six dietary treatment groups. The birds received a diet with either two levels of vitamin C (100 or 200 mg L-ascorbic acid/ kg) or three levels of vitamin E (125, 250 or 500 mg DL-α-Tocopheryl acetate/kg). Sahin and Kucuk (2001) stated that the increase in digestibility of nutrients in their study could have been due to positive effects of vitamin C and or vitamin E, alleviating the negative effects of heat stress.

CLA

CLA is a poly-unsaturated fatty acid containing 18 carbon atoms and *cis* and *trans* double bonds (Pettigrew and Esnaola, 2001). As described in section 2.2.1 of this chapter, an increase in the U/S ratio of a diet results in an increased fat digestibility (Wiseman et al. 1990; 1998). According to Zollitsch et al. (1997) as described in Azain (2004) higher levels of unsaturated fatty acids in the diet relative to saturated fatty acids, leads to an improved nutrient digestibility.

2.3 Meat and carcass quality

2.3.1 Feedstuff and lipid type

In a review on the effect of swine nutrition on pork quality, Pettigrew and Esnaola (2001) stated that the dietary fat composition reflects the body fat composition in pigs to some extent. Different dietary fat sources will therefore result in a different fat composition of pork cuts. According to Wood et al. (2003), the meat quality characteristics influenced by the fatty acid profile are fat tissue firmness, shelf life and flavour. The effect of the fatty acid profile on flavour is described in Section 2.6 of this Chapter.

Wenk et al. (1990) found that increased PUFA levels together with monounsaturated fatty acids (MUFA) could change the texture of the fat with it becoming soft, greasy and oily.

In a study by Irie and Sakimoto (1992), 64 pigs with an average body weight of 81.4 kg received either a control diet or one of three test diets containing 2, 4 or 6% fish oil. The pigs were fed *ad libitum* for a total of 4 weeks until slaughter at an average body weight of 107.8 kg. In this study, the hardness of the body fat was measured with a texturometer, and the hardness was decreased by increasing levels of dietary fish oil.

According to Wood et al. (2003) fatty acid composition has an important effect on firmness or softness of the fat in meat due to differences in melting points. Melting points decline and colour changes when unsaturation increases (Wood et al. 2003). Groups of fat cells containing solidified fat with a high melting point appear whiter than liquid fat with a lower melting point (Wood et al. 2003).

2.3.2 Supplement

A summary of the effects of the supplementation of Se, vitamin E, vitamin C and CLA on meat and carcass quality found in previous work comparable to the current study is given in Table 2.1.2.1.

Sanovite™

In the study by Janz et al. (2008) and Morel et al. (2008) no statistically significant effects of supplementary Sanovite™ were found on carcass weight, fat depth or dressing percentage.

As shown in Table 2.1.2.1, Janz et al. (2008) found that the ultimate pH (pHu) of the longissimus muscle (LM) was lower for samples from pigs that received the animal-based diet supplemented with Sanovite™ ($P<0.04$). Janz et al. (2008) also found a decrease in drip loss (in the semimembranosus muscle (SM)) and expressed juice (in the LM) in samples from pigs fed the animal-based diet supplemented with Sanovite™ before pH adjustment ($P<0.01$), however differences were not significant after pHu adjustment ($P<0.07$).

As described in Table 2.1.2.1, Morel et al. (2008) found that inclusion of Sanovite™ increased the Se and vitamin E content ($P<0.001$) and the intramuscular fat percentage of the LM ($P<0.05$). Supplementation of Sanovite™ increased levels of CLA ($P<0.001$) and stearic acid ($P<0.05$) but lowered the levels of oleic acid ($P<0.001$) in different tissues in the study.

Selenium

Mahan et al. (1999) did not find any effect of organic Se (Sel-Plex; Alltech, Nicholasville, KY) on the growth performance or carcass characteristics of pigs. Howerver, adding organic Se to the diet did increase the Se content in muscle tissue.

Vitamin E

In the study by Hasty et al. (2002), supplementation of α-tocopheryl acetate had no effect ($P>0.45$) on carcass yield or carcass characteristics. Asghar et al. (1991) and Cannon et al. (1996) also reported no differences in carcass yield or carcass characteristics in pigs supplemented with vitamin E compared to control pigs.

Similarly, Hoving-Bollink et al. (1998) found no effect of dietary vitamin E on lean meat percentage or meat quality.

As shown in Table 2.1.2.1, increased dietary vitamin E levels resulted in higher vitamin E levels in different tissues in the studies by Asghar et al. (1991), Cannon et al. (1996), Hoving-Bolink et al. (1998), and Hasty et al. (2002). Concentrations of α-tocopherol acetate in blood plasma also increased ($P<0.05$) in the study by Asghar et al. (1991).

Vitamin E supplementation reduced thiobarbituric acid (TBA) values in the studies by Cannon et al. (1996) and Hoving-Bolink et al. (1998). Colour stability in the longissimus muscle (LM) after six days of storage was improved when diets were supplemented with vitamin E in the study by Hoving-Bolink et al. (1998). In the study by Hasty et al. (2002), vitamin E supplementation tended ($P<0.07$) to increase b* values, which indicate relative yellowness, linearly ($P<0.06$).

Vitamin E and vitamin C

Eichenberger et al. (2004) found no treatment effects of vitamin E and vitamin C supplementation on carcass characteristics.

Eichenberger et al. (2004) found that vitamin E levels increased in all tissues (liver, spleen, heart, kidney, backfat outer layer, ham and LM) when diets were supplemented with vitamin E. The supplementation of vitamin C also tended to enhance vitamin E levels in these tissues, but not in ham samples. A clear effect of vitamin C supplementation on the vitamin C concentration in different tissues could not be found. Vitamin C supplementation increased the vitamin C content of LM and spleen, but not in any of the other tissues analysed. The correlation between vitamin E and vitamin C was positive in LM, ham and kidney, but negative in the heart. No correlation between the two vitamins was found in liver and spleen.

Vitamin E supplementation had a positive effect on the oxidative stability (Thiobarbituric acid reactive substances, TBARS) measured in the outer layer of the backfat in the study by Eichenberger et al. (2004). Vitamin C on the other hand, decreased oxidative stability in all analysed tissues.

CLA

As described in Pettigrew and Esnaola (2001), Pettigrew (1999) reviewed data from seven experiments on the effect of CLA supplementation on carcass quality. In all the experiments, CLA supplementation reduced backfat thickness and increased the percentage lean meat in carcasses in the studies that reported this value (six out of seven). Thiel-Cooper et al (2001) found that carcass fat measurements from pigs fed CLA decreased quadratically, with the biggest decreases occurring when the CLA level in the diet was 0.50% or less.

In this study, CLA supplementation decreased 10th rib backfat (P<0.05), fat depth over the loin eye at the 10th rib (P<0.05), intramuscular fat (P<0.001) and subcutaneous fat (P<0.05). Supplementation of CLA increased belly firmness linearly as the concentration of CLA increased (lean side up: P<0.001; lean side down: P<0.05).

In the study by Dugan et al. (1997), CLA supplementation decreased the deposition of subcutaneous fat by 6.8% (P = 0.01) and increased lean gain by 2.3% (P = 0.03).Increasing the CLA content increased the lean content of commercial cuts by 2.7% (P = 0.008), and supplementing CLA reduced the amount of subcutaneous fat (SCF) by 6.6% (P = 0.002).

2.4 Fatty acid profile

2.4.1 Feedstuff and lipid type

As mentioned in Section 2.3.1, the dietary fat composition reflects the body fat composition in pigs to some extent (Pettigrew and Esnaola, 2001).

In a 50 day study with 70 gilts (61.8 ± 5.2 kg BW) by Realini et al. (2010), the effect of dietary fat source on carcass fatty acid composition was studied. The dietary treatments consisted of a barley and soybean diet supplemented with 10% fat. Additionally, one group received a semi-synthetic diet containing no fat (NF). The experimental diets contained the following fat sources or combinations of fat sources: beef tallow (T), high-oleic acid sunflower (HOSF), sunflower oil (SFO), linseed oil (LO), fat blend (FB: 55% T, 35% SFO and 15% LO) or oil blend (OB: 40% fish oil, 60% LO). The gilts had *ad libitum* access to their dietary treatment and water. The fatty acid contents of the experimental diets in this study are shown in Table 2.4.1.1.

Table 2.4.1.1: Fatty acid content of experimental diets in the study by Realini et al. (2010)

Dietary fatty acids (g/kg feed)	T	HOSF	SFO	LO	FB	OB	NF
SFA	57.2	14.3	18.8	12.6	36.0	20.0	0.6
MUFA	36.2	87.4	33.2	21.1	30.5	20.5	0.7
PUFA	16.1	22.8	74.5	73.4	45.2	70.8	1.6
n-3 FA	1.9	1.3	1.3	47.2	19.0	48.3	0.2
n-6 FA	14.2	21.5	73.3	26.2	26.1	22.5	1.4
PUFA/SFA	0.3	1.6	4.0	5.8	1.3	3.5	2.7
n-6/n-3	7.5	16.5	56.4	0.6	1.4	0.5	7.0

The fat sources were selected to achieve diets with different fatty acid composition. The beef tallow diet (T) was high in saturated fatty acids (SFA). The diets containing high-oleic acid sunflower oil (HOSF), sunflower oil (SFO) and linseed oil (LO) were high in oleic, linoleic and linolenic acids, respectively. The diet formulated with the fat blend (FB) contained intermediate levels of palmitic, stearic, oleic, linoleic and linolenic acids. The diet formulated with the oil blend (OB) contained a high amount of long-chain PUFA.

In the study by Bryhni et al. (2002) 48 crossbred growing-finishing pigs (barrows and gilts) were fed six different diets from an initial weight of 28 kg to an average weight at slaughter of 104 kg. Dietary treatments were combinations of two basic diets containing either a low (31%) or a high level of PUFA (50% of total fat content) and three levels of fish oil (0, 0.2, 0.4% of concentrated commercial capelin oil).

Animal

Longissimus muscle (LM), loin subcutaneous fat and belly cut from pigs fed diets containing animal feedstuffs in the study by Morel et al. (2008) contained higher levels of CLA and DHA than when plant feedstuffs were fed.

As shown in Table 2.4.1.2, Realini et al. (2010) found that carcasses from gilts fed diets supplemented with 10% beef tallow had a high degree of saturation. The fatty acid profile of the carcasses was similar to that of pigs fed the semi-synthetic diet containing no fat.

Table 2.4.1.2: Fatty acid content of carcasses from gilts fed experimental diets in the study by Realini et al. (2010)

Carcass fatty acid profile (%)	T	HOSF	SFO	LO	FB	OB	NF
SFA	35.70^{b}	29.30^{d}	30.30^{d}	31.00^{d}	34.70^{b}	32.80^{c}	40.60^{a}
MUFA	50.90^{b}	56.70^{a}	38.80^{de}	37.90^{e}	44.20^{c}	39.30^{d}	51.00^{b}
PUFA	13.50^{d}	14.00^{d}	30.80^{a}	31.10^{a}	20.90^{c}	27.90^{b}	8.32^{e}
n-3 FA	1.43^{d}	1.08^{e}	1.12^{e}	16.60^{a}	6.72^{c}	14.80^{b}	0.73^{f}
n-6 FA	12.20^{d}	13.00^{cd}	30.00^{a}	14.70^{b}	14.45^{b}	13.20^{c}	7.53^{e}
PUFA/SFA	0.38^{e}	0.49^{d}	1.03^{a}	1.01^{a}	0.61^{c}	0.85^{b}	0.20^{f}
n-6/n-3	8.45^{d}	11.90^{b}	26.60^{a}	0.87^{f}	2.11^{e}	0.88^{f}	10.20^{c}

$^{a-f}$ Within a row, means lacking a common superscript letter differ (P<0.05)

Plant

In the study by Morel et al. (2008), fatty acid profiles were analysed in LM, loin subcutaneous fat and belly cut. These three tissues contained lower concentrations of SFA and MUFA and higher concentrations of PUFA (except for CLA and DHA) when animals were fed diets containing plant feedstuffs compared to when animal feedstuffs were fed. Therefore, the PUFA:SFA ratio was greater in tissues from pigs fed the plant diets.

Feeding diet HOSF in the study by Realini et al. (2010) resulted in a high percentage of MUFA in the carcasses, while carcasses form gilts fed SFO and LO showed high percentages of n-6 and n-3 FA, respectively (Table 2.4.1.2).

In the study by Bryhni et al. (2002), the level of PUFA in the diet was highly correlated with PUFA in backfat (R^2 = 0.80). There was also a correlation between linoleic acid (C18:2) and α-linolenic acid (C18:3) in feed and backfat (R^2 = 0.80 and R^2 = 0.81, respectively).

Animal + plant

In the study by Realini et al. (2010), the carcasses from gilts fed diets supplemented with 10% fat blend or oil blend contained intermediate percentages of most fatty acids while the amount of n-3 fatty acids was higher in carcasses from pigs fed the oil blend than for pigs fed a fat blend as shown in Table 2.4.1.2.

Fish oil

In the study by Bryhni et al. (2002) it was found that backfat from pigs fed 0.4% of fish oil contained significantly more (P<0.001) docosapentaenoic acid (C22:5) than backfat from pigs in treatment groups without fish oil.

2.4.2 Supplement

Sanovite™

In the study by Morel et al. (2008), supplementing experimental diets with Sanovite™ resulted in greater tissue contents of CLA. The increase was evident when the supplement was added to the diet containing plant feedstuffs for both the subcutaneous and intramuscular fat.

Longissimus muscle, loin subcutaneous fat and the belly cut from pigs fed diets supplemented with Sanovite™ in the study by Morel et al. (2008) contained lower levels of C18:1 and C20:1, and greater proportions of C14:0, C16:0 and C18:0. Morel et al. (2008) assumed that these effects were the result of CLA in the supplement, but effects of other constituents in the supplement can not be ruled out. According to Lee et al. (1998) as described in Morel et al. (2008), CLA has an inhibitory effect on stearoyl-CoA desaturase, which may explain the increase in concentrations of SFA at the expense of MUFA.

CLA

In a review by Raes et al. (2004) it was stated that incorporation of CLA in to pork is mainly achieved by inclusion of CLA rich oils in swine diets.

Thiel-Cooper et al (2001) found that CLA supplementation of the diet led to increased CLA levels in pig tissue. The CLA concentration in subcutaneous fat and lean tissue increased linearly when dietary CLA levels increased (P<0.001).
Raes et al. (2004) stated that CLA supplementation in swine diets resulted in increased deposition of SFA (C14:0, C16:0 and C18:0) and decreased deposition of MUFA (mainly C18:1). These conclusions are in line with the findings of Morel et al. (2008).

2.5 Adequate intake of EPA, DPA and DHA

2.5.1 Feedstuff and lipid type

As mentioned in Section 2.4.2, the fatty acid profile of pork can be altered by dietary manipulation. According to Coates et al. (2009), meat and eggs can be enriched with LC n-3 PUFA by feeding appropriate sources of these fatty acids to monogastric animals.

2.5.2 Health benefits

According to Howe et al. (2007), the risk of coronary heart disease can be reduced by a reduced consumption of saturated fat. Long-chain omega-3 polyunsaturated fatty acids EPA, DPA and DHA offer a wide range of potential health benefits and are increasingly recognised as essential nutrients in the human diet according to Simopoulos (1991) as stated in Sioutis et al. (2008). As described in Coates et al. (2009), long-chain n-3 PUFA have been implicated in the prevention of cardio vascular disease (Harris, 2007), chronic inflammatory disorders (Calder, 2006) and mental health conditions (Assisi et al., 2006).

In a human study with 33 (16 female and 17 male) healthy adults by Coates et al. (2009), participants consumed either n-3 enriched or regular pork (1000 g/week) for 12 weeks. Fasting blood samples were collected every 4 weeks and analysed for serum lipids, maximally stimulated thromboxane production and erythrocyte fatty acid composition. The study found that consumption of n-3 enriched pork significantly elevated the n-3 content erythrocytes, and decreased serum TAG and thromboxane production.

As described in Howe et al. (2007), the National Health & Medical Research Council recommended AIs for the sum of EPA, DPA and DHA of 90 and 160 mg/day and SDTs of 430 and 610 mg/day for women and men, respectively.

2.6 Palatability and meat processing

It is relatively easy to alter the fatty acid profile of pork to create a healthier product for consumers (Enser et al., 2000), but an altered fatty acid profile can cause problems in the meat processing industry due to changes in fat texture and shelf life (Wenk et al., 1990).

2.6.1 Feedstuff and lipid type

As mentioned in section 2.3.1, increased PUFA and monounsaturated fatty acids (MUFA) levels could change the texture of the fat with it becoming soft, greasy and oily (Wenk et al., 1990). Melting points decline when unsaturation increases (Wood et al. 2003). In the study by Irie and Sakimoto (1992), the hardness of the fat, as measured by texturometer, decreased as dietary fish oil levels increased.

The change in shelf life and fat texture could have consequences for the meat processing industry as suggested by Prabucki (1991) as referenced by Hadorn et al. (2008).

Wenk et al. (1990) found a positive relationship between increased PUFA levels in pork and possibly higher occurrence of oxidation and rancidity.

Fish oil

Bryhni et al. (2002) found significantly more (P<0.05) fishy and rancid odours, higher TBA values, and less meat odour in samples of meat and fat from pigs fed high PUFA diets compared to samples form pigs fed the low PUFA diets after 1 month of freezer storage.

Jaturasitha et al. (2009) used 600 crossbred pigs in a study of deposition of n-3 fatty acids from tuna oil, with 56 pigs randomly selected for studies on carcass and pork quality. Pigs received either a control diet containing 0% tuna oil, 1% tuna oil fed from 35-90kg of BW or 3% tuna oil in the early (35-60 kg BW) or late (75-90 kg BW) stage of fattening. The trial was set-up so that all pigs would receive an equal amount of fish oil during their lifetime (approximately 1.6 kg per pig). Jaturasitha et al. (2009) did not find any treatment effects on the texture of the loin, or in the firmness and melting properties of the backfat. Six trained panellists in the sensory assessment for this study did not detected off or fishy flavours, but shelf life and scores given for sensory flavour and overall acceptability were superior when tuna oil was supplemented in the early stage of fattening.

A withdrawal period for fish oil was recommended in previous studies (Jaturasitha et al., 2009). As described in Jaturasitha et al. (2009), Fraser et al. (1934) recommended a withdrawal period of 4 weeks while Melton (1990) suggested a withdrawal period of at least 2 weeks.

2.6.2 Supplement

Sanovite™

In the study by Janz et al. (2008) a rancid odor was detected more often in samples from pigs that received a plant based diet supplemented with Sanovite™ compared with samples from pigs fed the un-supplemented plant based diet (25% vs. 12%, respectively).

Vitamin E

As described by Sheard et al. (2000), feeding PUFA to pigs increases the susceptibility of pork to oxidation. According to Wood et al. (2003), α-tocopherol could be used to delay lipid and colour oxidation and thereby extend shelf life. Asghar et al. (1991a) also stated that higher levels of dietary vitamin E can lead to a decreased susceptibility to oxidation.

CLA

Supplementing pig diets with CLA leads to an alteration of the fatty acid profile in pork as described in Section 2.4.2 and therefore the statements in Section 2.6.1 also apply.

2.7 References

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CHAPTER III

MATERIALS AND METHODS

3.1 Animals

All animals were managed according to the Massey University Animal Ethics Committee and the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Forty-eight female pigs (PIC hybrids, mean live weight 16.19 kg ± 1.56 SD) were obtained from a single commercial operation in the North Island of New Zealand. The pigs were transported to the University Pig Biology Unit, rank ordered by weight and assigned to one of six dietary treatment groups as shown in Table 3.2.1.

The pigs were kept in pens of six, but fed individually twice daily. Water was available at all times. Individual feed intakes were measured daily and live weight recorded weekly.

3.2 Experimental design

In this experiment, the effects of (1) lipid type (soy bean oil, tallow and fish oil), (2) the period the fish oil was provided and (3) a dietary supplement containing conjugated linoleic acid (CLA), selenium (Se), vitamin E and vitamin C on pig performance and pork quality were studied.

The experiment follows on from those of Janz et al. (2008) and Morel et al. (2008) in which the influence of diets supplemented with Sanovite^{TM}, with or without animal protein, on the growth performance, meat quality and pork fatty acid profile from female pigs was studied.
The experiment was designed to measure planned differences between dietary treatment groups using the groups and linear contrast shown in Table 3.2.1.

Table 3.2.1: Dietary experimental groups[a] and linear contrast

	Experimental Groups					
Diets	AT	PO	POS	PTS	PFSe	PFSl
Feedstuff	Animal	Plant	Plant	Plant	Plant	Plant
Lipid Type	Tallow	Soy	Soy	Tallow	Fish (early)	Fish (late)
Sanovite + Vit. C	-	-	+	+	+	+
Contrast						
Animal vs Plant	1	-1				
Sanovite		1	-1			
Tallow vs Soy			1	-1		
Fish vs Soy			-2		1	1
Early/Late Fish					-1	1

[a] *The six dietary treatments:*

AT: Animal feedstuffs and tallow over the whole experiment (84 days)

PO: Plant feedstuffs, soybean and linseed oil over the whole experiment (84 days)

POS: Plant feedstuffs, soybean and linseed oil with dietary supplement over the whole experiment (84 days)

PTS: Plant feedstuffs and tallow with dietary supplement over the whole experiment (84 days)

PFSe: Plant feedstuffs and fish oil with supplement between days 1 and 35 and then diet POS up to day 84.

PFSl: Diet POS between days 1 and 35; plant feedstuffs and fish oil with supplement between days 36 and 56 and then diet POS up to day 84.

3.3 Diets and feeding regimen

The diet base was either a combination of animal and plant feedstuffs (AT and PTS), plant feedstuffs only (PO, POS) or plant feedstuffs combined with fish oil (PFS). The diets also differed depending on the presence or absence of the nutritional supplement Sanovite™ and Vitamin C. Sanovite™ is a trademarked dietary supplement containing CLA (BASF, Auckland, New Zealand), organic Selenium (Alltech Inc., Nicholasville, KY) and vitamin E (Morel et al., 2008). Diet POS, PTS and PFS contained Sanovite™ and Vitamin C. Pigs fed diet PFSe received plant feedstuffs and fish oil with supplement between days 1 and 35 and then diet POS up to day 84. Pigs fed diet PFSl received diet POS between days 1 and 35; plant feedstuffs and fish oil with supplement between days 36 and 56 and then diet POS up to day 84. Pigs in group PFSe and PFSl both received the same total amount of fish oil per pig (2.52 l/ 2.31 kg).

The composition of the different grower and finisher diets is given in Table 3.3.1. The grower diets were fed between days 1 and 56 of the experiment and the finisher diets between days 57 and 84 of the experiment.

Table 3.3.1: Composition of the experimental diets

Ingredient in % of diet	Grower					Finisher			
	AT	PO	POS	PTS	PFS	AT	PO	POS	PTS
Barley	55.95	67.37	67.37	67.37	67.37	55.95	67.37	67.37	67.37
Wheat	10	-	-	-	-	10	-	-	-
Broll	6	6	6	6	6	7.6	7.6	7.6	7.6
Soybean meal	7	16	16	16	16	7	16	16	16
Blood meal	3	-	-	-	-	3	-	-	-
Meat and bone meal	13	-	-	-	-	13	-	-	-
Tallow	4.4	-	-	4.4	-	2.8	-	-	2.8
Soybean oil	-	3.3	3.3	-	-	-	2.1	2.1	-
Linseed oil	-	1.1	1.1	-	-	-	0.7	0.7	-
Fish oil[a]	-	-	-	-	4.4	-	-	-	-
Lysine	-	0.37	0.37	0.37	0.37	-	0.37	0.37	0.37
Methionine	0.15	0.26	0.26	0.26	0.26	0.15	0.26	0.26	0.26
Threonine	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2
Dicalcium phosphate	-	3	3	3	3	-	3	3	3
Limestone	-	1.6	1.6	1.6	1.6	-	1.6	1.6	1.6
Sodium chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Disodium phosphate	-	0.4	0.4	0.4	0.4	-	0.4	0.4	0.4
Vitamin-mineral grower	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Conjugated linoleic acid	-	-	0.250	0.250	0.250	-	-	0.250	0.250
Selplex	-	-	0.024	0.024	0.024	-	-	0.024	0.024
Vitamin E	-	-	0.040	0.040	0.040	-	-	0.040	0.040
Vitamin C	-	-	0.300	0.300	0.300	-	-	0.300	0.300

[a] *To prevent oxidation, the fish oil was stored in a fridge and added to the mash diet freshly every feed by the use of a syringe. Water was added to the mixture afterwards.*

The pigs were fed increasing amounts according to a fixed feeding schedule (Table 3.3.2). Pigs in group PFSe and PFSl both received the same total amount of fish oil per pig (2.52 liter / 2.31 kg). Because the fish oil was added with a syringe, the weight in gram was converted into milliliters (1liter of fish oil = 920 gram).

Table 3.3.2: Feeding schedule and amount of oil fed over the total experimental period

Week	Planning	Fish oil (ml) per pig	
	Feed Offered Daily (gram)	PFSe	PFSl
1	1000	336	0
2	1250	420	0
3	1500	504	0
4	1750	588	0
5	2000	672	0
6	2250	0	756
7	2500	0	840
8	2500	0	924
9	2650	0	0
10	2650	0	0
11	2200	0	0
12	2400	0	0

3.4 Feed intake and growth

A set amount of feed was weighed out once a day, with pigs receiving 2/3 in the afternoon (at approximately 3.30 pm) and 1/3 in the morning (at approximately 8 am). The feeding schedule is shown in Table 3.3.2. Enough water was added to dampen the mash diet so that it was slightly moist. Refusals were weighed out after every feeding and a sample of the total refusal was collected. Each week, samples of the refusals of each pig were mixed, sub sampled and dried in a convection oven on 105 ^{o}C for 24 hours. The Total Feed Intake was calculated according to the dry matter (DM) percentage.

Each pig was weighed weekly after feeding. The scale consisted of a cage, a set of (2) loadbars and a weighing indicator (True-Test AG500). The cage was fully enclosed by bars and had see-trough gates that could be locked. The loadbars were installed under the floor of the cage.

The electronics averaged the weight to minimize the effect of animal movement. The weighing indicator was accurate to 0.5 kg. The scales were tared after each measurement.

3.5 Feed quality

All diets were analysed by the nutrition laboratory of the Institute of Food, Nutrition and Human Health (Massey University, Palmerston North, New Zealand) for gross energy (GE), fatty acid profile, neutral-detergent fibre (NDF), protein, fat, dry matter, ash, acid-detergent fibre (ADF), lignin, hemicellulose and cellulose.

The GE was determined by bomb calorimetry. The fatty acid profile was determined by Fames, GC separation. The amount of NDF was determined as reported by Robertson, J.B., Van Soest, P.J. (1981).

The following AOAC (2000) procedures were used for analyses: protein (Leco, total combustion method, AOAC 968.06), fat (Soxtec extraction, AOAC 991.36), moisture (convection oven 105 ^{o}C, AOAC 930.15, 925.10), ash (Furnace 550 ^{o}C, AOAC 942.05), ADF, lignin, hemicelulose and cellulose (AOAC Method 973.18).

3.6 Carcass quality

The pigs were slaughtered at Land Meat New Zealand Limited, Wanganui at day 85 of the experiment. Carcass quality characteristics determined at the abattoir included carcass weight and backfat thickness as measured at the end of the slaughter line. Fat depth was measured at P2 (65mm from mid dorsal line at last rib as described in Wood et al.,1989).

3.7 Digestibility

3.7.1 Faeces

Faeces were collected once a day during two days (day 32 and 33) in week five of the trail. Pigs were separated into individual feeders in the morning, were fed and released after they defecated. From five days prior to (day 27), and during the days of collection the pigs received 0.4% titanium dioxide (TiO_2) through their diet.

3.7.2 Digestibility determination

The faecal samples of each pig were frozen, freeze-dried and ground. From the ground samples, approximately 5 g was submitted to the Nutrition Laboratory, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand where the samples were analysed.

The TiO_2 percentage was determined by a Sulphuric acid digestion assay followed by colorimetric determination.

The digestibility parameters where then calculated as follows:

$$y = \frac{\left(Xd/Tid\right) - \left(Xe/Tie\right)}{\left(Xd/Tid\right)}$$

y = *Digestibility of DM or OM or Ash*
Xd = *DM or OM or Ash in diet*
Tid = *TiO_2 in diet*
Xe = *DM or OM or Ash in excreta*
Tie = *TiO_2 in excreta*

3.8 Meat quality

The carcasses were chilled for 40 hours (± 3 hours) before they were boned. The SM and LM from each pig were taken during the cut-up process at Land Meat New Zealand Limited, Wanganui. Meat quality assessments were performed on the semimembranosus muscle (SM) from one of the topside cuts of each pig. This muscle was chosen for meat quality analysis because the longissimus muscles (LM) were exported to Singapore for fatty acid analysis and sensory evaluation as part of a different study. The meat quality results were corrected for ultimate pH (7 days post-mortem) by covariance. The semimembranosus muscles used for meat quality measurements were frozen (-30^{o}C) after ageing for 7 days at a temperature of 2-3^{o}C. The meat quality assessments were conducted within 3.5 months after the samples were frozen.

3.8.1 Colour

A 10-15 mm thick slice from the proximal end of each SM was used for the determination of colour. An internal sample of approximately 40x40 mm was taken from the pale end of the slice and frozen. The frozen samples were defrosted and exposed to the air in the chiller for 50-70 min before measurements started. The samples were refrozen in order to be able to conduct all the colour measurements at once. The colour was measured using a Minolta CR-200 chroma-meter with a 10 mm diameter aperture (where L^* depicted relative lightness, a^* indicated relative redness and b^* represented relative yellowness).

3.8.2 Cooking loss and tenderness

To determine cooking loss and tenderness, four steaks of 25 mm thickness were cut. All samples were weighed individually before cooking. The samples were cooked by suspending in a plastic bag in water. Two out of the four steaks were cooked for 90 minutes at 60°C while the other two were cooked for 90 minutes at 70°C. After cooking, the excess liquid was poured off from the bag and the samples were chilled overnight at 1-2°C before reweighing and slicing. Samples were dabbed dry before reweighing to calculate the cooking loss. A Warner-Bratzler shear force (WBSF) machine was then used to measure meat tenderness. The method as described in Purchas and Aungsupakorn (1992) was used for WBSF measurements. Slicing devices were in good condition and sharp. Samples were cut into cores (six per sample) along the grain of the muscle with 13x13 mm cross sections. Any cores that were not uniform were discarded. Each core was sheared once at a 1/3 and once at 2/3 along its length to give 12 shear values.

Fat firmness is not addressed in this study because it was assumed that the fatty acid analysis would provide enough information on fat firmness according to published data.

3.8.3 pH

For pH measurement, an internal sample of 2-2.5 g was taken. The sample was diced, placed into a plastic container and homogenized for several seconds in 10 ml of chilled distilled water, and then pH was measured with a pH meter with a spear tip combination electrode and automatic temperature compensation. The pH meter was calibrated at pH of 7.0 and 4.0 before measurements of the samples each day.

3.8.4 Water-holding capacity

To measure the water-holding capacity (WHC) of the meat samples, an internal sample of 500 mg ± 20 SD was taken. The sample was placed on a Whatman No1 filter paper (11cm diameter). The filter paper was stored over saturated potassium chloride (KCl) for a constant water content. The meat sample was placed in the middle of a filter paper on a Perspex plate, another Perspex plate was placed on top of that and the two were pressed together. A weight of 10 kg was added and left for five minutes. After five minutes, both the weight and the Perspex plates were removed, the area of squashed meat was marked and the sample was reweighed. To measure the area of the expressed juice and the area of squashed meat, a Placom KP-90N Digital Planimeter was used. The planimeter measured the area by pulse count, and expressed juice values were calculated in three ways as follows:

1. Expr. juice % loss in weight = [(W1 – W2) x 100] / W1
2. Expr. juice Outside area (cm^2/g) = OA / W1
3. Expr. juice Outside – Inner area (cm^2/g) = (OA – IA) / W1

W1 = *Initial weight (g)*
W2 = *Squashed weight (g)*
OA = *Outside area (cm^2)*
IA = *Inner area (cm^2)*

3.8.5 Myofibrillar fragmentation index

Myofibrillar fragmentation indexes (MFIs) were measured using a modification of the method of Johnson et al. (1990) as described in Purchas et al. (1997).

The proportion of muscle fragments that passed trough a 231 µm filter after the sample had been homogenised was measured. In order to do this, a 5 g ± 0.5 SD sample of diced (c. 6 mm^3 pieces) thawed meat was added to 50 ml of physiological saline (0.85% sodium chloride (NaCl)) in a 50 ml graduated cylinder. Before homogenising, 5-10 drops of antifoam A emulsion (Sigma Chemical Co) was added to the cylinder.

An Ultra-Turrax homogeniser with an 18 mm diameter shaft at circa ¼ speed was used for this assessment. Each sample was homogenised for two 30-second periods separated by a 30-second rest period. Any homogenate remaining on the shaft was rinsed off into the cylinder using circa 20 ml of saline. The total was poured into a pre-weighed filter made from a 60 mm length of plastic pipe (52 mm internal diameter) with stainless steel mesh (231x231 µm holes) glued to one end. After the dripping (2-3 hours), the filters were dried in an incubator for 40 hours at 28-30^{0}C before being re-weighed. The MFI values were calculated as 100 minus the percentage of the initial meat sample weight that remained on the filter.

3.8.6 Sarcomere length

For the sarcomere length (SL) measurements, methods as described in Bouton et al (1973) and Purchas and Aungsupakorn (1992) were used. A sample from the dark part of the Semimembranosus, parallel to the muscle fibres was taken. A small slither (8-10 mm along the direction of the fibre and 1x1 mm in cross section) of muscle was prepared with a scalpel blade and transferred to a microscope slide. The muscle was teased out and 2-3 drops of distilled water were added. The sample was squashed with another microscope slide before the measurements were carried out. The prepared sample was placed in the holder. A helium-neon (He-Ne) laser was passed through it and the holder was moved around until clear bands were apparent. A total of 12 measurements were made of the distance between first order diffraction bands and the mean distance was calculated. To calculate the sarcomere length (µm), the following equation was used:

$$SL = 0.6328 * \left[\sqrt{\left(\left(x / 10 * 2 \right)^2 * 100 \right)} \right] / \left(x / 10 * 2 \right)$$

$x =$ *Calculated mean distance between first-order diffraction bands (mm)*

3.8.7 Selenium content lean meat

The selenium concentration in the lean meat was analysed in Singapore by J. Leong. Samples of the longissimus muscle (2 g) were grounded and weighed into a teflon vessel, 7 ml of nitric acid (65%) was added followed by 1 ml of hydrogen peroxide (30%). The sample and the acids were mixed by gently swirling the vessel. The vessel was placed into the rotor of a microwave digestion system (Ethos 1600, Milestone, USA) for 10 minutes to reach a temperature of 200 ^{o}C, this temperature was maintained for another 10 minutes. After the teflon vessel had cooled down to room temperature, the vessel was opened and the solution was transferred into a marked volumetric flask and topped up with ultrapure water to 100 mL.

An ICPE spectrometer (Shimadzu model ICPE-9000) was used for the analysis using Argon as a cooling, plasma, and carrier gas at flow rates of 14.0, 1.2, 0.7 L/minute respectively.

3.9 Fatty acid profile of loin and backfat

These measurements were conducted in Singapore by Mrs. J. Leong (MSc). The loins were frozen (-20 ^{o}C) 48 hours after slaughter and exported to Singapore. The backfat samples from over the loin were used in these measurements.

The fatty acid profile was determined by the direct fatty acid methyl esters (FAME) synthesis method of O'Fallon et al.(2007). Fatte acid methyl ester synthesis was conducted in the presence of up to 33% water. Wet tissues were permeabilised and hydrolysed for 1.5 hours at 55 ^{o}C. Hexane was then added to the reaction tube, which was vortex-mixed and centrifuged. The hexane was pipetted into a gas chromatography vial for subsequent gas chromatography (O'Fallon et al., 2007).

The fatty acids (and their abbreviations) as used in this study are described in Table 3.9.1.

Table 3.9.1: Description of fatty acids and abbreviations as used in this study

Saturated fatty acids (SFA)	Monounsaturated fatty acids (MUFA)[a]	Polyunsaturated fatty acids (PUFA)[a]
C6:0, caproic	C14:1-*cis*-9, myristoleic	C18:2-*trans*-9,12, linolelaidic
C8:0, caprylic	C15:1-*cis*-10, pentadecenoic	C18:2 n-6 *cis*-9,12, linoleic
C10:0, capric	C16:1-*cis*-9, palmitoleic	C18:2-*cis*-9, *trans*-11, CLA
C11:0, undecanoic	C17:1-*cis*-10, heptadecenoic	C18:2-*trans*-10, *cis*-12, CLA
C12:0, lauric	C18:1-*cis*-9, oleic	C20:2 n-6 *cis*-11,14, eicosadienoic
C13:0, tridecanoic	C18:1-*trans*-9, elaidic	C22:2-*cis*-13,16, docosadienoic
C14:0, myristic	C18:1-*trans*-11, vaccenic	C18:3 n-6 *cis*-6,9,12, γ-linolenic
C16:0, palmitic	C18:1 -*cis*-11, vaccenic	C18:3 n-3 *cis*-9,12,15, α-linolenic
C17:0, margaric	C20:1-*cis*-11, eicosenoic	C20:3 n-6 *cis*-8,11,14, eicosatrienoic
C18:0, stearic	C22:1-*cis*-13, erucic	C20:3 n-3 *cis*-11,14,17, eicosatrienoic
C20:0, arachidic	C24:1-*cis*-15, nervonic	C20:4 n-6 *cis*-5,8,11,14, arachidonic
C21:0, heneicosanoic		C20:5 n-3 *cis*-5,8,11,14,17, EPA
C22:0, behenic		C22:5 n-3 cis-7,10,13,16,19, DPA
C23:0, tricosanoic		C22:6 n-3 *cis*-4,7,10,13,16,19, DHA
C24:0, lignoceric		

[a] Abbreviations for unsaturated fatty acids, Omega-3 PUFA and Omega-6 PUFA are UFA, n-3 PUFA and n-6 PUFA, respectively

3.10 Fatty acid profile of diets

The fatty acid (FA) profile in the diets was determined in the nutrition laboratory of the Institute of Food, Nutrition and Human Health (Massey University, Palmerston North, New Zealand). The fatty acid profile was determined by the FAME synthesis method as purchased from Sigma-Aldrich Co. For the determination of the FA profile, a Shimadzu GC-17A Gas chromatograph was used. The gas chromatograph was equipped with a flame ionization detector (FID) and fitted with a Supelco^{TM}-2560 Capillary Column (100m x 0.25mm x 0.2um film thickness). The oven temperature was programmed to hold at 140^{o}C for 5 minutes then to increase to 240^{o}C at the rate of 4^{o}C per minute, and hold at this temperature for 38 minutes. The injector temperature was 250^{o}C, the detector temperature 255^{o}C.

3.11 Adequate intake of EPA, DPA, DHA

The combined (EPA+DPA+DHA) content in loin and backfat was determined as a percentage of the total fatty acid content. To calculate the concentration of (EPA+DPA+DHA) (g/g) in the loin muscle, the intramuscular fat concentration (g/g) was multiplied by the concentration of (EPA+DPA+DHA) relative to total fatty acids (g/g). These calculations were based on the assumption that fatty acids made up 100% of intramuscular fat.

To calculate the concentration of (EPA+DPA+DHA) (g/g) in the backfat, the concentration of fat in backfat (g/g) was multiplied by the concentration of (EPA+DPA+DHA) in the total of all fatty acids (g/g). The fat percentage of the backfat was not determined, but estimated to be 72% based on that determined for loin subcutaneous fat as shown in the study by Morel et al. (2008).

The following equation was used for the calculations:

INT = AI / (((LN%/100) x LN-N3) + ((LN%/100) x BF%-N3))

Where:

INT = required intake of the pork product (g/d) to obtain 160 mg of (EPA+DPA+DHA)

AI = adequate intake of long-chain n-3 fatty acids (EPA+DPA+DHA)

LN% = percentage of lean loin in the pork product

LN-N3 = concentration of (EPA+DPA+DHA) in the lean loin (g/g)

BF% = percentage of backfat in the pork product

BF-N3 = concentration of (EPA+DPA+DHA) in the backfat (g/g)

For the calculations in Table 4.7.1, the backfat was used as fat source and the balance was made up with loin muscle (lean meat content). Calculations were made in the same way as for Figure 4.7.1. Calculations were based on fatty acid levels in uncooked pork.

The following equation was used for the calculations:

INT = TAR / (((LN%/100) x LN-N3) + ((LN%/100) x BF%-N3))

Where:

INT = required intake of the pork product (g/d)

TAR = adequate intake (AI) or suggested dietary targets (SDT) for (EPA+DPA+DHA)

LN% = percentage of lean loin in the pork product

LN-N3 = concentration of (EPA+DPA+DHA) in the lean loin (g/g)

BF% = percentage of backfat in the pork product

BF-N3 = concentration of (EPA+DPA+DHA) in the backfat (g/g)

There is a difference in levels recommended for adequate intake (AI) in gram per day and suggested dietary targets (SDT) in gram per day. For the calculations in Table 4.7.1, an AI of 160 mg and a SDT of 610 mg of (EPA+DPA+DHA) per day were used.

3.12 Statistical analysis

A linear model with dietary treatment as a fixed effect was fitted to the growth performance, digestibility, fatty acid and meat quality data. Ultimate pH was used as a covariate for the meat quality data, and the homogeneity of the slope was tested with the interaction between the covariate (pH) and the fixed effect (dietary treatment). Statistical analyses were performed using the GLM procedure (SAS Inst. Inc., Cary, NC). A non-orthogonal set of contrasts were tested for significance as set out in Table 3.2.1.

3.13 References

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CHAPTER IV

RESULTS

The results are presented in separate tables for growth performance and carcass quality characteristics, digestibility estimates, meat quality characteristics and fatty acid profiles for backfat and the loin muscle (m. longissimus lumborum).

One pig in group PFSe (as described in Table 3.2.1) was excluded from the study after the digestibility trial because she suffered a fracture of the right femur.

4.1 Growth performance and carcass quality

As shown in Table 4.1.1, no significant differences (P>0.05) in average daily gain, feed intake or feed conversion ratio were observed over the experimental period. The growth performance of the pigs was adequate, with the average daily gain (ADG) from week 1-12 (mean ± SD) being 851(± 56) gram per day. The average total feed intake was 168.0 (± 7.4) kg and the overall average feed conversion ratio (FCR) was 2.36 (± 0.14).

Table 4.1.1: Least-squares means for the growth data of pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Growth Data	Treatment Group								
	AT	PO	POS	PTS	PFSe	PFSI	Effect (P value)	R^2(%)[b]	RSD[b]
Week 1-5 (Grower 1)									
BW Start, kg	18.6	18.7	18.9	19.0	19.0	18.9	1.00	0.6	1.83
BW End, kg	42.8	44.4	45.4	45.4	45.3	45.9	0.37	11.9	3.09
ADG, g	689	733	758	755	752	772	0.22	15.1	68.5
DFI, g	1402	1454	1472	1467	1411	1469	0.11	19.1	62.0
FCR	2.07	1.99	1.95	1.94	1.90	1.91	0.36	12.0	0.16
Week 6-8 (Grower 2)									
BW End, kg	63.1	65.7	68.0	66.9	66.2	68.8	0.15	17.5	4.25
ADG, g	970	1015	1074	1024	997	1086	0.32	12.8	114.8
DFI, g	2258	2322	2404	2354	2240	2340	0.39	11.6	163.6
FCR	2.36	2.32	2.25	2.33	2.26	2.16	0.71	6.6	0.27
Week 9-12 (Finisher)									
BW End, kg	88.1	89.0	92.6	89.9	88.6	93.5	0.21	15.5	5.12
ADG, g	891	833	877	822	801	884	0.21	15.5	84.6
DFI, g	2439	2442	2476	2472	2401	2470	0.46	10.3	80.0
FCR	2.77	2.96	2.84	3.06	3.01	2.81	0.32	12.9	0.30
Week 1 -12 (Total)									
ADG, g	827	837	877	845	829	888	0.14	17.9	54.0
DFI, g	1962	2001	2040	2023	1948	2020	0.26	14.2	86.4
FCR	2.37	2.40	2.33	2.41	2.35	2.28	0.48	10.1	0.14

[a] A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).
[b] Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD).

As shown in Table 4.1.2 no significant differences (P>0.05) were observed in carcass weight, fat depth at P2 and dressing-out percentage. The pigs had an overall average of 72.1 kg (± 4.7 SD) carcass weight, 10.3 mm (± 1.9 SD) of fat depth and a dressing-out percentage of 79.8 percent (± 1.9 SD).

Table 4.1.2: Least-squares means for carcass weight, backfat thickness and dressing-out percentage for pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Slaughter Data	Treatment Group								
	AT	PO	POS	PTS	PFSe	PFSl	Effect (P value)	R^2(%)[b]	RSD[b]
Carcass weight, kg	70.05	70.80	74.63	72.05	69.94	74.70	0.12	18.4	4.47
Backfat thickness, mm	10.30	10.30	10.15	10.70	10.06	10.10	0.99	1.3	1.98
Dressing-out perc., %[c]	79.57	79.52	80.65	80.14	78.89	79.89	0.59	8.4	1.90

[a] A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).
[b] Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD)
[c] Dressing-out percentage was calculated as carcass weight versus last live weight

4.2 Digestibility

Dietary treatment had a significant effect on DDM, DOM and DA (P<0.001) as shown in Table 4.2.1. All contrasts for DDM and DOM were significant (P<0.001), except for AT vs. PO. The contrast for DA was significant for all groups. Diet PO had a significantly higher DA then diet AT (0.44 compared to 0.30). Diet POS had the highest digestibility for DDM, DOM and DA.
Diet POS had a significantly higher digestibility for DDM (0.82) than diet PO (0.74), PTS (0.79) or PFS (0.79). Diet POS had a significantly higher digestibility for DOM (0.84) than diet PO (0.77), PTS (0.81) or PFS (0.82). Diet POS had a significantly higher digestibility for DA (0.62) than diet PO (0.44), PTS (0.54) or PFS (0.52).

Table 4.2.1: Least-squares means and contrasts for the digestibility characteristics dry matter (DDM), organic matter (DOM) and ash (DA) of pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Digestibility	Treatment Group								Contrast (P values)[b]			
	AT	PO	POS	PTS	PFS	Effect (P value)[b]	R^2 (%)[c]	RSD[c]	AT vs PO	PO vs POS	POS vs PTS	POS vs PFS
DDM	0.74	0.74	0.82	0.79	0.79	**<.0001**	84.7	0.015	0.262	**<.0001**	**<.0001**	**<.0001**
DOM	0.77	0.77	0.84	0.81	0.82	**<.0001**	81.9	0.015	0.530	**<.0001**	**<.0001**	**<.0001**
DA	0.30	0.44	0.62	0.54	0.52	**<.0001**	89.4	0.042	**<.0001**	**<.0001**	**<.0001**	**<.0001**

[a] A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).
[b] Significant effects (P<0.05) are shown in bold
[c] Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD)

4.3 Diet analyses

Composition experimental diets

The dietary composition of all experimental diets were analysed (Table 4.3.1).

Table 4.3.1: Dietary composition of experimental diets on as-received basis

Dietary composition on as-received basis	Grower					Finisher			
	AT	PO	POS	PTS	PFS	AT	PO	POS	PTS
Dry Matter, g/kg	894	892	896	892	895	893	888	890	890
Ash, g/kg	60	67	83	72	69	65	71	66	73
Crude Protein, g/kg	219	149	173	164	155	221	180	183	167
Fat, g/kg	61	45	63	56	54	61	48	50	49
Neutral Detergent fibre, g/kg	186	141	148	159	139	241	210	199	186
Acid Detergent Fibre, g/kg	26	26	33	35	32	39	47	42	43
Lignin, %	9	10	9	9	9	13	10	11	10
Gross Energy, MJ/kg	17.4	16.3	16.7	16.6	16.7	16.9	16.3	16.3	16.4

Fatty acid profile experimental diets

Table 4.3.2 shows means for groups of dietary fatty acids and ratios between those groups. This data has not been analysed statistically. Diets PO and POS contained the lowest level of saturated fatty acids/100 g FA. Diet PFS contained the lowest amount of MUFA. Diet PO and POS contained the highest levels of unsaturated FA. Animal-based diets AT and PTS contained the lowest PUFA and n-3 FA levels. The n-6 FA level was lower in diets AT, PTS and PFS than in diets PO and POS. The PUFA/saturated FA ratio was the highest in plant based diets PO and POS. The n-6/n-3 ratio on the other hand was highest in the animal based diets AT and PTS.

The complete fatty acid profile of the experimental diets is shown in Table 4.3.3. Fish oil was the main source of EPA, DPA and DHA. Lower levels of EPA and DPA were also found in grower diet AT. Fish oil was the only dietary source of eicosatrienoic acid (C20:3n3-cis 11,14,17). CLA was found in diet AT and in the supplemented diets.

Table 4.3.2: Means for groups of dietary fatty acids (g/100g FA) and ratios between those groups

Dietary fatty acids	Grower					Finisher				Fish oil
	AT	PO	POS	PTS	PFS[a]	AT	PO	POS	PTS	
SFA, g/100 g FA	39.21	14.70	15.24	35.55	38.52	37.62	15.22	15.69	32.16	43.60
MUFA, g/100 g FA	42.08	42.40	42.45	40.48	28.19	41.77	38.80	33.63	38.51	32.13
PUFA, g/100 g FA	18.71	42.90	42.31	23.97	33.29	20.62	45.98	50.68	29.33	24.28
UFA, g/100 g FA	60.79	85.30	84.76	64.45	61.48	62.38	84.78	84.31	67.84	56.40
n-3 FA, g/100 g FA	1.70	14.25	13.88	2.05	14.92	1.85	14.80	14.00	2.53	18.23
n-6 FA, g/100 g FA	17.01	28.64	26.74	20.28	16.06	18.37	31.18	34.10	24.06	3.79
PUFA/SFA, ratio	1.55	5.80	5.56	1.81	1.60	1.66	5.57	5.37	2.11	1.29
n-6/n-3, ratio	9.99	2.01	1.93	9.89	1.08	9.92	2.11	2.44	9.51	0.21

[a] *Calculated from the fatty acid profile in the grower diet and the fatty acid profile in the fish oil according to the following equation:* $FA_{Calculated} = (FA_{Grower\ diet} + (0.044 \times FA_{Fish\ Oil})) / 1.04$

Table 4.3.3: Fatty acid profile of experimental diets in g/100 g FA

Fatty Acids g / 100 g FA	Grower					Finisher				fishoil
	AT	PO	POS	PTS	PFS[a]	AT	PO	POS	PTS	
C6:0, caproic	-	-	-	-	-	-	-	-	-	-
C8:0, caprylic	-	-	-	-	-	-	-	-	-	-
C10:0, capric	-	-	-	-	-	-	-	-	-	-
C11:0, undecanoic	-	-	-	-	-	-	-	-	-	-
C12:0, lauric	-	-	-	-	0.04	-	-	-	-	0.06
C13:0, tridecanoic	-	-	-	-	0.02	-	-	-	-	0.03
C14:0, myristic	1.44	-	-	1.12	4.80	1.51	0.19	0.18	1.00	6.48
C16:0, palmitic	24.20	11.27	11.44	23.13	18.53	22.71	11.25	11.57	21.46	17.52
C17:0, margaric	0.89			0.69	0.59	0.91	-	-	0.61	0.80
C18:0, stearic	12.62	3.09	3.09	10.45	3.58	12.34	2.92	3.16	8.86	3.90
C20:0, arachidic	0.06	0.35	0.39	0.15	0.14	0.14	0.37	0.34	0.12	0.19
C21:0, heneicosanoic	-	-	-	-	-	-	-	-	-	-
C22:0, behenic	-	-	0.19	-	0.06	-	0.35	0.32	0.11	0.08
C23:0, tricosanoic	-	-	-	-	0.00	-	-	-	-	-
C24:0, lignoceric	-	-	0.13	-	10.76	-	0.14	0.13	-	14.54
C14:1-*cis*-9, myristoleic	0.20	-	-	0.22	-	0.22	-	-	0.17	-
C15:1-*cis*-10, pentadecenoic	-	-	-	-	-	-	-	-	-	-
C16:1-*cis*-9, palmitoleic	4.11	-	-	3.80	4.79	4.03	0.19	0.18	3.68	6.47
C17:1-*cis*-10, heptadecenoic	-	-	-	-	-	-	-	-	-	-
C18:1-*cis*-9, oleic	35.17	40.82	40.86	33.85	16.53	34.61	36.56	31.71	32.37	16.59
C18:1-*trans*-9, elaidic	0.26	-	-	0.26	0.09	0.29	-	-	0.17	0.12
C18:1-*trans*-11, vaccenic	1.06	-	-	0.78	0.52	1.16	-	-	0.63	0.70
C18:1 -*cis*-11, vaccenic	1.27	1.58	1.59	1.27	1.96	1.20	1.40	1.24	1.20	2.42
C20:1-*cis*-11, eicosenoic	-	-	-	0.30	3.48	0.26	0.58	0.50	0.29	4.70
C22:1-*cis*-13, erucic	-	-	-	-	0.35	-	-	-	-	0.47
C24:1-*cis*-15, nervonic	-	-	-	-	0.48	-	0.07	-	-	0.64
C18:2-*trans*-9,12, linolelaidic	-	-	-	-	-	-	-	-	-	-
C18:2 n-6 *cis*-9,12, linoleic	17.01	28.64	26.74	20.28	14.92	18.27	31.18	34.10	24.06	2.25
C18:2-*cis*-9, *trans*-11, CLA	-	-	0.92	1.11	0.39	0.40		1.36	1.57	-
C18:2-*trans*-10, *cis* - 12, CLA	-	-	0.77	0.54	0.25	-	-	1.22	1.17	-
C20:2 n-6 *cis*-11,14, eicosadienoic	-	-	-	-	0.20	-	-	-	-	0.27
C22:2-*cis*-13,16, docosadienoic	-	-	-	-	0.00	-	-	-	-	-
C18:3 n-6 *cis*-6,9,12, γ-linolenic	-	-	-	-	0.11	-	-	-	-	0.15
C18:3 n-3 *cis*-9,12,15, α-linolenic	1.70	14.25	13.88	2.05	2.13	1.85	14.80	14.00	2.53	0.95
C20:3 n-6 *cis*-8,11,14, eicosatrienoic	-	-	-	-	0.08	-	-	-	-	0.10
C20:3 n-3 *cis*-11,14,17, eicosatrienoic	-	-	-	-	0.15	-	-	-	-	0.20
C20:4 n-6 *cis*-5,8,11,14, arachidonic	-	-	-	-	0.75	0.10	-	-	-	1.01
C20:5 n-3 *cis*-5,8,11,14,17, EPA	-	-	-	-	0.02	-	-	-	-	0.03
C22:5 n-3 cis-7,10,13,16,19, DPA	-	-	-	-	1.67	-	-	-	-	2.26
C22:6 n-3 *cis*-4,7,10,13,16,19, DHA	-	-	-	-	12.62	-	-	-	-	17.05

[a] *Calculated from the fatty acid profile in the grower diet and the fatty acid profile in the fish oil according to the following equation:* $FA_{Calculated} = (FA_{Grower\ diet} + (0.044 \times FA_{Fish\ Oil})) / 1.044$

4.4 Meat quality

The meat quality data from one pig from group PTS was excluded because the meat had a high pH which was an outlier 6.69 SD away from the mean (calculated without that value).

Another pig from group PTS was excluded from the MFI data, because its MFI was 10.43 SD away from the mean (calculated without that value) and was therefore also taken as an outlier.

There were no significant effects for any variable when pH-squared was entered as a second covariate in the statistical model to test for curvilinearity.

As shown in Table 4.4.1, pH had a significant effect on all the Warner-Bratzler shear force measurements, except for the difference between peak force and yield force. There were no significant differences for four out of five contrasts. There was a significant contrast between PFSe and PFSl for the difference between peak and yield force at 70°C.The differences between the Warner-Bratzler shear force measurements and cooking losses at 70 and 60°C were tested by paired t-tests and all were significantly higher for the 70°C temperature ($P < 0.001$) except the difference between peak force and yield force, which was not significantly different ($P = 0.308$).

For the different colour parameters shown in Table 4.4.2, pH value had a significant effect on only the relative lightness (L*). The relationship between pH and L* was significantly negative ($P<0.005$).There were no significant contrasts between treatments for colour.

The meat quality characteristics shown in Table 4.4.3 (myofibrillar fragmentation index (MFI), sarcormere length, and measures of water-holding capacity) showed a significantly negative pH effect only for the cooking loss at 70^{o}C.There were significant contrasts for MFI, sarcomere length, expressed juice percentage loss in weight and cooking loss at 70^{o}C. For MFI all contrasts were significant except for PFSe vs PFSl. The MFI was significantly higher for group AT in contrast to group PO. Group POS had a significantly higher MFI value in contrast to group PO, PTS, PFSe and PFSl. The contrast for sarcomere length was significant for POS vs PFSe+PFSl and PFSe vs PFSl. Group POS had a lower sarcomere length in contrast to group PFSe and PFSl. Group PFSe had a significantly higher sarcomere length in contrast to group PFSl. Group PFSe had a significantly higher expressed juice percentage loss in weight in contrast to group PFSl. The cooking loss at 70^{o}C was significant for the contrast between all groups except for AT vs PO and PFSe vs PFSl. Group POS had a significantly higher cooking loss at 70^{o}C in contrast to groups PO, PTS, PFSe and PFSl.

As shown in Table 4.4.3, the selenium concentration in the lean meat was significantly higher in samples from pigs fed diet POS compared to samples from pigs fed diet PO.

Table 4.4.4 shows the linear correlation coefficients based on data from all animals (n = 46) between five measures of water-holding capacity. There were highly significant positive correlations between all three measurements of expressed juice, and there was a significant positive correlation between cooking loss at 60 and 70^{o}C ($P<0.01$) but correlations between expressed juice values and cooking loss were not significant.

Table 4.4.1: Least-squares means and contrasts for the Warner-Bratzler shear force parameters of pork from pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Warner-Bratzler (WB)	Treatment Group										Contrast (P values)[b]				
parameters (N)	AT	PO	POS	PTS	PFSe	PFSl	Effect (P value)[b]	R^2 (%)[c]	RSD[c]	P value pH[b]	AT vs PO	PO vs POS	POS vs PTS	POS vs PFSe + PFSl	PFSe vs PFSl
WB-Mean 70 ^{0} C	22.39	22.04	22.95	23.13	25.64	24.11	0.719	24.3	4.577	**0.002**	0.878	0.696	0.942	0.497	0.524
WB-Yield Force 70 ^{0} C	51.76	49.52	51.00	57.20	54.60	59.50	0.594	18.0	12.343	**0.021**	0.722	0.815	0.342	0.390	0.449
WB-Peak Force 70 ^{0} C	62.77	59.55	62.15	67.59	71.15	70.12	0.606	22.1	14.894	**0.005**	0.671	0.732	0.488	0.295	0.895
Peak Force -Yield Force 70 ^{0} C	11.01	10.02	11.15	10.39	16.55	10.62	0.170	28.2	5.005	**0.005**	0.699	0.659	0.774	0.317	**0.028**
WB-Mean 60 ^{0} C	17.50	18.64	20.95	19.49	20.72	18.71	0.551	19.9	4.100	**0.024**	0.585	0.274	0.500	0.654	0.351
WB-Yield Force 60 ^{0} C	35.86	39.16	43.78	44.27	41.64	38.14	0.537	19.0	10.124	**0.038**	0.522	0.373	0.926	0.860	0.509
WB-Peak Force 60 ^{0} C	44.99	47.07	55.61	53.10	55.87	49.02	0.324	24.4	11.542	**0.014**	0.723	0.152	0.679	0.831	0.260
Peak Force -Yield Force 60 ^{0} C	9.13	7.91	11.84	8.83	14.23	10.88	0.087	24.9	4.350	0.071	0.583	0.082	0.194	0.878	0.146

[a] A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).
[b] Significant effects (P<0.05) are shown in bold
[c] Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD)

Table 4.4.2: Least-squares means and contrasts for pork colour parameters measured by reflectance spectrometry for pork from pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Colour	Treatment Group										Contrast (P values)[b]				
Parameters	AT	PO	POS	PTS	PFSe	PFSl	Effect (P value)[b]	R^2 (%)[c]	RSD[c]	P value pH[b]	AT vs PO	PO vs POS	POS vs PTS	POS vs PFSe + PFSl	PFSe vs PFSl
L^* (lightness)	51.62	51.37	51.51	51.47	50.16	51.54	0.660	21.5	1.786	**0.005**	0.781	0.877	0.966	0.399	0.144
a^* (redness)	6.05	5.66	6.03	6.44	5.66	5.51	0.559	9.0	1.056	N.A.	0.457	0.481	0.464	0.974	0.786
b^* (yellowness)	5.95	5.81	5.73	6.08	5.50	5.92	0.771	5.9	0.757	N.A.	0.727	0.821	0.378	0.856	0.293

[a] A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).
[b] Significant effects (P<0.05) are shown in bold
[c] Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD)

Table 4.4.3: Least-squares means and contrasts for selected meat quality parameters of pork from pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Meat quality parameters	Treatment Group										Contrast (P values)[b]				
	AT	PO	POS	PTS	PFSe	PFSl	Effect (P value)[b]	R^2 (%)[c]	RSD[c]	P value pH[b,d]	AT vs PO	PO vs POS	POS vs PTS	POS vs PFSe + PFSl	PFSe vs PFSl
pH	5.55	5.49	5.56	5.50	5.44	5.47	0.426	11.2	0.128	N.A.	0.365	0.326	0.368	0.122	0.650
MFI	98.18	97.64	98.22	97.14	97.86	97.89	**0.004**	34.4	0.333	N.A.	**0.002**	**0.001**	**0.005**	**0.005**	0.890
Sarcomere Length, µm	1.92	1.98	1.91	2.04	2.21	1.86	**0.044**	24.1	0.212	N.A.	0.576	0.497	0.234	**0.026**	**0.003**
Expr. Juice % loss in weight	0.38	0.37	0.39	0.37	0.41	0.35	**0.025**	26.6	0.030	N.A.	0.551	0.212	0.374	0.864	**0.001**
Expr. Juice Outside area, cm^2/gram	34.76	34.18	34.68	36.38	37.11	32.82	0.136	18.3	3.146	N.A.	0.716	0.753	0.303	0.147	**0.012**
Expr. Juice Outside - Inner area, cm^2/gram	26.50	25.29	25.81	28.69	28.73	24.45	0.134	18.4	3.613	N.A.	0.508	0.776	0.131	0.078	**0.027**
Cooking loss 70 Deg., %	30.08	30.49	32.47	29.64	29.69	30.66	**0.002**	44.9	1.302	**0.001**	0.532	**0.005**	**0.0002**	**<.0001**	0.156
Cooking loss 60 Deg., %	21.78	22.35	22.52	21.68	22.26	22.48	0.754	6.2	1.379	N.A.	0.413	0.801	0.243	0.372	0.759
Intramuscular fat (loin), %	1.96	1.91	1.91	1.96	1.83	1.91	0.610	8.3	0.160	N.A.	0.530	0.980	0.490	0.860	0.320
Se concentration (lean meat)[e], ug/g	0.232	0.297	0.397	0.405	0.412	0.410	0.077	87.9	0.411	N.A.	0.135	**0.002**	0.886	0.760	0.981

[a] *A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).*
[b] *Significant effects (P<0.05) are shown in bold*
[c] *Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD)*
[d] *N.A. stands for not applicable, P-value pH was removed from model when shown to be non significant.*
[e] *Se concentration in lean meat (ug/g) was determined in Singapore by Mrs J. Leong MSc (personal communication,2010)*

Table 4.4.4: Linear correlation coefficients based on data from all animals (n = 46) between five measures of water-holding capacity

Item	Correlation with the item			
	Expr. Juice % loss in weight	Expr. Juice Outside area	Expr. Juice Outside - Inner area	Cooking Loss 70 Deg.
Expr. Juice Outside area	0.82***			
Expr. Juice Outside - Inner area	0.73***	0.96***		
Cooking Loss 70 Deg.	0.17	0.16	0.16	
Cooking Loss 60 Deg.	0.13	0.16	0.18	0.44**

* *P < 0.05 = r ≥ 0.280*
** *P < 0.01 = r ≥ 0.372*
*** *P < 0.00 1= r ≥ 0.465*

4.5 Fatty acid profile backfat

Table 4.5.1: Least-squares means and contrasts for the concentration of fatty acids (% of total fatty acids) in lipid from the backfat from pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Fatty acids backfat	Treatment Group									Contrast (P values)[b]				
	AT	PO	POS	PTS	PFSe	PFSl	Effect (P value)[b]	R^2 (%)[c]	RSD[c]	AT vs PO	PO vs POS	POS vs PTS	POS vs PFSe + PFSl	PFSe vs PFSl
C14:0, myristic	1.75	1.33	1.56	1.95	1.96	1.77	**0.000**	43.1	0.271	**0.004**	0.097	**0.006**	**0.014**	0.202
C16:0, palmitic	18.70	19.25	17.74	19.59	17.84	15.16	**0.005**	32.9	2.251	0.622	0.185	0.107	0.219	**0.027**
C18:0, stearic	9.53	9.65	10.89	12.07	9.43	11.43	0.189	16.1	2.501	0.924	0.329	0.349	0.681	0.129
C20:0, arachidic	0.19	0.19	0.22	0.20	0.20	0.21	0.644	7.6	0.045	0.974	0.157	0.350	0.593	0.782
C16:1-*cis*-9, palmitoleic	1.34	0.76	0.83	1.13	1.11	0.64	**0.004**	33.2	0.367	**0.003**	0.709	0.118	0.792	**0.019**
C18:1-*cis*-9, oleic	47.99	43.01	39.58	39.12	37.80	41.19	**<.0001**	47.0	3.803	**0.012**	0.079	0.807	0.959	0.093
C18:1-*trans*-9, elaidic	0.52	0.32	0.25	0.41	0.24	0.21	**<.0001**	69.7	0.077	**<.0001**	0.080	**<.0001**	0.557	0.457
C18:1-*trans*-11, vaccenic	0.33	0.33	0.34	0.41	0.36	0.31	0.073	21.1	0.065	0.970	0.754	0.053	0.690	0.146
C18:1-*cis*-11, vaccenic	2.90	2.99	2.66	3.55	3.25	3.22	0.115	18.8	0.634	0.786	0.311	**0.008**	**0.045**	0.932
C20:1-*cis*-11, eicosenoic	0.06	0.02	0.04	0.05	0.01	0.04	**0.003**	34.6	0.024	**0.003**	0.107	0.838	0.098	**0.019**
C18:2 n-6 *cis*-9,12, linoleic	11.99	15.26	15.93	15.93	15.63	14.08	**0.004**	33.9	2.118	**0.004**	0.533	1.000	0.255	0.164
C18:2-*cis*-9, *trans*-11, CLA	0.71	0.26	0.73	1.03	0.89	1.04	**0.018**	27.4	0.464	0.061	0.051	0.202	0.248	0.540
C18:2-*trans*-10, *cis* - 12, CLA	0.16	0.19	0.45	0.37	0.42	0.41	**<.0001**	54.8	0.111	0.635	**<.0001**	0.153	0.418	0.871
C18:3 n-6 *cis*-6,9,12, γ-linolenic	0.14	0.25	0.27	0.16	0.21	0.21	**0.004**	33.5	0.066	**0.003**	0.560	**0.003**	0.061	0.831
C18:3 n-3 *cis*-9,12,15, α- linolenic	1.18	3.25	5.92	1.27	5.06	4.12	**<.0001**	87.4	0.726	**<.0001**	**<.0001**	**<.0001**	**0.000**	**0.017**
C20:2 n-6 *cis*-11,14, eicosadienoic	0.50	0.57	0.45	0.55	0.63	0.58	0.156	17.2	0.135	0.304	0.097	0.155	**0.012**	0.491
C20:3 n-6 *cis*-8,11,14, eicosatrienoic	0.19	0.16	0.14	0.23	0.16	0.17	0.493	9.9	0.096	0.472	0.735	0.063	0.514	0.899
C20:3 n-3 *cis*-11,14,17, eicosatrienoic	0.21	0.73	0.69	0.32	0.66	0.50	**<.0001**	48.0	0.221	**<.0001**	0.722	**0.002**	0.252	0.168
C20:4 n-6 *cis*-5,8,11,14, arachidonic	0.16	0.14	0.11	0.16	0.13	0.12	0.091	20.0	0.036	0.312	0.137	**0.016**	0.296	0.554
C20:5 n-3 *cis*-5,8,11,14,17, EPA	0.12	0.13	0.11	0.13	0.71	0.89	**<.0001**	90.2	0.114	0.914	0.735	0.675	**<.0001**	**0.004**
C22:5 n-3 *cis*-7,10,13,16,19, DPA	0.29	0.37	0.33	0.44	1.08	1.28	**<.0001**	65.5	0.306	0.608	0.816	0.470	**<.0001**	0.212
C22:6 n-3 *cis*-4,7,10,13,16,19, DHA	0.14	0.16	0.15	0.11	1.33	1.57	**<.0001**	94.5	0.160	0.845	0.872	0.619	**<.0001**	**0.008**

[a] *A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).*

[b] *Significant effects (P<0.05) are shown in bold*

[c] *Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD)*

The following fatty acids showed significant (P<0.05) contrasts between treatments for backfat as shown in Table 4.5.1:

Myristic acid (C14:0)
Pigs fed diets containing tallow or fish oil show higher levels of myristic acid in their fatty acid profile. Pigs in group AT showed a significantly higher level of myristic acid in contrast to pigs from group PO. Group PTS had significant higher levels in contrast to group POS. Group PFSe and PFSI showed significantly higher levels in contrast to diet POS.

Palmitic acid (C16:0)
Group PFSe contained a significantly higher level of palmitic acid in contrast to pigs in group PFSI.

Palmitoleic acid (C16:1-cis 9)
The fatty acid profile of pigs fed diet AT contained significantly higher levels of palmoleic acid in contrast to pigs fed diet PO. Group PFSe contained a significantly higher level of palmitoleic acid in contrast to pigs in group PFSI.

Oleic acid (C18:1-cis 9)
The fatty acid profile of pigs fed diet AT contained significantly higher levels of oleic acid in contrast to pigs fed diet PO.

Elaidic acid (C18:1- trans 9)
Pigs fed diets containing tallow showed the highest levels of elaidic acid in their fatty acid profile. Group AT contained significantly higher levels in contrast to group PO. Group PO contained significantly higher levels in contrast to group POS and group PTS showed significantly higher levels in contrast to group POS.

Vaccenic (C18:1- trans 11)
The contrast between diet POS and diet PTS approached significance (P=0.053), the amount of trans 11 vaccenic acid found in the loin was higher in pigs fed diet PTS then in pigs fed diet POS.

Eicosenoic acid (C20:1- cis 11)
The fatty acid profile of pigs fed diet AT contained significantly higher levels of eicosenoic acid in contrast to pigs fed diet PO. Group PFSI contained significantly higher levels of eicosenoic acid in contrast to group PFSe.

Linoleic acid (C18:2- cis 9,12)

The fatty acid profile of pigs fed diet PO contained significantly higher levels of linoleic acid in contrast to pigs fed diet AT.

CLA 9c11t (C18:2)

There was a significant group effect, but there were no significant effects in the contrasts. The contrast between PO and POS approached significance (P = 0.051), the amount of CLA 9c11t found in the backfat was higher in pigs fed diet POS in contrast to pigs fed diet PO.

CLA 10t12C (C18:2)

The fatty acid profile of pigs fed diet POS contained significantly higher levels of CLA 10t12c in contrast to pigs fed diet PO.

γ-Linolenic acid (C18:3n6-cis 6,9,12)

Pigs fed diets containing tallow showed the lowest levels of γ-linolenic acid in their fatty acid profile. Group PO showed significantly higher levels of γ-linolenic acid in contrast to diet AT and diet POS showed significantly higher levels in contrast to diet PTS.

α-Linolenic acid (C18:3n-3 cis 9,12,15)

Group PO had showed higher levels in contrast to group AT. Group POS showed higher levels in contrast to group PO. Group POS showed higher levels in contrast to group PTS. Group POS showed higher levels in contrast to the pigs fed diet PFSe and diet PFSl. PFSe showed higher levels in contrast to PFSl.

Eicosatrienoic acid (C20:3n3-cis 11,14,17)

Pigs fed diets containing tallow showed the lowest levels of eicosatrienoic acid in their fatty acid profile. The amount of eicosatrienoic acid in the backfat was significantly higher in group PO in contrast to the amount found in group AT. Group POS had significantly higher levels in contrast to group PTS.

EPA (C20:5n3-cis 5,8,11,14,17), DPA (C22:5-cis 7,10,13,16,19) and DHA (C22:6n3-cis 4,7,10,13,16,19)

EPA, DPA and DHA are fatty acids present in fish oil. The results show that the amounts of these fatty acids found in the backfat were significantly higher in the diets containing fish oil (PFSe and PFSl). There was a significantly higher level of EPA and DHA in the backfat of the PFSl group relative to that of the PFSe group.

Table 4.5.2 shows means for groups of fatty acids and ratios between those groups in lipid from the backfat from pigs fed diets containing animal, plant, and fish products with or without a dietary supplement.

Table 4.5.2 Means for groups of fatty acids (g/100g FA) and ratios between those groups in lipid from the backfat from pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Backfat fatty acids	Treatment Group									Contrast (P values)[b]				
	AT	PO	POS	PTS	PFSe	PFSl	Effect (P value)[b]	R^2 (%)[c]	RSD[c]	AT vs PO	PO vs POS	POS vs PTS	POS vs PFSe + PFSl	PFSe vs PFSl
SFA, g/100 g FA	30.16	30.42	30.40	33.81	29.43	28.58	0.068	21.5	3.348	0.877	0.990	**0.048**	0.349	0.627
MUFA, g/100 g FA	53.14	47.44	43.72	44.66	42.78	45.62	**0.000**	42.9	4.227	**0.010**	0.086	0.658	0.795	0.201
PUFA, g/100 g FA	15.78	21.46	25.28	20.70	26.92	24.97	**<.0001**	61.3	3.144	**0.001**	**0.020**	**0.006**	0.630	0.237
UFA, g/100 g FA	68.93	68.89	68.99	65.36	69.70	70.59	0.073	21.1	3.389	0.984	0.955	**0.038**	0.442	0.613
n-3 FA, g/100 g FA	1.94	4.64	7.20	2.28	8.84	8.36	**<.0001**	89.1	1.034	**<.0001**	**<.0001**	**<.0001**	**0.004**	0.376
n-6 FA, g/100 g FA	12.97	16.37	16.89	17.02	16.77	15.16	**0.005**	32.3	2.236	**0.004**	0.640	0.909	0.349	0.171
PUFA/SFA, ratio	0.52	0.72	0.84	0.62	0.92	0.90	**<.0001**	51.6	0.151	**0.011**	0.128	**0.006**	0.307	0.766
n-6/n-3, ratio	6.91	3.59	2.35	7.75	1.95	1.82	**<.0001**	91.9	0.761	**<.0001**	**0.002**	**<.0001**	0.168	0.733

[a] A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).
[b] Significant effects (P<0.05) are shown in bold
[c] Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD)

The total amount of MUFA and the n-6/n-3 ratio in the backfat was significantly higher in pigs fed diet AT in contrast to pigs fed diet PO. The total amount of PUFA, n-3 FA, n-6 FA and the PUFA/saturated FA ratio was higher in pigs fed diet PO in contrast to pigs fed diet AT.

Pigs fed diet POS had a higher amount of PUFA and n-3 fatty acids in the backfat in contrast to pigs fed diet PO. The backfat from pigs fed diet PO had a higher n-6/n-3 ratio in contrast to pigs fed diet POS.

The total amount of PUFA, unsaturated FA, n-3 FA and the PUFA/saturated FA ratio was higher in pigs fed diet POS in contrast to pigs fed diet PTS. The amount of saturated FA and n-6/n-3 ratio on the other hand, was higher in pigs fed diet PTS in contrast to pigs fed diet POS.

Backfat samples from pigs fed diets PFSe and PFSl contained higher levels of n-3 FA then samples from pigs fed diet POS.

4.6 Fatty acid profile loin

Table 4.6.1: Least-squares means and contrasts for the concentration of fatty acids (% of total fatty acids) of intramuscular fat from the longissimus muscle of the loin from pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Fatty acids loin	Treatment Group									Contrast (P values)[b]				
	AT	PO	POS	PTS	PFSe	PFSl	Effect (P value)[b]	R^2 (%)[c]	RSD[c]	AT vs PO	PO vs POS	POS vs PTS	POS vs PFSe + PFSl	PFSe vs PFSl
C14:0, myristic	1.48	1.28	1.39	1.39	1.36	1.37	0.074	21.1	0.122	**0.002**	0.073	0.994	0.663	0.914
C16:0, palmitic	22.06	19.39	20.79	20.80	20.56	18.17	**0.004**	33.8	1.852	**0.006**	0.139	0.986	0.088	**0.017**
C18:0, stearic	14.14	13.19	13.67	15.18	13.77	11.98	**0.005**	32.3	1.511	0.217	0.530	0.052	0.238	**0.028**
C20:0, arachidic	0.25	0.38	0.33	0.31	0.22	0.26	0.195	15.9	0.131	0.057	0.437	0.762	0.132	0.508
C16:1-*cis*-9, palmitoleic	1.57	0.84	1.43	1.42	1.87	1.62	**<.0001**	50.3	0.332	**<.0001**	**0.001**	0.970	**0.036**	0.167
C18:1-*cis*-9, oleic	43.12	42.80	38.79	38.26	34.93	38.89	**<.0001**	61.7	2.324	0.783	**0.001**	0.646	0.071	**0.002**
C18:1-*trans*-9, elaidic	0.21	0.22	0.29	0.14	0.23	0.25	0.086	20.3	0.092	0.712	0.177	**0.004**	0.250	0.756
C18:1-*trans*-11, vaccenic	0.25	0.32	0.29	0.39	0.35	0.30	0.175	16.5	0.107	0.181	0.567	0.080	0.479	0.334
C18:1-*cis*-11, vaccenic	3.41	2.57	2.43	4.06	2.92	2.88	**<.0001**	47.9	0.620	**0.009**	0.668	**<.0001**	0.093	0.902
C20:1-*cis*-11, eicosenoic	0.30	0.36	0.31	0.12	0.28	0.36	0.404	11.3	0.242	0.596	0.661	0.133	0.930	0.520
C18:2 n-6 *cis*-9,12, linoleic	8.46	11.23	12.85	11.74	12.72	11.90	**<.0001**	63.5	1.191	**<.0001**	**0.010**	0.069	0.302	0.191
C18:2-*cis*-9, *trans*-11, CLA	0.17	0.09	0.27	0.21	0.36	0.49	**<.0001**	63.0	0.107	0.166	**0.002**	0.256	**0.002**	**0.025**
C18:2-*trans*-10, *cis* - 12, CLA	0.27	0.29	0.33	0.45	0.32	0.36	0.340	12.5	0.167	0.730	0.702	0.149	0.898	0.634
C18:3 n-6 *cis*-6,9,12, γ-linolenic	0.18	0.17	0.15	0.22	0.20	0.22	0.304	13.2	0.071	0.709	0.675	0.065	0.066	0.616
C18:3 n-3 *cis*-9,12,15, α- linolenic	0.82	2.92	3.18	1.97	3.18	2.84	**<.0001**	75.2	0.524	**<.0001**	0.321	**<.0001**	0.462	0.215
C20:2 n-6 *cis*-11,14, eicosadienoic	0.46	0.58	0.50	0.51	0.46	0.45	0.724	6.5	0.181	0.197	0.394	0.915	0.566	0.914
C20:3 n-6 *cis*-8,11,14, eicosatrienoic	0.14	0.11	0.13	0.16	0.09	0.19	0.334	12.6	0.094	0.456	0.555	0.650	0.872	**0.037**
C20:3 n-3 *cis*-11,14,17, eicosatrienoic	0.25	0.53	0.51	0.24	0.55	0.40	**<.0001**	48.7	0.141	**0.000**	0.845	**0.000**	0.546	0.052
C20:4 n-6 *cis*-5,8,11,14, arachidonic	0.46	0.39	0.27	0.55	0.45	0.45	**0.015**	28.3	0.145	0.361	0.099	**0.000**	**0.007**	0.957
C20:5 n-3 *cis*-5,8,11,14,17, EPA	0.26	0.34	0.43	0.18	1.18	1.45	**<.0001**	82.2	0.243	0.514	0.467	**0.047**	**<.0001**	**0.037**
C22:5 n-3 *cis*-7,10,13,16,19, DPA	0.30	0.59	0.55	0.40	1.38	1.71	**<.0001**	80.5	0.279	**0.043**	0.750	0.285	**<.0001**	**0.025**
C22:6 n-3 *cis*-4,7,10,13,16,19, DHA	0.14	0.19	0.19	0.18	1.36	2.22	**<.0001**	93.9	0.219	0.615	0.989	0.931	**<.0001**	**<.0001**

[a] A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).

[b] Significant effects (P<0.05) are shown in bold

[c] Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD)

The following fatty acids showed significant (P<0.05) contrasts between treatments for loin fat as shown in Table 4.6.1:

Palmitic acid (C16:0)

The loin of pigs fed diet AT contained significantly higher levels of palmitic acid in contrast to pigs fed diet PO. The loins of pigs fed diet PFSe contained a significantly higher level of palmitic acid in contrast to pigs in group PFSI.

Stearic acid (C18:0)

Pigs from group PFSe contained a significantly higher level of stearic acid in contrast to pigs in group PFSI. The contrast between POS and PTS approached significance (P = 0.052), and the amount of stearic acid found in the loin was higher in pigs fed diet PTS then in pigs fed diet POS.

Palmitoleic acid (C16:1-cis 9)

The fatty acid profile of pigs fed diet AT contained significantly higher levels of palmoleic acid in contrast to pigs fed diet PO. Group POS had significantly higher levels in contrast to group PO. The pigs in groups PFSe and PFSI contained a higher level of palmitoleic acid in contrast to group POS.

Oleic acid (C18:1-cis 9)

The fatty acid profile of pigs fed diet PO contained significantly higher levels of oleic acid in contrast to pigs fed diet POS. Group PFSI contained higher levels in contrast to group PFSe.

Vaccenic (C18:1- trans 11)

Pigs fed diets containing tallow showed the highest levels of vaccenic acid in their fatty acid profile. Group AT showed significantly higher levels in contrast to group PO and group PTS showed significantly higher levels in contrast to group POS.

Linoleic acid (C18:2- cis 9,12)

The fatty acid profile of pigs fed diet PO contained significantly higher levels of linoleic acid in contrast to pigs fed diet AT. Group POS contained significantly higher levels in contrast to group PO.

CLA 9c11t (C18:2)

Pigs fed diets containing Sanovite™ showed higher levels of CLA 9c11t in their fatty acid profile relative to pigs fed diets without Sanovite™. The diets containing fish oil showed the highest amount of CLA 9c11t. Group POS contained a significantly higher level in contrast to group PO. Groups PFSe and PFSl showed higher levels in contrast to group POS. Group PFSl showed significantly higher levels in contrast to group PFSe.

α-Linolenic acid (C18:3n-3 cis 9,12,15)

Pigs fed diets containing tallow showed the lowest levels of *α*-linolenic acid in their fatty acid profile. Group PO showed higher levels in contrast to group AT. Group POS showed higher levels in contrast to group PTS.

Eicosatrienoic acid (C20:3n3-cis 11,14,17)

Pigs fed diets containing tallow showed the lowest levels of eicosatrienoic acid in their fatty acid profile. The amount of eicosatrienoic acid in the backfat was significantly higher in group PO in contrast to in group AT. Group POS had significantly higher levels in contrast to group PTS.

Arachidonic acid (C20:4n6-cis 5,8,11,14)

Pigs fed diet PO contained significantly higher levels of arachidonic acid in contrast to pigs fed diet POS. Group PTS contained significantly higher levels in contrast to group POS and the groups PFSe and PFSl contained higher levels in contrast to pigs in group POS.

EPA (C20:5n3-cis 5,8,11,14,17), DPA (C22:5-cis 7,10,13,16,19) and DHA (C22:6n3-cis 4,7,10,13,16,19)

The results show that these fatty acids found in the backfat were significantly higher in the diets containing fish oil (PFSe and PFSl). The loins of pigs in groups PFSe and PFSl contained higher levels of these fatty acids in contrast to pigs in group POS. The pigs in group PFSl contained higher levels in the loin in contrast to pigs in group PFSe.

Table 4.6.2 shows means for groups of fatty acids and ratios between those groups in lipid from the loin from pigs fed diets containing animal, plant, and fish products with or without a dietary supplement.

Table 4.6.2: Means for groups of fatty acids (g/100g FA) and ratios between those groups in intramuscular fat from the longissimus muscle of the loin from pigs fed diets containing animal, plant, and fish products with or without a dietary supplement[a]

Loin fatty acids	Treatment Group									Contrast (P values)[b]				
	AT	PO	POS	PTS	PFSe	PFSl	Effect (P value)[b]	R^2 (%)[c]	RSD[c]	AT vs PO	PO vs POS	POS vs PTS	POS vs PFSe + PFSl	PFSe vs PFSl
SFA, g/100 g FA	37.92	34.23	36.17	37.68	35.91	31.79	**<.0001**	54.9	2.063	**0.001**	0.067	0.151	**0.014**	**0.000**
MUFA, g/100 g FA	48.86	47.11	43.54	44.40	40.58	44.30	**<.0001**	56.2	2.448	0.161	**0.006**	0.490	0.309	**0.006**
PUFA, g/100 g FA	11.90	17.44	19.37	16.80	22.23	22.68	**<.0001**	88.4	1.413	**<.0001**	**0.009**	**0.001**	**<.0001**	0.540
UFA, g/100 g FA	60.76	64.55	62.91	61.20	62.81	66.98	**<.0001**	55.2	2.039	**0.001**	0.117	0.100	0.032	**0.000**
n-3 FA, g/100 g FA	1.77	4.57	4.87	2.98	7.64	8.63	**<.0001**	91.5	0.787	**<.0001**	0.459	**<.0001**	**<.0001**	**0.020**
n-6 FA, g/100 g FA	9.70	12.48	13.91	13.17	13.92	13.22	**<.0001**	63.7	1.171	**<.0001**	**0.019**	0.215	0.505	0.253
PUFA/SFA, ratio	0.31	0.51	0.54	0.45	0.62	0.72	**<.0001**	86.8	0.053	**<.0001**	0.273	**0.001**	**<.0001**	**0.001**
n-6/n-3, ratio	5.78	2.74	2.97	4.53	1.83	1.57	**<.0001**	75.9	0.895	**<.0001**	0.614	**0.001**	**0.002**	0.572

[a] A dietary supplement (Sanovite™) was added at 0.614% as fed. Sanovite™ is a dietary supplement containing CLA, vitamin E (BASF, Auckland, New Zealand), and organic Se (Alltech, Inc., Nicholasville, KY).
[b] Significant effects (P<0.05) are shown in bold
[c] Measures of the goodness of fit of the model are given by coefficients of determination (R^2(%)) and residual standard deviations (RSD)

The total amount of saturated fatty acids and the n-6/n-3 ratio in the loin was significantly higher in pigs fed diet AT in contrast to pigs fed diet PO. The total amount of PUFA, unsaturated fatty acids, n-3 FA, n-6 FA and the PUFA/saturated FA ratio was higher in pigs fed diet PO in contrast to pigs fed diet AT.

Pigs fed diet POS tended (P = 0.067) to have a higher amount of saturated fatty acids and had a higher amount of PUFA and n-6 fatty acids in the loin in contrast to pigs fed diet PO. The loin from pigs fed diet PO contained a higher amount of MUFA in contrast to pigs fed diet POS.

The total amount of PUFA, n-3 FA and the PUFA/saturated FA ratio was higher in pigs fed diet POS in contrast to pigs fed diet PTS. The n-6/n-3 ratio on the other hand, was higher in pigs fed diet PTS in contrast to pigs fed diet POS.

The loin from pigs fed diet POS contained higher levels of saturated FA and had a higher n-6/n-3 ratio in contrast to pigs fed diets PFSe and PFSl. Loin samples from pigs fed diets PFSe and PFSl contained higher levels of PUFA, unsaturated FA, n-3 FA and had a higher PUFA/saturated FA ratio then samples from pigs fed diet POS.

The total amount of saturated fatty acids in the loin was higher in samples from pigs fed diet PFSe in contrast to pigs fed diet PFSl. The total amount of MUFA, unsaturated FA, n-3 FA and the PUFA/saturated FA ratio was higher in pigs fed PFSl in contrast to pigs fed diet PFSe.

4.7 Adequate intake of EPA, DPA and DHA

As described in Sections 4.5 and 4.6 of this chapter, pork from pigs fed different diets contained different levels of EPA, DPA and DHA. Feeding diets containing fish oil (PFSe and PFSI) increased the EPA, DPA and DHA content of loin muscle and backfat. These fatty acids play an important role in human nutrition.

Figure 4.7.1 shows the amount of pork products (g/d) with varying proportions of lean meat content that would need to be consumed to reach a daily intake of 160 mg EPA, DPA and DHA.

A higher percentage of backfat in the pork product results in higher levels of (EPA+DPA+DHA) per gram of pork product. As shown in Figure 4.7.1, leaner products require higher amounts of daily intake to reach the target of 160mg of (EPA+DPA+DHA) per day.

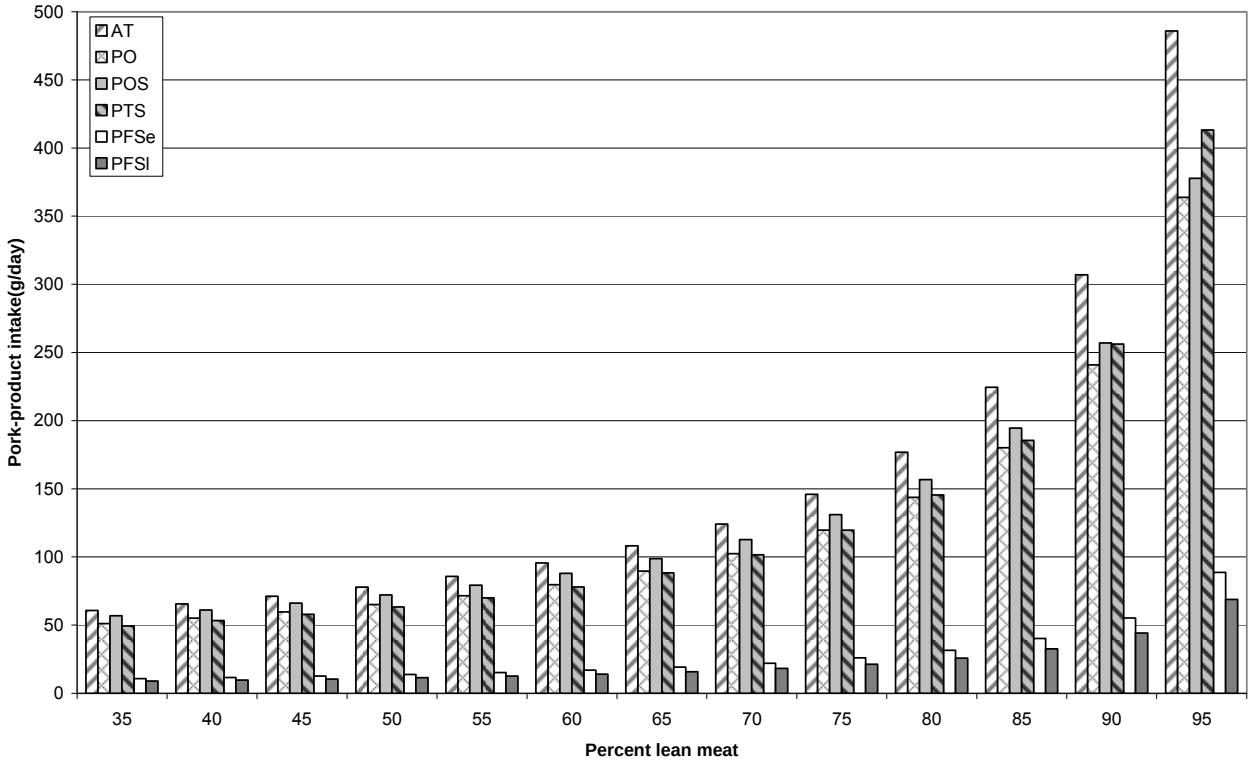

Figure 4.7.1: The intake of a pork product (g/day) required to achieve an intake of 160 mg of (EPA+DPA+DHA)/day as the percentage of lean loin in that product increases from 35% to 95%, with the remainder of the product being backfat

If pork products with 35% lean loin meat from pigs fed diet PFSe or PFSl were consumed, then a daily intake of 10.8 g or 9.0 g, respectively, would be sufficient to achieve an intake of 160 mg of (EPA+DPA+DHA)/day. Greater amounts would need to be consumed if pork products with 35% lean meat from pigs fed AT (60.8 g/d), PO (51.1 g/d), POS (56.8 g/d) or PTS (49.5 g/d) diets were consumed.

If pork products with 95% lean loin meat from pigs fed diet PFSe or PFSl were consumed, then a daily intake of 88.6 g and 68.9 g, respectively, would be required to achieve an intake of 160 mg of (EPA+DPA+DHA)/day. Greater amounts would need to be consumed if pork products with 95% lean meat from pigs fed AT (485.9 g/d), PO (363.9 g/d), POS (377.8 g/d) or PTS (413.2g/d) diets were consumed.

Table 4.7.1 shows the fat content of different raw pork products and the amount of (EPA+DPA+ DHA) per 100 g of product. It also shows the percentage of the AI and SDT that is achieved by consuming a 100 g portion of product and the amount of pork product that needs to be consumed on a daily basis to reach 100% AI and SDT.

Table 4.7.1: Combined (EPA+DPA+DHA) content for different pork products, % AI and SDT and required consumption per product per day to reach AI and SDT targets

Product	Fat %	Diet	(EPA+DPA+DHA) gram per 100 gram	% of AI[a]	% of SDT[a]	Weight of pork for 100% AI[b] (g/d)	Weight of pork for 100% SDT[b] (g/d)
Ham sliced lean only uncooked	3.4	AT	0.03	16.74	4.39	597.20	2276.81
	3.4	PO	0.04	23.00	6.03	434.82	1657.75
	3.4	POS	0.04	22.48	5.90	444.75	1695.63
	3.4	PTS	0.03	19.46	5.10	513.99	1959.59
	3.4	PFSe	0.15	91.03	23.88	109.86	418.83
	3.4	PFSl	0.19	**119.29**	31.29	**83.83**	319.59
Pork mince (raw)	7.5	AT	0.04	26.57	6.97	376.30	1434.64
	7.5	PO	0.06	34.49	9.05	289.93	1105.34
	7.5	POS	0.05	32.70	8.58	305.85	1166.04
	7.5	PTS	0.05	31.62	8.29	316.26	1205.73
	7.5	PFSe	0.23	**146.83**	38.51	**68.11**	259.66
	7.5	PFSl	0.30	**185.64**	48.69	**53.87**	205.37
Pork sausage (uncooked)	25.2	AT	0.11	69.01	18.10	144.91	552.45
	25.2	PO	0.13	84.11	22.06	118.89	453.28
	25.2	POS	0.12	76.78	20.14	130.24	496.55
	25.2	PTS	0.13	84.13	22.07	118.86	453.15
	25.2	PFSe	0.62	**387.72**	**101.70**	**25.79**	**98.33**
	25.2	PFSl	0.76	**472.09**	**123.83**	**21.18**	**80.76**

[a] *% AI and SDT >100% are shown in bold*
[b] *When consumed pork per day is <100 gram, results are shown in bold*

If mince or sausages, as shown in Table 4.7.1 from pigs fed diet PFSe and PFSl were consumed then a daily intake of 100 gram per day would be beyond AI requirements. Daily intake of 83.3 grams of ham from pigs fed diet PFSl contains enough EPA, DPA and DHA to reach AI requirements. For uncooked sausages, suggested dietary targets would be reached by consuming less then 100 gram from pigs fed diet PFSe and PFSl. Nether of the pork products shown in Table 4.8.1 from pigs fed any of the other diet are sufficient for AI or SDT when 100 gram per day is consumed.

CHAPTER V

DISCUSSION

5.1 Growth performance and carcass quality

Lipid type and the supplement Sanovite™ containing selenium (Se), vitamin E, and conjugated linoleic acid (CLA) had no significant effects on growth performance in the current study. This is in general agreement with earlier reports as shown in Table 5.1.2.1.

5.1.1 Feedstuff and lipid type

A significant effect of the treatments on performance was not expected. All diets used in this study had a similar nutrient content and the number of observations per group was not high enough to detect a statistically significant effect when differences were small.

5.1.2 Supplement

Table 5.1.2.1 summarises the results from experiments that have evaluated the effects of dietary supplementation of Se, vitamin E, vitamin C and CLA. In all studies pigs were fed experimental diets from the grower phase to slaughter, except for the studies by Hasty et al. (2002) and Dugan et al. (1997) where the trial started in the finisher phase and the study by Janz et al. (2008) and Morel et al. (2008) where pigs were fed experimental diets from the weaner phase through until slaughter.

Sanovite™

Adding the same supplement to similar diets in a previous study by Morel et al. (2008) had no significant effect on growth performance. In the study by Morel et al. (2008), 64 female weaner pigs (BW 6.7 ± 1.5 kg) Duroc x (Large white x Landrace) were used. The pigs were slaughtered at an average body weight of 101.9 ± 3.8 kg. The pigs were divided into 4 dietary treatment groups, the diets fed were either a combination of animal and plant feedstuffs or plant feedstuffs only with or without the supplement Sanovite™.

In the study by Janz et al. (2008) and Morel et al. (2008) the dietary concentrations of selenium, vitamin E and CLA in were calculated on an as-fed basis. In the current study, dietary concentrations were not analysed but the levels are assumed to be similar since the same supplement (Sanovite™) was used in the same concentration. The mean dietary concentrations in the un-supplemented and supplemented diets, as described in Janz et al. (2008), were 0.3 and 0.7 mg/kg diet for selenium, 38.5 and 164.4 mg/kg for vitamin E and 0.22 and 1.64% of total fatty acids for CLA respectively. In the study by Janz et al. (2008) and Morel et al. (2008) no statistically significant effects of supplementary Sanovite™ were found on carcass weight, fat depth or dressing percentage.

Table 5.1.2.1: A summary of results from studies into the effects of vitamin E, vitamin C, selenium and CLA on growth performance, and carcass or meat quality characteristics

Reference	Animals	Dietary treatments	Effects on Growth Performance	Effects on carcass, or meat quality (LM = longissimus muscle, SM = semimembranosus muscle, PM= psoas major muscle)
Sanovite™				
Janz et al. (2008)	59 gilts grown from an average of 11.4 kg to 101.9 kg on diets with or without animal products	Contr.: • 38.5 mg/kg Vit. E • 0 mg/kg Vit. C • 0.3 mg/kg Se • 0 % CLA in diet Suppl.: • 164.4 mg/kg Vit. E • 2700 mg/kg Vit. C • 0.7 mg/kg Se • 0.25 % CLA in diet	No effect	• Ultimate pH (pHu) of LM lower for samples from pigs that received an animal-based diet containing the supplement (P<0.04) • Drip loss (SM) and Expr. Juice (LM) lower for samples from pigs that received an animal-based diet containing the supplement (P< 0.01) but not after adjustments for pHu (P<0.07). • Rancid odour was more often detected in samples from pigs that received a plant based diet containing the supplement compared with control plant group (25 vs 12%)
Morel et al. (2008)	60 gilts grown from an average of 11.4 kg to 101.9 kg on diets with or without animal products	Contr.: • 38.5 mg/kg Vit. E • 0 mg/kg Vit. C • 0.3 mg/kg Se • 0 % CLA in diet Suppl.: • 164.4 mg/kg Vit. E • 2700 mg/kg Vit. C • 0.7 mg/kg Se • 0.25 % CLA in diet	No effect	• Inclusion of the supplement increased Se and vitamin E content in LM (P<0.001) • Inclusion of suppl. increased LM intramuscular fat (P<0.05), increased the CLA level in different tissues (P<0.001), lowered contents of oleic acid (P<0.001) and increased contents of stearic acid (P<0.05) • Sensory evaluation as per Janz et al. (2008)
Nuijten et al. (2010) [The current study]	47 gilts grown from an average of 18.9 kg to 90.3 kg on diets with or without animal products or fish oil	Contr.: • 38.5 mg/kg Vit. E • 0 mg/kg Vit. C • 0.3 mg/kg Se • 0 % CLA in diet Suppl.: • 164.4 mg/kg Vit. E • 2700 mg/kg Vit. C • 0.7 mg/kg Se • 0.25 % CLA in diet	No effect	• Fatty acid profile differed between pigs fed different diets with or without supplement. Increased CLA levels in loin and backfat were detected when supplement was added. • Organic selenium in diet increased Se content in muscle. • Feeding fish oil had a negative effect on the sensory characteristics of pork (J. Leong 2010, personal communication).
Selenium				
Mahan et al. (1999)	351 pigs grown from an average of 20.4 kg to 105.0 kg on basal diets	Contr.: • 0 mg/kg Se Suppl: • 0.05, 0.1, 0.2, 0.3 mg/kg Se	No effect	• Organic selenium in diet increased Se content in muscle tissue.

(continued overleaf)

Table 5.1.2.1 (continued)

Reference	Animals	Dietary treatments	Effects on Growth Performance	Effects on carcass, or meat quality (LM = longissimus muscle, SM = semimembranosus muscle, PM= psoas major muscle)
Vitamin E				
Asghar et al. (1991)	60 pigs (barrows and gilts) grown from an average of 29.0 kg to 103.7 kg on grower diets	Contr.: • 10 mg/kg Vit. E[a] Suppl.: • 100, 200 mg/kg Vit. E[a]	Higher levels of vitamin E (100 or 200 mg/kg) improved ADG and FCR (P<0.05) in early growth phase.	• Concentrations of α-Tocopherol in blood plasma and different tissues increased (P<0.05) with increasing levels of dietary vitamin E.
Cannon et al. (1996)	30 pigs (24 barrows and 6 gilts) grown from an average of 40.3 kg to 110.6 kg on grower/finisher diets	Contr.: • 0 mg/kg Vit. E Suppl.: • 100 mg/kg Vit. E	No effect	• Supplementation of vitamin E resulted in higher α-Tocopherol concentrations (P<0.05) in longissimus muscle and lower TBA values during extended retail display (P<0.05). • Palatability ratings were more desirable when vitamin E was supplemented (P<0.05).
Hoving-Bolink et al. (1998)	72 pigs (32 castrates and 40 gilts) grown from an average of 44.0 kg to 111.0 kg on tapioca based diets	Contr.: • 8 mg/kg Vit. E Suppl.: • 208 mg/kg Vit. E	No effect	• Vitamin E levels were 5 times higher in LM and PM muscles of treated group. • Vitamin E treatment reduced TBA- values in both muscles and improved colour stability in LM after 6 days of storage.
Hasty et al. (2002)	240 pigs (barrows and gilts) grown from an average of 87.0 kg to 129.5 on basal diets	Contr.: • 0 mg/kg Vit. E Suppl.: • 75, 150, 300, 600 mg/kg Vit. E	Vitamin E increased (P<0.05) ADFI linearly (P<0.05) and tended to affect ADG and FCR (P<0.10)	• Vitamin E tended (P<0.07) to increase b* values linearly (P<0.06). • Vitamin E supplementation increased tissue concentrations of vitamin E linearly (P<0.001).

[a] *Supplemented with DL-α-tocopheryl acetate, 1 IU of vitamin E is equivalent to 1 mg of with DL-α-tocopheryl acetate*

(continued overleaf)

Table 5.1.2.1 (continued)

Reference	Animals	Dietary treatments	Effects on Growth Performance	Effects on carcass, or meat quality (LM = longissimus muscle, SM = semimembranosus muscle, PM= psoas major muscle)
Vitamin E and C				
Eichenberger et al. (2004)	40 barrows grown from an average of 25.0 kg to 106.0 kg on basal diets	Contr.: • 0 mg/kg Vit. E Suppl.: • 200 mg/kg Vit. E and/or • 300 mg/kg Vit. C	No effect	• Supplementation of vitamin E led to significantly higher concentrations of vitamin E in tissues. • Supplementation of vitamin C tended to enhance vitamin E levels in tissues, except for ham samples. • Differences in vitamin C content in tissues were only significant in LM and spleen • The correlation between vitamin E and vitamin C in tissue content was positive in LM, ham and kidney, but negative in heart. • In the outer layer of backfat, oxidative stability (TBARS) was positively influenced by dietary vitamin E supplementation. • TBARS were higher in diets supplemented with vitamin C.
CLA				
Dugan et al. (1997)	108 pigs (54 barrows, 54 gilts) grown from an average of 61.5 kg to 106.0 kg on cereal based diets containing either CLA or sun flour oil	Contr.: • 0 % CLA in diet Suppl.: • 2 % CLA in diet	CLA suppl. tended to reduce FI (P=0.07) and improve FCR (P=0.06)	• Pigs fed CLA deposited less subcutaneous fat (P=0.01) and gained more lean (P=0.03)
Dugan et al. (2001)	216 barrows grown from an average of 36.0 to 115.0 kg on diets containing different levels of dietary CLA and total oil	Contr.: • 0 % CLA in diet Suppl.: • 0.25, 0.5 % CLA in diet	No effect	• An increased CLA content increased the lean content of commercial cuts by 2.7% (P = 0.008). • Supplementing CLA reduced the amount of subcutaneous fat (SCF) by 6.6% (P = 0.002)
Thiel-Cooper et al. (2001)	40 barrows grown from an average of 26.3 kg to 114.0 kg on basal diets	Contr.: • 0 % CLA in diet Suppl.: • 0.12, 0.25, 0.5, 1.0 % CLA in diet	ADG and FCR improved linearly when CLA level increased (P<0.05)	• CLA supplementation decreased both 10^{th} rib backfat (P<0.05), fat depth over loin eye at the 10^{th} rib (P<0.05), intramuscular fat (P<0.001) and subcutaneous fat (P<0.05). • Supplementation of CLA increased belly firmness linearly as the concentration of CLA increased (lean side up: P<0.001; lean side down: P<0.05). • CLA was incorporated into pig tissues, CLA concentration increased linearly in both subcutaneous fat and lean tissue (P<0.001)

Selenium

Mahan et al. (1999) did not find any effect of organic Se (Sel-Plex; Alltech, Nicholasville, KY) on the growth performance or carcass characteristics of pigs in a study with 351 crossbred pigs with an average BW of 20.4 kg at the start and slaughtered at 105 kg BW. The Se levels fed in the study by Mahan et al. (1999) were lower then the levels fed in the current study. The experimental diet with the highest level of supplemented Se in the study by Mahan et al. (1999) contained 2.3 times less organic Se then the supplemented diets in the current study.

Vitamin E

Different studies have found different results when it comes to the effect of vitamin E on growth performance. Cannon et al (1996) and Hoving-Bolink et al. (1998) did not find an effect, Asghar et al. (1991) and Hasty et al. (2002) on the other hand found a positive effect of vitamin E on growth performance.

In the 84 day trial by Cannon et al. (1996), thirty crossbred pigs (24 barrows and 6 gilts) with an initial BW of 40.3 kg (SEM 0.43 kg) were used. The pigs received either a control diet containing no vitamin E or a diet formulated to contain 100 mg of vitamin E/kg feed. Growth performance, slaughter characteristics, and proximate composition of the longissimus muscle did not differ ($P>0.05$) between groups, but the vitamin E levels fed were lower then the levels in the current study.

In the study by Hoving-Bolink et al. (1998), seventy-two crossbred pigs (32 barrows and 40 gilts) were used. The average BW at the start of the trial was 44 kg. The pigs were slaughtered after an 84 day trail at approximately 111 kg. The pigs were divided into two treatment groups, one group received a tapioca based diet (vitamin E: 8 mg/kg diet). The other group received the same diet supplemented with an extra 200 mg vitamin E per kg feed (208 mg/kg). The extra dietary vitamin E supplementation had no effect on ADG, FCR, lean meat percentage, or meat quality.

The levels of vitamin E in the unsupplemented diets in the current study were higher than the levels of the control diet in the study by Hoving-Bolink et al. (1998). The levels of vitamin E in the supplemented diets on the other hand were higher in the study by Hoving-Bolink et al. (1998) compared to the current study.

In contrast, Asghar et al. (1991) found that vitamin E supplementation resulted in improved growth rates. In this study, 60 pigs (barrows and gilts) with an average BW of 29 kg and slaughtered at 103.7 kg, were assigned to either a grower diet containing a basic level of 10 mg/kg vitamin E (control) or diets supplemented with 100 mg/kg or 200 mg/kg vitamin E. Growth rates in the early growth phase improved by 4% and 6%, respectively compared to the control diet.

Hasty et al. (2002) found a linear increase in average daily feed intake when diets were supplemented with different levels of vitamin E. In this six week study, the effect of vitamin E supplementation on pork quality of two different genotypes was evaluated. The supplementation of α-tocopheryl acetate in the study tended ($P<0.10$) to affect ADG and FCR inconsistently. The supplementation of vitamin E increased ($P<0.05$) the ADFI linearly.

In the study of Hasty et al. (2002), supplementation of α-tocopheryl acetate had no effect ($P>0.45$) on carcass yield or carcass characteristics. In agreement with these results, Asghar et al. (1991) and Cannon et al. (1996) reported no differences in carcass yield or carcass characteristics in pigs supplemented with vitamin E compared to control pigs either.

Vitamin E and vitamin C

Eichenberger et al. (2004) investigated the effect of vitamin E and vitamin C supplementation on vitamin content and oxidative stability of both vitamins in pork tissue and/or any interactions between vitamin E and vitamin C supplementation. In that study, 49 barrows with an initial live weight of 25 kg were assigned to 4 different treatment groups. The control group was fed a basal diet. The other groups received either the control diet with 200 mg/kg vitamin E, control diet with 300 mg/kg vitamin C, or the control diet supplemented with 200 mg/kg vitamin E and 300 mg/kg vitamin C. Pigs were slaughtered at an average weight of 106 kg. There were no treatment effects on growth performance and carcass characteristics.

CLA

Different studies have found different results when it comes to the effect of CLA on growth performance. On the other hand, supplementation of CLA had a significant effect on carcass quality in all the studies mentioned in this section.

Thiel-Cooper et al. (2001) found improvements in ADG and FCR, and improvements in FCR were also found by Dugan et al. (1997). On the other hand, a later study by Dugan et al. (2001) found no effect of CLA on either ADG or FCR.

Thiel-Cooper et al. (2001) used 40 barrows with an initial average BW of 26.3 kg in a study with diets containing 0, 0.12, 0.25, 0.50 or 1.0% CLA. They found that an increase in CLA level resulted in a linear increase in ADG ($P<0.05$). The concentration of CLA in the diet had no effect on the ADFI so this resulted in a linear decrease in FCR ($P<0.05$). Carcass fat measurements from pigs fed CLA decreased quadratically, with the biggest decreases when the CLA level in the diet was 0.50% or less. In this study, CLA supplementation decreased 10^{th} rib backfat ($P<0.05$), fat depth over loin eye at the 10^{th} rib ($P<0.05$), intramuscular fat ($P<0.001$) and subcutaneous fat ($P<0.05$). Supplementation of CLA increased belly firmness linearly as the concentration of CLA increased (lean side up: $P<0.001$; lean side down: $P<0.05$).

In the study by Dugan et al. (1997) 108 pigs (54 gilts and 54 barrows) were used. The initial body weight of the pigs at the start of the trial was 61.5 kg, and they were slaughtered at 106 kg live weight. The different treatment groups received a cereal-based diet containing either 2% CLA or 2% sunflower oil. They found that pigs fed CLA compared to pigs fed sunflower oil tended to have reduced FI (-5.2%, $P = 0.07$) and improved FCR (-5.9, $P = 0.06$), but similar growth rates. The CLA group pigs also had less subcutaneous fat (-6.8% P 0.01) deposition and more lean (+2.3%, $P = 0.03$) gain then pigs fed sunflower oil. In the study by Dugan et al. (1997), CLA supplementation decreased the deposition of subcutaneous fat with 6.8% ($P = 0.01$) and increased lean gain with 2.3% ($P = 0.03$).

In a later study, however, Dugan et al. (2001) found no effects of CLA supplementation on growth performance. For this study, Dugan et al. (2001) used 216 barrows to study the effects of feeding different levels of CLA and total oil (TO). The initial body weight of the pigs at the start of the trial was 36 kg, and the pigs were slaughtered at 115 kg live weight.

The diets contained different levels of CLA (0, 0.25 and 0.5%) and dietary TO (2 and 5% made up with canola oil). No treatment effect was found on weight gain from 0-4 weeks (36 to 61 kg) or from 4 weeks to slaughter (61 to 115 kg) ($P>0.05$). Feed intake was not affected by feeding CLA ($P=0.70$) in this study, but increasing dietary CLA levels increased the lean content in commercial cuts by 2.7% ($P=0.008$). In the study by Dugan et al. (2001) increasing the CLA content increased the lean content of commercial cuts by 2.7% ($P = 0.008$). Supplementing CLA reduced the amount of subcutaneous fat (SCF) with 6.6% ($P = 0.002$)

5.2 Digestibility

In the un-supplemented diets digestibility characteristics for dry matter (DDM) and organic matter (DOM) were the same for the animal-based (AT) diet and the plant-based diet (PO). In the supplemented diets, DDM and DOM were greater when soybean and linseed oil (POS) were used instead of Tallow (PTS) or fish oil (PFS). A 1.5 % increase in digestibility for soybean oil in comparison to tallow has been reported by Jorgensen and Fernandez (2000). Individual fatty acid (FA) digestibility increases with an increase in the degree of unsaturation, and a decrease in length (Duran-Montgé *et al.,* 2007). This shows that digestibility of fat sources are a function of their FA content (Duran-Montgé *et al.,* 2007). An increase in the ratio of unsaturated fatty acid to saturated fatty acid (U/S) has been shown to result in an increased fat digestibility (Wiseman *et al.,* 1990; 1998), and consequently an increase in DDM and DOM could be expected. In this study the U/S was greater in diet POS (4.71) than in the PTS or PFS diets (2.03 and 2.56, respectively), thus matching the difference observed in DDM and DOM.

Statistically significant differences were also observed between dietary lipid types for the digestibly characteristics of ashes (DA), which represents mainly mineral digestibility. Information on the effect of lipid types on mineral digestion and absorption in pig is limited. However, in other species such as the rat and human, the effect of essential fatty acids on mineral absorption, especially in the case of calcium, are well documented (Kruger *et al.,* 1997), but these effects are complex and interactions exist between different types of FA (Kelly *et al.,* 2003). Van Dokkum *et al.* (1983) showed that increased linoleic acid from soybean oil had no effect on faecal digestibility of calcium or magnesium but a negative effect on iron in young men.

In this study, statistically significant differences in DA were observed between the POS (0.62), PTS (0.54) and PFS (0.52) diets which differed both in term of U/S ratio and in linoleic acid content (1.9 % vs 1.4% and 1.3%, respectively). In the un-supplemented diets DA was higher in the plant based diet PO (0.44) than in the animal based diet AT (0.30).

The main difference in DDM, DOM and DA was between the PO and POS diets, thereby suggesting a positive effect of Vitamin E and selenium supplementation on nutrient digestibility. In this study, with grower pigs, the extra 0.4 ppm of selenium (in an organic form) and 200 ppm of vitamin E provided resulted in a 0.008 point increase in DDM and a 0.018 point increase in DA (Table 4.2.1). Adkins and Ewan (1984) reported a 0.003 point increase in dry matter digestibility when diets with 0 or 0.10 ppm of selenium were fed to weaner pigs.

However, Tian *et al.* (2006) did not find any difference in dry matter or ash digestibility between diets containing 0.16 ppm or 0.36 ppm of selenium in grower pigs. Increasing the fat soluble vitamins (A, D, E and K) content in the diet from 100% to 150% of the NRC (1998) requirement had a positive effect on dry matter digestibility (+ 0.022 points) and calcium digestibility (+ 0.043 points) and phosphorus (+ 0.09 points) in grower pigs (Lohakare *et al.,* 2006).

In Japanese quail reared under chronic heat stress, Sahin and Kucuk (2001) reported that an increased dietary level of vitamin E (+125 ppm) improved dry matter digestibility by 0.017 points and that an extra +0.1 ppm selenium improved DDM by 0.005 points, resulting in combined improvement in DDM of 0.022 point. Overall it seems that vitamin E has a greater influence on nutrient digestibility than selenium.

5.3 Meat quality

The myofibrillar fragmentation index (MFI) results in this study were overall quite high compared with values reported for beef using the same method. Similar results with higher MFI values for pork than beef were found in a study by Koohmaraie et al. (1991) using the MFI method of Culler et al. (1978).
In previous studies by Purchas et al. (1997) and Purchas et al. (2002) values ranged from 78% (when no fragments passed through the filter) to 100% (when all fragments passed through). The effect of ageing (1 or 20 days) on the MFI(%) of beef was reported in Purchas et al (1997). MFI (%) increased with ageing from 1 to 20 days as expected (for example, Morgan et al. 1993).
In the current study, the pork was stored at 3°C (±1°C) in a chiller for 7 days before it was frozen.

Increased cooking temperatures reduced tenderness (higher mean, peak force, yield force and peak force – yield force) and increased cooking loss. These effects of increased cooking temperatures are similar to results found for pork by Purchas et al. (1988). In a study by Crawford et al. (2010) average peak shear force (N) and cooking loss (%) were determined at three different cooking temperatures (62°C, 71°C and 79°C). In Crawford et al. (2010) both Warner-Bratzler shear force (WBSF) and cooking loss increased as cooking temperature increased ($P<0.001$). They found that when cooking temperature increased from 62°C to 71°C the average peak sheer force (N) increased by 11.6% and cooking loss increased by 26.1%.

In the current study, when cooking temperature increased from 60°C to 70°C average peak shear force (N) increased by 28.7% and cooking loss increased by 37.7%. These are greater increases than those reported by Crawford et al. (2010), possibly due to the methods used and the slightly different temperatures. A lower cooking temperature is likely to result in improved tenderness

because cooking losses will be lower and consequently a given cross-sectional area of meat sample will contain more water and less structural components (Purchas, 1990).
According to Hamm (1953) as described in Briskey et al. (1959), a lower pH causes the water in the meat to be released, the structure to become more dense and the light rays to be reflected from the surface layers and make the muscle appear light in colour. Therefore, a negative relationship between pH and L* was expected since meat with a high pH tends to be darker than meat with a low pH.

According to Wood et al. (2003) groups of fat cells containing solidified fat with a high melting point appear whiter than liquid fat with a lower melting point. The fatty acid ratio on which this statement is based was not mentioned in the publication by Wood et al. (2003). In this study, pork from pigs fed diets containing tallow (containing the highest level of saturated fat) did not appear whiter than pork from pigs fed diets containing soybean oil. The colour measurements in this study did not show any of the contrasts to be significant.

The significant treatment differences for MFI, sarcomere length, WHC and cooking loss at 70 ^{0}C were unexpected and no studies were found in which comparisons were made between similar dietary treatments for meat quality parameters. The effects found in this study were not very large and unlikely to have an effect on consumer acceptance.

All contrasts were significant for MFI except for PFSe vs PFSI. The contrasts for sarcomere length were significant for POS vs PFSe+PFSI and PFSe vs PFSI. The significant contrasts for MFI and sarcomere length were not expected since MFI and sarcomere length are both correlated to tenderness and no significant differences were found in WB- shear force parameters. A higher MFI indicates greater meat tenderness as described in Crouse et al. (1991). According to Cross et al. (1981) and Howard et al. (1968) a longer sarcomere length results in a lower resistance to shear, differences in sarcomere length in the current study were small.

Measurements for WHC were significantly higher in group PFSe than PFSI, but there is no apparent reason why this should have been the case.

The contrasts for cooking loss at 70 ^{0}C were significant for all contrasts except for AT vs PO and PFSe vs PFSI. For all other contrasts, the cooking loss at 70 ^{0}C was significantly higher in group POS. There is no apparent reason why this should have been the case.

5.4 Fatty acid profile loin and backfat

As described in Wood and Enser (1997), dietary fatty acids are absorbed unchanged from the intestine and incorporated into tissue lipids in pork and poultry, hence meat fatty acid composition can easily be changed via the diet. Linoleic and α-linolenic acids cannot be synthesised, so dietary levels have a major influence on tissue concentration of these fatty acids (Wood and Enser, 1997). In the current study, the fatty acid profile of the loin and the backfat resembled the fatty acid profile of the diets.
Feeding diets rich in α-linolenic acid results in increased levels of this fatty acid in pork (Wood and Enser, 1997). According to Wood and Enser (1997) increased deposition of α-linolenic acid could lead to increased synthesis of EPA and DHA. However, in human studies by Burdge et al. (2002, 2006) it was found that the ability of man to convert α –linolenic acid into DHA was very limited.

Linoleic acid is mainly found in soybean oil, and α-linolenic acid is mainly found in linseed oil. As expected, pigs fed diets without soybean or linseed oil had the lowest levels of linoleic acid and α - linolenic acids in backfat. Pigs fed diet PFSe had higher levels than pigs fed diet PFSl, possibly because pigs in group PFSe were fed a diet with soybean and linseed oil (POS) closer to slaughter in the second part of the grower phase.

A more effective way to increase tissue concentrations of EPA and DHA is to supplement the diet with fish oils which are good sources of these fatty acids (Wood and Enser, 1997). EPA, DPA and DHA are omega-3 long chain poly unsaturated fatty acids (PUFAs) found in fish oil (Witold et al., 2007). As expected, the levels of EPA, DPA and DHA were significantly higher in diets containing fish oil (PFSe and PFSl). The time during the study when fish oil was fed had an effect on the EPA, DPA and DHA levels. Higher levels were present in backfat and loins of pigs fed fish oil in the second part of the grower phase.

Table 5.4.1 shows an overview of the deposition of EPA, DPA and DHA (% of total fatty acids) in loin and backfat of pigs fed dietary fish oil in the studies mentioned below. All studies showed that an increased level of fish oil in the diet resulted in higher levels of EPA, DPA and DHA in loin and backfat. EPA, DPA and DHA levels found in loin and backfat decreased with withholding period.

Table 5.4.1: A summary of results from studies where measurements have been made of the deposition of EPA, DPA and DHA (% of total fatty acids) in loin and backfat of pigs fed dietary fish oil.

Reference	No. animals per treatment	Sex[a]	Duration	Withdrawal period (days)	Diet	Concentration fish oil	Total Fish oil (kg)	Loin			Backfat		
								EPA	DPA	DHA	EPA	DPA	DHA
Nuijten, 2010	8	G	18.9-92.6kg	0	Plant feedstuffs and	0.0	0.00	0.43	0.55	0.19	0.11	0.33	0.15
[Current study]	7	G	19.0-45.3kg	28	Sanovite™	4.4	2.31	1.18	1.38	1.36	0.71	1.08	1.34
	8	G	45.9-68.8kg	28		4.4	2.31	1.45	1.71	2.22	0.89	1.28	1.57
Jaturasitha	14	G + B	35-90kg	0	Maize, soybean meal,	0.0	0.00	0.30	-	0.72	0.07	-	0.36
et al., 2008	14	G + B	35-90kg	0	broken rice, rice bran,	1.0	1.60	0.65	-	0.95	0.18	-	0.91
	14	G + B	35-60kg	50[b]	fish meal	3.0	1.60	0.48	-	0.78	0.13	-	0.74
	14	G + B	75-90kg	0		3.0	1.60	0.64	-	0.99	0.21	-	0.96
Irie and	16	-	81.4-107.8kg	0	Basal diet	0.0	0.00	-	-	-	0.55[c]	0.34[c]	0.45[c]
Sakimoto, 1992	16	-	81.4-107.8kg	0		2.0	1.16[c]	-	-	-	0.42[c]	0.75[c]	0.66[c]
	16	-	81.4-107.8kg	0		4.0	2.32[c]	-	-	-	0.96[c]	0.96[c]	1.08[c]
	16	-	81.4-107.8kg	0		6.0	3.48[c]	-	-	-	1.19[c]	1.08[c]	1.27[c]
Bryhni et al., 2002	15	G + B	28-104kg	0	Factorial design + Low	0.0	0.00	-	-	-	-	0.10[e]	0.10[e]
	15	G + B	28-104kg	0	(31%) or High (50%)	0.2	0.37[d]	-	-	-	-	0.20[e]	0.00[e]
	16	G + B	28-104kg	0	PUFA	0.4	0.76[d]	-	-	-	-	0.20[e]	0.10[e]

[a] *In this Table, B stands for barrows, G stands for gilts only and G + B stands for gilts and barrows.*
[b] *Withdrawal period days calculated*
[c] *Total amount fish oil (kg) calculated by total growth and an FCR of 2.2.*
[d] *Total amount fish oil (kg) calculated by total growth and FCR average for high and low PUFA*
[e] *Average means for outer and inner layer of backfat*

In the study by Bryhni et al. (2002) 48 crossbred growing-finishing pigs (barrows and gilts) were fed six different diets from an initial weight of 28 kg to an average weight at slaughter of 104 kg. Dietary treatments were combinations of two basic diets containing either a low (31%) or a high level of PUFA (50% of total fat content) and three levels of fish oil (0, 0.2, 0.4% of concentrated commercial capelin oil). The level of PUFA in the diet was highly correlated with PUFA in backfat (R^2 = 0.80). There was also a correlation between C18:2 and C18:3 in feed and backfat (R^2 = 0.80 and R^2 = 0.81, respectively). Backfat from pigs fed 0.4% of fish oil contained significantly more (P<0.001) C22:5 than backfat from pigs in treatment groups without fish oil.

In the current study, the level of C22:5 (DPA) in backfat was significantly lower in pigs fed diet POS than in pigs that received fish oil during the grower phase (groups PFSe and PFSl).

Irie and Sakimoto (1992) showed that n-3-PUFA of backfat could be changed by feeding diets rich in those fatty acids, and that the n-3-PUFA increased linearly with additions of fish oil.
In the study, 64 pigs with an average body weight of 81.4 kg received either a control diet or one of three test diets containing either 2, 4 or 6% fish oil. The pigs were fed *ad libitum* for a total of 4 weeks until slaughter at an average body weight of 107.8 kg.

The levels of EPA and DHA in all fat tissues in the carcass samples increased as the level of dietary fish oil increased. Oleic and linoleic acids on the other hand, tended to decrease with increases in EPA and DHA.

The only dietary source of C20:3 n-3 in the current study was the fish oil (Table 3.5.1), and it was not detected in diets AT, PO, POS or PTS. This fatty acid was however detected in the loin and backfat samples of all groups. There were significant contrasts for group AT vs PO and POS vs PTS. The levels of C20:3 n-3 were significantly higher in samples from animals fed the plant-based diets rather than the animal-based diets.

Similar results were found in previous work by Ahn et al. (1996), Juárez et al. (2010) and Realini et al. (2010). Ahn et al. (1996) found higher levels of C20:3 n-3 in phosphatidylethanolamine from pork loin muscles when dietary α-linolenic acid levels increased. In the study by Juárez et al. (2010), dietary C18:3 n-3 (from flaxseed) increased C20:3 n-3 levels in pork backfat.

In a 50 day study with 70 gilts (61.8 ± 5.2 kg BW) by Realini et al. (2010), the effect of dietary fat source on carcass fatty acid composition was studied. The dietary treatments consisted of a barley and soybean diet supplemented with 10% fat. Additionally, one group received a semi-synthetic diet containing no fat (NF). The experimental diets contained the following fat sources or combinations of fat sources: beef tallow (T), high-oleic acid sunflower (HOSF), sunflower oil (SFO), linseed oil (LO), fat blend (FB: 55% T, 35% SFO and 15% LO) or oil blend (OB: 40% fish oil, 60% LO)

As shown in Table 5.4.2, Realini et al. (2010) found that higher levels of dietary C18:3 n-3 resulted in higher levels of C20:3 n-3 in the carcass.

Table 5.4.2: Fatty acid profile of diet and carcass in the study by Realini et al. (2010)

Fatty acid profile	T	HOSF	SFO	LO	FB	OB	NF
Dietary C18:3 n-3, g/kg feed	1.90	1.29	1.26	47.10	18.90	31.20	0.18
Carcass composition C20:3 n-3, %	0.16^{d}	0.11^{d}	0.11^{d}	1.60^{a}	0.67^{c}	0.92^{b}	0.10^{d}

[a-d] *Within a row, means lacking a common superscript letter differ (P<0.05)*

5.5 Adequate intake of EPA, DPA and DHA

There is a difference in the levels of intake of the long-chain n-3 fatty acids (EPA, DPA, & DHA) recommended for humans for “adequate intake” (AI) and “suggested dietary targets” (SDT) (Howe et al. 2007). Suggested dietary targets are set to achieve health benefits, and as a result are between 3.8 and 4.8 times higher (respectively for females and males) than levels for AI targets (Howe et al. 2007). Different sources give different recommendations, however in this study the nutrient reference values, set by The National Health & Medical Research Council for Australia and New Zealand were used. The National Health & Medical Research Council recommended AIs for the sum of EPA, DPA and DHA are 90 and 160 mg/day and recommended SDTs are 430 and 610 mg/day for woman and men, respectively. The AI for men was used in calculating the values for Figure 4.8.1, because men require the higher amount of EPA, DPA and DHA per day.

The current study shows that pork from pigs fed different diets contains different levels of EPA, DPA and DHA. A higher fat content in pork products results in higher levels of EPA, DPA and DHA per gram. In order to determine if consuming pork enriched with LC omega-3 PUFA could be used to reach AI targets it is important to know the composition of retail pork cuts and consumer preferences with regard to pork products.

According to Levy and Hanna (1994) as described in Brewer et al. (2001), the amount of visible fat is the strongest visual clue for consumers that ''pork is bad for you''. Brewer et al. (2001) evaluated the purchase intent of pork with low (1.05% fat), medium (2.33% fat) and high (3.46% fat) amounts of marbling. Brewer et al. (2001) found that the purchase intent means were higher for lean (41.55% of consumers) and medium (40.14%) marbled pork chops than for highly (18.31%) marbled chops.

Based on conclusions reached by Levy and Hanna (1994) and Brewer et al. (2001) it is unlikely that consumers would be willing to eat or buy pork with high amounts of visible fat. In order to reach AI requirement of the long-chain n-3 fatty acids large amounts of lean pork need to be eaten on a daily basis if animal diets are not enriched with LC omega-3 PUFA. If pork products with 85% lean loin meat from pigs fed diet PFSe or PFSl were consumed, then the daily intake required to reach AI requirements is reduced by 79.4% and 83.3%, respectively, compared to when lean loin meat from pigs fed diet POS was consumed. Enriching animal diets with LC omega-3 PUFA therefore helps in achieving standards for adequate intake of these fatty acids.

Greenfield et al. (2009), in a study of the nutrient composition of Australian retail pork cuts determined the proximate fat composition of different pork cuts. They reported that the fat content of the lean section of all raw cuts was low (1.1-2.2%) except for Scotch roast which had a fat content of 7.6%. Raw pork mince and strips and diced pork had fat levels ranging from 2.3 to 9.4%. In Table 4.8.1, fat levels are based on fat levels from New Zealand pork and processed pork products as described by Duncan et al. (1999). For the calculations in Table 4.8.1, the backfat was used as a fat source and the balance was made up with loin muscle (lean meat content). The EPA, DPA and DHA levels in the pork products as mentioned in Table 4.8.1 were calculated, they were not determined so actual levels could be different. Table 4.8.1 is used to show the difference in fatty acid content of pork from pigs fed diets with and without dietary fish oil. Calculations made in the current study are theoretical and based on fat levels in un-cooked pork products.

5.6 Palatability and meat processing

Although palatability characteristics by sensory panel and meat processing characteristics were not investigated in this study, it is important to consider the possible effects of changing the fatty acid profile of pork on these aspects of meat quality. The loins from the pigs in this study were frozen and exported to Singapore for sensory evaluation by a consumer panel. The results from the panel showed that feeding fish oil had a negative effect on the sensory characteristics of pork as assessed by consumers in Singapore (J. Leong, 2010, personal communication).

A human study on blood cholesterol and lipid profile of blood when consuming pork from pigs in dietary treatment groups AT, POS and PFSl will be performed by the Institute of Food Nutrition and Human Health at Massey University Palmerston North.

It is relatively easy to alter the fatty acid profile of pork to create a healthier product for consumers (Enser et al., 2000), but an altered fatty acid profile can cause problems in the meat processing industry due to changes in fat texture and shelf life (Wenk et al., 1990). The change in shelf life and fat texture could have consequences for the meat processing industry as suggested by Prabucki (1991) as referenced by Hadorn et al. (2008).

According to Bryhni et al. (2002), although fish oil and high PUFA levels in the diet of pigs might contribute to a more healthy meat, their undesirable affects on palatability would limit their use. Bryhni et al. (2002) found significantly more (P<0.05) fishy and rancid odours, higher TBA values, and less meat odour in samples of meat and fat from pigs fed high PUFA diets compared to samples form pigs fed the low PUFA diets after 1 month of freezer storage.

According to Jonsdottir et al. (2003) as described in Jaturasitha et al. (2009), LC n-3 PUFA are susceptible to oxidation. The fishy off flavours found in pork with increased levels of LC n-3 PUFA are at least partly a result of increased oxidative susceptibility (Jonsdottir et al., 2003).
The 6 trained panellists in the sensory assessment for the study of Jaturasitha et al. (2009) did not detect off or fishy flavours.

Shelf life and scores for sensory flavour and overall acceptability in the study by Jaturasitha et al. (2009) were better in pork from pigs fed tuna oil in the early fattening phase. According to Fraser et al. (1934) and Melton (1990) as described in Jaturasitha et al. (2009) a withdrawal period for fish oil of 4 weeks and 2 weeks respectively was recommended.

In the study by Janz et al. (2008), the sensory panel did not find any differences between treatment groups, except for the supplemented plant group compared to the control plant group. Rancid odour was detected more often in samples from the supplemented plant group than in samples from the control plant group (25 and 12% respectively).

In the study by Bryhni et al. (2002) increasing levels of dietary fish oil from 0 to 0.4% did not have any effect on sensory quality after one or eight months of frozen storage. Poly-unsaturated fatty acids and fish oil showed a significant interaction between TBARS values and rancid odour. The highest TBARS values and rancid odour were shown for pork from pigs fed the diets with high PUFA and 0.4% fish oil. This indicates that a diet with a combination of high PUFA and fish oil can result in product with undesirable sensory traits. Recommendations by Bryhni et al. (2002) to reduce oxidative problems for finishing pigs, included feeding less than 50 g PUFA/kg feed and having no more than a maximum of 23% PUFA in backfat.

Wenk et al. (1990) found a relationship between increased PUFA levels in pork and possibly higher occurrence of oxidation and rancidity. According to Wood et al. (2003), α-tocopherol could be used to delay lipid and colour oxidation and thereby extend shelf life.

Wenk et al. (1990) also found that increased PUFA levels together with monounsaturated fatty acids (MUFA) could change the texture of the fat with it becoming soft, greasy and oily. According to Wood et al. (2003) fatty acid composition has an important effect on firmness or softness of the fat in meat due to differences in melting points. Melting points decline when unsaturation increases (Wood et al. 2003). In the study by Irie and Sakimoto (1992) the hardness of the fat was measured with a texturometer, the hardness was decreased by increased levels of dietary fish oil.

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CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

In this Chapter, the main results of the research are summarized. First, the sub-questions are answered and recommendations are given. At the end of this chapter, the main question is answered as a definitive conclusion for the entire thesis.

1. ***What is the effect of feedstuffs and lipid type and Sanovite™ on growth performance and carcass quality?***

In this study feedstuffs, lipid type and Sanovite™ had no significant effect on growth performance and carcass quality. Findings in other studies regarding the effect of CLA on growth performance were not consistent. In order to determine if supplementing diets with CLA has a subtle effect on growth performance further study with a higher number of observations per group is required.

2. ***What are the effects of feedstuffs, lipid type and Sanovite™ on the digestibility of nutrients?***

In this study there were no differences in DDM and DOM in the un-supplemented animal (AT) and plant based (PO) diets.

Lipid type had a significant effect on DA and an increased ratio of unsaturated fatty acid to saturated fatty acid resulted in increases in DDM and DOM. DDM and DOM increased when soybean and linseed oil (POS) were used instead of Tallow (PTS) or fish oil (PFS).

The main differences in DDM, DOM and DA were observed between diets PO and POS. A positive effect of selenium, vitamin E and CLA supplementation is hereby suggested. There was no literature found on the effect of the different ingredients of the supplement on digestibility; further study is required.

3. What are the effects of feedstuffs, lipid type and Sanovite™ on the quality of pork?

The contrasts for cooking loss at 70 °C were significant for all contrasts except for AT vs PO and PFSe vs PFSI. For all other contrasts, the cooking loss at 70 °C was significantly higher in group POS than for groups PO, PTS and PFSe+PFSI.

Peak force – Yield force at 70°C was significantly higher in samples from group PFSe than for samples from group PFSI.

The MFI was significantly higher for group AT in contrast to group PO. Group POS had a significantly higher MFI value in contrast to groups PO, PTS and PFSe and PFSI.

Group POS had a lower sarcomere length in contrast to groups PFSe and PFSI. Group PFSe had a significantly higher sarcomere length in contrast to group PFSI.

Group PFSe had a significantly higher expressed juice percentage loss in weight in contrast to group PFSI.

The treatment effects found in this study were not expected and were not in line with the literature. Therefore further study is required to determine if they are repeatable.

Supplementing Sanovite™ increased the Se content in lean meat as analysed by J. Leong (2010, personal communication).

General meat quality conclusions:
Increased cooking temperatures reduced tenderness (higher mean, peak force, yield force and peak force – yield force) and increased cooking loss. As expected, there is a relationship between pH and relative lightness (L^*), with the pH value having a significant negative effect on L^*.

There were highly significant positive correlations between all three measurements of expressed juice, and there was a significant positive correlation between cooking loss at 60 and 70°C ($P<0.01$), but correlations between expressed juice values and cooking loss were not significant.

4. ***What are the effects of feedstuffs, lipid type and Sanovite^{TM} on the fatty acid profile of loin muscle and backfat?***

In this study it was found that feedstuffs, lipid type and the supplementation of CLA in diets for growing-finishing pigs had several effects on the fatty acid profile of pork. In general it was concluded that an increase in the ratio of unsaturated fatty acid to saturated fatty acid (U/S) in the diet resulted in higher levels of unsaturated fatty acids in loin and backfat. The fatty acid profile in the diet reflected the fatty acid profile of pork.

Backfat of pigs fed diets including soybean and linseed oil contained higher levels of linoleic and α-linolenic acid.

The use of fish-oil as a lipid type resulted in the highest levels of EPA, DPA and DHA in loin and backfat. Loin and backfat of pigs fed fish-oil in the second part of the grower phase (PFSl) contained higher levels of EPA, DPA and DHA than pigs fed fish-oil in the first part of the grower phase (PFSe).

Diets PO and POS were used to establish the effect of the supplementation of CLA. The backfat of pigs fed diet POS contained higher levels of CLA (C18:2-*trans*-10, *cis*-12) and α-linolenic acid than pigs fed diet PO. The loin of pigs fed diet POS contained higher levels of palmitoleic and linoleic acid and CLA (C18:2-*cis*-9, *trans*-11) and lower levels of oleic acid then pigs fed diet PO.

5. ***Is it possible to increase the EPA, DPA and DHA content of pork by diet manipulation sufficiently to create pork providing adequate intake levels of these fatty acids for human consumption?***

Yes, it is possible to increase the EPA, DPA and DHA content of pork by diet manipulation. Enriching pork with LC omega-3 PUFA will contribute to achieve standards for adequate intake. Pork enriched with LC omega-3 PUFA could be used to reach AI targets, but might not be suitable to reach SDT targets. It is more efficient to use fish-oil capsules or other dietary sources of LC omega-3 PUFA to reach SDT targets.

Further study is required to determine the consumer preferences when it comes to purchase intent and consumption of pork. Calculations made in the current study are theoretical and based on fat levels in un-cooked pork products. Further study is required to determine levels of EPA, DPA and DHA in cooked pork products.

6. What is the effect of feedstuffs, lipid type and Sanovite™ on palatability and meat processing?

A sensory evaluation with a consumer panel in Singapore showed that feeding fish oil had a negative effect on the sensory characteristics of pork as assessed by consumers in Singapore (J. Leong, 2010, personal communication).

In order to determine the effect of feedstuffs, lipid type and Sanovite™ on meat processing further study is required.

After answering the sub-questions, the main question can be answered. The main question in this thesis was:

Is it possible to change the composition of pork by dietary manipulation without compromising pig performance and meat quality?

Yes, in this study was found that it is possible to change the pork composition by dietary manipulation without compromising pig performance and meat quality. The dietary treatments, as used in this study, did not have a significant effect on growth performance or carcass quality. There were a few significant effects from treatments on meat quality characteristics reported in this study, but differences were small and relatively unimportant.