Animal (2009), **3:8**, pp 1196–1204 © The Animal Consortium 2009
doi:10.1017/S1751731109004455

animal

Effect of supplementation of the maternal diet with fish oil or linseed oil on fatty-acid composition and expression of Δ5- and Δ6-desaturase in tissues of female piglets

J. Missotten[1], S. De Smet[1], K. Raes[1,2] and O. Doran[3†]

[1]*Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium;* [2]*Research Group EnBiChem, Department of Industrial Engineering and Technology, University College West-Flanders, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium;* [3]*School of Life Sciences, University of the West of England, Frenchay Campus, Coldharbour Lane, Bristol BS16 1QY, UK*

(Received 30 September 2008; Accepted 28 January 2009; First published online 17 April 2009)

The present study investigated whether enrichment of the pig maternal diet with n-3 polyunsaturated fatty acids (PUFA) affects the fatty-acid composition of female piglets via enhancing of expression of the lipogenic enzymes Δ5-desaturase (Δ5d) and Δ6-desaturase (Δ6d). The sows (50% Landrace × 50% Large White) were fed a control diet or one of the experimental diets starting at day 45 in gestation. The experimental diets were supplemented either with linseed oil or fish oil, whereas the control diet contained palm oil. Expression of Δ5d and Δ6d, and fatty-acid composition was determined by Western blotting and gas-liquid chromatography, respectively, in muscle, subcutaneous adipose tissue and liver. The highest Δ5d protein expression was observed in the piglets' muscle, followed by subcutaneous adipose tissue, with the lowest level in the liver. Expression of Δ6d in the piglets' tissues followed an opposite pattern, and was highest in the liver, followed by subcutaneous adipose tissue, with the lowest level in muscle. Supplementation of the maternal diet with fish oil or linseed oil increased the level of n-3 PUFA of the piglets in a tissue-specific manner. The response of Δ6d and Δ5d protein expression in female piglets, with average birth weight 2.4 kg, to the dietary manipulation was also tissue-specific. It is suggested that the increase in n-3 PUFA content in the progeny was related, at least partially, to the activation of Δ6d and Δ5d expression.

Keywords: pig, diet, fatty-acid composition, lipogenic enzyme, protein expression

Implications

This study has demonstrated that pig maternal diet supplemented with n-3 polyunsaturated fatty acids, mediates its effects on female piglets' fatty-acid composition, at least partially, via regulation of expression of the lipogenic enzymes Δ5-desaturase and Δ6-desaturase. Moreover, the response of progeny to manipulations of the maternal dietary composition was tissue-specific. The present paper contributes to understanding of mechanisms by which pig maternal diets regulate the fatty-acid composition of progeny. The results of this study might contribute to designing of pig feeding strategies and improving quality of pork.

Introduction

The type of fatty acids in the diet has a strong impact on human health. Particular attention has been paid to the dietary long-chain n-3 polyunsaturated fatty acid (n-3 PUFA), such as eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3). These fatty acids are known to be crucial for normal growth and development (reviewed by Ruxton *et al.*, 2004). They also reduce risk of cardio-vascular disease (reviewed by Givens and Gibbs, 2008), decrease incidence of age-related eye disease (SanGiovanni *et al.*, 2008), positively alter human red blood cell fatty-acid composition (Barcelo-Coblijn *et al.*, 2008), decrease risk of cancer and have a number of other health benefits (Berquin *et al.*, 2008; Li *et al.*, 2008; Mozaffarian, 2008).

The current EPA and DHA consumption in many European countries is only about 25% of a recommended daily intake (reviewed by Givens and Gibbs, 2008). One way to enhance daily intake of long-chain PUFA is to increase the levels of these fatty acids in animal-derived foods and in meat in particular. Pork is one of the most consumed meats in Europe (Mourot and Hermier, 2001). It is well documented, that amounts of EPA and DHA in pork can be increased via inclusion of EPA- and/or DHA- rich oils in animals' diets

† E-mail: olena.doran@uwe.ac.uk

(Moghadasian, 2008; Wood *et al*., 2008). Moreover, the inclusion of n-3 PUFA in the maternal diet can influence the fatty-acid composition of offspring. It is generally accepted that the effect of the maternal diet on the fatty-acid composition of progeny is due to direct incorporation of the fatty acids (Rigau *et al*., 1995; Rooke *et al*., 1998; Lauridsen and Jensen, 2007). However, animal tissues also have the ability to synthesise long-chain fatty acids from dietary precursors (Brenner, 1974; Sprecher, 2000; Moghadasian, 2008). Thus, 18:2n-6 and 18:3n-3 can be used for tissue biosynthesis of long chain n-6 PUFA and n-3 PUFA, respectively. The key enzymes involved in the formation of long chain PUFA are Δ6-desaturase (Δ6d) and Δ5-desaturase (Δ5d). It is known that the fatty-acid composition of the maternal diet can affect the postnatal expression of Δ6d and Δ5d in human and rats (Neuringer *et al*., 1988; Xiang *et al*., 2006). However, whether the maternal diet has effects on these enzymes in piglets remains unclear.

The main objective of this study is to determine whether the effects of n-3 PUFA enriched pig maternal diets on fatty-acid composition of offspring is mediated via regulation of Δ6d and Δ5d protein expression. The study also aims to determine the tissue distribution of Δ6d and Δ5d proteins in piglets, and the tissue-specificity of the dietary effects.

Material and methods

Animals and diets

The animal trials were conducted at two farms of Danis n.v. in Belgium, and were approved by the Ethical Committee of the Faculty of Veterinary Sciences and Bioscience Engineering of Ghent University (approval number EC 2006/119).

Four groups of multiparous sows (50% Landrace × 50% Large White, 64 to 84 animals per group) were used in the experiment with two groups per farm (one group fed the control diet and one group fed an experimental diet). The experiment started at day 45 in gestation. A group of sows was allocated to one of the experimental diets and five sows per group of second or third parity were chosen from which, one piglet per litter was sacrificed for further analysis.

There were three diets in the trial: (1) a control diet (on both farms five sows of parity 2); (2) a diet supplemented with fish oil (INVE n.v., Dendermonde, Belgium; two sows of parity 2 and three of parity 3); and (3) a diet supplemented with linseed oil (three sows of parity 2 and two of parity 3). Diets fed during gestation and lactation slightly differed to meet the specific nutrient requirements of pregnant and lactating sows, respectively. The control diets were formulated to minimise inclusion of n-3 fatty acids by using palm oil. All diets were formulated to contain a similar amount of linoleic acid (13 g/kg), and were supplemented with the antioxidant α-tocopherol acetate (150 mg/kg diet). The dietary oil supplementation, analysed nutrient composition and fatty-acid composition of the gestation diets are given in Table 1. All three diets contained: beet pulp (250 kg/t), barley (215 kg/t), wheat (250 kg/t), maize (200 kg/t), molasses (20 kg/t), soy beans (10 kg/t), chalk (38% Ca; 5.75 kg/t), mono calcium phosphate (10 kg/t), salt (3.5 kg/t) and a vitamin and mineral mix (5 kg/t). The diets were supplemented with following amino acids: L-lysine HCl (1.5 kg/t), choline chloride (75%; 0.7 kg/t) and L-threonine (0.16 kg/t). The sows were fed the control or experimental diets by dump feeder from day 45 of gestation to meet their daily requirements.

Seven days before the expected farrowing date, each group of pregnant sows was transferred to a room in part-slatted farrowing house accommodation, with 10 farrowing crates per room. The sows then received 2.5 kg/day of the lactation diet until the day of farrowing. The lactation diet composition is given in Table 2. All three lactation diets contained: beet pulp (65 kg/t), barley (300 kg/t), wheat (240 kg/t), maize (195 kg/t), soy beans (134.6 kg/t), chalk (38% Ca; 17.6 kg/t), mono calcium phosphate (6 kg/t), natuphos 5000 FTU/g (0.1 kg/t), salt (3.6 kg/t) and a vitamin and mineral mix (5 kg/t). The diet contained added amino acids: L-lysine HCl (2.5 kg/t), choline chloride (75%; 0.7 kg/t), DL-methionine (0.32 kg/t) and L-threonine (0.74 kg/t). The farrowing was not induced and there was minimal intervention during farrowing. After farrowing, feed allowance of the sows was increased from 2 kg/day by approximately 1 kg/day until *ad libitum* feed intake was reached. The sows were subsequently offered feed according to appetite.

On an average age of 5.6 days (±0.3 s.e.), one female piglet from each of the five litters within each experimental group was sacrificed. Piglets were selected with a weight close to the average piglet weight of the litter. Average weights of piglets in groups were similar ($P > 0.05$) and were 2.3, 2.5, 2.4 and 2.3 kg for control group on farm 1, control group on farm 2, fish oil group and linseed oil group, respectively. Piglets were anaesthesised by inhalation of the halothane analogue Isoflurane (Isoba®; Schering-Plough Animal Health, Middlesex, UK) and immediately killed by bleeding. Samples of the liver, *longissimus dorsi* (LD; muscle) and subcutaneous fat (SF) were removed immediately after slaughter and divided into two pieces. One piece of each tissue (approximately 10 g) was immediately frozen in liquid nitrogen for further analyses of enzyme expression. These samples were stored at −80°C. The other piece of each tissue (approximately 70 g of liver, 30 g of LD and 5 g of SF) was transported to the laboratory on ice within 1 h and then stored vacuum packed at −20°C for fatty-acid analysis.

Feed and tissue samples analyses

Feed samples were analysed for dry matter, ash (ISO 5984, 2002), crude protein (ISO 5983-1, 2005) and crude fat (ISO 6492, 1999). Lipids were extracted from 5 g of feed and muscle, from 3 g of liver and from 1 g of fat (Folch *et al*., 1957). Nonadecanoic acid (C19:0) was added as an internal standard. After methylation of the fatty acids with NaOH/MeOH (0.5 M NaOH in MeOH) followed by HCl/MeOH (1 : 1; v/v), the fatty acid methyl esters (FAME) were analysed by gas-liquid chromatography (HP6890, Agilent, Brussels, Belgium) using a CP Sil88 column for FAME (100 m × 250 μm × 0.25 μm) (Chrompack, Middelburg, The Netherlands)

Table 1 *Composition of the sow diets fed from day 45 of gestation (means ± s.e.)*

	Control diet ($n = 16$)	Linseed oil diet ($n = 5$)	Fish oil diet ($n = 9$)
Oil supplementation (kg/t of feed)			
Palm oil	25	0	0
Soy oil	4	5	5
Linseed oil	0	20	0
Fish oil	0	0	20
Analyzed composition (% fresh matter)			
Dry matter	88.7 ± 0.1	88.7 ± 0.1	88.8 ± 0.1
Ash	6.9 ± 0.1	6.9 ± 0.1	7.0 ± 0.2
Ether extract (total fat)	5.0 ± 0.1	5.7 ± 0.1	5.8 ± 0.1
Total protein	12.6 ± 0.1	12.5 ± 0.1	12.6 ± 0.3
NE[1]	8.55	8.52	8.54
Fatty-acid composition (g/100 g fat)			
C16:0	22.35 ± 0.58	12.46 ± 0.51	16.80 ± 0.41
C18:0	2.41 ± 0.14	2.65 ± 0.02	2.67 ± 0.09
C18:1c9	22.66 ± 0.36	18.71 ± 0.18	15.14 ± 0.30
C18:2n-6	42.24 ± 0.75	38.72 ± 0.23	33.68 ± 0.37
C18:3n-6	0.008 ± 0.001	0.008 ± 0.002	0.09 ± 0.01
C20:3n-6	0.006 ± 0.001	0.010 ± 0.001	0.05 ± 0.01
C20:4n-6	0.020 ± 0.003	0.026 ± 0.003	0.38 ± 0.02
C22:4n-6	0.042 ± 0.002	0.037 ± 0.004	0.18 ± 0.01
C18:3n-3	4.52 ± 0.44	22.46 ± 0.83	3.50 ± 0.06
C18:4n-3	0.03 ± 0.01	0.025 ± 0.008	0.87 ± 0.04
C20:5n-3	0.17 ± 0.04	0.13 ± 0.04	5.85 ± 0.27
C22:5n-3	0.03 ± 0.01	0.031 ± 0.005	0.71 ± 0.03
C22:6n-3	0.12 ± 0.03	0.09 ± 0.03	4.03 ± 0.17

[1]Net energy value of the feed (KJ/kg feed) as calculated from the formulation.

Table 2 *Composition of the sow diets fed from day 7 before farrowing and throughout lactation (mean ± s.e.)*

	Control diet ($n = 5$)	Linseed oil diet ($n = 4$)	Fish oil diet ($n = 3$)
Oil supplementation (kg/t of feed)			
Palm oil	26	9.14	3.5
Soy oil	2.8	0	0.7
Linseed oil	0	20	0
Fish oil	0	0	20
Analyzed composition (% fresh matter)			
Dry matter	88.9 ± 0.1	89.1 ± 0.2	89.4 ± 0.6
Ash	5.8 ± 0.1	5.9 ± 0.1	5.6 ± 0.1
Ether extract (total fat)	4.9 ± 0.2	5.7 ± 0.1	6.5 ± 0.2
Total protein	14.5 ± 0.1	14.4 ± 0.2	13.6 ± 1.0
NE[1]	9.39	9.43	9.48
Fatty-acid composition (g/100 g fat)			
C16:0	27.08 ± 0.95	17.61 ± 0.56	20.99 ± 1.26
C18:0	2.97 ± 0.08	3.09 ± 0.08	3.22 ± 0.07
C18:1c9	26.02 ± 0.71	21.95 ± 0.14	18.59 ± 0.95
C18:2n-6	36.04 ± 1.46	30.05 ± 0.80	30.29 ± 2.02
C18:3n-6	0.006 ± 0.001	0.006 ± 0.001	0.06 ± 0.01
C20:3n-6	0.004 ± 0.001	0.012 ± 0.001	0.04 ± 0.01
C20:4n-6	0.012 ± 0.003	0.013 ± 0.003	0.31 ± 0.01
C22:4n-6	0.029 ± 0.004	0.019 ± 0.003	0.14 ± 0.02
C18:3n-3	2.96 ± 0.15	21.62 ± 0.79	2.90 ± 0.25
C18:4n-3	0.021 ± 0.004	0.031 ± 0.009	0.71 ± 0.01
C20:5n-3	0.11 ± 0.03	0.16 ± 0.06	4.91 ± 0.12
C22:5n-3	0.020 ± 0.001	0.020 ± 0.007	0.60 ± 0.03
C22:6n-3	0.10 ± 0.02	0.12 ± 0.04	3.42 ± 0.03

[1]Net energy value of the feed (KJ/kg feed) as calculated from the formulation.

(Raes *et al.*, 2001). The gas chromatography conditions were: injector: 250°C, detector: 280°C and H_2 as carrier gas. Temperature program: 150°C for 2 min followed by an increase by 1.5°C/min up to 200°C. After this the temperature was increase by 5°C/min up to 215°C. Peaks were identified by comparing the retention times with those of the corresponding standards (Sigma, Belgium; Nu-Chek Prep., Mn, USA).

Isolation of microsomes

Δ6d and Δ5d are microsomal enzymes. Samples of pig liver (approximately 3.5 g), LD muscle and subcutaneous adipose tissue (approximately 7.5 g) were homogenised in a Tris-sucrose buffer containing 250 mM sucrose and 10 mM Tris-HCl (pH 7.4), and the microsomal fraction was isolated by differential centrifugation as described earlier (Doran *et al.*, 2006). The microsomal pellets were re-suspended in a Tris-KCl buffer (150 mM KCl and 10 mM Tris-HCl, pH 7.4), supplemented with inhibitors of proteolytic enzymes (1.5 μM pepstatin, 1.5 μM antipain and 2 μM leupeptin). The protein level in isolated microsomes was determined by the Bradford method (Bradford, 1976). The average final concentration of microsomal protein for liver, muscle and SF were 16.3, 11.5 and 5.8 mg/ml, respectively.

Enzyme expression analyses

Expression of Δ6d and Δ5d proteins in isolated microsomes was determined by Western blotting. The microsomal proteins (12 μg) were separated by SDS-PAGE and transferred onto a nitrocellulose membrane at 100 V for 1 h. The membrane was probed with the corresponding primary antibody for 1.5 h. Δ6d and Δ5d antibodies were custom-produced by Sigma Genosys Ltd (Cambridge, UK) in rabbits against the synthetic peptides containing amino-acid sequences from the regions which are conserved in the rat, pig and human (near C-terminus of the corresponding proteins). The synthetic peptides were linked to Keyhole Limpet Haemocyanin. The antibodies were demonstrated to cross-react with the porcine Δ6d and Δ5d proteins. After probing with primary antibody, the nitrocellulose membrane was washed with PBST (phosphate-buffered saline–Tween) and incubated with horseradish peroxide linked donkey anti-rabbit IgG as the secondary antibody (Amersham, Buckinghamshire, UK) for 1 h. The protein bands were visualised with an enhanced chemiluminescence detection system (Amershan). The intensity of the protein bands was quantified using the ImageQuant program (Molecular Dynamics, GE HealthCare UK Ltd, Buckinghamshire, UK). A microsomal preparation from one particular sample was present on all the blots and the intensity of the Δ6d or Δ5d signals of this sample was taken as 100% (reference sample). The optimum of microsomal protein required for Δ6d or Δ5d analyses was detected by calibration.

Statistical analyses

The effect of dietary group ($n = 4$) on the fatty-acid composition was analysed separately in each tissue by one-way ANOVA. The relative expression of Δ6d and Δ5d proteins was initially analysed by a linear model including the effect of dietary group ($n = 4$), tissue ($n = 3$) and the dietary group × tissue interaction term. One-way ANOVA was performed per group to compare the relative expression of Δ6d or Δ5d between tissues. *Post-hoc* comparison of means was done using the Tukey method. $P < 0.05$ was considered statistically significant. For all the variables (except small changes in C22:4n-6 and Δ6d in liver, and saturated fatty acids (SFA) in SF) there was no significant difference between control groups on different farms. Therefore, an effect of farm on the parameters studied was considered to be negligible. The statistical program used was S_PLUS 6.1 for Windows (Insightful Corporation, Seattle, WA, USA).

Results

Effect of maternal diet supplemented with fish oil on polyunsaturated fatty acids in piglet's tissues

Fish oil supplementation caused a two-fold increase in the content of total hepatic n-3 PUFA, mainly due to an increase in 20:5n-3 and 22:6n-3 ($P < 0.001$; Table 3). The content of total and individual hepatic n-6 PUFA, such as 20:4n-6 and 22:4n-6 was significantly decreased by the fish-oil diet ($P < 0.001$ and $P < 0.001$, respectively). No significant changes were observed in the major n-6 fatty acid, 18:2n-6. As a result of the opposite effect of fish oil on n-3 and n-6 PUFA, the total PUFA content did not change significantly, and the n-6/n-3 ratio was significantly reduced ($P < 0.001$).

In muscle, as in liver, the fish-oil diet resulted in a significant increase (three-fold) in the content of total n-3 PUFA ($P < 0.001$; Table 4), mainly on the account of long-chain PUFA (20:5n-3, 22:5n-3 and 22:6n-3). However, the effect of fish-oil diet on muscle n-6 PUFA content was less pronounced when compared to the hepatic n-6 PUFA content. Total n-6 PUFA content in muscle did not change significantly, although there were some significant changes in the content of 22:4n-6 ($P < 0.001$).

As in liver and muscle, the fish oil-supplemented diet significantly increased individual (18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3) and total n-3 PUFA content in subcutaneous adipose tissue ($P < 0.001$; Table 5). The content of total and individual n-6 PUFA in SF was not affected.

Effect of maternal diet supplemented with linseed oil on polyunsaturated fatty acids in piglet's tissues

The linseed diet resulted in a significant increase in the content of some individual n-3 PUFA, mainly 18:3n-3 and 20:5n-3, which caused a five- and four-fold increase, respectively, in liver ($P < 0.001$; Table 3). The total content of n-3 PUFA increased in this tissue ($P < 0.001$), but there was no effect on the content of 22:6n-3. The total content of the hepatic n-6 PUFA was not affected by the diet. However, there were relatively small but significant decreases in the individual n-6 PUFA contents (20:4n-6 and 22:4n-6, $P < 0.01$ and $P < 0.001$, respectively).

Table 3 *Effects of a maternal diet supplemented with different oils (control, fish or linseed oil) on polyunsaturated fatty acids content (mg/100 g tissue) in liver tissue of piglets (means* n = *5)*

Fatty acids‡	Control farm 1	Fish oil diet farm 1	Control farm 2	Linseed oil diet farm 2	RMSE§	P_{diet}
SFA	982.4	808.2	972.9	821.2	81.0	0.291
MUFA	501.8	322.3	449.9	455.9	52.1	0.130
C18:2n-6	309.1	244.0	320.7	323.3	27.6	0.182
C18:3n-6	7.4	4.1	12.3	7.1	3.4	0.425
C20:3n-6	21.0	18.1	21.1	14.7	2.3	0.192
C20:4n-6	365.8^{a}	219.0^{b}	333.3^{a}	223.1^{b}	22.1	<0.001
C22:4n-6	14.2^{a}	2.5^{c}	10.1^{b}	2.1^{c}	0.9	<0.001
Σn-6 PUFA	717.5^{a}	487.7^{b}	697.4^{a}	570.3^{ab}	50.8	0.017
C18:3n-3	6.1^{a}	6.5^{a}	7.7^{a}	39.1^{b}	2.2	<0.001
C18:4n-3	1.2^{ab}	1.9^{b}	1.1^{a}	1.1^{a}	0.2	0.005
C20:5n-3	5.4^{a}	71.2^{b}	7.9^{a}	31.0^{c}	1.8	<0.001
C22:5n-3	40.4	52.9	44.2	52.4	5.3	0.286
C22:6n-3	115.3^{a}	208.3^{b}	107.2^{a}	98.9^{a}	10.6	<0.001
Σn-3 PUFA	168.5^{a}	340.8^{b}	168.2^{a}	222.5^{a}	16.7	<0.001
n-6/n-3	4.3^{a}	1.4^{b}	4.1^{a}	2.6^{c}	0.1	<0.001
Σn-6 + Σn-3	886.0	828.5	865.6	792.7	66.3	0.764
Total FA	2385.3	1970.3	2301.1	2087.4	193.9	0.432

‡SFA, MUFA, PUFA = saturated, monounsaturated and polyunsaturated fatty acids, respectively.
§RMSE = root mean square error.
^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
Σn-6 PUFA was calculated as: 18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6.
Σn-3 PUFA was calculated as: 18:3n-3 + 18:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3.

Table 4 *Effects of a maternal diet supplemented with different oils (control, fish or linseed oil) on polyunsaturated fatty acids content (mg/100 g tissue) in muscle tissue of piglets (means* n = *5)*

Fatty acids‡	Control farm 1	Fish oil diet farm 1	Control farm 2	Linseed oil diet farm 2	RMSE§	P_{diet}
SFA	1120.5	1295.5	1977.0	1734.2	236.6	0.069
MUFA	1029.9	1125.5	1910.7	1842.6	323.0	0.146
C18:2n-6	436.4	504.0	736.3	827.8	114.8	0.086
C18:3n-6	5.2	5.5	7.0	7.6	1.1	0.330
C20:3n-6	14.6	15.7	17.8	19.8	2.1	0.337
C20:4n-6	108.1^{ab}	84.6^{b}	112.7^{a}	103.0^{ab}	6.7	0.044
C22:4n-6	10.6^{a}	6.0^{b}	9.0^{ab}	6.5^{b}	0.8	0.004
Σn-6 PUFA	575.0	615.8	882.9	964.7	124.0	0.103
C18:3n-3	21.2^{a}	30.6^{a}	51.7^{a}	242.7^{b}	31.4	<0.001
C18:4n-3	0.4^{a}	1.5^{ab}	0.7^{a}	2.8^{b}	0.3	<0.001
C20:5n-3	5.1^{a}	35.9^{b}	9.3^{a}	30.2^{b}	2.9	<0.001
C22:5n-3	22.2^{a}	53.1^{bc}	32.3^{ab}	57.9^{c}	5.5	<0.001
C22:6n-3	14.1^{a}	59.5^{b}	16.3^{a}	25.1^{a}	3.4	<0.001
Σn-3 PUFA	63.0^{a}	180.6^{a}	110.3^{a}	358.7^{b}	40.2	<0.001
n-6/n-3	9.1^{a}	3.3^{b}	8.0^{a}	2.8^{b}	0.2	<0.001
Σn-6 + Σn-3	638.0^{a}	796.4^{ab}	993.2^{ab}	1323.4^{b}	159.6	0.043
Total FA	2813.8	3209.9	4921.9	4960.2	716.7	0.100

‡SFA, MUFA, PUFA = saturated, monounsaturated and polyunsaturated fatty acids, respectively.
§RMSE = root mean square error.
^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
Σn-6 PUFA was calculated as: 18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6.
Σn-3 PUFA was calculated as: 18:3n-3 + 18:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3.

In piglets' muscle, the maternal linseed oil supplemented diet affected all the individual n-3 PUFA (except C22:6n-3), which were significantly increased (Table 4). As a result, the content of the total n-3 PUFA was elevated by more than three-fold ($P < 0.05$). There was no significant effect of the linseed diet on either individual or total n-6 PUFA in piglets' tissues.

In adipose tissue, as in liver and muscle, the linseed diet significantly increased the content of total n-3 PUFA (by approximately four-fold, $P < 0.001$), mainly on the account of

Table 5 *Effects of a maternal diet supplemented with different oils (control, fish or linseed oil) on polyunsaturated fatty acids content (mg per 100 g tissue) in subcutaneous adipose tissue of piglets (means* n = *5)*

Fatty acids‡	Control farm 1	Fish oil diet farm 1	Control farm 2	Linseed oil diet farm 2	RMSE§	P_{diet}
SFA	9294.0[a]	13273.0[ab]	15427.0[b]	10741.0[ab]	1291.0	0.019
MUFA	13848.0	17104.0	21334.0	17566.0	2346.2	0.206
C18:2n-6	3842.4	5543.6	5726.0	6001.6	639.4	0.113
C18:3n-6	49.1	73.3	58.1	59.0	7.0	0.146
C20:3n-6	88.2	88.9	99.9	88.2	13.1	0.898
C20:4n-6	192.7	187.8	207.0	163.8	19.1	0.469
C22:4n-6	13.5	12.1	11.8	22.4	3.2	0.096
Σn-6 PUFA	4185.9	5905.7	6102.8	6335.0	675.0	0.139
C18:3n-3	286.1[a]	489.8[a]	548.1[a]	2375.6[b]	110.9	<0.001
C18:4n-3	20.4	22.3	16.3	22.0	2.1	0.219
C20:5n-3	20.4[a]	178.4[b]	21.9[a]	76.8[a]	16.5	<0.001
C22:5n-3	70.1[a]	255.9[b]	106.6[a]	167.8[ab]	27.0	0.001
C22:6n-3	36.1[a]	295.2[b]	46.2[a]	63.3[a]	27.4	<0.001
Σn-3 PUFA	433.0[a]	1241.7[b]	739.3[ab]	2705.6[c]	153.3	<0.001
n-6/n-3	9.7[a]	4.9[b]	8.5[a]	2.3[c]	0.3	<0.001
Σn-6 + Σn-3	4618.9[a]	7147.4[ab]	6842.0[ab]	9040.5[b]	796.7	0.011
Total FA	27979.0	37830.0	43927.0	37286.0	4358.2	0.119

‡SFA, MUFA, PUFA = saturated, monounsaturated and polyunsaturated fatty acids, respectively.
§RMSE = root mean square error.
[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
Σn-6 PUFA was calculated as: 18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6.
Σn-3 PUFA was calculated as: 18:3n-3 + 18:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3.

18:3n-3 and 20:5n-3 (Table 5). The diet did not significantly affect the individual and total n-6 PUFA in this tissue.

Effect of the maternal diet supplemented with fish-oil or linseed oil on Δ5d and Δ6d protein expression in piglets' tissues

We have detected the presence of Δ5d and Δ6d immunoreactive proteins in piglets' liver, LD muscle and subcutaneous adipose tissues by Western blot analyses. Examples of the blots are given in Figure 1. The sizes of the immunoreactive bands for both enzymes were approximately 50 kDa, which is consistent with the molecular weights of Δ5d and Δ6d proteins reported in other species (Cho *et al*., 1999a and 1999b). Across diets, the average expression of Δ5d protein was the highest in muscle, followed by subcutaneous adipose tissue, with the lowest level in the liver (Table 6). The picture was different for Δ6d (Table 7). This enzyme was mostly expressed in liver and subcutaneous adipose tissue, with the lowest level in muscle.

Fish oil supplementation in the diet significantly increased the expression of Δ5d in muscle tissue ($P < 0.01$; Table 6). The effect was higher in the case of Δ5d when compared to Δ6d (71.9% and 33.7% increase, respectively). In contrast to muscle, the fish-oil diet did not affect Δ6d or Δ5d protein expression in piglets' liver or subcutaneous adipose tissue.

In the case of linseed diet, there were no significant changes in Δ6d levels in the liver, muscle or subcutaneous adipose tissue. There was a non-significant increase in Δ5d protein expression in all three tissues under these conditions. Lack of statistical significance might be related to large variations between individual animals and a low number of samples per each group.

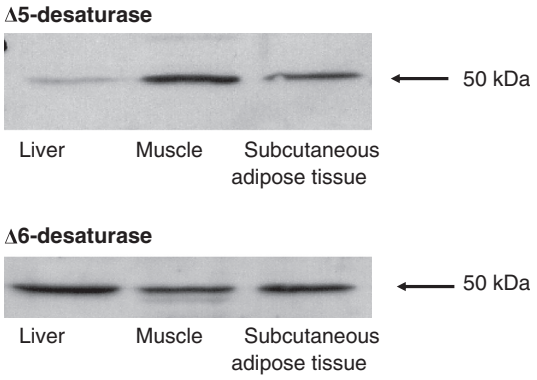

Figure 1 Representative blots of Δ5-desaturase and Δ6-desaturase protein expression in liver, muscle and subcutaneous adipose tissue of piglets. Enzyme expression was analysed in cytosol isolated from liver, *longissimus dorsi* muscle and subcutaneous adipose tissue of piglets from control group. A total of 12 μg of cytosolic protein was used for Western blot analysis in all the cases.

Discussion

Fish oil and linseed oil are good sources of n-3 PUFA. Fish oil is particularly high in long-chain n-3 PUFA, whilst linseed oil is high in 18:3n-3, which is a precursor for tissue biosynthesis of long-chain n-3 PUFA, such as 20:5n-3 and 22:6n-3 (Cleland *et al*., 2003; Leaf *et al*., 2003;

Table 6 *Effect of maternal diet supplemented with different oils (control, fish oil or linseed oils) on the relative expression of Δ5-desaturase in liver, muscle and subcutaneous adipose tissue of the piglets (means* n = *5)*

Tissue	Control diet farm 1	Fish oil diet farm 1	Control diet farm 2	Linseed oil diet farm 2	RMSE	P_{diet}
Liver	$^{y}52.4$	$^{y}67.0$	$^{y}66.8$	$^{y}110.0$	14.7	0.068
Muscle	$^{z}360.7^{a}$	$^{z}620.1^{b}$	$^{z}325.4^{a}$	$^{z}408.4^{a}$	49.2	0.003
SAT	$^{z}267.5$	$^{x}267.9$	$^{y}175.6$	$^{y}220.9$	37.1	0.275
P_{tissue}	<0.001	<0.001	<0.001	<0.001		

RMSE = root mean square error; SAT = subcutaneous adipose tissue.
^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
^{x,y,z}Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

Table 7 *Effect of maternal diet supplemented with different oils (control, fish oil or linseed oils) on the relative expression of Δ6-desaturase in liver, muscle and subcutaneous adipose tissue of the piglets (means* n = *5)*

Tissue	Control diet farm 1	Fish oil diet farm 1	Control diet farm 2	Linseed oil diet farm 2	RMSE	P_{diet}
Liver	$^{y}131.4^{a}$	$^{y}105.7^{ab}$	$^{yz}73.3^{b}$	$^{yz}73.9^{b}$	12.9	0.009
Muscle	$^{z}58.7^{ab}$	$^{z}78.5^{b}$	$^{z}55.4^{a}$	$^{z}60.5^{ab}$	5.2	0.028
SAT	$^{z}89.9^{ab}$	$^{z}70.1^{b}$	$^{y}104.0^{a}$	$^{y}101.3^{a}$	7.5	0.023
P_{tissue}	0.001	0.005	0.011	0.018		

RMSE = root mean square error; SAT = subcutaneous adipose tissue.
^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
^{y,z}Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

Vanschoonbeek *et al*., 2003). The tissue biosynthesis of long-chain n-3 PUFA from 18:3n-3 involves a chain of reactions catalysed by Δ6d, Δ5d and elongases, together with the β-oxidation of 24:6n-3 to 22:6n-3 (Sprecher, 2000). Therefore, in principle, dietary-induced changes in the fatty-acid composition could be the result of not only direct incorporation of the fatty acid into the tissues, but also a result of activation/inhibition of lipogenic enzyme expression.

In the present paper, the fish oil supplementation to the diets of pregnant and lactating sows resulted in a significant increase in long-chain n-3 PUFA in piglets' liver, muscle and subcutaneous adipose tissue, namely in 20:5n-3, 22:5n-3 and 22:6n-3. The main reason for this increase is likely to be direct incorporation of the maternal dietary fatty acid into the foetal tissue during pregnancy and/or to the piglet's tissues during lactation. This is consistent with the report that modulation of dietary fatty acids of the sow milk influences the fatty-acid composition of piglets' liver and adipose tissue (Lauridsen and Jensen, 2007). In respect to manipulation of the pig diet during gestation, some authors reported that only a small amount of the dietary fatty acids can be transferred to the foetus (Thulin *et al*., 1989; Ramsay *et al*., 1991). However, a later study demonstrated that maternal dietary fat has a significant input in the fatty-acid composition of plasma and tissues in the developing foetus (Leskanich and Noble, 1999). Interestingly, the present work has established a tissue-specific response to the fish oil-supplemented diet. The degree of the increase in 20:5n-3 and 22:6n-3 varied between piglets' organs. The maximum increase of 20:5n-3 (13-fold) was observed in the liver, with only 7-fold increase in muscle and 8.7-fold increase in SF. At the same time the liver had minimal increase in 22:6n-3 (only 1.8-fold), when compared to muscle and adipose (4-fold and 8.1-fold, respectively). Our results are consistent with the tissue-specific changes in the piglet's fatty-acid composition in response to fish oil supplementation in the maternal diet reported by other authors (Arbuckle and Innis, 1993; Fritsche *et al*., 1993; Rooke *et al*., 2001; Lauridsen and Jensen, 2007).

In the present study, the tissue-specific response was observed in not only the amount, but also in the type of fatty acids. Piglets' liver, SF and muscle responded similarly to the fish-oil diets only in terms of 20:5n-3 and 22:6n-3, which were significantly increased in all three organs. However, muscle and SF also had an elevated level of 22:5n-3, whilst the level of this fatty acid in the liver remained unchanged. In addition, the fish-oil diet led to a significant increase of 18:3n-3 in subcutaneous adipose tissue. An increase in adipose tissue 18:3n-3 is of particular interest, because it cannot be attributed to incorporation of this fatty acid from the maternal diet. The levels of this fatty acid in the fish oil-supplemented diet did not differ significantly from the control diet (Tables 1 and 2). Reasons for the above increase are not clear. An increase in 20:5n-3 and 22:6n-3 in piglets' muscles under maternal fish oil-supplemented diet might be attributed not only to incorporation of these fatty acids from the diet, but also to possible activation of their tissues biosynthesis. As mentioned above, the key enzymes involved in 20:5n-3 and 22:6n-3 biosynthesis are Δ6d and Δ5d. The present work reports the presence of Δ6d and Δ5d immunoreactive proteins in piglets' liver, muscle and subcutaneous adipose tissue. Interestingly, in our study, Δ6d and Δ5d expression patterns in the piglets' tissues follow opposite directions. The highest expression of Δ5d was observed in the muscles, followed by subcutaneous adipose tissue, with the lowest

level of this protein in the liver. In contrast to Δ5d, the highest expression of Δ6d was observed in piglets' liver and the lowest level of this enzyme was in muscles. These results suggest that the mechanisms regulating Δ6d and Δ5d expression in pigs are tissue-specific. The existence of tissue-specific mechanisms is in agreement with another finding of the present study, namely, with tissue-specific responses of Δ5d and Δ6d expression to the oil-supplemented diet. Tissue-specific responses of lipogenic enzymes to nutritional regulation are well known for other species. For example, Girard *et al*. (1994) and Iritany *et al*. (1996) established differences in responses of rat hepatic and adipose tissue acetyl-CoA carboxylase, fatty acid synthase, ATP citrate-lyase, malic enzyme and glucose-6-phosphate dehydrogenase to food deprivation and to a high-carbohydrate diet.

Some studies have also been conducted on pigs. However, to our knowledge, research on pigs has been limited to enzymes catalysing SFA and monounsaturated fatty-acid biosynthesis. Thus, Doran *et al*. (2006) reported differences in responses of pig muscle and adipose tissue Δ9-desaturase (but not acetyl-CoA carboxylase or fatty acid synthase) to a reduced protein diet.

The activatory effect of fish oil on Δ5d and Δ6d expression in muscle, observed in the present study, is not in agreement with the data of literature on other species, which report repression of lipogenic enzyme-encoding genes by PUFA (Garg *et al*., 1988; Clarke and Jump, 1994). However, we have to keep in mind that the inhibitory effect of PUFA was mainly reported in liver and fat (Shimomura *et al*., 1997) but not in muscle. We hypothesise that the activatory effect of fish oil on Δ5d and Δ6d protein expression in piglets' muscle might be related to the low expression, or to the lack of sterol-regulatory element binding protein-1 (SREBP-1) in this tissue. This SREBP-1 and the PPARα (peroxisome proliferator-activated receptor-α) play a key role in regulation of the human and mice Δ5d and Δ6d (Matsuzaka *et al*., 2002; Wang *et al*., 2006). Interestingly, distribution of SREBP-1 mRNA in mice and human is tissue specific, with the highest level in lipogenic tissues (liver and fat) and a very low level or absence in muscle (Gondret *et al*., 2001; Matsuzaka *et al*., 2002). It is unknown whether SREBP-1 is present in pig muscles. Moreover, dietary PUFA (mainly DHA and EPA) were demonstrated to downregulate the expression of desaturases via suppression of SREBP-1 (Matsuzaka *et al*., 2002).

As in the case with fish oil, supplementation of the maternal diets with linseed oil also resulted in an increase of total n-3 PUFA in piglets' liver, muscle and subcutaneous adipose tissue. This was mainly due to a significant increase in 18:3n-3 and 20:5n-3 in all three tissues, and an increase in 22:6n-3 in piglets' muscles. An increase in 18:3n-3 is likely to be the consequence of the direct incorporation of this fatty acid from the maternal diet and it is consistent with data of literature (D'Arrigo *et al*., 2002; Nuernberg *et al*., 2005). However, the elevated 20:5n-3 and 22:6n-3 content in the case of linseed-supplemented diet cannot be explained by dietary incorporation, because the levels of these fatty acids in the control and linseed-supplemented diets were similar. One possible explanation for an increase in the tissue 22:6n-3 and 20:5n-3 could be activation of their biosynthesis in piglets' tissues. This suggestion is consistent with the fact that the linseed oil diet supplies a high level of substrate, namely 18:3n-3, for 20:5n-3 and 22:6n-3 biosynthesis. Although we did not observe any significant changes in expression of enzymes involved in 20:5n-3 and 22:6n-3 biosynthesis (Δ5d and Δ6d protein levels were similar), we cannot dismiss a possible direct effect of the dietary fatty acids on enzyme activities. It cannot be excluded that dietary-induced fatty-acid conversion takes place in the maternal organism and is passed to the offspring. Including the fish oil or linseed oil to the maternal diet resulted not only in an increase in n-3 PUFA but also in a decline in n-6 PUFA in the tissues of the progeny. A similar pattern in the response of n-3 and n-6 PUFA to the maternal diet supplemented with salmon oil was reported by other authors (Arbuckle and Innis, 1993; Fritsche *et al*. 1993; Rooke *et al*., 2001; Lauridsen and Jensen, 2007).

In conclusion, the main findings of the present study are:

(i) A maternal diet supplemented with fish oil or linseed oil increases the level of n-3 PUFA in muscle, SF and liver of the piglets in a tissue-specific manner.
(ii) The increase in n-3 PUFA level in the offspring under the fish oil-supplemented diet is partially related to the activation of Δ6d and Δ5d protein expression.
(iii) This study is a first communication characterising tissue-specific distribution of Δ6d and Δ5d proteins in pigs.

Acknowledgements

This research was funded by the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT), Brussels, Belgium (Project IWT 050650) and the Commission Scientific Research (CWO), Faculty of Bioscience Engineering of Ghent University, Ghent, Belgium. The enzyme expression experiments were conducted at the facilities of the Department of Clinical Veterinary Science, University of Bristol.

References

Arbuckle LD and Innis SM 1993. Docosahexaenoic acid is transferred through maternal diet to milk and to tissues of natural milk-fed piglets. Journal of Nutrition 123, 1668–1675.

Barcelo-Coblijn G, Murphy EJ, Othman R, Moghadasian MH, Kashour T and Friel JK 2008. Flaxseed oil and fish-oil capsule consumption alters human red blood cell n-3 fatty acid composition: a multiple-dosing trial comparing 2 sources of n-3 fatty acids. American Journal of Clinical Nutrition 88, 801–809.

Berquin IM, Edwards IJ and Chen YQ 2008. Multi-targeted therapy of cancer by omega-3 fatty acids. Cancer Letters 269, 363–377.

Bradford MM 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248–254.

Brenner RR 1974. The oxidative desaturation of unsaturated fatty acids in animals. Molecular and Cellular Biochemistry 3, 41–52.

Cho HP, Nakamura M and Clarke SD 1999a. Cloning, expression, and fatty acid regulation of the human Δ5-desaturase. Journal of Biological Chemistry 274, 37335–37339.

Cho HP, Nakamura M and Clarke SD 1999b. Cloning, expression and nutritional regulation of the mammalian Δ6-desaturase. Journal of Biological Chemistry 274, 471–477.

Clarke SD and Jump D 1994. Dietary polyunsaturated fatty acid regulation of gene transcription. Annual Review of Nutrition 14, 83–98.

Cleland LG, James MJ and Proudman SM 2003. The role of fish oils in the treatment of rheumatoid arthritis. Drugs 63, 845–853.

D'Arrigo M, Hoz L, Lopez-Bote CJ, Cambero I, Pin C and Ordonez JF 2002. Effect of dietary linseed oil on pig hepatic tissue fatty acid composition and susceptibility to lipid peroxidation. Nutrition Research 10, 1189–1196.

Doran O, Moule SK, Teye GA, Whittington FM, Hallett KG and Wood JD 2006. A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneous adipose tissue: relationship with intramuscular lipid formation. British Journal of Nutrition 95, 609–617.

Folch J, Lees M and Sloane-Stanley GH 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226, 497–509.

Fritsche KL, Huang S-C and Cassity NA 1993. Enrichment of omega-3 fatty acids in suckling pigs by maternal dietary fish oil supplementation. Journal of Animal Science 71, 1841–1847.

Garg ML, Sebokova E, Thomson AB and Clandinin MT 1988. Delta 6-desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or omega 3 fatty acids. Biochemical Journal 249, 351–356.

Girard J, Perdereau D, Foufelle F, Prip-Buus C and Ferre P 1994. Regulation of lipogenic enzyme gene expression by nutrients and hormones. The FASEB Journal 8, 36–42.

Givens ID and Gibbs RA 2008. Current intakes of EPA and DHA in European populations and the potential of animal-derived foods to increase them. Proceedings of Nutrition Society 67, 273–280.

Gondret F, Ferre P and Dugail I 2001. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. Journal of Lipid Research 42, 106–113.

Iritany N, Fukusa H and Tada K 1996. Nutritional regulation of lipogenic enzyme gene expression in rat epididymal adipose tissue. Journal of Biochemistry 120, 242–248.

ISO 6492 1999. International Organization for Standardization. Animal feeding stuffs. Determination of fat content. ISO, Geneve, Switzerland.

ISO 5984 2002. International Organization for Standardization. Animal feeding stuffs. Determination of crude ash. ISO, Geneve, Switzerland.

ISO 5983-1 2005. International Organization for Standardization. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. Part 1: Kjeldahl method. ISO, Geneve, Switzerland.

Lauridsen C and Jensen SK 2007. Lipid composition of lactational diets influences the fatty acid profile of the progeny before and after suckling. Animal 1, 952–962.

Leaf A, Xiao YF, Kang JX and Billman GE 2003. Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. Pharmacology and Therapeutics 98, 355–377.

Leskanich CO and Noble RC 1999. The comparative roles of polyunsaturated fatty acids in pig neonatal development. British Journal of Nutrition 81, 87–106.

Li JJ, Huang CJ and Xie D 2008. Anti-obesity effects of conjugated linoleic acid, docosahexaenoic acid, and eicosapentaenoic acid. Molecular Nutrition and Food Research 52, 631–645.

Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Yoshikawa T, Hasty AH, Tamura Y, Osuga JI, Okazaki H, Iizuka Y, Takahashi A, Sone H, Gotoda T, Ishibashi S and Yamada N 2002. Dual regulation of mouse Δ5- and Δ6-desaturase gene expression by SREBP-1 and PPARα. Journal of Lipid Research 43, 107–114.

Moghadasian MH 2008. Advances in dietary enrichment with n-3 fatty acids. Critical Reviews in Food Science and Nutrition 48, 402–410.

Mourot J and Hermier D 2001. Lipids in monogastric meat (review article). Reproduction Nutrition Development 41, 109–118.

Mozaffarian D 2008. Fish and n-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death. American Journal of Clinical Nutrition 87, 1991S–1996S.

Neuringer M, Anderson GJ and Connor WE 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Annual Review of Nutrition 8, 517–541.

Nuernberg K, Fischer K, Nuernber G, Kuechenmeister U, Klosowska D, Eliminowska-Wenda G, Fiedler I and Ender K 2005. Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. Meat Science 70, 63–64.

Raes K, De Smet S and Demeyer D 2001. Effect of double-muscling in Belgian Blue bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. Animal Science 73, 253–260.

Ramsay TG, Karousis J, White ME and Wolverton CK 1991. Fatty acid metabolism by the porcine placenta. Journal of Animal Science 69, 3645–3654.

Rigau AP, Lindemann MD, Kornegay ET, Harper AF and Watkins BA 1995. Role of dietary lipids on fetal tissue fatty acid composition and fetal survival in swine at 42 days of gestation. Journal of Animal Science 73, 1372–1380.

Rooke JA, Bland IM and Edwards SA 1998. Effect of feeding tuna oil or soyabean oil as supplements to sows in late pregnancy on piglet tissue composition and viability. British Journal of Nutrition 80, 273–280.

Rooke JA, Sinclair AG, Edwards SA, Cordoba R, Pkiyach S, Penny PC, Penny P, Finch AM and Horgan GW 2001. The effect of feeding salmon oil to sows throughout pregnancy on pre-weaning mortality of piglets. Animal Science 73, 489–500.

Ruxton CH, Reed SC, Simpson MJ and Millington KJ 2004. The health benefit of omega-3 polyunsaturated fatty acids: a review of the evidence. Journal of Human Nutrition and Dietetics 17, 449–459.

SanGiovanni JP, Chew EY, Agron E, Clemons TE, Ferris FL 3rd, Gensler G, Lindblad AS, Milton RC, Seddon JM, Klein R and Sperduto RD 2008. The relationship of dietary omega-3 long-chain polyunsaturated fatty acid intake with incident age-related macular degeneration: AREDS report no. 23. Archives of Ophthalmology 126, 1274–1279.

Shimomura I, Shimano H, Horton JD, Goldstein JL and Brown MS 1997. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. The Journal of Clinical Investigation 99, 838–845.

Sprecher H 2000. Metabolism of highly unsaturated n-3 and n-6 fatty acids. Biochimica et Biophysica Acta 1486, 219–231.

Thulin AJ, Allee GL, Harmon DL and Davis DL 1989. Utero-placenta transfer of octanoic, palmitic and linoleic acids during late gestation in gilts. Journal of Animal Science 67, 738–745.

Vanschoonbeek K, de Maat MP and Heemskerk JW 2003. Fish oil consumption and reduction of arterial disease. The Journal of Nutrition 133, 657–660.

Wang Y, Botolin D, Xu J, Christian B, Mitchell E, Jayaprakasam B, Nair M, Peters JM, Busik J, Olson LK and Jump DB 2006. Regulation of hepatic fatty acid elongase and desaturase expression in diabetes and obesity. Journal of Lipid Research 47, 2028–2034.

Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI and Whittington FM 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Science 78, 343–358.

Xiang M, Rahman MA, Ai H, Li X and Harbige LS 2006. Diet and gene expression: delta-5 and delta-6 desaturases in healthy Chinese and European subjects. Annals of Nutrition and Metabolism 50, 492–498.