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

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Abstract

The objectives of this study were to investigate the effect of organically certified feed (KRAV) with regard to different fatty acid n-6/n-3 dietary ratios, on the fatty acid composition of muscle neutral and polar lipids. Forty-four pigs of two sexes (castrated males and females) were given two feeds with two different n-6/n-3 ratios (9.33 and 7.13). The results showed that the dietary n-6/n-3 ratio influenced the muscle n-6/n-3 ratio of polar and neutral lipids. In general, female pig muscle had a significantly higher level of polyunsaturated fatty acids (PUFA) in both lipid classes, than muscles from castrated males. However, whereas PUFA levels were generally higher in the neutral lipids, levels of highly unsaturated fatty acids (HUFA) were higher in the polar lipids of female pig muscle. Negative correlations were found between PUFA level in both neutral and polar lipids and carcass fatness parameters, even though the correlations were stronger in the neutral lipids.

Keywords: Organic feed; Lipid classes; Lipid oxidation; Pork; Sex; Tocopherol

1. Introduction

The fatty acid (FA) composition of the porcine intramuscular fat (IMF) has been shown to be affected by feed composition as reviewed by Bosi (1999), Nürnberg, Wegner, and Ender (1998), Vanoeckel and Boucque (1992), Wood, Enser, Fisher, Nute, Richardson, and Sheard, (1999) and Wood and Enser (1997). Högberg, Pickova, Babol, Andersson, and Dutta (2002) showed recently that polar and neutral lipids were affected differently by different contents of 18:2 n-6 in the feed. The importance of n-6/n-3 ratio is recognised since the FA 18:2 n-6 and 18:3 n-3 are precursors of their longer homologues, as well as of different eicosanoids, which have been shown to be of physiological importance (Horrocks & Yeo, 1999; Sardesai, 1992). Furthermore, there is also competition between PUFA n-3 and PUFA n-6 for the desaturation and elongation enzymes (Sargent, 1995).

In an earlier study (Högberg et al., 2002), we suggested a conservation of PUFA n-3 to take place in the polar lipids, possibly caused by the rather high dietary n-6/n-3 ratio, in comparison with the dietary ratio in other studies (Enser, Richardson, Wood, Gill, & Sheard, 2000). A possible lack of PUFA n-3 was suggested to be one additional cause. It was therefore of interest to investigate the effect of the KRAV feed used in the rearing system as its dietary n-6/n-3 ratio was lower.

It has been suggested that sex can affect the FA composition of pig meat (Högberg, et al., 2002; Högberg, Pickova, Dutta, Babol, & Bylund, 2001; Koch, Pearson, Magee, Hoefer, & Schweigert, 1968; Nilzén, Babol, Lundeheim, Enfält, & Lundström, 2001; Villegas, Veum, McFate, & Bailey 1973; Warnants & van Oeckel, 1998). These studies showed that the FA composition of the muscle lipids of the female pig was more polyunsaturated than those of castrated males. This was suggested to be an indication of a difference in lipid metabolism (Högberg et al., 2002, 2001). However, an interaction between sex and other parameters included

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in those studies was found in Högberg et al. (2001) (re. interaction with rearing conditions) and Högberg et al. (2002) (re. interaction with RN genotype).

In general, it is known that the FA composition of tissues changes with increasing total lipid content, due to the smaller contribution of polar lipids in total fat. Cameron and Enser (1991) showed that the FA composition of IMF was influenced by the content of IMF. Correlations between other carcass composition parameters and FA composition of various tissues have been indicated in several studies (Cameron, Warriss, Porter, & Enser, 1990; Nürnberg & Ender, 1989; Whittington, Prescott, Wood, & Enser, 1986; Wood & Enser, 1997).

The objective of the present study was to investigate the effects of organic and conventional feed, with regard to different dietary n-6/n-3 ratios, on the FA composition of neutral and polar lipids in muscle of female and castrated male pigs. The present study was conducted in pigs reared in an indoor environment and only with pigs that were non-carriers of the RN genotype, in order to simplify interpretation of the results. Furthermore, we aimed to investigate how the FA composition of different lipid classes correlated with carcass composition parameters, such as carcass fatness and proportion of lean meat (expressed as meat %).

2. Materials and methods

2.1. Animals and treatments

The pigs were raised at the Swedish University of Agricultural Sciences from 28 to 107 kg live weight. The animals studied were a 44 animal subsample selected from a total of 240 crossbreeds [(Hampshire)×(Swedish Landrace×Swedish Yorkshire)]. The samples used in the present study were chosen randomly for each sex and feed group within the non-carriers of the RN^- allele (rn^+rn^+).

Pigs were divided into two groups and fed two different diets, one conventional (feed 1; 12 castrated males, 11 females) and one organic diet (KRAV; feed 2; 11 castrated males, 10 females) (Table 1). All pigs were reared indoors and fed restrictedly in accordance with the Swedish standard feeding regimen for growing pigs. The daily feed allowances for the pigs, in MJ of ME (metabolisable energy), were 16.5, 19.0, 24.1, 29.0 at 25, 30, 40, 50, 60 kg live weight, respectively, and 34.1 MJ from 60 kg until slaughter. The amount of feed was adjusted every second week, depending on the mean weight of each group of eight animals.

The pigs were slaughtered at a commercial abattoir (Scan Farmek, Skara, Sweden). The carcasses underwent

Table 1
Feed composition ,nutritive value, fatty acid composition and antioxidant content of feeds 1 and 2

Composition	Feed 1	Feed 2	Fatty acid (mg/100g)	Feed 1	Feed 2
	Conventional	Organic		Conventional	Organic
Barley	8.1	–	12:0	31.7	8.4
Rye wheat	27.8	–	14:0	26.6	8.7
Wheat bran	10.0	–	16:0	727	507
Middling	12.0	–	16:1tr	3.4	1.4
Dried beet with molasses	9.0		16:1	13.9	6.8
Rape seed	4.0		17:0	5.8	3.9
Wheat	10.0	41.0	18:0	165	106
Peas	10.0	12.0	18:1tr	4.8	nd
Rapeseed meal	6.2	3.7	18:1n-9	1791	917
Oats	–	30.0	18:1n-7	102	47.2
Soya meal		7.5	18:2 n-6	1692	1655
Meat meal	–	0.2	18:3 n-3	237	177
Vegetable fat	0.5	0.3	20:0	15.9	10.4
Molasses	–	0.3	20:1	37.1	17.7
Potato protein	–	2.3	22:0	10.2	9.6
Vitamins and minerals	2.2	2.7	22:1	9.2	2.6
Synthetic amino acids	0.2	–	24:0	7.8	5.5
			SAFA	999	660
MJ/kg	12.0	12.4	MUFA	1954	991
			PUFA	1930	1832
Crude protein (%)	15.2	17.9	PUFA n-6/ PUFA n-3	7.1	9.3
				(μg/g)	(μg/g)
Fat content	5.9	3.6	α-tocopherol	25.7	19.5
Lysine	0.85	0.88	γ-tocopherol	8.8	5.7
Methionine	0.24	0.28			
Threonine	0.56	0.64			

SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detectable; tr, trans.

standard slaughter procedures. The estimation of carcass meat (%) is based on two linear measurements of back fat thickness and one measure of thickness of *M. longissimus dorsi*. The measurements were made with the Hennessy Grading Probe. The first measurement of back fat was taken behind the last rib, 80 mm beside the midline of the split carcass (fat 1). The second measurement was taken 120 mm ahead the first site, 60 mm beside the midline (fat 2). Muscle thickness was measured at this second site. The carcasses were divided into joints 48 h after slaughter. The carcasses were further dissected and the weights on the subcutaneous fat, the whole ham and *M. Longissimus dorsi* muscle were recorded. Samples were chosen randomly within each sex and feeding regime. Samples from the front part of *M. Longissimus dorsi* were frozen and stored at −80 °C (6 months) until analysis. Samples for analysis of malondialdehyde (MDA) were collected from the same muscle and stored at −20 °C for 12 months.

2.2. Analytical procedures

All analyses were performed with duplicate samples. The assumed RN genotype of each individual pig was determined by enzymatic determination of the concentration of residual glycogen (including glucose and glucose-6-phosphate) in homogenised muscle tissue (Dalrymple & Hamm, 1973). Animals with a concentration of 30 μmol/g *M. Longissimus dorsi* were deemed to be not carrying the RN allele, while animals with a concentration above 40 μmol/g glycogen were considered as carriers of the RN^- allele. Pigs with glycogen concentration between 30 and 40 mol/g were not included in the study.

For fat content extraction the method of Hara and Radin (1978) was performed. 2×15 g of muscle were homogenised and the fat content was recorded. Before FA analysis the total lipids were separated with SPE (Solid Phase Extraction) into neutral lipid and polar lipid fractions according to the method of Prieto, Ebri and Collar (1992). Fatty acid methyl esters (FAME) from both neutral and polar lipids were prepared before gas chromatography (GC) as described by Dutta and Appelqvist (1989). The peaks of GC chromatograms were analysed by comparing them with authentic standards (GLC-68 A, Nu-Chek Prep, Elysian, USA) as described by Högberg et al. (2001). The trans fatty acids were compared with standards from Larodan Fine Chemicals AB, Malmö, Sweden. The identification of trans fatty acids was performed by comparing retention times for sample fatty acids with standards. In the case of 18:1 trans, the trans fatty acids 18:1 n-9 trans and 18:1 n-7 trans were not separable in the column used.

Tocopherols and MDA were determined by HPLC, using a Merck Hitachi L6200A pump, a FL Lt480 detector and an As-2000 A autosampler. The HPLC column was a 4.0×250 mm RP-18 LiChroCART. Identification and quantification were done by using external standards.

To analyse α- and γ-tocopherol, a method described by Högberg et al. (2002) was used. Two 1-g muscle were cut into pieces and homogenised in two tubes and 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, tocopherols were extracted in 2×4 ml of hexane. The hexane–vitamin solution was evaporated under nitrogen gas and diluted with the mobile phase. The mobile phase used consisted of 95% methanol:acetonitrile (1:1) and 5% chloroform with a flow rate of 1.2 ml/min. Tocopherols were detected with excitation wavelengths of 290 and 344 nm, respectively, and with emission wavelengths of 327 and 472 nm, respectively.

For MDA analysis we used the same method as previously used by Högberg et al. (2002). 2x1 g muscle were homogenised and, after preparation, 300 μl of MDA/butanol solution was diluted (1:10) with the mobile phase. HPLC analysis was performed according to the method of Öhrvall, Tengblad, Ekstrand, Siegbahn, and Vessby (1994) (excitation 532 nm and emission 553 nm). A mobile phase consisting of 60% potassium phosphate buffer (50 mM, pH 6.8) and 40% methanol, with a flow rate of 0.7 ml/min, was used.

The analyses of the feed were performed as follows: energy analysis was determined by the method of Boisen and Fernandez (1997). The FA composition of the feed was analysed as described earlier after grinding the feed. Tocopherols of feeds 1 and 2 were extracted according to the method of Børsting, Engberg, Jakobsen, Jensen, and Andersen (1994) and analysed by HPLC.

2.3. Statistical analysis

Statistical evaluation was carried out with Minitab statistical software for Windows 95 and NT (ver. 12) (Minitab, 1998) on the mean values of the duplicate samples. Feed and sex were regarded as fixed effects of the experiment 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 with 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. When a covariate or an interaction failed to reach significance ($P > 0.05$), the term was excluded from the model. To visualise the difference between sexes, multivariate statistics (Unscrambler 7.5) were used (Esbensen, Schönkopf, Mitdgaard, & Guyot, 1998). The procedure for choosing variables was as follows. Individual fatty acids,

important groups of fatty acids and carcass composition parameters were used as variables in principal component analysis (PCA). The model was validated by cross validation and all variables were weighted with 1/standard deviation and centred. The recorded measurements of carcass fatness (fat 1, fat 2, weight of the fat in the whole ham, and weight of the fat of *M. Longissimus dorsi*), except IMF, correlated well and were therefore weighted by PCA with 1/ standard deviation, and the scores for principal component 1 (PC1) were used as replacements for the four carcass fatness parameters (Fat Variables). In polar lipids the sex effect appeared in PC1 after correction of mean values (GLM procedure) for feed effect in all the variables of interest. In the neutral lipids, the sex effect appeared in PC1 without any correction and was therefore used immediately for further data interpretation. Furthermore, an analysis of variance (see earlier) was calculated on the PC1 scores for both polar and neutral lipids. Overall and partial correlation coefficients were calculated between carcass composition parameters and certain individual and important groups of fatty acids in polar and neutral lipids. The partial correlations were calculated after correcting for feed only, or feed and sex. Finally, an overall correlation was calculated between MDA and -tocopherol.

3. Results

No general differences in slaughter weight, carcass composition or IMF were evident between the two feeds (Table 2). The castrated male pigs had a fatter carcass composition than the females ($P = 0.04$; Table 2) and tended to have a higher IMF (Table 2).

3.1. Correlation between carcass composition parameters and fatty acids

In the neutral lipids, positive correlations were observed between SAFA and IMF ($r = 0.46$) and between SAFA and fat variables ($r = 0.66$) (Table 3). Corresponding negative correlations between PUFA, HUFA and IMF ($r = -0.48$ and -0.53 respectively) and PUFA, HUFA and fat variables ($r = -0.54$ and -0.62 respectively) (Table 3) were also obtained. Finally, fat variables correlated negatively with HUFA ($r = -0.50$) in the polar lipids (Table 4).

3.2. Muscle fatty acid composition depending on feed

In the neutral lipids of muscle we found higher levels of 18:3 n-3 ($P = 0.001$) and PUFA n-3 ($P = 0.001$) in the pigs fed feed 1 than in pigs fed feed 2 (Table 5).

Table 2
Daily weight gain and carcass composition (least squares mean and standard error) of castrated male and female pigs fed feeds 1 and 2

	Feed 1	Feed 2	SE	*P*-value	Castrated males ($n = 23$)	Females ($n = 21$)	SE	*P*-value
	Conventional ($n = 23$)	Organic ($n = 21$)						
Daily weight gain (g)	770	748	15	0.32	769	750	15	0.39
Carcass weight (kg)	78.5	77.9	0.5	0.34	78.2	78.3	0.5	0.91
Fat variables[a]	0.01	−0.06	0.35	0.88	−0.50	0.55	0.35	0.04
Meat%	57.5	57.4	0.5	0.88	56.6	58.2	0.5	0.03
IMF (%)	2.37	2.40	0.14	0.89	2.56	2.20	0.15	0.10

[a] Scores from principal component 1 as calculated by principal component analysis. See Section 2.

Table 3
Overall and partial correlations (after correction for feed as fixed effect and for feed and sex) between groups of fatty acids of importance and carcass composition parameters in the neutral lipids of porcine muscle[a]

Neutral lipids	Global correlation			Partial correlation (feed)			Partial correlation (feed and sex)		
	IMF	Meat %	Fat variables	IMF	Meat %	Fat variables	IMF	Meat %	Fat variables
IMF		–	–		–	–		–	–
Meat %	–		−0.73	–		−0.73	–		−0.69
Fat variables	–	−0.73		–	−0.73		–	−0.69	
18:2 n-6	−0.47	0.40	−0.53	−0.47	0.40	−0.54	−0.42	0.30	−0.47
18:3 n-3	−0.34	–	−0.33	−0.41	0.31	−0.44	−0.36	–	−0.37
SAFA	0.46	−0.46	0.66	0.43	−0.46	0.67	0.41	−0.38	0.62
HUFA	−0.53	0.56	−0.62	−0.53	0.56	−0.63	−0.49	0.49	−0.58
PUFA	−0.48	0.41	−0.54	−0.48	0.41	−0.55	−0.43	0.32	−0.48
Trans	−0.41	0.35	−0.34	−0.45	0.38	−0.40	−0.39	–	–

Abbreviations: See Table 1. HUFA, highly polyunsaturated fatty acids (18 carbon atoms or more, except for 18:2 n-6 and 18:3 n-3). Trans: 16:1 9 tr, 18:2 (9,12tr), 18:1 tr.

[a] Correlation > 0.29; $P < 0.05$, only significant values shown in table; $n = 44$.

Table 4
Overall and partial correlations (after correction for feed as fixed effect and for feed and sex) between groups of fatty acids of importance and carcass composition parameters in the polar lipids of porcine muscle[a]

Polar lipids	Global correlation			Partial correlation (feed)			Partial correlation (feed and sex)		
	IMF	Meat %	Fat variables	IMF	Meat %	Fat variables	IMF	Meat %	Fat variables
18:3 n-3	–	–	–	–	–	0.32	–	–	–
20:4 n-6	–	0.36	−0.49	–	0.42	−0.53	–	0.32	−0.46
22:6 n-3	–	0.35	−0.45	–	0.37	−0.50	–	–	−0.41
MUFA	0.36	–	–	0.56	–	–	0.54	–	–
HUFA	–	0.32	−0.50	−0.29	0.33	−0.51	–	–	−0.44
Trans	–	–	–	–	–	–	−0.30	–	–

Abbreviations: See Tables 1 and 3.
[a] Correlation > 0.29; $P < 0.05$, only significant values shown in table; $n = 44$.

Table 5
Fatty acid composition (% least squares mean and standard error) of neutral and polar lipids in intramuscular fat from pigs fed feeds 1 and 2

Fatty acid	a. Neutral lipid				b. Polar Lipid			
	Feed 1 conventional (n = 23)	Feed 2 organic (n = 21)	SE	p-value	Feed 1 conventional (n = 23)	Feed 2 organic (n = 21)	SE	p-value
14:0	1.37	1.35	0.03	0.69	Trace	Trace		
Unknown	nd	nd			1.94	3.64	0.33	0.001
16:0	23.2	23.7	0.24	0.18[a]	17.0	15.8	0.37	0.03
16:1 9 tr	0.37	0.33	0.01	0.004[a]	0.30	0.44	0.04	0.01
16:1 n-7	2.86	3.10	0.10	0.10	0.31	0.29	0.02	0.66
17:0	0.19	0.21	0.01	0.03[a]	0.19	0.28	0.01	0.001
18:0	12.1	12.3	0.24	0.59	9.00	9.32	0.16	0.17
Unknown	nd	nd			3.89	2.87	0.32	0.03
18:1 tr	0.27	0.22	0.01	0.01	nd	nd		
18:1 n-9	43.0	42.6	0.41	0.52	8.89	7.02	0.22	0.001
18:1 n-7	4.31	4.51	0.08	0.07	2.04	1.97	0.04	0.27[a]
18:1 n-5	0.24	0.24	0.01	0.47	2.64	1.59	0.21	0.001
18:2 9,12tr	Trace	Trace			0.32	0.21	0.03	0.01
18:2 n-6	8.62	8.25	0.35	0.50[a]	28.2	29.8	0.36	0.004
18:3 n-6	Trace	Trace			0.27	0.27	0.01	0.89
18:3 n-3	1.02	0.70	0.04	0.001[a]	0.96	0.67	0.03	0.001
20:0	0.20	0.21	0.004	0.12	nd	nd		
20:1	0.74	0.75	0.02	0.79	Trace	Trace		
20:2 n-6	0.35	0.38	0.02	0.78[a]	0.34	0.44	0.01	0.001
20:3 n-6	Trace	Trace			1.14	1.13	0.02	0.54
20:4 n-6	0.31	0.35	0.02	0.85[a]	10.9	11.9	0.21	0.002
20:3 n-3	0.16	0.12	0.01	0.001	0.27	0.28	0.01	0.78
20:5 n-3	Trace	Trace			1.14	0.74	0.03	0.001
22:4 n-6	Trace	Trace			1.15	1.27	0.04	0.001
22:5 n-3	Trace	Trace			2.27	2.09	0.05	0.02
22:6 n-3	Trace	Trace			0.90	0.73	0.04	0.003
SAFA	37.2	37.8	0.43	0.30[a]	26.3	25.5	0.41	0.20
MUFA	51.1	51.2	0.48	0.80	13.9	11.0	0.24	0.001[a]
HUFA	1.33	1.27	0.05	0.35[a]	18.8	19.3	0.28	0.19
PUFA	11.0	10.2	0.45	0.25[a]	47.6	49.5	0.27	0.001
PUFA n-6	9.58	9.24	0.41	0.56[a]	42.1	45.0	0.28	0.001
PUFA n-3	1.39	0.98	0.05	0.001[a]	5.55	4.51	0.08	0.001
Trans	0.72	0.62	0.01	0.001[a]	0.62	0.63	0.30	0.84
PUFAn-6/ PUFAn-3	6.93	9.35	0.10	0.001[a]	7.64	10.1	0.17	0.001
PC1	0.55	−0.52	0.45	0.10	0.05	0.04	0.41	0.98

Abbreviatons: See Tables 1 and 3. PC1: Scores from principal component 1 as calculated by principalcomponent analysis. Trace: identified fatty acids below 0.15% are shown as trace but included in sum of SAFA, PUFA n-6 and PUFA n-3, respectively.
[a] Intramuscular fat content was included as covariate ($P < 0.05$).

Furthermore, we found higher levels of 18:3 n-3 ($P=0.001$) and of PUFA n-3 ($P=0.001$) in the muscle polar lipids of the pigs fed feed 1 compared with pigs fed feed 2 (Table 5). By contrast, there were higher levels of 18:2 n-6 ($P=0.004$) and PUFA n-6 ($P=0.001$) in the polar lipids of pigs fed feed 2 than in these fed feed 1. Finally, the differences in PUFA n-6 and PUFA n-3 fatty acids also caused higher n-6/n-3 ratios in both polar and neutral lipids of pigs fed feed 2 ($P=0.001$).

3.3. Muscle fatty acid composition depending on sex

Sex-related differences were found in both neutral and polar lipids. In the neutral lipids, higher levels of 18:2 n-6 ($P=0.03$) and 20:4 n-6 ($P=0.03$) were found in muscle of female pigs than of the castrated males (Table 6). Furthermore, slightly higher levels of PUFA n-3 ($P=0.04$) were found in muscle of female pigs than the castrated males (Table 6). In the polar lipids, a higher level of HUFA was found in female pig muscle. Differences were found in 20:4 n-6 ($P=0.01$), 20:5 n-3 ($P=0.01$) and 22:6 n-3 ($P=0.001$) (Table 6).

In the PCA plot for the neutral lipids, the scores for the castrated male pigs were grouped more to the left along principal component one (PC 1), while the females grouped more to the right (Fig. 1). In the variable plot for the neutral lipids, IMF, other fat variables and SAFA were grouped to the left with the castrated

Table 6
Fatty acid composition (% least squares mean and standard error) of neutral and polar lipids in intramuscular fat from castrated male and female pigs

Fatty acid	a. Neutral lipid				b. Polar lipid			
	Castrated males ($n=23$)	Females ($n=21$)	SE	P-value	Castrated males ($n=23$)	Females ($n=21$)	SE	P-value
14:0	1.39	1.33	0.03	0.21	Trace	Trace		
Unknown	nd	nd			2.77	2.81	0.35	0.94
16:0	23.9	22.9	0.24	0.01[a]	16.5	16.4	0.37	0.79
16:1 9 tr	0.32	0.38	0.01	0.001[a]	0.37	0.37	0.04	0.93
16:1 n-7	3.05	2.91	0.10	0.35	0.33	0.27	0.02	0.05
17:0	0.20	0.20	0.01	0.88[a]	0.25	0.21	0.01	0.02
18:0	12.4	12.0	0.24	0.16	9.15	9.17	0.16	0.93
Unknown	nd	nd			3.35	3.41	0.32	0.90
18:1 tr	0.23	0.26	0.01	0.04	nd	nd		
18:1 n-9	42.8	42.8	0.41	0.52	8.13	7.77	0.23	0.26
18:1 n-7	4.46	4.36	0.08	0.36	1.99	2.02	0.04	0.63[a]
18:1 n-5	0.24	0.23	0.01	0.16	2.20	2.03	0.21	0.55
18:2tr	Trace	Trace			0.27	0.27	0.03	0.99
18:2 n-6	7.78	9.08	0.39	0.03[a]	29.0	29.0	0.36	0.91
18:3 n-6	Trace	Trace			0.27	0.27	0.01	0.88
18:3 n-3	0.81	0.92	0.04	0.05[a]	0.85	0.79	0.03	0.15
20:0	0.21	0.20	0.01	0.19	nd	nd		
20:1	0.76	0.73	0.02	0.27	Trace	Trace		
20:2 n-6	0.35	0.38	0.01	0.13[a]	0.39	0.39	0.01	0.82
20:3 n-6	Trace	Trace			1.14	1.15	0.02	0.92
20:4 n-6	0.31	0.35	0.01	0.03[a]	11.0	11.9	0.21	0.01
20:3 n-3	Trace	Trace			0.27	0.28	0.01	0.20
20:5 n-3	Trace	Trace			0.89	1.00	0.03	0.01
22:4 n-6	Trace	Trace			1.38	1.24	0.04	0.02
22:5 n-3	Trace	Trace			2.11	2.25	0.06	0.07
22:6 n-3	Trace	Trace			0.71	0.92	0.04	0.001
SAFA	38.2	36.8	0.44	0.04[a]	26.0	25.8	0.41	0.82
MUFA	51.3	50.9	0.48	0.58	12.6	12.3	0.24	0.46[a]
HUFA	1.23	1.37	0.05	0.05[a]	18.4	19.6	0.28	0.004
PUFA	9.82	11.4	0.46	0.03[a]	48.0	49.2	0.27	0.003
PUFA n-6	8.71	10.1	0.42	0.03	43.1	44.0	0.28	0.04
PUFA n-3	1.11	1.26	0.05	0.04[a]	4.83	5.23	0.08	0.001
trans	0.62	0.72	0.01	0.001[a]	0.64	0.61	0.03	0.53
PUFAn-6/ PUFAn-3	8.13	8.15	0.10	0.93[a]	9.13	8.57	0.17	0.03
PC1	−0.99	1.01	0.44	0.003	−1.01	1.01	0.41	0.001

Abbreviatons: See Tables 1 and 3.
[a] Intramuscular fat content was included as a covariate ($P<0.05$).

male pigs and PUFA, HUFA, meat% and n-6/n-3 ratio to the right with the female pigs (Fig. 2). The PCA score plot for the polar lipids (not shown) revealed a similar pattern to the score plot of the neutral lipids. In the PCA variable plot for the polar lipids, the results were somewhat different. Along PC 1, 18:3 n-3, 18:2 n-6 and n-6/n-3 were grouped on the castrated male side to the left along with IMF, other fat variables, SAFA and MUFA whereas HUFA and meat % were grouped on the female pig side to the right (Fig. 3). Moreover, using the scores obtained from the multivariate statistical analysis for the neutral and polar lipids, as a variable in

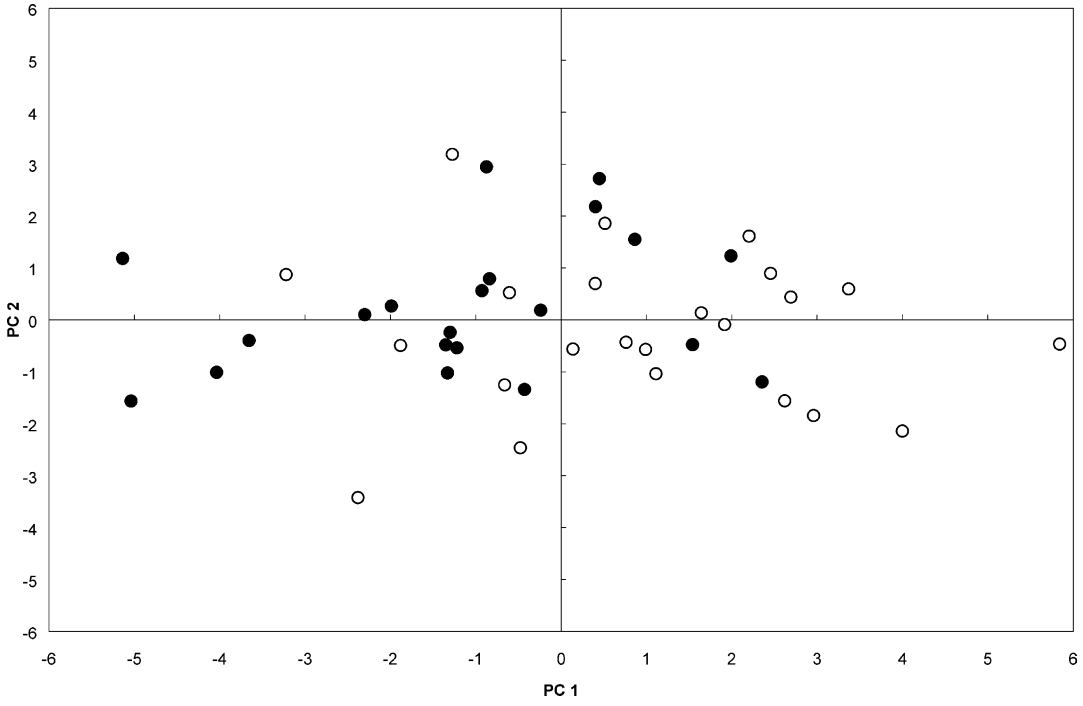

Fig. 1. PCA plot of scores in neutral lipids illustrating the sex difference between muscle of castrated male pigs (filled dots) and females. The variables used in the PCA calculation can be seen in Fig. 2.

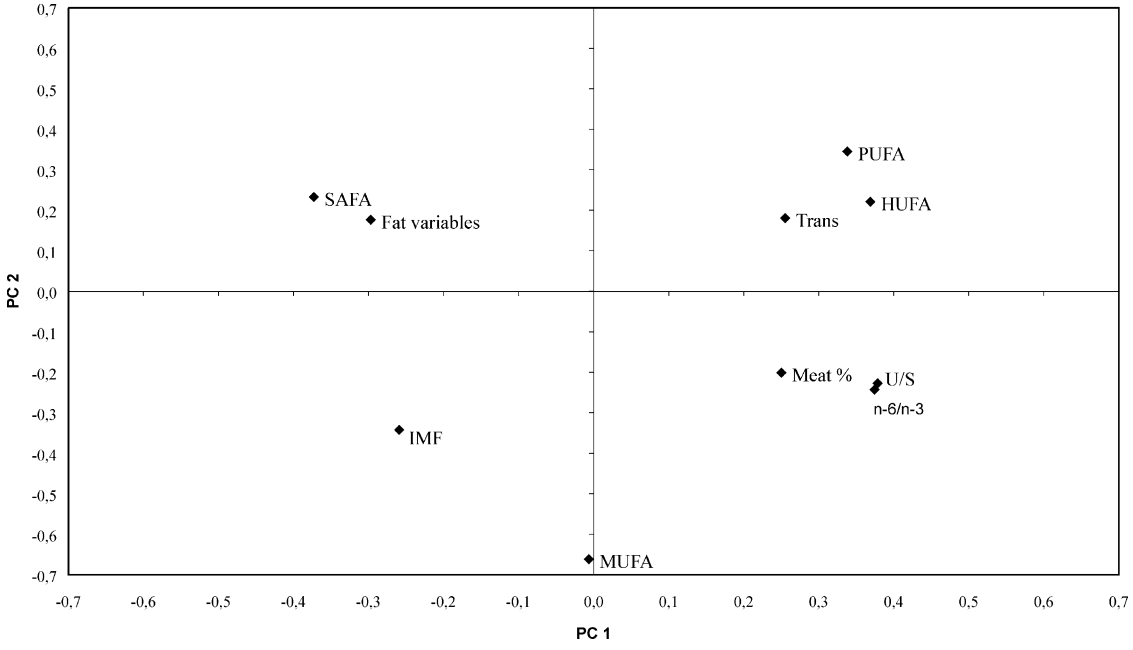

Fig. 2. PCA plot of fat variables and groups of fatty acids of importance in neutral lipids of pig muscle. Explained X variance in PC 1, 55% and PC2, 20%.

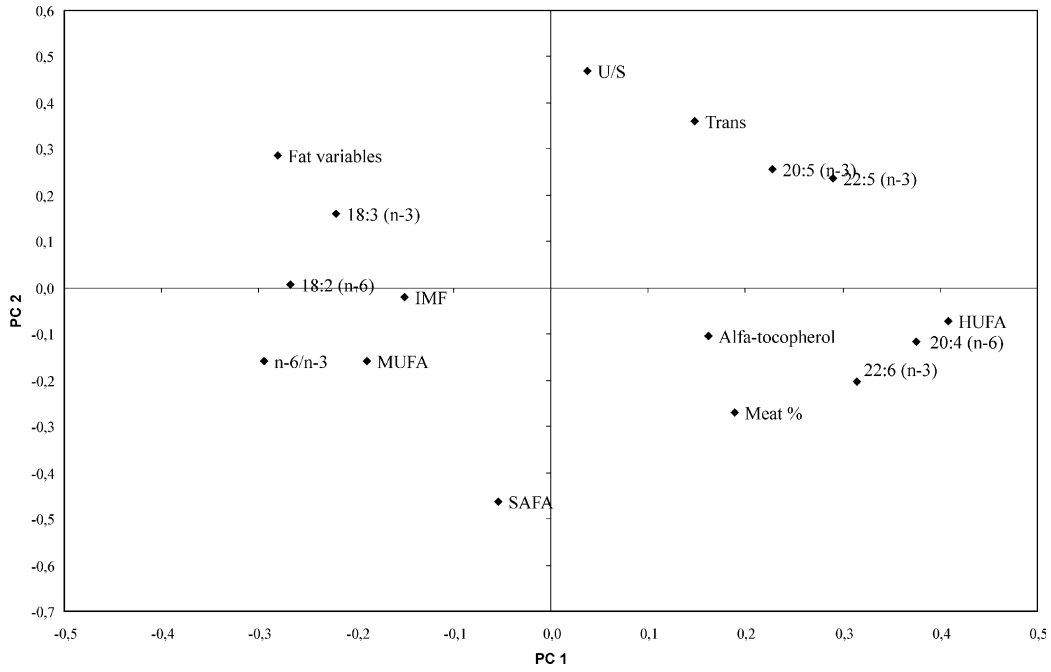

Fig. 3. PCA plot of fat variables and groups of fatty acids of importance in polar lipids of pig muscle. Explained X variance in PC 1, 30% and PC2, 18%.

Table 7
Antioxidant and malondialdehyde (MDA) contents (least squares mean and standard error) in muscle from pigs fed feeds 1 and 2 and from castrated male and female pigs

Antioxidant	Feed				Sex			
	Feed 1 conventional ($n=23$)	Feed 2 organic ($n=21$)	SE	*P*-value	Castrated males ($n=23$)	Females ($n=21$)	SE	*P*-value
μg/100 g meat								
α-tocopherol	108	112	6.7	0.43	98.8	124	6.7	0.01
γ-tocopherol	5.6	5.9	1.0	0.87	6.0	5.5	1.0	0.72
μg/g intramuscular fat								
α-tocopherol	60.9	52.6	4.7	0.22	46.8	66.7	4.7	0.001
γ-tocopherol	3.2	2.69	0.5	0.44	2.9	2.9	0.5	0.97
MDA (*ng/g meat*)	87.1	88.4	9.7	0.92	93.7	81.7	9.7	0.39

an ANOVA analysis, the sex-related difference was again observed (Table 6). However, this difference was even more pronounced, especially in the neutral lipids, when scores from a PCA were used in the ANOVA (Table 6).

3.4. Antioxidants and MDA

MDA in pig muscle did not differ between dietary regimes or sexes (Table 7). A higher content of α-tocopherol was found in muscle of female pigs than that of the castrated males ($P=0.01$) (Table 7). Furthermore, a negative correlation was found between MDA and α-tocopherol ($r=-0.37$; $P=0.01$) and a positive correlation between α-tocopherol and HUFA in the polar lipids of pig muscle ($r=0.36$; $p=0.02$).

4. Discussion

The correlation between FA composition and carcass fatness parameters has been reported in several studies. It has been shown that intramuscular fat became more unsaturated as carcass lean weight increased (Cameron et al., 1990; Martin, 1972). Whittington et al. (1986) found that 18:2 n-6 decreased as backfat thickness increased with correlation coefficients ranging between −0.6 and −0.4. Several investigators showed that subcutaneous fat became more saturated as fat deposition increased (Johns, 1940; Koch, Parr, & Merkel, 1968; Nürnberg et al., 1989; Wood & Enser, 1997). However, Wood, Brown, Nute, Whittington, Perry, Johnson et al. (1996) found that the level of 18:3 n-3 was not affected by fat thickness. In the present study, a positive corre-

lation between SAFA and IMF and a negative correlation between PUFA, HUFA and IMF were obtained in neutral lipids, which is in agreement with the investigations above. Furthermore, we obtained results similar to those of Cameron et al. (1990), where the meat% was correlated positively to HUFA and PUFA and negatively to SAFA in this lipid fraction. We also found a negative correlation between 18:2 n-6 and 18:3 n-3 in neutral lipids and carcass fatness parameters, in contrast to Wood et al. (1996). In the polar lipids, the correlations between fatty acids and carcass composition measurements were less pronounced than for the neutral lipids. As the neutral lipid fraction accounts for the main part of the total lipid, the effects of carcass parameters on the composition of this fraction should be more similar to the effects obtained in the investigations on total lipid, as shown in the different studies earlier.

The difference between lipid classes is most likely a consequence of their respective roles in the living animal. The polar lipids are important constituents of membranes and the C20 fatty acids of the membranes function as precursors in eicosanoid metabolism, whereas neutral lipids serve mainly as a depot used as energy source (Henderson, 1987). Recently, it has been suggested that PUFA n-3, in particular, can regulate energy balance by enhancing thermogenesis, thereby keeping fatty acids away from triglyceride synthesis and directing them toward FA oxidation (Clarke, 2000). The negative correlation between PUFA of the neutral and polar lipids and carcass fatness parameters might be a result of such a regulatory mechanism, as reviewed by Clarke (2000), and therefore of metabolic importance for the pig.

In the present study, the n-6/n-3 ratio was rather high in both muscle neutral and polar lipids in pigs fed feed 2 (organic feed) and thereby also in total lipids of the meat. Gerster (1998) recommended that the dietary n-6/n-3 ratio should not exceed 4–6. In our previous study (Högberg et al., 2002) we found that, in the pigs given the feed with the highest dietary n-6/n-3 ratio, this ratio in the polar lipids was lower than in the diet (11.4 vs. 13.4). In the present study, the result was the converse, as the muscle polar lipid n-6/n-3 ratio was higher than the feed n-6/n-3 ratio (10.1 vs. 9.3). We therefore believe that the polar lipid fraction is affected by the n-6/n-3 ratio in the feed but only to a certain degree. If the n-6/n-3 ratio is too high, the n-6 fatty acids will not be incorporated into the membranes, or the n-3 fatty acids will be retained in the membranes.

In several studies the sex of pigs has been suggested to be of importance for the FA composition of various porcine tissues (Högberg et al., 2001,2002; Koch Pearson et al., 1968; Nilzén et al., 2001; Villegas, 1973; Warnants & van Oeckel, 1996; Warnants et al., 1998). The present study showed a similar pattern, that the female pig muscle had higher levels of PUFA than the muscle of castrated males. By using multivariate statistics we found that the essential fatty acids, 18:2 n-6 and 18:3 n-3, of the polar lipids were grouped with the castrated male pigs alone, and that the desaturation and elongation products of these fatty acids were grouped with the female pigs. This might be an indication that desaturation and elongation of 18:2 n-6 and 18:3 n-3 in castrated males were affected by the loss of sexual functions as an effect of castration.

Despite differences in FA composition, due to diet in both lipid classes in this study, no effect on oxidation stability was found. α-Tocopherol is regarded as one of the most important antioxidants, preventing lipid oxidation in meat (Jensen, Lauridsen, & Bertelsen, 1998). Since the higher level of HUFA in both lipid fractions of the female muscle is accompanied by a higher content of α-tocopherol, the female muscle is the spite this difference in FA composition, as stable towards lipid oxidation as that of castrated males. We conclude, that the organic feed resulted in a higher PUFA n-6 level in the muscle polar lipid fraction as well as a higher n-6/n-3 ratio, in both lipid classes, and thereby also in total lipids of the produced meat. The fatty acid profile in the organic feed could be further optimised to give a composition, in the meat, which will fulfil human nutritional requirements with regard to PUFA n-3 contents.

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