Fatty Acids, Tocopherols and Lipid Oxidation in Pig Muscle

Effects of feed, sex and outdoor rearing.

Anders Högberg

Department of Food Science
Uppsala

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Slutligen uppmanar jag er alla att betrakta vad som är
kunskapens sanna mål, så att ni inte söker den för nöjes, eller
tävlingens, eller överlägsenhetens, eller vinstens, eller
berömmelsens, eller maktens, eller något annat värdelösts skulle
utan för livets fördel och nytta.

Francis Bacon 1561-1626

Abstract

The objective of this thesis was to investigate the effect of rearing conditions, sex, and RN genotype on the fatty acid composition in polar and neutral lipids, content of antioxidants, and lipid oxidation in pig muscle. Furthermore, the effect of different dietary levels of various polyunsaturated fatty acids (PUFA) on rat performance was investigated.

Pigs were reared in outdoor or indoor rearing systems and fed different diets of conventional and organic origin. All pigs were slaughtered at approximately 105 kg live weight and samples were collected from *M. longissimus dorsi* and *M. Biceps femoris* for lipid analysis.

Outdoor rearing increased the level of PUFA n-3 in the neutral lipids of pig muscle. The increase was rather small and the effect on oxidative stability can be considered as minor, especially as outdoor pigs had a higher content of tocopherols. In addition, our studies showed a more pronounced increase of PUFA level in females and entire males, due to diet and outdoor rearing, compared with castrated males. This difference in PUFA level is suggested to depend on grazing behaviour, incorporation of PUFA into fat tissues or a slight alteration of PUFA metabolism, possibly mediated by light.

The level of PUFA in neutral lipids was higher in entire males compared with females and castrated males, which can partly be explained by lower body fat accumulation. In polar lipids the level of highly unsaturated fatty acids was higher in entire male and female pigs compared with castrated males, which indicates that the desaturation and elongation of PUFA are more pronounced in sexes with an intact reproductive function.

The RN genotype was found to influence the fatty acid composition in the polar lipids of pig muscle suggesting involvement of PUFA in glycogen metabolism or that the RN genotype itself affects PUFA metabolism.

Finally, feeding rats for approximately 39 days with linseed oil or sunflower oil diet improved wound healing. There was a positive correlation between PUFA n-3 fatty acids in liver and wound healing. Thus, the higher levels of PUFA in tissues covariating with the diet and the demonstrated increased wound healing, revealed a potential of PUFA in affecting the performance of animals.

Keywords: Castrated male pigs, Entire male pigs, Female pigs, Lipid oxidation, Neutral lipids, Polar lipids, Pork, PUFA, Rearing condition, RN genotype

Author´s address: Anders Högberg, Department of Food Science, P.O. Box 7051, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden

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I. Högberg, A., Pickova, J., Dutta, P.C., Babol, J., and Bylund A.C. 2001. Effect of rearing system on muscle lipids of gilts and castrated male pigs. *Meat Science* 58, 223-229.

II Högberg, A., Pickova, J., Babol, J., Andersson, K. and Dutta, P. C. 2002. Muscle lipids, vitamins E and A, and lipid oxidation as affected by diet and RN genotype in female and castrated male Hampshire crossbred pigs. *Meat Science* 60, 411-420.

III. Högberg, A., Pickova, J., Andersson, K. and Lundström K. 2002. Fatty acid composition and tocopherol content of muscle in pigs fed organic and conventional feed with different n6/n3 ratios respectively. Food Chemistry (submitted)

IV. Högberg, A., Pickova, J. Stern, S., Lundström, K. and Bylund. A.C. Fatty acid composition and tocopherol concentrations in muscle of entire male, castrated male and female pigs, reared in an indoor or outdoor housing system. (Manuscript)

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Appendix

List of abbreviations used in the text

BPI	Base peak intensity
C	Length of fatty acid chain
CLA	Conjugated linoleic acids
GST	Glutathione-S-transferace
GSH	Glutathione
GSH-PX	Glutathione peroxidase
CV	Coefficient of variation
GC	Gas chromatograph
HOSO	High oleic sunflower oil
HUFA	Highly unsaturated fatty acids
IMF	Intramuscular fat
LW	Live weight
MDA	Malondialdehyde
MUFA	Monounsaturated fatty acids
NL	Neutral lipids
PCA	Principal component analysis
PC 1	Principal component one
PL	Polar lipids
P/S	PUFA/SAFA
PUFA	Polyunsaturated fatty acids
SAFA	Saturated fatty acids
SOD	Superoxide dismutase
RT	Retention time
TBA	Thiobarbituric acid

Introduction

General background

In Sweden pig meat is the most common food of animal origin on the consumer table. The Swedish pig meat industry with its production of 260 000 tons of meat (2001) represents almost 45 % of the total meat production in Sweden (Jordbruksverket, 2001). The total consumption of meat per capita in Sweden in 1999 was 73.6 kg, of which 35.9 kg was pig meat. Thus, pork accounts for almost 50 % of the yearly consumption of meat, most of which originates from Swedish farmers (Jordbruksverket, 2000).

The diet of the countries in the Western world tends to contain more and more meat and meat products. Sweden is no exception to the rule and its consumption of pig meat has increased from 24.4 kg in 1950 to 35.9 kg in 1999. This increase makes pig meat, along with poultry meat, the meat dish that has increased the most during this time period (Jordbruksverket, 2000). However, an increased consumer concern has recently been observed regarding pig management, the environmental effects of meat production and the health value of pork (Bosi, 1999). In Sweden these public concerns have lead to an increase in research in the area of sustainable food production (Food 21), which covers a broad range of research areas from the farm to the consumer (Bylund et al., 1997). The quality aspects of meat are diverse and quality management is complicated, as many of the aspects valued by the consumer are difficult to measure in the final meat product. This is especially the case if the focus should be concentrated on animal health and natural behaviour as suggested by Bylund et al. (1997). Therefore, researchers have divided the meat quality concept into two separate parts, product quality and production quality, to differentiate between meat and the production conditions under which meat is produced (Hofmann, 1994).

Consumer concerns regarding product quality, e.g. the health value of pig meat, have made it more important to determine how the composition of meat is influenced by different factors. Several factors have been identified that affect the composition of meat, e.g. genetic disposition (sex and breed), feeding strategies (diet and maintenance), physiological parameters (fatness, age, weight and hormones) and handling as reviewed by Nürnberg et al. (1998). It has been argued that feeding together with genetic improvement and management are fundamental to adapting pig production to the market (Bosi, 1999) and also to meeting human dietary guidelines (Lough et al., 1992).

One important parameter of meat quality that is highly variable depending on the above factors is the fat content and composition. The variability in fat content and composition has considerable impact on the product quality of both fresh meat and processed meat products. The effect of feed on fat parameters, e.g. fatty acid composition, antioxidant content and storage stability of meat has previously been investigated (Wood and Enser, 1997; Nürnberg et al., 1998; Jakobsen, 1999; Lauridsen et al., 1999b; Lauridsen et al., 1999c).

Fat has diverse functions in animals and is an essential part of diets. Many meat products contain a considerable amount of saturated fat, which is considered to increase the risk of developing disease. Therefore the alteration of the fatty acid composition in meat has attracted considerable interest, i.e. making meat products more nutritionally favourable (Hartog et al., 1987; Madsen et al., 1992; Enser, 2000). Furthermore, the role of meat as a functional food has been discussed (Jimenez-Colmenero et al., 2001). Special interest has been focused on the PUFA and specially the PUFA n-3 fatty acids. These have been shown to have a positive impact on human health and are essential for growth and development throughout the human life cycle (Simopoulos, 1991; Horrocks and Yeo, 1999; Simopoulos, 1999; Cunnane, 2000; Simopoulos, 2000).

Meat quality

The concept of meat quality implies different properties depending on whether it is applied by the producers, industries or consumers. In the broad sense the quality concept can contain parameters like high daily gain or high feed conversion ratio. However, as the retailers have become more sensitive to consumer demands, new brands have evolved that consider other aspects, such as animal welfare. Hoffman (1994) has suggested that 'Meat quality is the sum of all sensory, nutritive, hygienic-toxicological and technological properties of meat' (Table 1). In this perspective, lipids in food possess unique physical and chemical properties, i.e. structure, melting properties, association ability and stability. However, subjective aspects such as consumer appreciation, as well as economical, physiological, ethical or political aspects, are of no doubt also important. In the previously mentioned Food 21 programme the additional quality parameter (excluding product quality) has been called 'production quality.' This concept has been proposed to encompass animal care and environmental conditions influenced by agriculture (Bylund et al., 1997).

Table 1. Product quality parameters (adapted from Hoffman (1994))

Sensory factors	Nutritive factors	Hygienic and toxicological factors	Technological factors
Colour	Proteins	Micro-organisms	Structure
Shape	Fat	Toxins	Texture
Smell	Fatty acids	Shelf-life	Consistency
Taste	Vitamins	Water activity	Viscosity
Flavour	Amino acids	pH	Water content
Marbling	Minerals	Additives	Water binding
Tenderness		Residues	pH
Juiciness			State of protein
Fat composition			State of fat

Fatty acid composition of different dietary lipid sources

Fat sources of plant and animal origin used in different studies on pigs vary considerably in their fatty acid composition. In general, it can be said that lipids of animal origin, with the exception of fish oil, are more saturated and less polyunsaturated compared with lipids of plant origin (Table 2). The highest content of MUFA has been shown in safflower oil, olive oil and in HOSO. Regarding PUFA the highest amount of PUFA n-6 fatty acids are found in sunflower oil whereas the highest amount of PUFA n-3 fatty acids are found in flaxseed. Finally, fish oils contain a high amount of HUFA n-3 (Table 2).

Table 2. Fatty acid composition of different fats and oils from plant and animal origin

	Tallow [1]	Lard [1]	Soya bean oil [1]	Sun flower oil [2]	Rape seed oil [2]	Saff lower oil [3]	Olive oil [4]	HOSO [3]	Flax seed [5]	Fish oil [6]
14:0	3.3	1.5	0.1	0.2	-	0.1	-	0.1	-	4.7
16:0	25.5	24.8	11.0	6.8	5.0	5.4	10.0	4.1	6.0	10.5
16:1 n-7	3.4	3.1	0.1	0.1	0.3	-	-	-		7.8
18:0	21.6	12.3	4.0	4.7	1.4	2.2	3.0	4.0	4.4	2.3
18:1 n-9	38.7	45.1	23.4	18.6	58.5	72.1	77.0	80.9	25	15.5
18:2 n-6	2.2	9.9	53.2	68.2	21.9	19.4	8.0	9.5	14	1.9
18:3 n-3	0.6	0.1	7.8	0.5	12.7	-	1.0	-	52	0.9
20:0	-	-	-	-	-	-	0.5	-	-	-
20:1 n-9	-	-	-	-	-	-	0.5	-	-	16.0
22:1	-	-	-	-	-	-	-	-	-	8.6
20:4 n-6	0.4	0.4	-	-	0.1	-	-	-	-	0.4
20:5 n-3	-	-	-	-	-	-	-	-	-	9.3
22:6 n-3	-	-	-	-	-	-	-	-	-	13.8

[1](Chow, 1992); [2] (Leskanich, 1999); [3] (Shackelford et al., 1990c); [4] (Leissner et al., 1989); [5] (Romans et al., 1995a); [6] (Nürnberg et al., 1999)

Lipids in animals

Lipids can be roughly defined as substances generally soluble in organic solvents. Christie (1987) defined lipids as 'fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds.'

Fatty acids of animal tissues

Animal lipids have been classically divided into structural fat and depot fat. In addition, lipids can be divided into two main classes, i.e. the neutral and polar lipids. The polar lipids are important constituents of membranes and they function as precursors in eicosanoid metabolism (structural fat), whereas the neutral lipids serve mainly as a depot of lipids used as an energy source (depot fat) (Henderson, 1987). Neutral lipids include the triacyl-, diacyl- and monoacyl-glycerols, cholesterol, cholesterol esters, free fatty acids and wax esters. Polar lipids are divided into glycerophosholipids, glyceroglycolipids and sphingo-lipids. The main glycerophosholipids in pig tissues are the phosphatidyl-cholines followed by

phosphatidyl-ethanolamines and –inositols, but the quantities vary depending on tissue examined.

Fatty acids, the main structural component of animal lipids, consist of carbon chains with a terminal carboxyl group (Figure 1). Saturated fatty acids lack double bonds, whereas unsaturated ones can contain up to six double bonds. Fatty acids with one double bond are referred to as MUFA. Fatty acids with two or more double bonds are referred to as PUFA, while C 18 (not including 18:2 n-6 and 18:3 n-3) or longer fatty acids with more than two double bonds are referred to as HUFA. The length of the carbon chain and the number of double bonds determine the characteristics of the fatty acid. In biological systems fatty acids are mainly involved in determining the physical and chemical properties and capacities of biological membranes. Fatty acids also serve as precursors in the synthesis of several different chemical messengers and hormones as well as other regulating factors.

The fatty acids 18:2n-6, 18:3n-3 are essential to most mammals, including humans, and are produced by terrestrial and aquatic plants (e.g. algae). Dietary sources of these fatty acids seem to be essential for normal growth and survival. Most mammals are capable of synthesizing the longer desaturation and elongation products (HUFA) from the essential fatty acids (Figure 2). It is not entirely clear which of the metabolic pathways, presented in Figure 2, is correct but most researchers are convinced that there is an elongation followed by a desaturation and a final peroxisomal retroconversion to 22:6 n-3, the so called 'Sprecher pathway' (Voss et al., 1991; Hansen, 1994; Sprecher et al., 1995; Petroni et al., 1998; Innis et al., 1999; Sprecher et al., 1999).

Number of carbon   Number of carbon atoms from the
                        last double bond to the terminal
                        methyl group

18:3 n-3

Number of double bonds

18:2 n-6
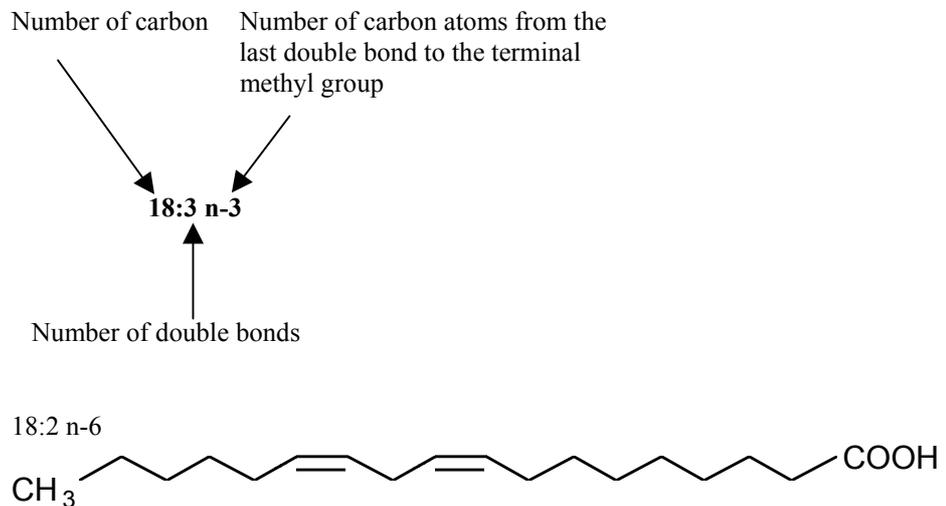
Figure 1. Principle nomenclature of a fatty acids.

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Desaturation of n-3 fatty acids

18:3 n-3

####÷-6 desaturase

Elongase

18:4 n-3 ⟶ 20:4 n-3

÷-5 desaturase

Elongase   Elongase

20:5 n-3 ⟶ 22:5 n-3 ⟶ 24:5 n-3

###############################÷-4 desaturase   ÷-6 desaturase

Oxidase

22:6 n-3 ⟵ 24:6 n-3
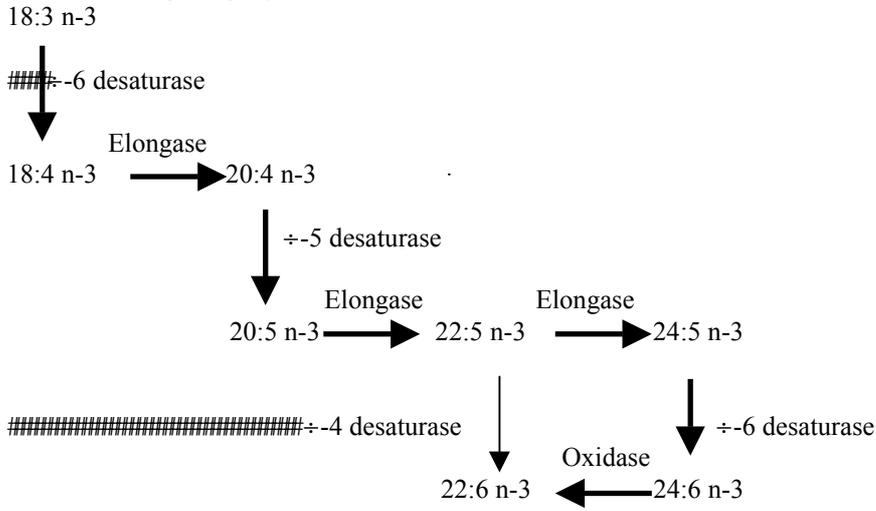
Figure 2a . Schematic presentation of the desaturation and elongation steps involved in the conversion of 18:3n-3 to 22:6n-3. Vertical and horizontal lines represent desaturation and elongation steps, respectively. The thicker line represents the Sprecher pathway (adapted from Lauritzen (2001).

Desaturation of n-6 fatty acids

18:2 n-6

#######÷-6 desaturase

Elongase

18:3 n-6 ⟶ 20:3 n-6

÷-5 desaturase

Elongase   Elongase

20:4 n-6 ⟶ 22:4 n-6 ⟶ 24:4 n-6

#############################÷-4 desaturase   ÷-6 desaturase

Oxidase

22:5 n-6 ⟵ 24:5 n-6
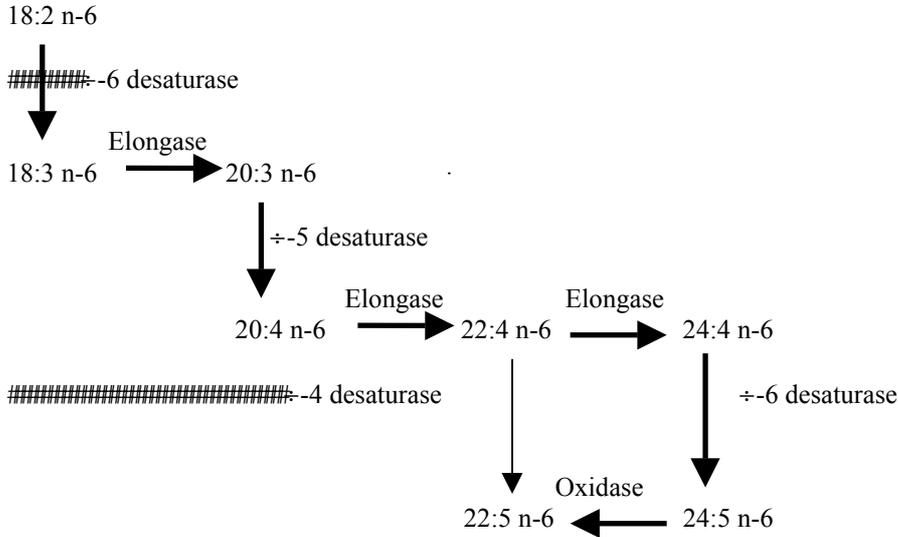
Figure 2b . Schematic presentation of the desaturation and elongation steps involved in the conversion of 18:2 n-6 to 22:5 n-6. Vertical and horizontal lines represent desaturation and elongation steps, respectively. The thicker line represents the Sprecher pathway (adapted from Lauritzen (2001)).

Absorption of dietary lipids in monogastric animals

Dietary fat is generally absorbed in the distal duodenum and jejunum part of the small intestine. In the intestine the triacylglycerols, phospolipids and cholesterolesters undergo hydrolysis to form monoglycerides, lysolecithin, cholesterol and free fatty acids. In addition, ζ-tocopherol acetate is hydrolyzed into free ζ-tocopherol. After intestinal absorption the free fatty acids longer than 10 carbon atoms are re-esterified into triacylglycerols, phospolipids and cholesterol ester and are transported together with the vitamins from the intestinal area as chylomicrons (lipoprotein rich in triglycerides) via the lymphatic system (Figure 3). The shorter fatty acids are absorbed directly into the portal blood attached with albumin and transported to the liver and other organs for processing or energy utilization.
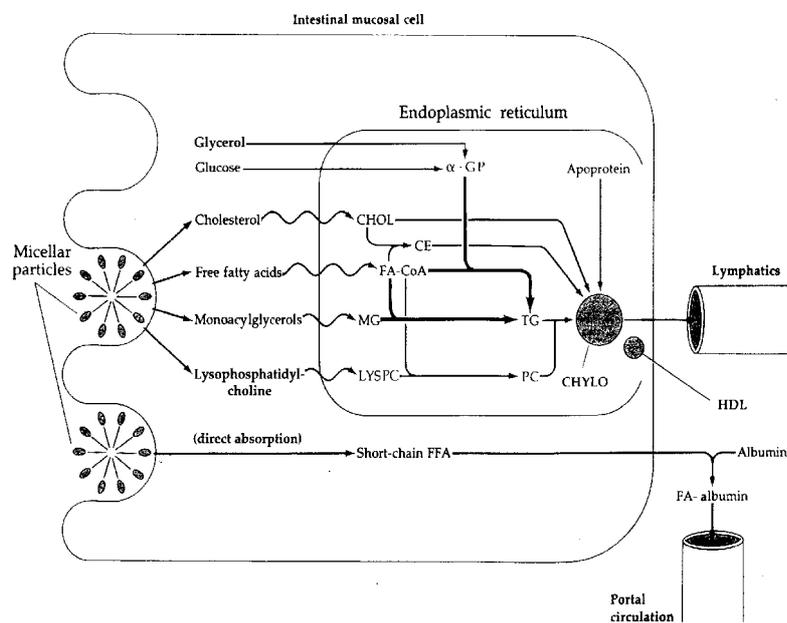

Figure 3. The uptake of fat from the intestine and further metabolism in the intestinal mucosal cell and final excretion into the lymphatic system and portal circulation. Abbreviations: CHOL, cholesterol; CE, cholesteryl ester; FA-CoA, CoA activated fatty acids; MG, monoacylglycerols, LYSPC, lysophosphatidyl-choline; ζ-GP, ζ-glycerophosphate; CHYLO, chylomicrones; TG, Triacylglycerols; PC, phosphatidylcholine. Adapted from Groff and Gropper (1999) (published by kind permission of Wadsworth Thomson Learning)

Muscle and depot fat variation in fatty acid composition and antioxidant content

The different muscles of mammals contain three different fibre types. Fibre type composition can vary considerably from muscle to muscle (Kiessling and

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Hansson, 1983). The type I fibre is a slow-twitch fibre that is more oxidative than glycolytic, has a high fat content and high myoglobin content. The muscles that contain high quantities of type I fibres are often referred to as dark muscles. The type II fibres can be divided into two different subgroups. Type II b is a fast-twitch fatigable fibre and has, as opposed to the type I fibre, a high glycolytic capacity, a low fat content and a low myoglobin content. This fibre fatigues rapidly because of low oxidative metabolism. The type II a fibre is a fast fatigue resistant fibre and is intermediate in glycolytic/oxidative capacity, fat content and myoglobin content and therefore has a higher oxidative metabolism compared with type II b. The muscles with more type II fibres are often referred to as light muscles.

Fatty acid composition has been shown to depend on fibre type composition. The dark muscles *M. adductores, M. Psoas major* and *M. quadriceps* have higher levels of PUFA n-6 and PUFA n-3 compared with the light *M. Longissimus dorsi* (Allen et al., 1967; Malmfors et al., 1978; Taugbøl and Saarem, 1995)*,* which partly can be explained by the larger amount of mitochondria in the dark muscle and thus more PUFA containing membranes. Dark muscles contain more vitamin E, e.g. *M Psoas major* contains more ζ-tocopherol compared with *M. Longissimus dorsi* (Jensen et al., 1997). Furthermore, both dark and light muscles seem to respond equally to changes in dietary levels of PUFA and vitamin E (Taugbøl and Saarem, 1995; Jensen et al., 1997).

Regarding depot fat, Irie and Sakimoto (Irie and Sakimoto, 1992) have shown that the anatomical location will influence the fatty acid composition. It has been shown in several studies that the level of polyunsaturation in the depot fat is as follows (Koch, 1968; Villagas et al., 1973; Malmfors et al., 1978; Warnants et al., 1995):

Outer subcutaneous fat layer > inner subcutaneous fat layer > perirenal fat

However, Irie and Sakimoto (1992) conclude that this is not valid for all PUFA as HUFA n-3 are higher in the perirenal fat compared with the subcutaneous fat. It has been shown that the inner subcutaneous fat layer contained more vitamin E compared with the outer layer (Morrissey et al., 1996).

Tissue accumulation of fatty acids and vitamin E

The factor that influences the fat composition in our domestic animals the most is diet. During the last few decades the incidence of diet related illnesses e.g. cardiovascular diseases have increased among humans. Diets high in saturated fat have been indicated to be partly responsible for this increase. Meat in general contains large amounts of saturated fat and meat consumption tends to increase with increasing welfare. In this perspective an alteration of the fatty acid composition in meat products is of general interest. Over the last few decades,

Table 3. n6/n3 and P/S ratios in IMF of *M. Longissimus dorsi* and subcutaneous fat of pigs from a selection of studies with different feed sources

Diet	Muscle (IMF)		Subcutaneous fat		Remark	Reference
	PUFA n6/ PUFA n3	P/S	PUFA n6/ PUFA n3	P/S		
15 % full fat soy beans In weeks before slaughter						(Warnants et al., 1999)
0 (2.5 % tallow)	15.27	0.39	13.04 [1,2]	0.29 [1]	[1] Inner layer [2] 18:2n-6/ 18:3 n-3	
2	14.96	0.39	11.15	0.37		
4	15.74	0.39	10.82	0.41		
6	13.54	0.39	10.76	0.50		
8	13.71	0.39	10.34	0.49		
3 % tallow	2.33 [1]	0.22 [1]	2.74	0.40	[1] Neutral lipids	(Monahan et al., 1992)
3 % soy oil 90-84 kg LW	4.06	0.43	4.88	0.88		
6% soy bean/ 2 % tallow			10.00	0.48		(Warnants and van Oeckel, 1998)
7 % soy bean/ 2 % tallow/ 1% soy oil			9.20	0.60		
3 % soy oil/ 1 % lard			8.90	0.74		
2 % tallow/ 7 % linseed (25-105 kg LW)			1.19	0.67		
Control	45.24 [1]	0.28	22.21 [1,2]	0.25 [2]	[1] 18:2n-6/ 18:3 n-3 [2] Bacon	(Leszczynski et al., 1992)
20 % extruded full fat soy beans	23.21	0.43	12.70	0.50		
4 % Tallow (6 weeks before slaughter)	33.14	0.33	16.74	0.29		
3 % tallow	12.67-11.07	0.35-0.33	9.15-8.92	0.35-0.30		(Pfalzgraf et al., 1995)
3 % soy oil (30-95 kg LW)	10.53-9.68	0.62-0.56	7.34-6.88	0.75-0.67		
Tallow	21.10	0.52				(Corino et al., 2002)
Corn oil	22.19	0.54				
Rape seed oil (3 % 25-110 kg LW 2.5 % 110- 160 LW)	13.91	0.46				

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Treatment					Notes	Reference
Control	7.31-6.39	0.42-0.34	6.65-6.37	0.31		(Omibi et al., 1998)
25 % full fat rape seed	4.99-3.63	0.65	3.27-3.24	0.89-0.80		
(50-90 kg. LW)						
Control	7.59[1,2]	0.13[2]	15.07[1]	0.26	[1] 18:2n-6/ 18:3 n-3	(Lauridsen et al., 1999c)
6 % rapeseed	4.53	0.29	4.26	0.61	[2] Neutral lipids	
25-100 kg LW						
Control	22.33		13.67			(Nürnberg, 1995)
Rape seed						
5 %	11.38		6.67			
7.5 %	10.44		6.14			
10.5 %	6.53		4.96			
% extruded rape seed						(Warnants and van Oeckel, 1996)
0	14.3	0.15				
5.25	10.2	0.17				
7	8.00	0.20				
8.75	8.63	0.24				
10.5	8.1	0.29				
(25-100 kg LW)						
Control	8.8	0.4	4.9	0.3		(Cherian and Sim, 1995)
Linseed						
10 %	2.4	0.5	1.4	0.6		
17.5 %	1.5	0.7	1.5	0.8		
25 %	1.0	0.7	1.0	0.8		
(25-100 kg LW)						
Linseed (g/kg feed)						(Van Oeckel et al., 1996)
1.91 % (4.0)	6.70-5.29	0.36-0.29	3.00-2.93	0.43-0.35		(Van Oeckel et al., 1997)
3.65 % (7.0)	3.56-3.46	0.42-0.32	1.89-1.88	0.55-0.44		
5.38 % (10.0)	2.83-2.75	0.42-0.32	1.38-1.28	0.58-0.50		
(36-107 kg LW)						
Control	9.21	0.25	15.52[1]	0.29[1]	[1] Middle layer	(Romans et al., 1995)
15 % flax seed	2.85	0.32	4.73	0.37		
(28 days before slaughter)						

Diet					Reference
4 % olive oil	9.31	0.30	9.34	0.38	(Fontanillas et al., 1998)
4 % linseed oil	1.14	0.67	1.00	0.77	(Fontanillas et al., 1997)
(82 days before slaughter)					
Control	9.23	0.63			(Ahn et al., 1996)
18:3 n-3 g/kg feed					
15	1.58	0.96			
25	1.07	1.04			
35	1.07	0.96			
(20-100 kg LW)					
/kg diet					(Enser et al., 2000)
15.5g 18:2 n-6 / 1.9g 18:3 n-3	9.02-8.61	0.51-0.49	9.08-8.91	0.61-0.54	
10.0g 18:2 n-6 / 4.0g 18:3 n-3	5.06-5.03	0.42-0.40	5.58-4.85	0.45-0.42	
(25-95 kg LW)					
1.5 % sunflower oil	6.33-5.33	0.31-0.29			(Rey et al., 2001)
1.5 % olive oil	5.09-3.70	0.28-0.20			
1.5 % sunflower + 0.5 % linseed oil	4.69-4.31	0.39-0.33			
1.5 % olive oil + 0.5 % linseed oil	3.55-3.51	0.31-0.26			
(42 days before slaughter)					
112 days before slaughter					(Øverland et al., 1996)
3% soy oil	7.85	0.64	28.78	0.80	
2 % soy oil + 1 % fish oil	5.14	0.59	15.5	0.69	
3 % fish oil	3.11	0.62	5.87	0.59	
148 days before slaughter					
2 % soy oil + 1 % fish oil	3.44	0.58	9.25	0.75	
3 % fish oil	1.47	0.60	1.47	0.49	
3 % Tallow:soybean (4:1)	7.3	0.8	8.5	0.4	(Leskanich et al., 1997)
2 % rape seed + 1 % fish oil	4.6-4.5	0.7	4.9-4.6	0.5	
(50-95 kg LW)					

Control	3.98	0.22	2.84 [1]	0.32 [1]	[1] Inner layer	(Irie and Sakimoto, 1992)
Fish oil 6%	1.66	0.35	1.68	0.32		
(4 weeks before slaughter)						
9-60 kg LW on experimental diets					[1] Biopsies	(Lauridsen et al., 1999a)
6 % Tallow	5.21 [1]	0.40 [1]	4.85 [1]	0.35 [1]		
3 % fish oil/ 3 % tallow	1.52	0.50	1.45	0.44		
6% fish oil	0.96	0.35	0.81	0.47		
4 % fish oil/ 2 % coconut fat	1.10	0.59	1.05	0.35		
42 days of 2 % tallow between 60-100 kg LW on 2 % tallow						
6 % tallow	6.63	0.28	5.09	0.24		
3 % fish oil/ 3 % tallow	1.99	0.30	3.48	0.28		
6% fish oil	1.39	0.35	1.56	0.30		
4 % fishoil/ 2 % coconut fat	1.77	0.42	1.80	0.29		

meat scientists have discussed how to achieve an increased nutritional quality of meat by feeding pigs different fat sources (Table 2). The strategies can be summarized into 4 main groups:

∉ Increased level of MUFA changing the monounsaturated to saturated ratio.
∉ Increased level of PUFA changing the polyunsaturated to saturated ratio (see Table 3).
∉ Increased level PUFA n-3 changing the n6 to n3 ratio (see Table 3).
∉ Other components, for example CLA.

Thus, the overall aim has been to produce pig meat with increased nutritional quality and thereby decrease the incidence of disease. However, feeding pigs with unsaturated fatty acids in high amounts might have negative effects on the technological and sensory quality due to oxidation. Therefore, additional feeding with vitamin E has an important role in preventing other quality losses when producing more nutritional pig meat.

Fatty acids

The fatty acid composition of tissues in monogastric animals is more sensitive to dietary modification compared with ruminants. In ruminants, the MUFA and PUFA have to pass the rumen, in which a substantial part of the unsaturated fatty acids is hydrogenated into saturated, branched-chained, and trans fatty acids (Harfoot). As much as 95 % of all PUFA was biohydrogenated by rumen microorganisms (Jenkins, 1993; Scollan et al., 2001b). However, it has been shown that ruminal micro-organisms do not biohydrogenate HUFA like 20:5 n-3 and 22:5 n-3 to any significant extent (Ashes et al., 1992; Doerau, 1994; Choi, 1997). However, studies in this area are inconsistent. (Scollan et al., 2001b) found that these highly unsaturated fatty acids were biohydrogenated to the same extent as 18:2 n-6 and 18:3 n-3. Thus, the fatty acid composition of the ruminant tissues will not reflect the dietary level PUFA as of monogastric animals. This does not exclude the possibility of affecting the fatty acid composition in ruminant meat as shown in beef (Choi et al., 2000; Scollan et al., 2001a) and lamb (Enser et al., 1998; Wood et al., 1999) as PUFA can partly be protected by cellular walls in grass or by the seed coats of oilseeds.

In pigs, several studies have been conducted to evaluate how the fatty acid composition of different tissues is affected by the addition of different dietary oil or oilseed. A linear increase of 22:6 n-3, 18:2 n-6 and 18:3 n-3 in subcutaneous fat and muscle have been shown when feeding different levels of fish oil (Valaja et al., 1992; Lauridsen et al., 1999a), rape seed cake (Nürnberg, 1995) and rape seed (Madsen et al., 1992; Warnants and van Oeckel, 1996). Fontanillas et al. (1998) fed 4 % linseed oil or hydrogenated oil for 82 days. They showed that the level of PUFA n-3, HUFA n-3 (except 22:6 n-3) and trans fatty acids in subcutaneous fat had reached approximately 70 % of the level at slaughter in 31 days and 90 % at 60 days. Regarding MUFA, Fontanillas et al. (1998) found that feeding 4 % pomace oil increased the level in back fat to a maximal level at day 17 and then remained constant until day 82. Warnants et al. (1999) showed that a maximum PUFA level was reached in both the inner and outer subcutaneous fat layer at 6

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weeks feeding of full fat soya. The greatest accumulation and depletion was suggested to occur during the first two weeks feeding full fat rapeseed (Warnants et al., 1999), also found feeding fish oil (Irie and Sakimoto, 1992). Thus, dietary PUFA will affect both subcutaneous fat and IMF, even though the change will probably occur earlier in the depot fat. The time and level of increase will be highly dependent on the PUFA level in the feed, the fatty acids that are to be raised or decreased, and the level of these fatty acids in the live pig before the start of the trial period.

Vitamin E

The levels of one of the most important antioxidants in pig muscle, ζ-tocopherol, have been shown to be affected by diet in several studies as reviewed by Wood et al. (1997) and Buckley et al. (1995). This effect has not only been shown in monogastric animals and poultry but also in ruminants as reviewed by Liu et al. (1995) and Leonhardt et al. (1997). Morrissey et al. (Morrissey et al., 1996) have shown that feeding 200 mg of ζ-tocopherol acetate/kg feed resulted in a different accumulation in pig tissues as follows:

M. Longissimus dorsi<adipose tissue<liver.

Feeding 200 mg of ζ-tocopherol acetate/kg feed to pigs for 126 days resulted in a greater content of ζ-tocopherol in all tissues examined, except in heart and plasma, compared with feeding 20 mg of ζ-tocopherol acetate/kg feed for 91 days followed 35 days of 200 mg of ζ-tocopherol acetate/kg feed (Morrissey et al., 1996). In plasma, there was a rapid increase during the first week of ζ-tocopherol feeding 200 (Morrissey et al., 1996) or 405 mg of ζ-tocopherol acetate/kg feed (Jensen et al., 1990). Soler-Velasquez et al. (1998) found that the blood ζ-tocopherol level reached its maximum at 35 days of feeding 125 mg all-rac-ζ-tocopherol acetate. Morrissey et al. (1996) reported that the largest difference between dietary regimes were found in subcutaneous fat (inner and outer layer), *M. Longissimus dorsi* and kidney fat. In addition, Bieri (1972) found that approximately one week was required for a 33 % depletion of the initial ζ-tocopherol concentration in plasma and liver. In pig muscle the depletion rate is slower. Feeding 405 all-rac ζ-tocopherol acetate/kg feed for 11 weeks, followed by a 7 weeks' withdrawal, depleted vitamin E reservoirs in muscles (*M. Biceps femoris, M. Longissimus dorsi*) to approximately 40 % of the original level (Jensen et al., 1988). Together, these results would indicate that muscle and fat tissues reflect the long-term nutritional history of ζ-tocopherol, in contrast to plasma and liver contents that reflect the immediate nutritional status of the animals.

Regarding muscle and depot fat accumulation Morrissey et al. (1996) found that the daily increase of ζ-tocopherol in muscle and adipose tissue feeding 200 mg of ζ-tocopherol acetate/kg feed were as shown in Table 4.

Table 4. Accumulation of ζ-tocopherol in different fat tissues of pigs fed 200 mg of ζ-tocopherol acetate/kg feed (Morrissey et al., 1996)

	Days 1-21	Days 22-126	
Muscle (LD)	0.24 σg/g/week	0.18 σg/g/week	

	Days 1-35	Days 36-91	Days 92-126
Subcutaneous fat (inner layer)	2.55 σg/g/week	1.14 σg/g/week	0.85 σg/g/week
Subcutaneous fat (outer layer)	0.98 σg/g/week	1.04 σg/g/week	0.83σg/g/week
Kidney fat	3.72 σg/g/week	1.07 σg/g/week	1.07 σg/g/week

In summary, ζ-tocopherol is strongly accumulated in different tissues. The rate and absolute amount of accumulation will depend on time of feeding and the content of supra nutritional ζ-tocopherol in the feed. The metabolic activity in tissues of interest and the level of total fat in the tissue are also important parameters to consider.

Lipid oxidation and antioxidants in animal tissues

Non enzymatic lipid oxidation

Autoxidation of lipids is initiated by a free radical reaction with a lipid component, e.g. unsaturated fatty acids. The most potent radicals involved are the hydoroxyl radical as well as singlet oxygen (Nakazawa et al., 1996). The attack of free radicals species on unsaturated fatty acids leads to oxidative chain reactions (1-5). The reactivity of the unsaturated fatty acids increases with their chain length and number of double bounds. For example, the risk of an oxidative attack causing a chain reaction is many times higher in 22:6n-3 than in 18:2 n-6 (Cosgrove et al., 1987). The autoxidation can be divided into three steps, initiation, propagation and termination:

Initiation: $LH + Initiator \Downarrow L^{.}$ (1)

Propagation: $L^{.} + O_2 \Downarrow LOO^{.}$ (2)
$LOO^{.} + LH \Downarrow LOOH + L^{.}$ (3)

Termination: $LOO^{.} + LOO^{.} \Downarrow LOOH + O_2$ (4)
$LOO^{.} + L^{.} \Downarrow LOOL$ (5)
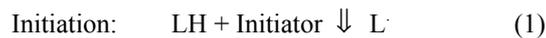
The initiation of lipid oxidation in meat products is generally believed to occur at the membrane level in the polyunsaturated phospholipid fraction (Gray et al., 1996). The initiators participating in this reaction can be heat, metals or light. The final termination step gives rise to a broad range of secondary oxidation products such as aldehydes, alkanes, conjugated diens and various other oxidation products. Larick et al. (Larick et al., 1992) have shown that the headspace volatiles found in pork meat in significant amounts are aldehydes, alcoholes, and saturated and unsaturated hydrocarbons of which hexanal exists in the largest quantities. These

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and other oxidation products can make the flavour and shelf life of meat unacceptable (Melton, 1990; Gray et al., 1996), can influence membrane structure (Monahan et al., 1994), and can also be regarded as a potential health risk (Guardiola et al., 1996).

Antioxidants

An antioxidant is a substance which, even at low concentrations, delays or inhibits the oxidation of various substrates (Halliwell, 1991). Some antioxidants function as radical scavengers or peroxide decomposers, while others quench singlet oxygen, remove catalytic metal ions, remove oxygen or inhibit enzymes. The cellular antioxidants in the muscle can be divided into enzymes, water-soluble and fat-soluble low molecular substances.

Enzymes and water-soluble antioxidants
Among the enzymes SOD, catalase, GSH-PX and GST are all active in skeletal muscle but have not been extensively studied with regard to meat quality aspects (Bertelsen, 2000).

SOD is a Cu- and Zn-containing enzyme in the cytoplasm and mitochondrial interspace and Mn containing in the mitochondrial matrix (Fridovich, 1989). SOD is known to protect cells against reactive oxygen species and the active centre catalyzes the disproportion of the superoxide anion (O_2^-) (6).

$$O_2^- + 2\,H^+ \Downarrow\ H_2O_2 \qquad\qquad (6)$$

Catalase is the best known H_2O_2-splitting enzyme catalyzing the splitting of H_2O_2 into water and oxygen (7). Catalase, similarly to SOD, is found in all major organs, but it is more concentrated in the liver and erythrocytes. The catalase activity is very limited in the mitochondria and the endoplasmatic reticulum but is strongly accumulated in the peroxisome (Kujumdzievasavova et al., 1991) and active in the cytosol (Toussaint et al., 1993).

$$H_2O_2 \Downarrow\ O_2 + 2\,H_2O \qquad\qquad (7)$$

A third enzyme that is also important for antioxidative properties in tissues is the selenium containing GSH-PX which both catalyzes the degradation of H_2O_2 (8) and lipid peroxides (9). GSH-PX is found in both the cytosol and the mitochondrial matrix (Flohe and Schengel, 1971), which allows a more efficient access to H_2O_2.

$$2\,GSH + H_2O_2 \Downarrow\ GSSG + 2\,H_2O \qquad (8)$$
$$2\,GSH + LOOH \Downarrow\ LOH + H_2O + GSSG \qquad (9)$$
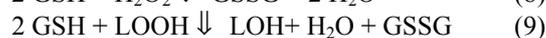
The non selenium containing GST is located in the cytosol and in a small amount in the mitochondrial membranes and endoplasmatic reticulum. This enzyme uses only lipid peroxides as substrate (9) but the peroxide must be cleaved out from the membrane by phospholipase A_2.

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Ascorbic acid is a water-soluble, cytosolic antioxidant that exists mostly in the form of ascorbate at physiological pH. This antioxidant is known to be one of the most efficient scavengers of radicals, including lipid radicals (Niki, 1991). Ascorbate can reduce metal ions, react with superoxide radicals, hydroperoxyl radicals, hydroxyl radicals and scavenge singlet oxygen (Halliwell, 1991). Another water-soluble antioxidant is free glutathione (GSH), found in the cytosol and the mitochondria (Halliwell, 1991). It serves as a scavenger for Superoxide and hydroxyl radical and singlet oxygen. GSH is reduced to GSSG, which is potentially dangerous as it inactivates a number of enzymes and therefore the reduction of GSSG by gluthathione reductase is of importance.

Fat-soluble antioxidants

Vitamin E is divided into the structurally different molecules tocopherols (Figure 4) and tocotrienols. Chemically the two forms are different in that tocotrienols have an unsaturated phytyl tail. There are four different forms of both tocopherols and tocotrienols, that is ζ, η, θ and ι. Among the tocopherols ζ-tocopherol has been recognized as the most important for preventing oxidation changes in meat (Jensen et al., 1998a). ν-Tocopherol is also a potent antioxidant (Lynch, 1991; Rey et al., 1998) but is not found in large amounts in meat or meat products (Piironen and et, 1985). To enhance the antioxidative status of meat, different forms of tocopherols are commercially used in feed mixtures. The most commonly used forms of ζ-tocoherol are the free form, ζ-tocopherol and the esterified form ζ-tocopherol acetate. In addition there is a commercial mixture that contains 8 different isomers of ζ-tocopherol ,all-rac-ζ-tocopherol, also in free and esterified form (Liu et al., 1995).
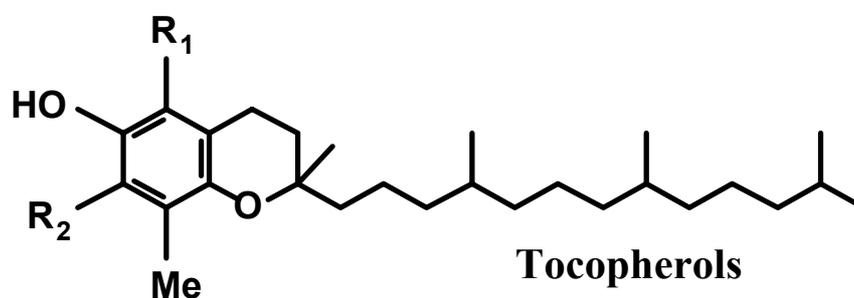
Tocopherols

Common Name	R$_1$	R$_2$	IU/mg	Activity based on Rat Assay Relative to ζ-T
ζ-tocopherol	Me	Me	1.49	100%
η-tocopherol	Me	H	0.75	50%
ν-tocopherol	H	Me	0.15	10%
ι-tocopherol	H	H	0.05	3%

Figure 4. Structures and methyl-positions of four natural forms of vitamin E and their biological activities (modified from Veris (1994)).

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Vitamin E reacts with a large number of molecules that enhance lipid oxidation in muscle in vivo and in meat in vitro. Among the molecules are superoxide radicals, hydroperoxyl radicals, hydroxyl radicals, hydrogen peroxide, singlet oxygen and finally, perhaps most important of all, lipid peroxy and alkoxy radicals. The basic principle of action of vitamin E is to reduce a lipid alkoxy and peroxy radical in the membrane and thus a less harmful state is rendered as the vitamin E radical has a lower reduction potential compared with the lipid radical. (10).

$$LOO^. + Vit.\ E \Downarrow\ LOOH + Vit.\ E^.\quad (10)$$

Finally, among the lipid-soluble antioxidants found in meat are carotenoids which can scavenge free radicals in biological systems and, hence, protect fatty acids and membranes against oxidation (Bertelsen, 2000). E.g. η- carotene is an efficient quencher of singlet oxygen, hydroxyl radicals, superoxide radicals and peroxyl radicals. Also retinol is readily oxidized but is generally not considered an antioxidant. However, dietary retinol has been indicated to interact with other fat-soluble vitamins, e.g. excessive intake of retinol has been shown to decrease ζ-tocopherol status in the plasma of chickens (Jensen et al., 1998b) and in various tissues of pigs (Hoppe et al., 1992).

The antioxidative defence system
The individual antioxidants described above are active in different parts of the cell depending on whether they are lipid- or water-soluble. The most important antioxidant in the membranes, which are highly susceptible to oxidation in vivo and postmortem, is the lipid-soluble antioxidant vitamin E. This antioxidant is incorporated in the membranes at a level of 1:1000 (Burton et al., 1983), thus 1 molecule of tocopherol protects 1000 of lipid molecules. When PUFA are oxidized in the membrane, a cascade of reactions occurs. Vitamin E reduces the lipid oxidation product, after which the vitamin E molecule is regenerated by ascorbate (vitamin C), although doubts have been raised regarding the role of ascorbate in vivo (Gille and Sigler, 1995). Another mechanism of regenerating vitamin E is by gluthathione but as with ascorbate this needs clarification (Gille and Sigler, 1995). Furthermore the resulting ascorbate radical formed from the regeneration of vitamin E is recycled by different enzyme systems like phospholipase A_2, glutathione peroxidase, or fatty acyl-coenzyme A.

The effect of different dietary sources of fat and levels of vitamin E on the quality of pig meat and pig meat products

Monounsaturated fatty acids
The effect of increased levels of monounsaturated fatty acids on meat quality in fresh meat and processed meat have been investigated in several studies. Feeding 10 % high oleic sunflower oil (HOSO) for 90 days (20-100 kg LW) (Miller et al., 1990) or 12 % for 55 days (102 kg LW) (Rhee et al., 1988a) resulted in a doubled monounsaturated to saturated ratio in IMF and subcutaneous fat compared to the control. Processing yield on pork chops were not negatively affected by HOSO (Miller et al., 1990). However, pigs fed HOSO had lower marbling scores,

firmness and texture scores of muscle and their fat became softer (Rhee et al., 1988a; Miller et al., 1990). The decreased softness might increase the ripening time in Parma ham (Bosi et al., 2000). 10 % inclusion of HOSO did not affect the sensory, physical or chemical characteristics in cooked boneless hams, bacon or various whole muscle products (Rhee et al., 1990a; Shackelford et al., 1990a; Shackelford et al., 1990d). In minced cooked products made from pigs fed HOSO the fat content of the products has proven to be important. A fat content between 15-35 % gave worse overall acceptability (Rhee et al., 1990b; Shackelford et al., 1990b; Shackelford et al., 1990c; Shackelford et al., 1991). Thus, inclusion of 10 % HOSO for 90 days or 12 % HOSO for 55 days will increase the level of MUFA in muscle and depot fat. It is possible that even a higher level can be included without any negative quality effects, but this will depend on processing and time of storage.

Polyunsaturated fatty acids

In order to monitor the negative quality effects of high PUFA diets on pig meat several thresholds have been suggested for the maximum level of PUFA in back fat and feed. It has been suggested that feed should not contain more than 12-21 % PUFA and back fat should not contain more than 12-22 % PUFA (Vanoeckel and Boucque, 1992; Stiebing et al., 1993; Warnants and van Oeckel, 1996; 1998). The suggested levels will be highly dependent on whether the meat is processed, storage conditions and intrinsic factors like antioxidants and major fatty acids. Feeding up to 10.5 % rape seed (18 g PUFA/kg feed) from 25 kg LW until slaughter did not have any negative effect on fresh and frozen back fat (Warnants et al., 1995; Warnants and van Oeckel, 1996; Corino et al., 2002). However, too much rape seed may affect the feed intake, carcass quality or performance (Warnants et al., 1995; Nürnberg et al., 1998). Adding rapeseed oil (canola) to diet resulted in contradictory results. Feeding 10 % oil for 90 days resulted in various quality problems in different meat products (Miller et al., 1990; Shackelford et al., 1990a; Shackelford et al., 1990c; Shackelford et al., 1990d; Miller et al., 1993). On the other hand St. John et al. (1986) and Rhee et al. (1988b) showed that feeding 20 % rapeseed oil for 100 days had no effect on sensory quality of meat.

Linseed and fish fat contain more PUFA n-3 compared with rape seed. Thus, meat quality problems can arise at lower feeding levels compared with rape seed. Feeding linseed (4 g/kg feed of 18:3 n-3) had no negative effect on pork products (Sheard et al., 2000). In fresh meat feeding up to 10 g/kg feed of 18:3 n-3 had no negative effects on back fat colour, back fat consistency, or sensory scores (Van Oeckel et al., 1996) but increased the lipid oxidation (Van Oeckel et al., 1997). Feeding 15-35 grams of 18:3 n-3/kg resulted in worse eating quality (Ahn et al., 1996). Feeding 10-15 % flax seed (25 days before slaughter) resulted in a more flavour-intense meat (Romans et al., 1995a; Romans et al., 1995b). For fish fat a maximum of 10 % inclusion of fishmeal (8-10 % crude fat) has been suggested for fresh meat (Valaja et al., 1992). For meat stored for 6 months Kjos et al. (1999) suggests no more than 3 g/kg feed of fish fat until slaughter or 5 g/kg until 60 kg.

In summary a back fat content of 22 % PUFA (mainly 18:2 n-6) does not seem to have any negative effects on fat consistency and oxidation stability. However, this

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level will have to be modified depending on the type of fatty acids and antioxidants included in diet, duration of feeding, processing and storage of the meat.

Vitamin E

Dietary vitamin E at supra nutritional levels to pigs and its effect on different quality parameters in meat have been investigated in numerous studies as reviewed by others (Morrissey et al., 1994; Buckley et al., 1995; Morrissey et al., 1998; Sahoo and Verma, 1999). The level of protection of vitamin E in different meat products is dependent on processing and storage conditions. In general the reduction of lipid oxidation in fresh and cooked meat and meat products stored at +4°C for 5-9 days (varying illumination and modified atmosphere) was between 22-94 % when feeding between 100-800 mg all-rac-α-tocopherol/kg feed (Jensen et al., 1998a). Similar investigations have been done on frozen meat and meat products stored for 13-16 weeks. The reduction in lipid oxidation was between 42-67 % (Jensen et al., 1998a). The dietary lipid source will affect the reduction in lipid oxidation caused by vitamin E. Monahan et al. (1992) have shown that feeding of 200 mg α-tocopherol acetate decreased lipid oxidation in pigs fed both soy oil and beef tallow. However, the decrease was greater in the soy oil fed pigs. Similar results have been shown by Rey et al. (2001) feeding sunflower oil, olive oil and linseed oil. Furthermore, the optimal level of dietary vitamin E needed to keep an acceptable lipid stability in meat products can be discussed. This will naturally depend on the fat source used, processing and storage. For example, Onibi et al. (2000) found that when feeding 25 % full fat rape seed, additional feeding above 200 mg/kg α-tocopherol acetate did not improve oxidation stability.

Objectives and experimental design of the individual studies

The overall aim of the work presented here was to study the effect of rearing conditions such as feed and outdoor environment, sex and RN genotype, on the content of antioxidants and the fatty acid composition in PL and NL of pig muscle. Furthermore, we aimed to investigate how the altered fatty acid composition affected the oxidative stability in frozen meat stored for one year at –20°C.

Study I

Objectives: To establish if and how outdoor rearing of pigs influences muscle (*M. Biceps femoris*) fatty acid composition in NL and PL, and to evaluate if there was a difference between female and castrated males in the fatty acid composition of these lipid classes.

Experimental design: The animals studied were a subsample of 24 animals selected from 120 Hampshire crossbreeds ((Hampshire) x (Swedish Landrace x Swedish Yorkshire)) (castrates and female pigs). The samples used in the present study were chosen randomly for each sex and rearing condition within the non carriers of the RN^4 allele ($rn^+ rn^+$). The same basal diet was used for both rearing groups and the outdoor group had access to growing peas, oats and barley which had been sown in a so called 'strip grazing' fashion.

Study II

Objectives: To investigate how fatty acids of NL and PL in pig muscle (*M. longissimus dorsi*) were affected by dietary fatty acids, RN genotype and sex, using castrated male and female pigs. In addition, the contents of retinol, ζ-tocopherol, ν-tocopherol and the level of lipid oxidation were studied.

Experimental design: The animals studied were a subsample of 40 animals selected from a total of 80 crossbreeds ((Hampshire) x (Swedish Landrace x Swedish Yorkshire)). The animals were reared in an indoor or outdoor production system and randomly chosen for each sex, feed and RN genotype.

Study III

Objectives: To investigate the effect of organic and conventional feed, with regard to different n-6/n-3 ratios in the diet, on the fatty acid composition in lipid classes and lipid oxidation of female and castrated male pig muscle (*M. longissimus dorsi*). Furthermore, we aimed to investigate how the fatty acid composition of different lipid classes correlated with carcass composition parameters such as subcutaneous fat thickness, lean meat percent and IMF content.

Experimental design: The animals studied were a subsample of 44 animals selected from a total of 232 crossbreeds ((Hampshire) x (Swedish Landrace x

Swedish Yorkshire)). The samples used in the study were chosen randomly for each sex and feed group within the non carriers of the RN^4 allele ($rn^+ rn^+$).

Study IV

Objectives: To investigate the difference in fatty acid composition in muscle (*M. longissimus dorsi*) PL and NL between entire male, female and castrated male pigs. Further, the aim was to follow up Study I and investigate the effect of outdoor rearing on the fatty acid composition of NL and PL in the 3 sexes.

Experimental design: The 39 animals used in this study were selected from a total of 140 crossbreeds ((Hampshire) x (Swedish Landrace x Duroc)), divided into two rearing conditions. The outdoor pigs, except the entire males, were raised together, in an enclosed field of about 6000 m^2 clay soil overgrown with grass. The entire males were reared on a field on their own outdoors and in a single box indoors after the weight of 60 kg. The animals were selected randomly for each fixed factor investigated within the carriers of the RN^4 allele ($RN^4 rn^+$).

Methodology

Analyses of the assumed RN genotype

In Study I the RN genotype was predicted by measuring the concentration of glucose + glucose-6-phosphate in meat juice from *M. longissimus dorsi* (LD) as described by Lundström and Enfält (1997). By using this concentration, each animal of the population was subordinated to a bimodal distribution. In this study the experimental dividing line and valley point between the two groups was set at 35 σmol glucose + glucose-6-phosphate/ml meat juice. Animals that revealed a glucose + glucose-6-phosphate concentration in the meat juice that was close to this value were not included in the study.

In Studies II and III, the assumed RN genotype of each individual pig was determined by the method of Dalrymple et al. (1973) by enzymatic determination of the concentration of residual glycogen (including glucose and glucose-6-phosphate) in homogenized muscle tissue. Animals with a concentration of less than 35 σmol/g of *M. Longissimus dorsi* were considered as not carrying the RN[4] allele, while animals with a concentration above 35 σmol/g were considered as carriers of the RN-allele. Since there is some overlap in the distribution of glycogen content between the carriers and non-carriers of the RN[4]allele, a diagnostic DNA test for the causative mutation (Milan et al., 2000) was carried out on some individuals with glycogen concentrations between 30 and 40 σmol to avoid misclassifications. In Study IV, the RN genotype was determined as in Studies II and III or by using RN homozygote fathers.

Fat content and fatty acid analyses

The intra muscular fat content (IMF) of *M. Longissimus dorsi* (LD) was analyzed using Soxhlet analysis (Soxtec System H^{+} equipment, Tecator AB, Höganäs, Sweden) or by using the method of Hara and Radin (1978). In the fat content analysis and in all analyses below, meat without visible connective tissue and subcutaneous fat was used. All solvents and chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany) unless otherwise stated.

For fatty acid analysis minced muscle, approximately 15 g in duplicate samples, was homogenized in hexan:isopropanol (3:2) and after filtration the nonlipids were removed by adding aqueous sodium sulfate (6.67 %) (Hara and Radin, 1978). The suspension of lipids were evaporated to dryness in a Büchi Rotavapor at 38 ºC. Total lipids were separated into neutral lipid and polar lipid fractions using a method described by Prieto et al. (1992). NL and PL were eluted in Solid Phase Extraction columns (Isolute; 500 mg, 6 ml). The columns were activated with 6 ml hexane and NL was eluted with 18 ml diethyl ether: acetic acid (100:0.2) solution. PL was eluted with 6 ml methanol. Samples were evaporated to dryness under nitrogen gas. Fatty acid methyl esters (FAME) from both NL and PL were prepared in two steps with NaOH in dried methanol and with BF_3 in methanol (Studies I, II and III) according to the method described by Dutta and Appelqvist (1989) or with NaOH in dried methanol alone as described by

Appelqvist (1968) (Study IV). Analytical TLC (thin-layer chromatography) was carried out to check the FAME preparation.

For the GC analysis, fused silica capillary column BPX 70 (SGE, Austin, Texas, USA), length 50 m, i.d. 0.22 mm, 0.25 μm film thickness, was used for the separation of FAME and helium was used as a carrier gas. The column was fitted in a Chrompack CP 9001 gas chromatograph (Chrompack, Middelburg, Netherlands) equipped with a flame ionization detector and split/splitless injector. The peak areas were analyzed using a Maestro 2 version 2.4 integrator (Chrompack, Middelburg, Netherlands). To assess the variation in detector response to different FAME, a reference sample mixture GLC-68 A (Nu-Chek Prep, Elysian, USA) was run. No response factors were applied when calculating the percentage distribution of the various FAME.

GC/MS identification of unknown compounds

The unknown compounds were analyzed using the same column and under the same conditions as described above. GC-MS analysis was performed on a GC 8000 Top Series gas chromatograph (ThermoQuest Italia S. p. A., Rodano, Italy) coupled to a Voyager mass spectrometer with the MassLab data system version 1.4V (Finnigan, Manchester, England). Helium was used as carrier gas at an inlet pressure of 150 kpa. The injector temperature was 230°C and the samples were injected in a split mode of injection. The mass spectra were recorded at an electron energy of 70 eV and the ion source temperature was 200°C.

Malondialdehyde and vitamin analyses

MDA, retinol and tocopherols were determined by HPLC using a Merck Hitachi L6200A pump, a FL L7480 detector and an As-2000 A auto sampler. The HPLC column was a 4.0 x 250 mm RP-18 LiChroCART. Identification and quantification were done by using external standards. For the analysis of retinol and tocopherols a modified method by Jensen et al. (1998b) was used. Meat weighing 2x1 grams was cut into pieces and homogenized in two tubes, after which 1.2 ml of 20 % ascorbic acid solution, 0.6 ml methanol and 1.2 ml of KOH-water (1:1) were added to each tube. After saponification and cooling, retinol and tocopherols were extracted in 2x4 ml of hexane. The hexane-vitamin solution was evaporated under nitrogen gas and diluted with the mobile phase. The mobile phase used for retinol and tocopherols consisted of 95 % metanol:acetonitrile (1:1) and 5 % chloroform with a flow rate of 1.2 ml/min. Tocopherols and retinol were detected with excitation wavelengths of 290 and 344 nm, respectively, and with emission wavelengths of 327 and 472nm, respectively.

For MDA (malondialdehyde) analysis the method of Draper et al. (1993) (method 4) was used for preparing the meat samples before HPLC analysis. Meat weighing 2x1 grams was homogenized and after preparation 300 σl of MDA/butanol solution was diluted 10 x with the mobile phase. HPLC analysis was performed according to the method of Öhrvall et al. (1994) (excitation 532 nm and emission 553 nm). A mobile phase consisting of 60 % potassium phosphate buffer (50mM, pH 6.8) and 40 % methanol with a flow rate of 0.7 ml/min was used.

Feed analyses

The energy analysis was performed using the method described by Boisen et al. (1997). The fatty acid composition of the feed samples was analyzed as described above after grinding of the feed. Retinol and tocopherols were extracted according to the method of Børsting et al. (1994) and analyzed by HPLC.

Statistical analysis

Statistical evaluation was carried out using Minitab statistical software for Windows 95 and NT (ver.12) (Minitab, 1998). Production system, feed, sex or RN genotype were regarded as fixed effects in the individual studies and were tested by analysis of variance (GLM procedure) and different groups were compared on the basis of least-squares means. Normal distributions were tested using the Andersson-Darling normality test. Intramuscular fat content (IMF) was used as covariate in both NL and PL. In the case of interaction between fixed effects, a pairwise comparison was made, using Tukey's method. If a covariate or an interaction failed to reach significance ($p>0.05$), the term was excluded from the model. In addition, for some of the fatty acids in Studies II and IV the interaction between rearing and sex was included to show the subgroup means in spite of non-significant interactions. Furthermore, Pearson overall and partial correlation coefficients were calculated between variables of interest.

For multivariate statistical analysis, Unscrambler 7.5 was used (Esbensen et al., 1998). The procedure for choosing variables was done as follows. Principal component analysis (PCA) were used and variables poorly explained by the first two principal components were excluded from further analysis. The models were validated by cross validation and all variables were weighted with 1/standard deviation and centred.

Results

Study I. Effect of rearing system on muscle lipids of gilts and castrated male pigs

The outdoor rearing of pigs is suggested to contribute to animal welfare as well as to an increased level of some health beneficial fatty acids in pig tissues. In this study a higher level of PUFA n-3 was seen in the muscle NL of free-range pigs. Rearing conditions affected the n-6/n-3 ratio in NL. Although both the indoor and the outdoor pigs exceeded the level for the recommended n-6/n-3 ratio (4-6), the ratio in the outdoor pig muscle was closer to the recommended level. Also a slightly higher level of total trans fatty acids was obtained in the NL of the outdoor pig muscle. Regarding PL there were significant interactions between rearing condition and sex showing a higher PUFA n-6 level in the muscle of outdoor compared with indoor female pigs whereas no differences occurred between the castrated male groups. It was shown that, independent of rearing condition, a higher level of C 20 PUFA in the muscle PL of female pigs was present. Our results could indicate that the differences between sexes in fatty acid composition found in membranes is caused by a difference in metabolism between castrates and female pigs.

Study II. Muscle lipids, vitamins E and A, and lipid oxidation as affected by diet and RN genotype in female and castrated male Hampshire crossbred pigs

In this study the pigs that received the highest PUFA diet had a higher level of these fatty acids in muscle NL. The level of C18:2 n-6 only increased in NL and not in PL even if this effect was more pronounced in the female pigs. The female pigs given the diet with more PUFA had a lower content of ζ-tocopherol, which resulted in a higher degree of oxidation in the meat from these pigs. This result was further strengthened by the negative correlation between MDA and ζ-tocopherol content. Thus, differences in metabolic activity between sexes, in addition to the relatively low content of ζ-tocopherol in the indoor female pig muscle, might contribute to the increased susceptibility of the indoor female pig muscle to postmortem oxidation. Differences in fatty acid composition between RN genotypes were found in PUFA n-3 and PUFA n-6 of PL, whereas no difference was found in NL. However, there was an interaction between sex and RN genotype in PL. The content of ν-tocopherol in pig muscle was relatively low compared with ζ-tocopherol in our study. No effect due to sex, RN genotype or rearing condition could be seen on the content of retinol in muscle.

Study III. Fatty acid composition and tocopherol content of muscle in pigs fed organic and conventional feed with different n6/n3 ratios respectively

A negative correlation between both 18:2 n-6, 18:3 n-3 and HUFA in NL and carcass composition parameters was found. However, in PL correlations was found between 20:4 n-6, 22:6 n-3 and carcass composition measurements. In both lipid classes in this study, 18:3 n-3 was affected by diet, but the muscle content of 18:2 n-6 differed only in the PL and not in the NL. These results suggest a complex relationship regarding how PUFA n-3 and PUFA n-6 are incorporated into muscle lipid fractions or used as an energy source. This study showed a sex difference in fatty acid composition in muscle PL and NL. The muscles of female pigs were found to have a higher PUFA level and a higher HUFA level. It can be suggested that the castration of male pigs affects the desaturation and elongation of 18:2 n-6 and 18:3 n-3. Despite differences in fatty acid composition due to feed in both lipid classes in the present study, no effect on oxidation stability was found. However, a negative correlation between MDA and ζ-tocopherol was obtained. Furthermore, a higher content of ζ-tocopherol was found in female pig muscle, accompanying the more unsaturated fatty acid content.

Study IV. Fatty acid composition and tocopherol concentrations in muscle of entire male, castrated male and female pigs, reared in an indoor or outdoor housing system

The outdoor pig muscle was richer in PUFA than the indoor pig muscle. However, the difference in 18:3 n-3 in NL and total PUFA n-3 in PL was only found between the outdoor and indoor entire males and not between the two female and castrated male groups. On the other hand, total HUFA n-3 in PL was higher in all outdoor pig muscle independent of sex. Furthermore, there was an increased level of 18:2 n-6 in the pig muscle PL of outdoor reared female pigs compared with the indoor female pig. The differences between the sexes in NL and PL are suggested to depend on either a difference in foraging behaviour or that the outdoor environment, e.g. light, influences the fatty acid metabolism. Independent of rearing condition, a difference between sexes in the level of PUFA in muscle lipids was found. In NL the entire males had overall more PUFA compared with castrated males and female pigs. In the polar lipids the levels of HUFA n-6 and HUFA n-3 were higher in the female and entire male pig muscle compared with the castrated male pig muscle. In addition, the muscle of castrated males had a lower 18:2 n-6/20:4 n-6 ratio in the NL and higher in the PL compared with entire males and female pigs. The 18:2 n-6/20:4 n-6 ratio is an indicator of activity of the enzyme responsible for the ÷-6 desaturation. The results may indicate a decreased desaturation activity due to castration or decreased demand for eicosanoids. The higher levels of PUFA and HUFA in the entire male pigs were not accompanied by a higher level of antioxidants. The highest content of ζ-tocopherol was found in the muscles of female pigs and the highest content of ν-tocopherol in the castrated male pigs.

Effect of different levels of PUFA on performance and fatty acid composition of rat liver

There are numerous studies that have investigated the effect of different fat sources on the fatty acid composition in different tissues of pigs and the end quality of the meat products. There have been recent suggestions that some PUFA, e.g. PUFA n-3, have an influence on many physiological parameters and metabolism in general (Horrocks and Yeo, 1999; Clarke, 2000; Lauritzen et al., 2001). Therefore feeding our domestic animals an increased level of dietary PUFA in order to produce healthier meat would probably result in some effects on performance. Thus, to investigate the possible effects on the performance of PUFA, a rat study was conducted feeding similar or lower levels of PUFA n-3 and PUFA n-6 fatty acids as described in Studies I-IV or by others (see above).

Table 5 a. Fatty acid omposition (%) of feeds and of liver NL in rats fed sunflower oil (1), control (2), linseed oil (3) diet (n= 12 in each dietary group) (least-squares means and standard error)

Fatty acid	Feed 1	2	3	NL 1	2	3	SE	p-value
18:2 n-6	49.96	36.35	38.84	14.16	12.24	12.10	0.72	0.09
18:3 n-6				0.19^a	0.16^{ab}	0.12^b	0.01	0.002
18:3 n-3	3.67	3.81	16.78	0.50^a	0.57^a	2.31^b	0.07	0.001
20:2 n-6	0.15	0.26	0.17	0.34	0.29	0.25	0.04	0.20
20:3 n-6				0.21	0.17	0.16	0.02	0.23
20:4 n-6				1.53^a	1.31^{ab}	1.05^b	0.13	0.03
20:5 n-3				0.08^a	0.09^a	0.45^b	0.02	0.001
22:4 n-6				0.71^a	0.54^{ab}	0.23^b	0.09	0.003
22:5 n-6				0.37^a	0.26^b	0.06^b	0.04	0.001
22:5 n-3				0.32^a	0.37^a	1.23^b	0.10	0.001
22:6 n-3				0.51^a	0.61^a	1.59^b	0.12	0.001
MUFA	25.21	31.22	22.47	41.16^a	43.87^b	40.84^a	0.72	0.009
HUFA				4.26	3.80	5.24	0.49	0.12
HUFA n-6				3.35^a	2.72^{ab}	1.88^a	0.31	0.007
HUFA n-3				0.91^a	1.08^a	3.37^b	0.24	0.001
PUFA	52.79	40.42	55.79	18.91	16.60	19.66	1.22	0.20
PUFA n-6	49.12	36.61	39.00	17.51^a	14.96^{ab}	13.98^b	0.99	0.047
PUFA n-3	3.67	3.81	16.78	1.40^a	1.64^a	5.67^b	0.30	0.001
HUFA n-6 /HUFAn-3				3.85^a	2.59^b	0.60^c	0.09	0.001
n-6/n-3	13.38	9.60	2.32	13.19^a	9.39^b	2.56^c	0.35	0.001

[abc] Values with different letters within a row are significantly different (p<0.05).

In the study, 36 male Sprague-Dawley (SD) rats were divided in 3 groups and fed 3 isoenergetic diets. The 3 diets contained either 1 % pork fat (control), 0.75 % sunflower oil and 0.25 % pork fat or 0.75 % linseed oil and 0.25 % pork fat. Fatty acid composition of the diets is shown in Table 5. The rats were fed the diets from weaning at 21 days until the age of 103 days. A skin wound healing test was performed at the age of 60 days. Two biopsies per rat were made with a 4 mm in diameter dermal biopsy punch, and the wounds were measured using a vernier calliper at the start of trial and thereafter every 24 hours for 7 days.

Table 5 b. Fatty acid composition (%) of liver PL in rats fed sunflower oil (1), control (2), linseed oil (3) diet (n= 12 in each dietary group) (least-squares means and standard error)

Fatty acid	PL 1	2	3	SE	p-value
18:2 n-6	12.16[a]	11.67[a]	13.43[b]	0.26	0.001
18:3 n-6	0.13	0.12	0.11	0.01	0.31
18:3 n-3	0.04[a]	0.04[a]	0.18[b]	0.01	0.001
20:2 n-6	0.44	0.40	0.41	0.02	0.34
20:3 n-6	1.39[a]	1.33[a]	1.60[b]	0.05	0.003
20:4 n-6	25.86[a]	25.84[a]	19.64[b]	0.54	0.001
20:5 n-3	0.18[a]	0.26[a]	1.77[b]	0.05	0.001
22:4 n-6	0.63[a]	0.55[a]	0.30[b]	0.02	0.001
22:5 n-6	1.04[a]	0.75[b]	0.19[c]	0.05	0.001
22:5 n-3	1.04[a]	1.15[a]	2.28[b]	0.09	0.001
22:6 n-3	5.14[a]	5.97[a]	8.29[b]	0.28	0.001
MUFA	9.61	10.24	9.79	0.26	0.22
HUFA	36.99[a]	36.54[a]	34.77[b]	0.43	0.002
HUFA n-6	30.49[a]	29.01[a]	22.25[b]	0.49	0.001
HUFA n-3	6.50[a]	7.53[b]	12.52[c]	0.26	0.001
PUFA	49.19	48.25	48.38	0.33	0.11
PUFA n-6	42.65[a]	40.68[b]	35.68[c]	0.48	0.001
PUFA n-3	6.53[a]	7.57[b]	12.70[c]	0.25	0.001
HUFA n-6/ HUFA n-3	4.74[a]	3.92[b]	1.80[c]	0.15	0.001
n-6/n-3	6.58[a]	5.47[b]	2.83[c]	0.20	0.001

[abc] Values with different letters within a row are significantly different (p<0.05).

No differences in live weights, growth performance or organ weights were found among the different dietary treatments. The level of PUFA in the liver PL and NL were affected by diet. Compared with the control diet, the rats fed sunflower oil had a higher level of n-6 fatty acids and the rats fed linseed oil had a higher level of n-3 fatty acids (Table 5 a and b). Other results that were shown in this study were an increased level of desaturation and elongation products of the 18:3 n-3 to HUFA n-3 in the linseed fed group.

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In the wound healing test we could see an improved wound healing after 7 days both in the linseed oil and the sunflower oil fed rats compared with the control (Figure 5). After 4 days of wound healing there were significant differences between the control group and the other two feeding regimes. The differences remained after 7 days. After 5 days the crust was still stuck to the wound in both wounds in 11 of 12 control animals. The corresponding number for the rats fed sunflower diet was 2 out of 12 and for linseed diet 3 out of 12.
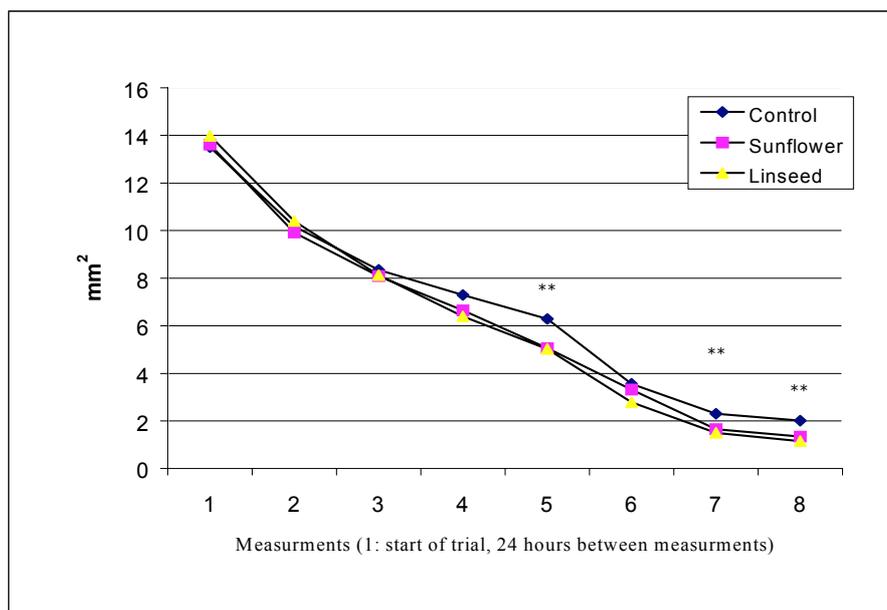
Figure 5. Wound healing during 7 days in rats fed a control, sunflower oil or linseed oil diet
** Significant difference between diets; p<0.01.

Multivariate analysis revealed a dietary effect along PC1 and that the loading for wound healing had a closer grouping to the PUFA n-3 fatty acids compared with PUFA n-6 fatty acids (Figure 6). Also, there was a positive correlation between wound healing, 18:3 n-3, 18:2 n-6 and HUFA n-3 in PL, whereas there was a negative correlation between 20:4 n-6 in PL and wound healing (Table 6). A similar trend could be seen between PUFA n-3 in NL and wound healing where a positive correlation was found. Finally, the coefficients of variations in NL of the rats fed the linseed oil diet were lower in almost all fatty acids and in the total sum of CV (Table 7) in comparison to the other two diets. Similar trends could be seen in PL especially in the total sum of CV.

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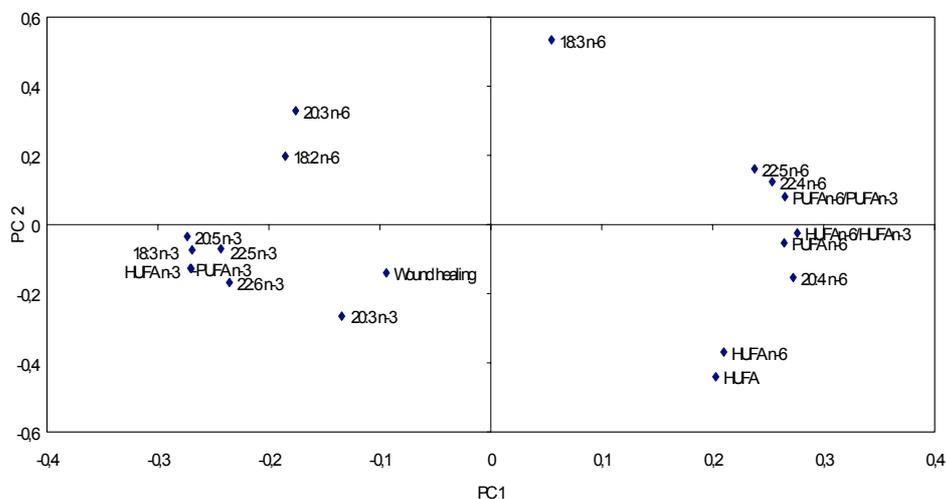
Figure 6. PCA plot of wound healing, individual and groups of PUFA in polar lipids of rat liver. Explained X variance in PC 1, 66 % and PC2, 10 %.

Table 6. Correlation coefficients between wound healing during 4-7 days and the level of important individual fatty acids and groups of fatty acids in PL and NL of rat liver

	PL				NL			
	4	5	6	7	4	5	6	7
18:2 n-6	0.39	0.34	0.33	0.35	---	---	---	---
18.3 n-3	0.34	0.35	0.33	0.32	0.37	0.37	0.35	0.36
20:4 n-6	-0.36	-0.36	-0.34	-0.27	---	---	---	---
20:5 n-3	0.28	0.30	0.28	0.27	0.37	0.32	0.32	0.36
22:5 n-3	0.36	0.34	0.34	0.32	0.43	0.37	0.37	0.42
22:6 n-3	0.26	0.33	0.29	0.25	0.41	0.40	0.40	0.43
MUFA	---	---	---	---	-0.43	-0.36	-0.36	-0.30
HUFA n-6	-0.33	-0.30	-0.29	-0.28	---	---	---	---
HUFA n-3	0.32	0.35	0.33	0.29	0.42	0.38	0.38	0.42
PUFA n-6/ PUFA n-3	-0.26	-0.31	-0.27	-0.22	-0.21	-0.30	-0.25	-0.32

Correlation (r-value) >0.32; p< 0.05. Correlation (r-value) >0.28; p< 0.10.

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Table 7. CV (%) in rat liver fatty acid composition (1:sunflower oil; 2: control; 3: linseed oil (n= 12 in each dietary group)

Fatty acid	NL			PL		
	1	2	3	1	2	3
18:2 n-6	22.43	20.21	13.56	8.16	8.03	5.66
18:3 n-6	24.72	29.37	13.95	27.83	20.85	19.53
18:3 n-3	28.37	21.31	16.91	23.50	15.66	11.08
20:2 n-6	47.01	43.41	27.39	20.64	12.45	11.35
20:3 n-6	48.60	38.69	22.41	17.41	14.04	6.64
20:4 n-6	37.92	33.08	21.05	6.46	7.44	9.82
20:5 n-3	53.82	47.12	30.08	37.84	26.35	14.35
22:4 n-6	61.43	60.69	37.42	15.25	11.99	9.21
22:5 n-6	54.21	40.27	41.65	21.25	24.86	17.61
22:5 n-3	68.71	64.47	41.78	19.15	27.14	16.70
22:6 n-3	50.65	36.14	38.43	11.99	17.15	14.34
OCV[1]	497.87	434.76	304.63	209.48	185.96	136.29

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General discussion

Methodological considerations

In Study IV as compared with Studies I, II and III the method for the preparation of the fatty acid methyl esters (FAME) was changed. In Studies I, II and III the method with BF_3 was used as the samples contained some amount of free fatty acids. However, samples in Study IV contained less free fatty acids and therefore BF_3 was not added.

In Studies I-IV some unidentified peaks occurred at RT 10.75, 15.08, 15.89, and 17.32 minutes (Figure 8). Surprisingly, the peak at 17.32 minutes was not as large when running the sample on GC-MS as in the ordinary GC analysis used in Studies I, II, III and IV, which makes the interpretation of the mass spectra difficult (Figure 9). The other three compounds have tentatively been identified from the mass spectra as dimethyl acetals formed from the phospholipid plasmalogen, i.e. alkenyl ethers (Christie, 2002). In bovine heart muscle 16 and 11 % of the total phospholipids was the alkenyacyl form of phosphatidylcholine and phosphatidylethanolamine respectively (Schmidt and Takahashi, 1968). The corresponding figures for the diacyl forms were 25 and 16 % (Schmidt and Takahashi, 1968). When plasmalogen is treated with acidic transesterification reagents, the vinyl ether bond is broken and aldehydes are generated that are immediately converted into dimethyl acetals (Figure 7) phases (Christie, 2002). These are almost exclusively saturated and monounsaturated (C16 and C18), and they tend to elute just before 16:0 and 18:0 methyl esters on most GC phases (Christie, 2002).

The mass spectra of these compounds have been found to have a base peak at m/z = 75 (Figures 10-12). The first compound eluted at 10.75 minutes was found to be the dimethyl acetal of hexadecanal (Figure 10). The first ion in the high mass range found in this compound was m/z = 255 (BPI % 6.11) but the molecular weight is 286 (BPI % 0.27). M/z 31 represents a loss of a methoxyl ion. The two compounds eluted at 15.08 and 15.89 have been found to be octadecanal and octadec-9-enal (Figure 11 and 12). The first ions in the high mass range of these compounds were m/z = 283 (BPI % 6.18) and m/z =281 (BPI % 9.58) The molecular weight on these compounds is 314 (BPI % 0.46) and 312 (BPI % 1.18) (Christie, 2002).
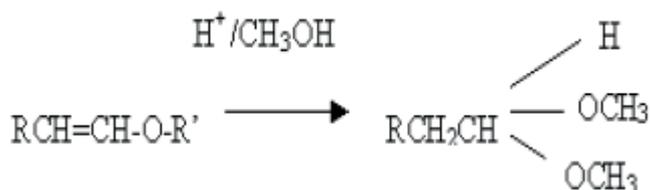
Figure 7. Reaction pattern of plasmalogen with methanol under acidic conditions (adapted from Christie (2002)).

40
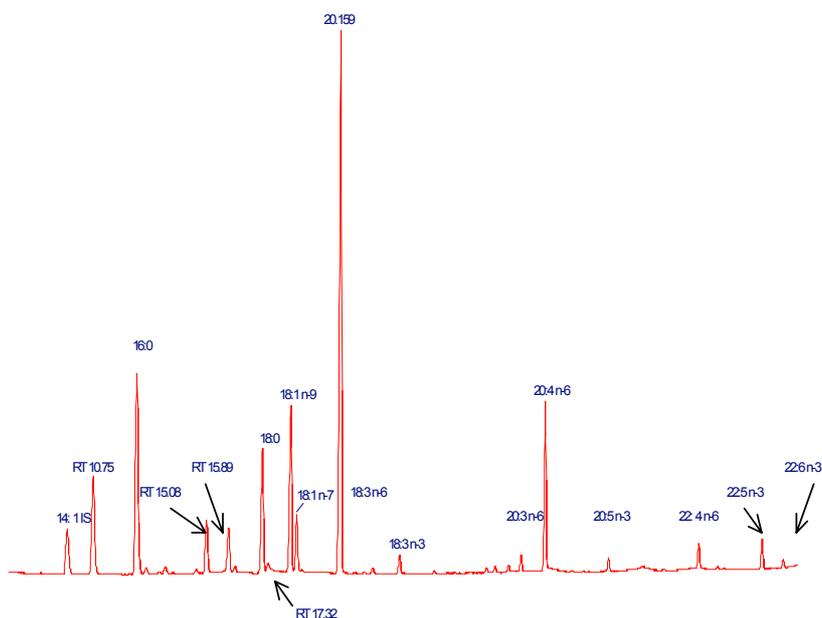
Figure 8. GC-MS chromatogram of fatty acid methyl esters in PL fraction of a sample in Study IV. Mass spectra of the tentatively identified diacetals at RT10.75, RT 15.08, RT 15.89 are shown below in Figure 10-12. Mass spectrum of unknown compound at RT 17.32 is shown in Figure 9.
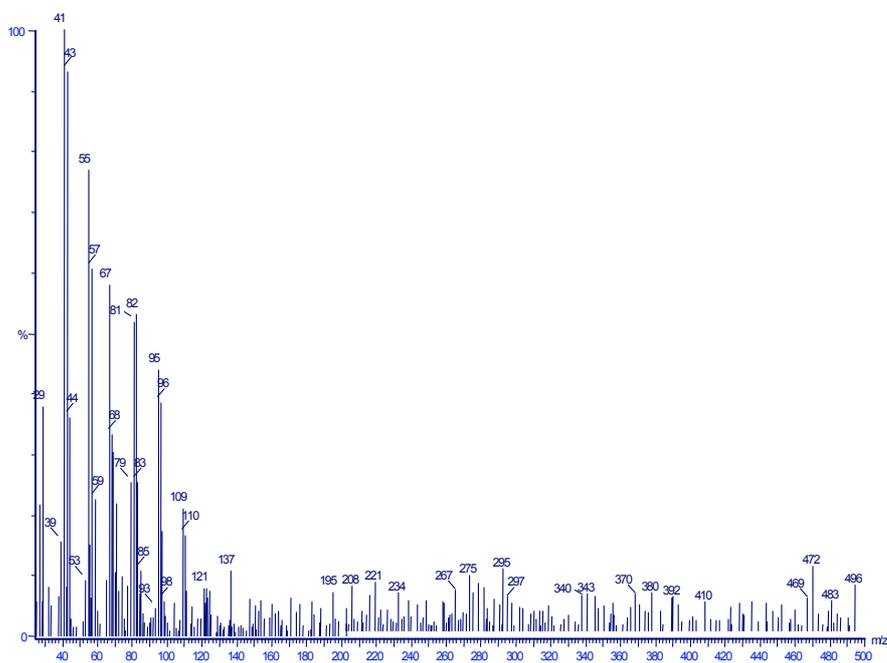
Figure 9. Mass spectrum of unknown fatty acid at retention time 17.32 minutes run with the GC method described under section Fat content and fatty acid analysis
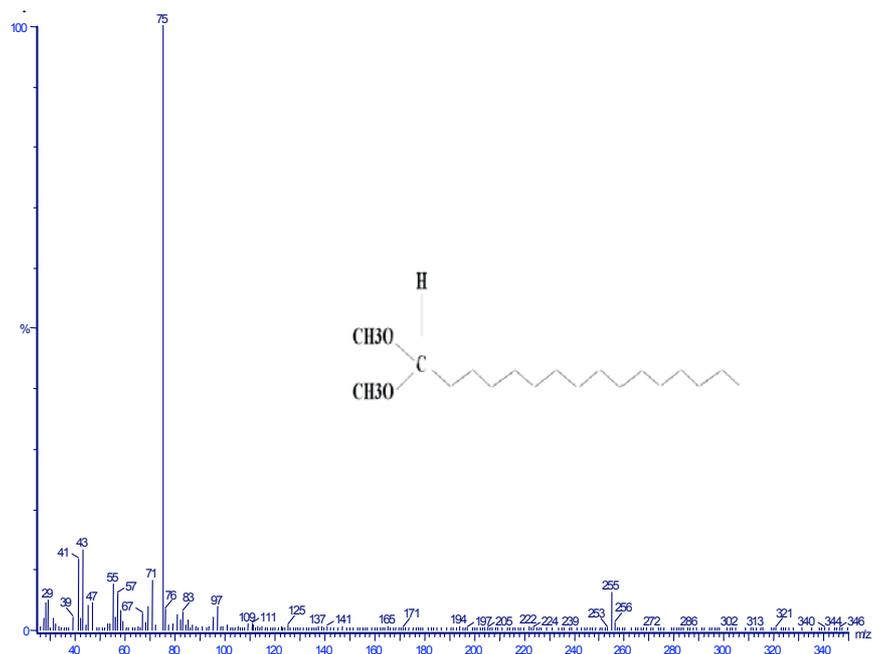
Figure 10. Mass spectrum of dimethyl acetal of hexadecanal with the retention time 10.75 minutes.
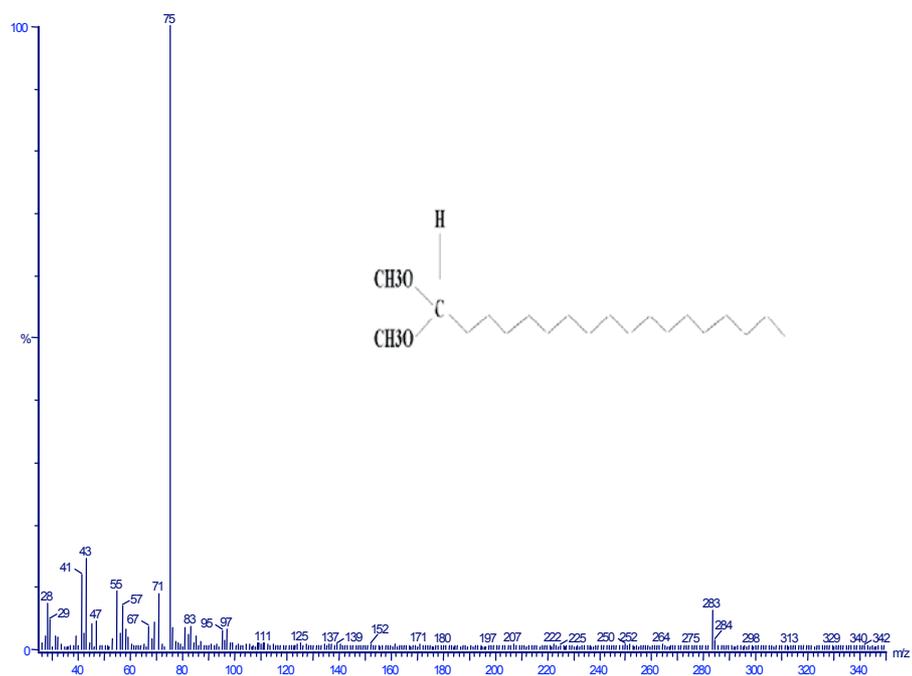
Figure 11. Mass spectrum of dimethyl acetal of octadecanal with the retention time 15.08 minutes.
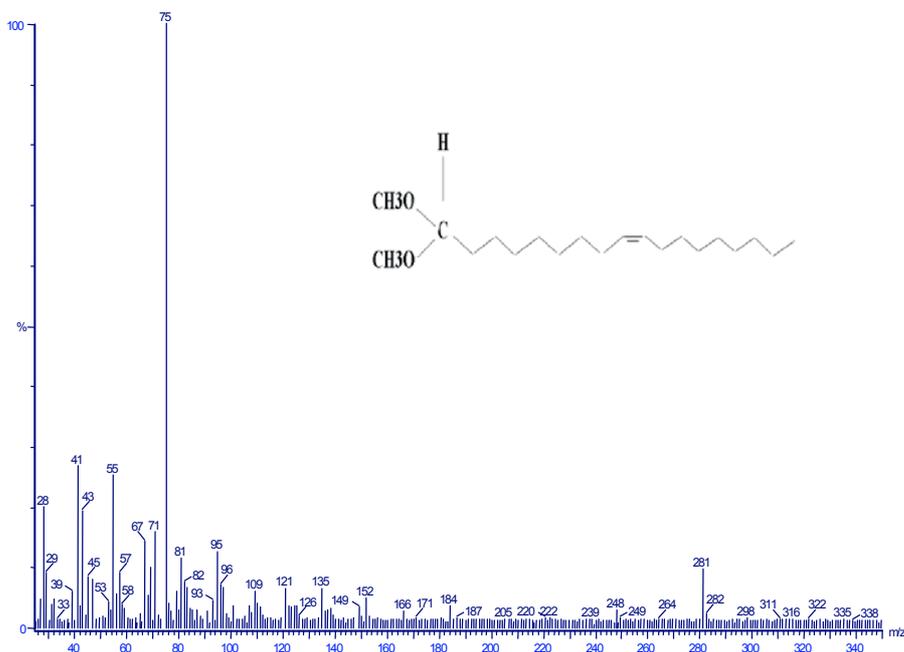
Figure 12. Mass spectrum of dimethyl acetal of octadec-9-enal with the retention time 15.89 minutes.

After final identification the diacetals should be added to the respective fatty acid, i.e. 16:0, 18:0 and 18:1 n-9, and as a consequence there will be higher levels of these fatty acids. For the interpretation of the data in Studies I-IV in PL the identification of these three substances will have a minor influence on the results discussed. In Studies I-III the tentatively identified diacetals is either found in the Tables as unknown or they have been included in the pool of unidentified peaks. In Study IV, the tentatively identified diacetals from 16:0, 18:0, 18:1 n-9 have been summed and are showed as such.

Effects of free range system and dietary PUFA on fatty acid composition and antioxidant content

The rearing of outdoor pigs has become more and more frequent in recent years. This type of rearing system is suggested to be better for the pig and therefore has become a demand that farmers must meet before selling the meat under certain ecological brand names. Moreover, since the health benefits of PUFA n-3 are recognized (Horrocks and Yeo, 1999) and because grass contains high levels of PUFA n-3 (Garton, 1965), meat from pigs having access to pasture might have nutritional benefits. In Study I, NL of muscles from outdoors pigs had a higher level of PUFA n-3, which previously have been indicated in back fat (Van der Wal et al., 1993; Jakobsen, 1995) and muscle total lipids (Nilzén et al., 2001). However, in Study IV the difference in 18:3 n-3 of muscle NL was only found

between the outdoor and indoor entire males. The levels of increased PUFA n-3 in both Studies I and IV were quite low which would indicate that pigs, even when they have access to fresh pasture, do not graze heavily. This has also been suggested recently in a study by Andresen and Stern (2002). In Study II the pigs that received the most PUFA n-6, PUFA n-3 and HUFA in the basal diet had a higher level of these in muscle NL. However, as in Study I and Study IV the effect of diet appeared in one sex and not in the other, i.e. the female pigs were affected by dietary PUFA and castrated males were not. It has previously been shown that feeding pigs with PUFA resulted in an increase in PUFA in both NL and PL of muscle (Monahan et al., 1992; Warnants and van Oeckel, 1996; Warnants et al., 1999; Enser et al., 2000) and that this increase was dependent on sex (Pfalzgraf et al., 1995)

It is difficult to explain the increased level of PUFA in females and entire males, due to diet and outdoor rearing, whereas no effect occurred in castrated males (Studies I, II and IV). This sex difference in PUFA level can be suggested to depend on differences in grazing behaviour, incorporation of PUFA into fat tissues or a slight alteration of PUFA metabolism, possibly mediated by light. Moreover, an overall higher level of ζ-tocopherol in the female pigs fed low PUFA was found (Study II). The female pigs with the highest level of postmortem ζ-tocopherol in muscle were reared outdoors. These pigs had access to grass which is generally rich in antioxidants, among them tocopherols. Moreover, Studies III and IV showed that female pigs had a higher content of ζ-tocopherol in muscle. Therefore it can be suggested that the high PUFA diet in Study II led to a higher turnover of ζ-tocopherol in the female pigs in vivo, resulting in a lower postmortem level. In summary, these results indicate that the response to dietary PUFA is greater in animals with an intact reproductive organ. The overall altered metabolism due to castration seems to change the PUFA metabolism and the content of antioxidants.

The increases in PUFA n-3 fatty acids in Studies I and IV in the outdoor pigs can be said to increase the nutritional value of the pig meat, even though the effect is small. The small increase will not have any negative effects on the oxidation stability (Nilzén et al., 2001) as muscle from outdoor pigs might contain higher amounts of antioxidants (Study II, Nilzén et al (2001)). However, increased PUFA in female pig muscle in combination with a lower content of ζ-tocopherol resulted in increased oxidation (Study II). Similar results have been shown earlier by Nilzén et al. (2001) in *M. Biceps femoris* and Pfalzgraf et al. (1995) in *M. longissimus dorsi*. Thus, the possible differences in metabolic activity and incorporation of PUFA, in addition to the relatively low content of ζ-tocopherol, contributed to the increased susceptibility of the indoor female pig muscle to postmortem oxidation. However, the difference in susceptibility to oxidation was rather small and did not exceed the threshold for detection in raw pork by a trained sensory panel (0.50 mg malondialdehyde equivalents/kg meat; reviewed by Jensen et al. (1998a)).

A slightly higher level of total trans fatty acids was obtained in the outdoor pigs than in indoor pigs (Study I). Trans fatty acids have been reported to have

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negative nutritional effects on humans (Kelly, 2001). The level of trans fatty acids in pig meat is in general low compared with ruminant meat. However, the trans fatty acid level in pig meat products can be increase due to diet, as shown in back fat (Fontanillas et al., 1998). In our study the explanation for the increased level of trans fatty acids in the pig meat can only be hypothesized, as there is no obvious source influencing the level. The effect is suggested to depend on the additional feeding of the outdoor pigs or increased production of trans fatty acids in the digestive tract of the free-range pigs earlier shown in ruminants (Harfoot) and in rats (Eyssen, 1974). It is known that the pig stomach and small intestine contain rather large amounts of microorganisms (Jonsson, 1985) and thus there is a possibility for a hydrogenation of unsaturated fatty acids. Thus, as there is an increasing interest in feeding pigs high PUFA oils, this possibility of trans fatty acid formation should be further investigated.

As discussed above, it was shown that the fatty acid composition of tissues, among them muscle NL and PL, is affected by different dietary fatty acids. In Study II and Study III different response patterns in NL and PL due to dietary 18: 2 n-6 were found whereas this was not the case for 18:3 n-3. It has been suggested that 18:3 n-3, if fed in low amounts, might be conserved in different tissues at the expenditure of 18:2 n-6 (Fu and Sinclair, 2000). This would mean that high dietary PUFA n-6/PUFA n-3 ratios will not be reflected in the tissue lipids of farmed animals as shown in Study II and by Fu et al. (2000). It can be suggested that there is a complex interaction regarding how PUFA n-3 and PUFA n-6 is incorporated into muscle lipid fractions or utilized as energy. As for PL, an optimal range of the levels of different fatty acids must be obtained to ensure its function, such as membrane fluidity, protein-lipid interactions and eicosaniod production (Lauritzen et al., 2001).

Importance of the sex of pigs for the fatty acid composition and antioxidant content

In several studies the sex of pigs has been suggested to be of importance to the fatty acid composition and antioxidant content of various porcine tissues (Table 8), (Koch, 1968; Villegas, 1973; Warnants and van Oeckel, 1996; 1998; Nilzén et al., 2001). These studies have all shown that the PUFA level is higher in females compared with castrated males in the investigated tissues. Enser et al. (2000) have shown that the entire male pigs had more PUFA in back fat compared with females but there was no difference in muscle and liver. Similar results have also been shown by others (Johns, 1940; Koch et al., 1968; Wood et al., 1986; Wood and Enser, 1988; Nürnberg and Ender, 1989; Cameron and Enser, 1991; Nürnberg and Ender, 1992). Malmfors et al. (1978) have shown that entire male pigs had a higher level of PUFA both in back fat and muscle compared with castrated males. In addition, the level of PUFA in the female pig muscle was intermediate between entire and castrated male pigs. The explanation for the higher degree of saturation in back fat and muscle of castrated males has been argued to depend partly on differences in fatness between the sexes. IMF became more unsaturated as carcass lean weight increased and back fat became more saturated as back fat thickness increased (Study III),

Table 8. Summary of the sex differences in Studies I-IV

Study	I	II	III	IV
ζ-tocopherol	Females > Castrated males	Females outdoors (OHL)	Females > Castrated males	Females>Entire males=Castrated males
ν-tocopherol	---	NS	NS	Castrated males > Entire males
PL 18:2 n-6	Females outdoors > Females indoors	NS	NS	Females outdoors > Females indoors
PL 18:3 n-3	NS	Castrated males > Females	NS	NS
PL HUFA	Females > Castrated males	Females > Castrated males	Females > Castrated males	Females=Entire males>Castrated males
NL 18:2 n-6	NS	Females fed high PUFA (OHL)	Females > Castrated males	Entire males> Females= Castrated males
NL 18:3 n-3	NS	Females fed high PUFA (OHL)	NS	Entire males out doors (OHL)
NL HUFA	NS	Females fed high PUFA> Castrated males fed low PUFA	NS	Entire males> Females= Castrated males

Abbreviations. ns: no significant effect between sexes; OHL: Overall highest level among the subgroups

(Johns, 1940; Koch et al., 1968; Martin, 1972; Whittington et al., 1986; Nürnberg and Ender, 1989; Cameron et al., 1990; Cameron and Enser, 1991; Wood and Enser, 1997). However, in Study IV IMF content explained 15 % of the total variation in 18:2 n-6 while 33 % of the variation was a pure sex effect. This would indicate that there are some other metabolic explanations for the difference in fatty acid composition between the sexes besides increased fat synthesis.

The higher level of 20:4 n-6 (Studies I-III) and thereby the raised 18:2 n-6/20:4 n-6 ratio (Study IV) in muscle PL would indicate that the castration of pigs affected fatty acid metabolism. The fatty acid composition of rat liver and serum has

previously been shown to be affected by castration, oestrogen and testosterone treatment (Cinci et al., 1993; Cinci et al., 2000). It is known that fatty acids in the n-6 and n-3 groups are precursors of eicosanoids, which have a documented effect on the reproductive system (Sardesai, 1992). In contrast to castrated males, females and entire males have an intact reproductive system. Thus, it can be suggested that the actual need for ecosanoids is lower in the castrated males and therefore a lower rate of elongation and desaturation of 18:2 n-6 and 18:3 n-3 can be seen in these pigs. Using multivariate statistics this conclusion was further strengthened as the essential fatty acids 18:2 n-6 and 18:3 n-3 in PL were grouped with the castrated males and the desaturation and elongation product with the females (Study III). However, it should be stressed that fat metabolism in different tissues is difficult to compare. Various tissues differ in their ability to desaturate (Lauritzen et al., 2001) and the incorporation and metabolism of fatty acids in muscle is slow in comparison to liver. Further investigations are needed to elucidate whether sex hormones and fatty acid metabolism interact in pigs as they do in rats and mice.

Regarding sex differences in the fatty acid composition of PL the results in the literature are rather inconsistent. Warnants et al. (1996) and Warnants et al. (Warnants et al., 1999) found no major sex differences in the composition of the polar lipids in pigs. By contrast, higher PUFA levels in PL have been found in heifer muscle (Malau-Aduli et al., 1998) as well as in female pigs (Studies I-IV) compared with castrated males (Table 8).

Finally the higher levels of PUFA and HUFA in the entire male pigs both in polar lipid and neutral lipids were not accompanied by a higher level of antioxidants. We found the highest content of ζ-tocopherol in the muscles of female pigs and the highest content of ν-tocopherol in the castrated male pigs. Whether this will lead to an increased susceptibility of entire male pig muscle remains to be investigated. However, as the difference in the content of antioxidants between sexes was rather small, this might be a question more of metabolic importance than of the oxidation stability of meat.

Effects of the RN genotype on fatty acid composition

Pigs carrying the dominant RN[4] allele have a higher muscle glycogen content, which will affect the muscle quality characteristics of the final meat products (Enfält et al., 1997). The results from Study II might indicate a metabolic relationship between PUFA and muscle glycogen content e.g. the RN genotype of the pig. Clarke (2000) hypothesizes that especially PUFA n-3 fatty acids play an essential role in the maintenance of energy balance and glucose metabolism. In a recent study by (Milan et al., 2000) it was shown that a mutation in the PRKAG3 gene and the subsequent decreased level of the enzyme adonesin monophosphate-activated protein kinase explain the higher level of muscle glycogen in pigs carrying the dominant RN[4] allele. Considering this, it can be suggested that: 1) PUFA is involved in the mechanism leading to the increased glycogen content of *M. Longissimus dorsi* in pig muscle 2) The mutation in the PRKAG3 gene affects the metabolism of PUFA and thereby the fatty acid composition of the muscle membranes.

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Physiological and nutritional importance of fatty acids

Major advances have been made in understanding the biochemistry of essential fatty acids, linolenic (18:3 n3) and linoleic acid (18:2 n6), and their interactions with metabolic pathways leading to their corresponding elongation and desaturation products (reviewed by Sargent (1995)). Dietary PUFA have been shown to be important for growth and development (Leskanich, 1999), energy metabolism (Clarke, 2000), reproduction (Mercure and Vanderkraak, 1995; Mattos et al., 2000), immune system (Calder, 1999), brain (Yehuda et al., 2000; Lauritzen et al., 2001) and eye development (Rotstein et al., 1997; Rotstein et al., 1998). In addition, dietary lipids have effects on gene expression, leading to changes in metabolism, growth and cell differentiation (Jump and Clarke, 1999). Specific fatty acid regulated transcription factors and receptors have been identified as involved in the control of lipid metabolism and eicosanoid production (Jump and Clarke, 1999). These are regulated by for example i) direct binding of fatty acids, fatty acyl-coenzyme A, oxidized fatty acids, ii) eicosanoid regulation. Finally, different balances of fatty acid groups in the feed have been shown to influence behaviour (Delion et al., 1996; Delion et al., 1997; Belzung et al., 1998; Chalon et al., 1998).

Our own studies (I, II, IV) have shown a complicated pattern regarding how the fatty acid composition of the PL is affected by sex and outdoor environment. Study II showed that the fatty acid composition of PL was affected by the RN genotype, indicating that PUFA is involved in glycogen metabolism. The negative correlation between PUFA of the neutral and polar lipids and carcass fatness parameters (Study III) might be a result of that PUFA, or PUFA n-3 in particular, can regulate energy balance by keeping fatty acids away from triglyceride synthesis and directing them towards fatty acid oxidation as reviewed by Clarke (2000). Thus, the results from our studies would indicate that different actions in the farming system would affect the pig metabolism, i.e. energy metabolism, in which fatty acids seem to be involved.

The major aim of increasing the level of unsaturated fatty acids in meat and meat products is to enhance the nutritional quality. It has been suggested that for humans to avoid dietary fat-related diseases, they should on average consume no more than 30 % of their energy as fat and that only 1/3 of this should be from saturated fat. It has also been suggested that fatty acids exert a controlling factor in the modulation of membrane fluidity and that the critical factor in fatty acid action and efficacy is not the absolute level but rather the ratio between various groups of fatty acids (Sargent, 1995; Yehuda et al., 1999; Lauritzen et al., 2001). The groups in mind are SAFA, MUFA, PUFA, PUFA n-3 and PUFA n-6. Therefore, a dietary P/S ratio between 0.4-1.0 and n-6/n-3 ratio between 4-6 has been suggested (Gerster, 1998). In meat and meat products the P/S ratio most often is to low whereas the n-6/n-3 ratio is exceeded. However, too low levels of n-6/n-3 have been shown to have adverse physiological and behavioural effects (Cheon et al., 2000).

As shown above several studies, including Studies I and II, have indicated that PUFA in general and especially PUFA n-3 can be increased in meat and meat

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products through various dietary regimes, thereby increasing the nutritional value. In Table 4 the PUFA n-6/PUFA n-3 ratios as well as P/S ratios from different studies are summarized. These studies show a large variation among ratios, e. g. PUFA n-6/PUFA n-3 ratios varied between approximately 1 and 20 and P/S between 0.2 and 0.9 in muscle and back fat. Considering the different physiological effects of PUFA, it is plausible that this large tissue variation of PUFA due to diet will effect pigs. The preliminary results from the study on rats included in this thesis suggest effects on the performance measured as wound healing in this study. There was a significant impact on wound healing as an effect of dietary alteration of fatty acids. Furthermore, the lower variation in fatty acid composition among individuals in the linseed diet group compared with sunflower and pork fat group, can be interpreted as PUFA n-3 is nutritionally favourable and hence does not cause large variation in lipid metabolism among individuals. Thus, it can be suggested that the impact of the change in dietary fatty acids was recognized both directly via the lipid metabolism and via other metabolic mechanisms resulting in an improved wound healing. However, analyses of other substances involved in PUFA regulated mechanisms as suggested by Jump and Clarke (1999) have to be included in the future. To mention some, insulin, corticosterone and thyroid hormone will be analysed. The underlying rational is that the expression rates of various proteins in de novo lipogenesis are induced by insulin, carbohydrates and thyroid hormone but PUFA overrides these stimulatory effects and exert dominant negative effect (Jump and Clarke, 1999). Thus, these processes are possibly regulated directly by fatty acids and their derivatives for example by chemical signalling or gene expression (Jump and Clarke, 1999). The level of 0.75 % linseed oil or 0.75 % sunflower oil in the study on rats (se above) is not an extreme level of oil in the diet. This level of linseed oil would be equivalent to the level of 18:3 n-3 given to pigs in the study of Enser et al. (2000) assuming that linseed contains 60 % 18:3 n-3. Thus, it would be possible to suggest using these dietary levels of PUFA in pigs, resulting in meat with increased nutritional quality for humans, which might also be beneficial to the wellbeing of the animal itself, in this case the pig.

However, the expected physiological effects on pigs due to feeding different fat sources, can be different compared with the effects on rats. In studies conducted on pigs, where different fat sources on a farm scale were fed, various performance measurements, e.g. daily gain, feed consumption, feed conversion ratio etc, were used as indicators of animal wellbeing. As far as we know different fat qualities alone will not affect these parameters if the essential fatty acids and other macro and micro nutrients exist in sufficient amounts, which was also found in our rat study. Instead, it has been shown that more fat in general, independent of fat quality, increases the daily gain and feed conversion ratio, which can be explained partly by the higher energy density in fat. The above performance parameters need of course to be monitored. However, it is plausible that other aspects will become more important to consider when producing pig meat in the future.

Finally, the efficiency of altering the fatty acid composition in meat, and thereby affecting human health, can be questioned. A better effect might be obtained if humans were to consume less meat and meat products, which might also result in decreased negative environmental effects caused by the intense animal production.

However, as there is an increasing demand for meat in the Western countries and eating habits tend to be rather difficult to change, the best option for the nearest future might be to alter the fatty acid composition of meat.

Conclusions

- Outdoor rearing applied had no major influence on the fatty acid composition of pig muscle depot fat. The implication for human nutrition can therefore be considered as minor, and other feeding regimes are suggested if PUFA n-3 is to be increased to a larger extent.

- The RN genotype was found to influence the fatty acid composition in the polar lipids of pig muscle.

- The level of HUFA was higher in entire male and female pigs compared with castrated males, which indicates that the desaturation and elongation of PUFA are more pronounced in animals with an intact reproductive function .

- Entire males had a higher level of PUFA in muscle NL compared with females and castrated males. The effect can partly be explained by fat deposition and partly was a pure sex effect.

- The various effects on muscle lipid composition and especially membranes indicate that the metabolic processes involving PUFA are diverse.

- The lower variation in fatty acid composition among individual rats fed linseed oil indicates that this dietary oil source causes less variation in lipid metabolism, which can be interpreted as linseed oil is more nutritionally favourable.

- The higher levels of PUFA in tissues covariating with the diet and the demonstrated increased skin wound healing, revealed a potential of PUFA in affecting animal performance.

Future research

- In order to optimize the fatty acid composition and antioxidant content in meat and meat products, further feeding experiments should be conducted. Storage stability as well as sensory, technological and nutritional quality, and their interactions should be considered.

- Further investigations should be conducted to establish how the mutation in the PRKAG3 affects fatty acid metabolism. These investigations might give further insights into PUFA involvement in glycogen metabolism.

- The conducted studies indicate that the level of HUFA is higher in pigs with intact reproductive organs. However, further investigation should be conducted to elucidate the relationship between sex hormones and the fatty acid metabolism in pigs. This includes investigations on organs more involved in fat metabolism than muscle and on enzymes involved in the desaturation and elongation of fatty acids.

- The outdoor environment has been shown to affect the fatty acid composition in PL. However, the mechanisms behind this effect are not clear, nor whether this is an indication that outdoor rearing is a production system better suitable for pigs.

- Dietary PUFA levels affected wound healing in rats. Therefore the importance of dietary PUFA and their effects on physiological processes as well as tissue fatty acid composition, especially the n-6/n-3 ratio, must not be neglected. Understanding for arising tasks in connection to inadequate feeding with regard to essential fatty acids during fast growing and similarly in neonatal nutrition has to be attended.

- As there is an increased interest in feeding pigs with dietary oils containing large levels of PUFA the possibility of hydrogenation in the pig digestive tract should be investigated.

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