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Linoleic and α-linolenic acid as precursor and inhibitor for the synthesis of long-chain polyunsaturated fatty acids in liver and brain of growing pigs

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Studies suggested that in human adults, linoleic acid (LA) inhibits the biosynthesis of n-3 long-chain polyunsaturated fatty acids (LC-PUFA), but their effects in growing subjects are largely unknown. We used growing pigs as a model to investigate whether high LA intake affects the conversion of n-3 LC-PUFA by determining fatty acid composition and mRNA levels of Δ5- and Δ6 desaturase and elongase 2 and -5 in liver and brain. In a 2 × 2 factorial arrangement, 32 gilts from eight litters were assigned to one of the four dietary treatments, varying in LA and α-linolenic acid (ALA) intakes. Low ALA and LA intakes were 0.15 and 1.31, and high ALA and LA intakes were 1.48 and 2.65 g/kg $BW^{0.75}$ per day, respectively. LA intake increased arachidonic acid (ARA) in liver. ALA intake increased eicosapentaenoic acid (EPA) concentrations, but decreased docosahexaenoic acid (DHA) (all $P < 0.01$) in liver. Competition between the n-3 and n-6 LC-PUFA biosynthetic pathways was evidenced by reductions of ARA (>40%) at high ALA intakes. Concentration of EPA (>35%) and DHA (>20%) was decreased by high LA intake (all $P < 0.001$). Liver mRNA levels of Δ5- and Δ6 desaturase were increased by LA, and that of elongase 2 by both ALA and LA intakes. In contrast, brain DHA was virtually unaffected by dietary LA and ALA. Generally, dietary LA inhibited the biosynthesis of n-3 LC-PUFA in liver. ALA strongly affects the conversion of both hepatic n-3 and n-6 LC-PUFA. DHA levels in brain were irresponsive to these diets. Apart from Δ6 desaturase, elongase 2 may be a rate-limiting enzyme in the formation of DHA.

Keywords: linoleic acid, linolenic acid, liver, brain, enzymes

Implications

Docosahexaenoic acid (DHA; *c*22:6 n-3) and eicosapentaenoic acid (EPA; *c*22:5 n-3) are important n-3 fatty acids to improve human health. DHA is important in brain for cognitive development. This study has been carried out with growing pigs as model for humans. The n-3 fatty acid such as α-linolenic acid (ALA; *c*18:3 n-3) is a precursor for DHA and EPA via enzymatic steps. EPA can be increased in the liver by dietary ALA. Feeding of linoleic acid (LA; *c*18:2 n-6), a precursor for a competitive pathway, inhibits the synthesis of EPA. The potential for increasing brain DHA via an increase in dietary ALA is limited.

Introduction

Arachidonic acid (ARA; *c*20:4 n-6), eicosapentaenoic acid (EPA; *c*20:5 n-3) and docosahexaenoic acid (DHA; *c*22:6 n-3) are long-chain polyunsaturated fatty acids (LC-PUFA) and well known to play important regulatory functions in the immune, nervous and cardiovascular system (Innis, 2007; Calder, 2009; Calder and Yaqoob, 2009; Russo, 2009). The n-6 LC-PUFA can be synthesized from linoleic acid (LA; *c*18:2 n-6) and those of the n-3 family from α-linolenic acid (ALA; *c*18:3 n-3). Synthesis of EPA and DHA from ALA is achieved by a sequence of desaturation and chain elongation steps, controlled by the enzymes Δ5- (FADS1) and Δ6 desaturase (FADS2) and presumably elongase 2 (ELOVL2) and -5 (ELOVL5) (see Figure 1). DHA can be generated from EPA that requires an additional chain-shortening step (i.e. β-oxidation) in the peroxisome (Sprecher, 2000; Igarashi *et al.*, 2007a). The conversion of n-6 LC-PUFA from LA (resulting in the production of ARA) sharing the same enzymes resulting in enzymatic competition between the biosynthetic pathways of n-3 and n-6 LC-PUFA.

The requirement of LC-PUFA for brain growth and development is of current interest. LC-PUFA, in particular DHA and ARA, accumulate rapidly during the brain growth spurt, the most critical stage of brain development that takes place during the later part of gestation and early postnatal life.

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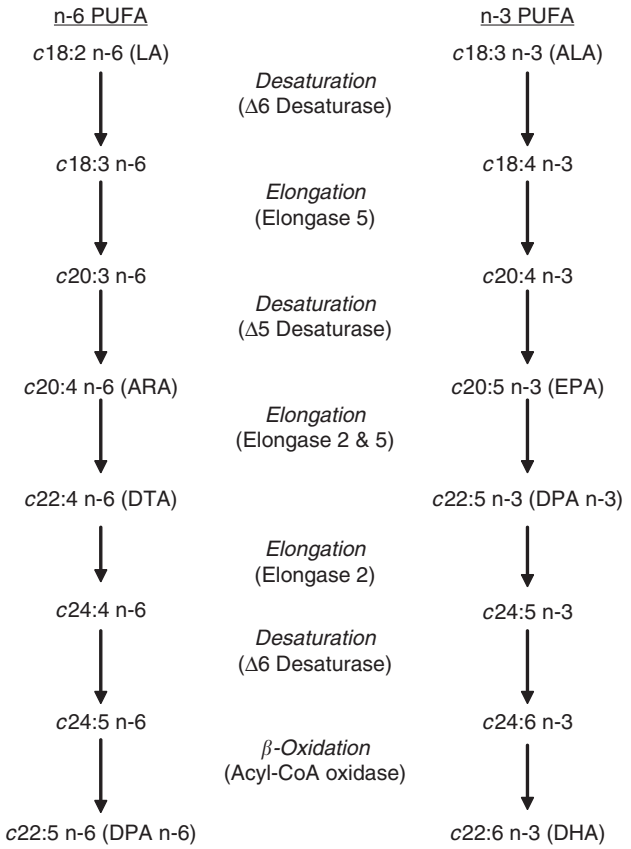

Figure 1 General metabolic pathway of n-6 and n-3 fatty acids (adapted from Igarashi *et al.*, 2007a); LA = linoleic acid; ALA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DTA = docosatetranoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

ARA is essential for normal brain growth, playing a role in synaptic transmission (Bazan, 2003; Sang and Chen, 2006). Changes in brain DHA concentrations have been demonstrated to be positively correlated with cognitive development (Innis, 2007). In addition, the dietary supplementation of DHA could improve the cognitive dysfunction due to aging (McCann and Ames, 2005; Cole and Frautschy, 2006). The regulatory mechanisms of the conversion of LC-PUFA into ARA, EPA and DHA, and the consequences of LA- and ALA-rich diets consumed during childhood remain to be elucidated. In adult humans, it has been estimated that the β-oxidation of ALA is $\pm$ 20% to 30%, which is much lower than that of LA, which was estimated to be 12% to 19% (DeLany *et al.*, 2000). The oxidative losses may partly explain why the conversion of ALA to EPA and DHA in adults is low (Burdge and Calder, 2005). In addition, human food sources such as cereals, sunflower and soybean oil are rich in LA (>50% of the fat) and have been dominant in the Western diet over the last decades. Consumption of LA-rich diets could also result in inadequate conversion of ALA to DHA and reduced concentrations of DHA in the developing brain (Russo, 2009). Dietary studies about the effect of LA are mostly carried out at an isocaloric basis. LA is then replaced by other energy sources such as oleic acid (18:1 n-9). However, it has been reported recently that oleic acid itself can reduce Δ6-desaturase activity (Portolesi *et al.*, 2008).

This study focuses on the interactions between the n-3 and n-6 LC-PUFA biosynthetic pathways in liver and brain using young, growing pigs as a model for human infancy. This animal model has three major advantages over rodents when studying lipid metabolism. First, brain anatomy and morphology and the timing of the brain growth spurt in pigs and humans are similar (Pond *et al.*, 2000; Duhaime, 2006). Second, the anatomy of the digestion system, including liver, stomach and intestine, and also many of the pathways of lipid metabolism in pigs are rather similar to human (Moughan *et al.*, 1991; Innis, 1993). Third, the risk for obesity in young, growing pigs is low, enabling large study contrasts in absolute intakes of LC-PUFA precursors, rather than exchanging for other nutrients to maintain isocaloric intake. Here, we have fed young, growing pigs either low or high amounts of LA and ALA, with equal difference between low and high in the intake within the respective fatty acid, and investigated the effect on the fatty acid composition in liver and brain. In the same tissues, we have determined the mRNA levels of Δ6- and Δ5 desaturase and ELOVL2 and ELOVL5 by quantitative PCR.

Material and methods

Animals and housing

In all, 32 female pigs were selected from eight litters. From each litter, four gilts were allotted to one of the four dietary treatments. On arrival, pigs had a body weight (BW) of 16 kg (s.d. = 1.8). The pigs were housed individually during an experimental period of 4 weeks. They were weekly weighed and feed intake was recorded daily. The experimental protocol was approved by the Animal Experimental Committee of Wageningen University, The Netherlands.

Diets

This study was carried out as a 2×2 factorial design with daily intakes of LA and ALA as independent variables. Differences between low and high intakes were designed to be identical for LA and ALA: low ALA and LA intakes were 0.16 and 1.32, and high ALA and LA intakes were 1.48 and 2.65 g/kg $BW^{0.75}$ per day, respectively. The dietary energy percentage from LA was 3.4, 3.3, 6.6 and 6.4 and those of ALA was 0.4, 3.7, 0.4 and 3.5 for the diets low LA/low ALA, low LA/high ALA, high LA/low ALA and high LA/high ALA, respectively. The size of the experimental contrasts was, in part, based on recent studies with humans showing an effect of dietary LA on EPA in plasma phospholipids (Goyens *et al.*, 2006). In addition, the low LA and ALA intakes were slightly above minimal requirement figures for pigs (National Research Council, 1998; Schellingerhout, 2002). The addition of LA and ALA was on top of a basal diet. The intakes of fatty acids are presented in Table 1. With the exception of LA and ALA, the intakes of SFA (saturated fatty acids) and MUFA (monounsaturated fatty acids) were kept constant. This was done by optimizing the dosages of analyzed sunflower oil, linseed oil, high-oleic acid

Table 1 *Experimental design: average intakes of DE, starch, ileal digestible lysine, fat and fatty acids[1] in g/kg BW$^{0.75}$ per day*

	Low LA		High LA	
	Low ALA	High ALA	Low ALA	High ALA
*c*18:2 n-6 (LA)	1.32	1.31	2.64	2.67
*c*18:3 n-3 (ALA)	0.15	1.49	0.16	1.47
*c*12:0	0.03	0.03	0.03	0.03
*c*14:0	0.02	0.02	0.02	0.02
*c*16:0	0.69	0.66	0.64	0.60
*c*16:1	0.01	0.01	0.01	0.01
*c*18:0	0.13	0.17	0.17	0.22
*c*18:1 n-9	1.34	1.32	1.31	1.31
*c*18:1 n-7	0.03	0.04	0.04	0.05
*c*20:0	0.01	0.01	0.02	0.02
*c*20:1 n-9	0.01	0.01	0.01	0.01
*c*22:0	0.02	0.01	0.03	0.03
*c*24:0	0.01	0.01	0.01	0.01
SFA	0.90	0.91	0.92	0.92
MUFA	1.39	1.38	1.37	1.38
Total fat	4.00	5.42	5.42	6.85
DE (MJ/kg BW$^{0.75}$ per day)[2]	1.24	1.30	1.30	1.35
Ileal digestible lysine[2]	0.89	0.89	0.89	0.89
Starch[2]	34.7	34.7	34.7	34.7

DE = digestible energy; LA = linoleic acid; ALA = α-linolenic acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.
[1]Based on analyzed values.
[2]Based on calculated values (CVB Table Pigs, 2007).

Table 2 *Dietary ingredients (in g/kg, relative to the low LA/low ALA diet) of the dietary treatments with differences in LA and ALA intakes*

	Low LA		High LA	
	Low ALA	High ALA	Low ALA	High ALA
Basal diet[1]	967.4	967.4	967.4	967.4
Sunflower oil	10.5	3.36	40.5	33.6
Linseed oil	1.92	34.1	1.85	34.2
HOSF oil	10.3	6.61	3.76	–
Palm oil	9.89	6.07	4.05	–
Total[2]	1000	1018	1018	1035

LA = linoleic acid; ALA = α-linolenic acid; HOSF = high-oleic sunflower oil.
[1]The basal diet contained (g/kg, as fed) corn starch, 160.1; wheat, 160.5; barley, 321.0; wheat bran, 53.5; molasses, 32.1; soybean meal, 64.2; peas, 53.5; potato protein, 53.5; wheat gluten, 53.5; limestone, 14.1; monocalcium phosphate, 13.7; salt, 5,4; vitamin-mineral mix, 10.7; L-lysine HCl 3.0; L-Threonine, 0.32; tryptophan, 0.32; DL-methionine, 0.54.
[2]The sum of feed ingredients adds up to 1000 g/kg for the low LA/low ALA diet only. A total exceeding 1000 reflects an increased feed intake of the respective treatment group as explained in the text.

sunflower oil and palm oil. The diets did not contain n-3 or n-6 LC-PUFA other than LA and ALA. The composition of the basal diet and the inclusion rates of the oils in the treatments are presented in Table 2. The low LA/low ALA diet was formulated to contain sufficient ileal digestible amino acids in relation to energy (CVB Table Pigs, 2007).

As the LA and ALA increments were not exchanged for other nutrients but dosed on top of the basal diet, digestible energy intake varied among experimental treatments between 2.6 and 2.8 × maintenance energy requirement. The diets were fed twice daily as mash. To allow pigs to express at least some natural foraging behavior, straw (20 g/day) was provided daily, which has been demonstrated to alleviate the stress of individual housing (De Jong *et al.*, 1998; Chaloupková *et al.*, 2007). Water was provided *ad libitum.*

Sampling and fatty acid analysis

At the start of the experimental period, blood sample was taken from each pig at 2.5 h after feeding. At the end of the experimental period, pigs were anesthetized 2.5 h after feeding with an administration of ketamine (10 mg/kg BW) and xylazine (1 mg/kg BW). Blood sample was collected using cardiac puncture before euthanasia was performed by an intracardiac administration of pentobarbital (100 mg/kg BW). Brain and liver were immediately removed after euthanasia. The total brain, frontal cortex and hippocampus were weighed. For gene expression measurements, samples (~1 mg) of liver and hippocampus were rapidly frozen in liquid nitrogen and stored at −80°C until analysis. Other parts of the collected tissues were stored at −20°C pending fatty acid analysis. Total lipids were extracted from diets, liver, frontal cortex and hippocampus with a choloroform : methanol (2 : 1, v/v) Folch mixture and then saponified and methylated to determine fatty acid composition by gas chromatography as described previously (Smink *et al.*, 2008).

Quantitative PCR

Total RNA was isolated from the hippocampus and liver tissue using TRIzol reagent (Invitrogen, Bleiswijk, The Netherlands). Reverse transcription of 1 μg of total RNA was performed in a 20-μl reaction using Superscript III reverse transcriptase (Invitrogen), dNTPs (Roche) and random hexamer primers (Roche, Almere, The Netherlands) for 1 h at 50°C according to the manufacturer's protocol (Invitrogen). Real-time PCR was performed on a LightCycler 2.0 Real-Time PCR System by using FastStart DNA Master SYBR Green I reagents (Roche). The primers used are presented in Table 3. All primer pairs, except for 18S RNA, were designed in such a way that they span an intron of their corresponding genomic sequence or that its sense or reverse primer anneals on an exon-intron junction. Templates were amplified after a preincubation for 10 min at 95°C, followed by amplification for 40 cycles (10 s at 95°C, 5 s at 60°C, 5 s at 72°C). PCR efficiencies for the genes were established to be between 97% and 100%. Expression levels of FADS1, FADS2, ELOVL2 and ELOVL5 were normalized using the corresponding values of 18S RNA. All reactions revealed a single product as determined using melting curve analysis and specificity of the primer sets were verified by sequencing of the generated amplicons.

Statistical analysis

The trial was conducted as a 2 × 2 factorial arrangement of treatments with eight replicates per treatment. The individually

Table 3 *Quantitative PCR primers*

Gene	Forward primer	Reverse primer
Δ5 desaturase	CAGGATGCTACGGATCCCTTT	GCTGCTCCGGAGACAGTTCT
Δ6 desaturase	CAGCACGATTACGGCCATCT	AGTTGGCAGAGGCACCCTTT
ELOVL2	TGACGCTGGTCATCCTGTTC	CGGCACGTCCGTATCTTTCT
ELOVL5	CCTCTCGGCTGGCTGTACTT	GAGAGGCCCCCTTCTTGTTG
18S RNA	GTTCAAAGCAGGCCCGAG	CGCCGCCGCATCGCCA

Table 4 *BW at the start and at the end of the trial and the effect of LA and ALA intakes on brain weight in growing pigs*

	Low LA		High LA		Pooled s.e.m.	*P*-values for effect		
	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA × ALA
BW start (kg)	16.3	16.4	16.1	16.4	0.68	0.85	0.75	0.85
BW end (kg)	28.5	28.8	29.3	29.7	0.63	0.20	0.61	0.96
Brain (g/BW end)	2.14	2.13	2.00	1.99	0.051	0.012	0.78	0.97
Frontal cortex (g/BW end)	0.48	0.48	0.46	0.44	0.019	0.13	0.56	0.67
Hippocampus (g/BW end)	0.076	0.073	0.076	0.070	0.002	0.37	0.046	0.63

BW = body weight; LA = linoleic acid; ALA = α-linolenic acid.

housed pig served as the experimental unit. The results were analyzed by ANOVA using the software program SAS version 9.1 (Statistical Analysis Systems Institute Inc., Cary, NC, USA). The fatty acid concentrations in all tissues and enzyme RNA concentrations were tested using the following model:

$$Y = \mu + \mathrm{LA}_i + \mathrm{ALA}_j + (\mathrm{LA\ x\ ALA})_{ij} + \mathrm{litter}_k + \mathrm{e}_{ijk},$$

where LA = daily LA intake i (i = low or high); ALA = daily ALA intake j (j = low or high); litter k ($k = 1, \ldots, 8$).

For fatty acid concentrations in blood plasma, the initial concentrations, measured at the start of the experiment were included as a covariate.

Homogeneity of variance was tested using the Shapiro–Wilk test. When model residuals were not distributed normally ($P < 0.05$), the statistical analysis was performed on transformed data. The level of statistical difference was preset at $P < 0.05$.

Results

All pigs remained healthy throughout the study and rates of BW gain were within the normal range. The effects of LA and ALA intakes on BW and brain weight are presented in Table 4. The average BW at slaughter was 29 kg and did not significantly differ among treatments. Fresh brain weights (g/kg BW) were significantly ($P = 0.012$) lower for the pigs fed the high LA diet. The hippocampus weight was lower ($P = 0.046$) for the high ALA groups.

The effects of LA and ALA intakes on the content of liver fatty acids are presented in Table 5. The results of the dietary treatments on the composition of plasma fatty acids largely resembled the effects on liver fatty acid composition (data not shown). Nearly all fatty acid contents were affected by LA and ALA intakes. High LA pigs had a significant ($P < 0.0001$) higher content of LA and ARA in liver. The content of n-3 fatty acids such as ALA, docosapentaenoic acid (DPA; *c*22:5 n-3) and DHA were decreased ($P < 0.01$) in the high LA pigs. A high ALA intake resulted in an increased ($P < 0.0001$) ALA and EPA contents in liver. The proportion of DHA, however, was decreased ($P = 0.01$) by high intake of ALA. High ALA pigs had increased content of LA in the liver but ARA and docosatetraenic acid (DTA; *c*22:4 n-6) contents decreased ($P < 0.0001$) by approximately 50%.

The fatty acid profiles of the frontal cortex and the hippocampus are presented in Tables 6 and 7, respectively. LA and ALA were hardly present in both brain tissues. ARA (9%), *c*22:4 n-6 (3% to 4%) and DHA (7% to 8%) were the main PUFA in the two brain tissues. High LA intake tended ($P < 0.1$) to increase the ARA content and significantly ($P < 0.05$) increased the proportion of *c*22:4 n-6 in both the hippocampus and frontal cortex. High LA intake resulted in a significant ($P < 0.001$) decrease of DPA and a tendency ($P = 0.09$) of a decreased DHA content in the frontal cortex. Increased ALA intake did not affect the ARA and *c*22:4 n-6 contents but lowered *c*22:5 n-6 in the frontal cortex. High ALA increased DPA, but did not affect DHA contents in the frontal cortex significantly. High LA intake resulted in a decreased content of DPA but did not affect that of DHA in the hippocampus. A high ALA intake significantly decreased all n-6 LC-PUFA, but increased the LA content in the hippocampus. High ALA intake increased the DPA content, but did not affect other n-3 fatty acids.

The change in mRNA expression levels of desaturase and elongase enzymes were measured in liver and hippocampus (Table 8). In liver, both LA and ALA intakes significantly ($P < 0.05$) increased the transcript level of ELOVL2. On the contrary, ELOVL5 mRNA was not significantly increased.

Table 5 *Effect of LA and ALA intakes on the fatty acid profile (% of total fatty acids) of the liver in growing pigs*

	Low LA		High LA		Pooled s.e.m.	*P*-values of effects		
Fatty acids	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA × ALA
*c*16:0	15.1	11.7	13.0	10.5	0.33	<0.0001	<0.0001	0.18
*c*17:0	1.31	1.17	1.29	1.03	0.040	0.49	0.07	0.55
*c*18:0	23.5	26.7	24.6	24.0	0.43	0.08	<0.01	<0.0001
SFA	40.3	39.6	38.9	35.6	0.40	<0.0001	<0.001	<0.01
*c*16:1	0.53	0.43	0.38	0.26	0.040	<0.001	0.013	0.75
*c*18:1 n-7	1.48	1.26	1.11	1.20	0.043	<0.001	0.17	<0.01
*c*18:1 n-9	13.5	10.8	10.7	9.51	0.31	<0.01	<0.001	0.79
MUFA	15.7	12.5	12.2	11.1	0.36	<0.0001	<0.0001	<0.01
*c*18:2 n-6 (LA)	16.4	16.8	21.2	24.3	0.33	<0.0001	<0.0001	<0.001
*c*20:3 n-6	0.81	0.77	0.68	0.80	0.047	0.28	0.38	0.10
*c*20:4 n-6 (ARA)	17.4	8.86	19.3	11.1	0.33	<0.0001	<0.0001	0.57
*c*22:4 n-6 (DTA)	0.73	0.13	1.02	0.19	0.036	<0.0001	<0.0001	<0.01
PUFA n-6	35.4	26.6	42.3	36.4	0.36	<0.0001	<0.0001	<0.001
LC n-6	19.0	9.77	21.0	12.1	0.32	<0.0001	<0.0001	0.62
*c*18:3 n-3 (ALA)[1]	0.48	5.09	0.44	4.18	0.030	<0.01	<0.0001	<0.05
*c*20:3 n-3	ND	0.87	ND	0.98	0.066	–	–	–
*c*20:5 n-3 (EPA)	0.58	8.13	0.28	5.28	0.155	<0.0001	<0.0001	<0.0001
*c*22:5 n-3 (DPA n-3)	2.42	3.23	1.67	2.66	0.079	<0.0001	<0.0001	0.26
*c*22:6 n-3 (DHA)	2.63	2.33	2.10	1.71	0.123	0.0001	0.010	0.07
PUFA n-3	6.16	19.7	4.50	14.8	0.25	<0.0001	<0.0001	<0.0001
LC n-3	5.67	14.6	4.06	10.6	0.181	<0.0001	<0.0001	<0.0001
*c*16:1/*c*16:0	0.035	0.037	0.029	0.024	0.0028	<0.01	0.56	0.24
*c*18:1 n-9/*c*18:0	0.58	0.40	0.44	0.40	0.019	<0.001	<0.001	<0.01

LA = linoleic acid; ALA = α-linolenic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; ARA = arachidonic acid; DTA = docosatetraenic acid; PUFA = polyunsaturated fatty acids; LC = long chain (fatty acids with 20 or 22 C-atoms); EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.
[1] *P*-value and s.e.m. are obtained from $Y = \log(1 + x)$ function.

Table 6 *Effect of LA and ALA intakes on the fatty acid profile (% of total fatty acids) of the frontal cortex in growing pigs*

	Low LA		High LA		Pooled s.e.m.	*P*-values of effects		
Fatty acids	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA × ALA
*c*16:0	20.0	19.4	19.2	19.3	0.234	0.06	0.30	0.20
*c*18:0	18.2	17.9	17.8	17.8	0.184	0.24	0.58	0.32
SFA	39.4	38.5	38.2	38.3	0.406	0.13	0.33	0.25
*c*16:1	0.89	0.94	0.90	0.92	0.018	0.76	0.07	0.26
*c*17:1	1.48	1.60	1.72	1.59	0.099	0.27	0.93	0.23
*c*18:1 n-7	4.91	4.93	5.03	5.02	0.097	0.30	0.97	0.23
*c*18:1 n-9	16.8	17.4	17.7	17.1	0.482	0.56	0.96	0.18
MUFA	24.9	25.8	26.3	25.4	0.733	0.48	0.97	0.89
*c*18:2 n-6 (LA)	0.69	0.80	0.79	0.90	0.030	<0.01	<0.01	0.91
*c*20:4 n-6 (ARA)	8.85	8.40	8.92	9.28	0.237	0.06	0.85	0.10
*c*22:4 n-6 (DTA)	3.30	3.04	3.39	3.36	0.086	0.027	0.10	0.21
*c*22:5 n-6 (DPA n-6)	1.18	0.59	1.02	0.61	0.094	0.46	<0.0001	0.34
PUFA n-6	14.5	13.4	14.6	14.7	0.330	0.015	0.17	0.08
LC n-6	13.8	12.6	13.8	13.8	0.330	0.07	0.09	0.08
*c*18:3 n-3 (ALA)	0.62	0.68	0.72	0.63	0.066	0.70	0.72	0.10
*c*22:5 n-3 (DPA n-3)	0.24	0.56	0.21	0.46	0.015	<0.001	<0.0001	<0.05
*c*22:6 n-3 (DHA)	7.77	8.14	6.81	7.69	0.393	0.09	0.13	0.52
PUFA n-3	8.56	9.39	7.68	8.74	0.356	0.044	0.015	0.74
LC n-3	8.16	8.91	7.20	8.30	0.388	0.06	0.027	0.65

LA = linoleic acid; ALA = α-linolenic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; ARA = arachidonic acid; DTA = docosatetraenic acid; DPA = docosapentaenoic acid; PUFA = polyunsaturated fatty acids; LC = long chain (fatty acids with 20 or 22 C-atoms); DHA = docosahexaenoic acid.

Table 7 *Effect of LA and ALA intakes on the fatty acid profile (% of total fatty acids) of the hippocampus in growing pigs*

	Low LA		High LA		Pooled s.e.m.	*P*-values of effects		
Fatty acids	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA × ALA
*c*14:0	0.46	0.44	0.45	0.43	0.0059	0.13	<0.05	0.90
*c*16:0	17.6	17.5	18.3	17.8	0.36	0.17	0.47	0.61
*c*17:0	0.21	0.21	0.21	0.20	0.0040	0.35	0.12	0.17
*c*18:0	18.5	18.4	18.6	18.6	0.14	0.42	0.60	0.73
SFA[1]	37.6	37.2	38.4	37.7	0.47	0.20	0.27	0.80
*c*16:1	0.83	0.86	0.80	0.52	0.011	<0.01	0.015	0.50
*c*17:1	1.15	1.28	1.06	1.17	0.073	0.17	0.12	0.91
*c*18:1 n-7	4.93	4.80	4.72	4.80	0.047	0.031	0.57	0.041
*c*18:1 n-9	18.5	19.0	17.6	18.3	0.54	0.15	0.29	0.80
*c*20:1 n-9	0.74	0.76	0.62	0.68	0.050	0.07	0.45	0.63
MUFA	26.5	27.0	25.0	26.0	0.72	0.12	0.31	0.74
*c*18:2 n-6 (LA)	0.53	0.70	0.56	0.94	0.10	0.19	0.010	0.29
*c*20:4 n-6 (ARA)	8.90	8.33	9.35	8.81	0.24	0.07	0.033	0.96
*c*22:4 n-6 (DTA)	4.18	3.87	4.42	4.12	0.098	0.018	<0.01	0.96
*c*22:5 n-6 (DPA n-6)	0.70	0.43	0.88	0.50	0.034	0.03	<0.0001	0.14
PUFA n-6	14.1	13.5	14.8	14.5	0.37	0.02	0.06	0.81
LC n-6	13.6	12.8	14.2	13.5	0.32	0.03	0.01	0.97
*c*18:3 n-3 (ALA)[1]	0.45	0.48	0.38	0.43	0.194	0.56	0.29	0.06
*c*20:3 n-3	0.04	0.04	0.03	0.06	0.027	0.86	0.59	0.82
*c*20:5 n-3 (EPA)	0.07	0.06	0.06	0.06	0.0051	0.13	0.25	0.67
*c*22:5 n-3 (DPA n-3)	0.29	0.67	0.26	0.54	0.011	<0.0001	<0.0001	<0.001
*c*22:6 n-3 (DHA)	6.38	6.65	6.96	6.93	0.31	0.18	0.71	0.63
PUFA n-3	7.23	7.90	7.69	8.02	0.29	0.32	0.10	0.55
LC n-3	6.78	7.42	7.31	7.58	0.31	0.29	0.17	0.56

LA = linoleic acid; ALA = α-linolenic acid; MUFA = monounsaturated fatty acids; ARA = arachidonic acid; DTA = docosatetraenic acid; DPA = docosapentaenoic acid; PUFA = polyunsaturated fatty acids; LC = long chain (fatty acids with 20 or 22 C-atoms); EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.
[1] *P*-value and s.e.m. are obtained from an inverse function.

Table 8 *Effects of LA and ALA intakes on the mRNAs of D5- and D6 desaturases and elongase 2 and -5 in liver and hippocampus of growing pigs*

	Low LA		High LA		Pooled s.e.m.	*P*-values for effect		
	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA × ALA
Liver								
ELOVL2	1.00	2.44	2.40	3.53	0.412	0.044	0.037	0.24
ELOVL5	1.00	1.13	1.20	1.24	0.108	0.11	0.27	0.92
Δ5 desaturase	1.00	1.08	1.60	1.78	0.219	<0.01	0.57	0.81
Δ6 desaturase	1.00	1.15	1.54	1.71	0.158	<0.01	0.33	0.96
Hippocampus								
ELOVL2	1.00	2.81	1.80	2.14	0.421	0.89	0.022	0.09
ELOVL5	1.00	1.59	1.02	1.22	0.248	0.50	0.13	0.43
Δ5 desaturase	1.00	1.60	1.14	1.05	0.393	0.58	0.51	0.36
Δ6 desaturase	1.00	1.73	1.00	1.14	0.264	0.27	0.12	0.27

LA = linoleic acid; ALA = α-linolenic acid.
mRNA levels were quantified by quantitative PCR and normalized to 18S ribosomal RNA. They are expressed relative to the Low LA/Low ALA diet group (1.00).

In addition, both hepatic transcript levels of Δ5- and Δ6 desaturase were increased following high LA intake ($P < 0.01$). In hippocampus, high ALA intake significantly ($P = 0.022$) increased the mRNA expression of ELOVL2, but not of ELOVL5, Δ6- and Δ5 desaturase. In contrast, variation in LA intake had no effect on the mRNA expression of these four genes.

Discussion

The formulated diets contained LA and ALA and were free of other n-3 or n-6 LC-PUFA. Once consumed, LA and ALA can be converted to other LC-PUFA by desaturation and elongation. The route and assumed enzymes involved in the biosynthesis of LC-PUFA are given in Figure 1.

In this study, we supplied LA or ALA maintaining identical intakes of other nutrients, an approach that allows independent evaluation of the effects of LA and ALA in liver and brain. Importantly, we did not observe a difference in BW of the gilts among the experimental groups.

Liver

A high LA intake increased the content of LA and n-6 LC-PUFA such as ARA in liver lipid. This agrees well with other studies in pigs (Romans *et al.*, 1995; Schellingerhout, 2002). Increasing the ALA intake by 1.3 g/kg $BW^{0.75}$ per day increased the EPA content in liver lipid from less than 1% to 8%. In contrast to other n-3 LC-PUFA, the DHA content was decreased by high ALA intake. Interestingly, a similar effect was seen on the content of ALA, EPA and DHA in plasma phospholipids in humans (Goyens *et al.*, 2006), indicating that the composition of these plasma lipids is a reflection of the lipid metabolism of the liver. Other earlier studies showed that plasma DHA level was not affected or marginally increased in humans by dietary ALA (Blank *et al.*, 2002; Burdge and Calder, 2005). In contrast to our study, these authors compensated the higher intake of ALA with a decrease in the intake of other fatty acids. We believe that caution should be taken in choosing this exchange approach, as there is evidence that other lipids such as LA, oleic acid and myristic acid (*c*14:0) can alter hepatic desaturase activity as well (Bézard *et al.*, 1994; Jan *et al.*, 2004; Portolesi *et al.*, 2008; Rioux *et al.*, 2008). These fatty acids may contribute to the observed opposite effects on plasma DHA in their studies.

This study shows that high LA intake increased levels of both Δ5- and Δ6-desaturase mRNA in liver. Similar effects have been found in rats (Bézard *et al.*, 1994) and piglets (Theil and Lauridsen, 2007). In contrast, in a human hepatoma cell line a suppression of Δ6-desaturase mRNA content was seen after the addition of LA or ALA (Portolesi *et al.*, 2008). Increased ALA intake had no effect on Δ5- and Δ6 desaturase. The rate-limiting enzyme Δ6 desaturase has been reported to have a higher affinity for ALA than for LA (Rodriguez *et al.*, 1998; Sprecher, 2002). Although not measured in our model, it is tempting to speculate that the surfeit of ALA in liver is preferentially converted above LA down the cascade (Figure 1) or alternatively catabolized (via β-oxidation) and that the LA-elevated expression of Δ6 (and Δ5)-desaturase may be necessary to metabolize the surplus of LA provided by the diet. Indeed, we found a strong increase in the EPA content in liver lipid in response to ALA intake, suggesting that the enzymatic activity of both desaturases and ELOVL5, at least for ALA, were not rate limiting.

In our study, high LA and high ALA intakes increased the transcript of ELOVL2 but not of ELOVL5 in the liver of growing piglets. These findings indicate that the conversion of ARA and EPA to longer elongation products became more efficient (see Figure 1). Indeed, we found an increased proportion of DPA and DTA in liver lipid in response to ALA and LA intakes, respectively. Unfortunately, we did not have the standards in order to establish whether this was paralleled by a positive effect on the content of *c*24:5 n-3 and *c*24:4 n-6 as well. Fatty acid-dependent changes in hepatic ELOVL2 expression have also been reported in rat and salmon (Igarashi *et al.*, 2007a; Morais *et al.*, 2009).

Interestingly, DHA levels were significantly reduced, whereas ELOVL2 mRNA expression was increased in response to ALA intake. This finding suggests a rate-limiting enzyme downstream of ELOVL2. As Δ6 desaturase has a higher affinity for ALA than for *c*24:5 n-3 (D'Andrea *et al.*, 2002; Portolesi *et al.*, 2007), it is conceivable that Δ6 desaturation of *c*24:5 n-3 is inhibited when ALA is abundant, preventing DHA synthesis. We would suggest that in the liver of piglets, Δ6 desaturase rather than ELOVL2 is the rate-limiting enzyme downstream of EPA in the biosynthesis of DHA. Whether ALA has any inhibitory effect on the peroxisomal β-oxidation of *c*24:6 n-3 remains to be elucidated.

Increased intake of LA decreased the proportion of total n-3 LC-PUFA and a high intake of ALA decreased the total n-6 LC-PUFA in liver lipid. This confirms the general concept that competition between ALA and LA occurs because of sharing of the same desaturation and elongation enzymes. In addition, this study illustrates that for EPA and DHA, the effects of LA intake are stronger at high ALA intake and the effects of ALA intake are stronger at low LA intake (interaction LA × ALA for EPA, DHA, $P < 0.001$ and $P = 0.07$, respectively). The first concern is the effect of high LA as the Western diet is generally rich in LA and poor in n-3 fatty acids. High LA intake in our study decreased the proportion of EPA and DHA in liver fat by >35% and >20%, respectively. A similar effect was also observed in blood plasma fat. On the other hand, high ALA intake drastically decreased the proportion of ARA by approximately 50%. This inhibitory effect provides a strong indication that ALA and LA competes for the same desaturases and elongases.

Taken together, our experimental design allows a direct comparison of the effects of identical increments in daily intakes of LA and ALA (both 1.3 g/kg $BW^{0.75}$), which is rarely found in literature. The effect of LA both as a substrate of the n-6 chain and as an inhibitor of the n-3 chain was much lower in comparison with that of ALA. Generally, the effect of ALA on LC PUFA appeared to be four times stronger than that of LA. Our findings in growing piglets are in agreement with human intervention studies showing a strong positive correlation of ALA intake and blood EPA, but weaker between LA intake and blood ARA (Mantzioris *et al.*, 1995).

Brain

The timing of the pig brain growth spurt is similar to that in humans (Dobbing and Sands, 1979; Pond *et al.*, 2000). Several trials with young piglets were conducted to study the effect of dietary fat on brain fatty acid composition (Arbuckle *et al.*, 1991; De la Presa-Owens *et al.*, 1998; Arbuckle and Innis, 2003). These nutritional intervention studies were mainly focused on whole brain. In this study, we selected the frontal cortex, representing a rather large region of the brain, with primary functions (Ng and Innis, 2003), and in addition, the hippocampus, which is involved in the memory storage and retrieval. Although it has been reported that deprivation

of essential fatty acids causes a decrease in brain weight (Odutuga, 1981), we found to our surprise a decreased brain weight in response to increased LA intake, irrespective of ALA intake. This is in contrast with the study by Hrboticky *et al*. (1990), showing that higher LA at the expense of oleic acid did not affect brain weight in young piglets. In our study, an increased LA intake tended ($P < 0.1$) to increase ARA and significantly increased *c*22:4 n-6 in fat extracts of both hippocampus and frontal cortex. Moreover, high ALA intake increased DPA, but not DHA concentrations in the fat of those brain tissues. Earlier studies in piglets demonstrated an increase in brain DHA by dietary ALA (Arbuckle *et al*, 1991). The difference in the effect on DHA between the latter and our study may be due to their use of younger piglets, knowing that younger piglets respond stronger to dietary PUFA (Cheon *et al*., 2000). However, the lack of the effect on brain DHA was also found in adult rats in which ALA intake exceeded the low ALA groups in our study (Bourre *et al*., 1993). The elongation of n-3 fatty acids in brain astrocytes did not extend beyond DPA (Innis and Dyer, 2002). Although high ALA intake drastically increased the ALA content in plasma lipids (an increase of the proportion from $\pm$ 1% to 10%), which is a reflection of the increase of ALA content in liver (Table 4), the ALA and DHA contents in frontal cortex and hippocampus did not increase (Tables 5 and 6). Other studies in rat brain showed that ALA after uptake from the circulation across the blood-brain barrier will be mainly oxidized and only a small fraction is converted to DHA (DeMar *et al*., 2005; Igarashi et al., 2007b). The activity of desaturation and elongation are higher in liver in comparison with brain (Igarashi *et al*., 2007a). This suggests that most DHA in the brain is derived from another source, such as liver, intestine (diet) and/or adipose tissue. Surprisingly, the DHA concentration in the pig liver lipid decreased with increasing ALA intake. High ALA intake resulted in a significant increase in ELOVL2 mRNA expression, which may relate to the strong increase in the content of DPA, a product of ELOVL2, in both frontal cortex and hippocampus. On the other hand, we did not observe an effect on mRNA expression of Δ6 and Δ5 desaturase and ELOVL5 in the hippocampus that agrees with studies in rat brain (Igarashi *et al*., 2007a).

Similarly to liver, high dietary ALA intake increased the concentration of LA in fat extracts of both frontal cortex and hippocampus. The proportion of ALA, however, was not increased. The latter may be due to a high rate of oxidation in brain (DeMar *et al*., 2005), or elongation and desaturation, resulting in increased concentrations of n-3 LC PUFA in the frontal cortex. The higher LA concentration in high ALA diets might be due to competition for enzymes and sparing of LA oxidation in the presence of ALA. The rate of oxidation of ALA is higher than that of LA (DeLany *et al*., 2000).

In conclusion, the effect of LA and ALA are important both as precursor and inhibitor for the synthesis of LC-PUFA. LA as a substrate is increasing mRNA expression of Δ6- and Δ5 desaturase and all n-6 PUFA in liver. High ALA intake did increase EPA in liver lipid but decreased the proportion of DHA. This study supports the idea that high dietary LA intake inhibits the conversion of ALA into n-3 LC-PUFA and that ALA inhibits the conversion of LA into n-6 LC-PUFA. The magnitude of the effect of ALA in liver is higher than that of LA. This suggests that manipulation of ARA and EPA availability by dietary interventions should be optimized varying both dietary LA and ALA. DHA in brain tissue is hardly affected by both dietary LA and ALA. mRNA expression of ELOVL2 was upregulated by dietary treatment and was, apart from Δ6 desaturase, identified as a potentially rate-limiting step.

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References

Arbuckle LD and Innis SM 2003. Docosahexaenoic acid in developing brain and retina of piglets fed high or low alpha-linolenate formula with and without fish oil. Lipids 27, 89–93.

Arbuckle LD, Rioux FM, Mackinnon MJ, Hrboticky N and Innis SM 1991. Response of (n-3) and (n-6) fatty acids in piglet brain, liver and plasma to increasing, but low, fish oil supplementation of formula. Journal of Nutrition 121, 1536–1547.

Bazan NG 2003. Synaptic lipid signaling: significance of polyunsaturated fatty acids and platelet-activating factor. Journal of Lipid Research 44, 222–233.

Bézard J, Blond JP, Bernard A and Clouet P 1994. The metabolism and availability of essential fatty acids in animal and human tissues. Reproduction Nutrition Development 34, 539–568.

Blank C, Neumann MA, Makrides M and Gibson RA 2002. Optimizing DHA levels in piglets by lowering the linoleic acid to α-linolenic acid ratio. Journal of Lipid Research 43, 1537–1543.

Bourre JM, Dumont O, Pascal G and Durand G 1993. Dietary α-linolenic acid at 1.3 g/kg maintains maximal docosahexaenoic acid concentration in brain, heart and liver of adult rats. Journal of Nutrition 123, 1313–1319.

Burdge GC and Calder PC 2005. α-Linolenic acid metabolism in adult humans: the effects of gender and age on conversion to longer-chain polyunsaturated fatty acids. European Journal of Lipid Science and Technology 107, 426–439.

Calder PC 2009. The relationship between the fatty acid composition of immune cells and their function. Prostaglandins, Leukotrienes and Essential Fatty Acids 79, 101–108.

Calder PC and Yaqoob P 2009. Omega-3 polyunsaturated fatty acids and human health outcomes. Biofactors 35, 266–272.

Chaloupková H, Illmann G, Neuhauserová K, Tománek M and Valis L 2007. Preweaning housing effects on behavior and physiological measures in pigs during the suckling and fattening periods. Journal of Animal Science 85, 1741–1749.

Cheon SH, Huh MH, Lee YB, Park JS, Sohn HS and Chung CH 2000. Effect of dietary linoleate/alpha-linolenate balance on the brain lipid composition, reproductive outcome and behavior of rats during their prenatal and postnatal development. Bioscience, Biotechnology and Biochemistry 64, 2290–2297.

Cole GM and Frautschy SA 2006. Docosahexaenoic acid protects from amyloid and dendritic pathology in an Alzheimer's disease mouse model. Nutrition and Health 18, 249–259.

CVB Table Pigs 2007. Chemical composition and nutritional value of feedstuffs and feeding standards. CVB series no 36, The Hague, The Netherlands.

D'Andrea S, Guillou H, Jan S, Catheline D, Thibault JN, Bouriel M, Rioux V and Legrand P 2002. The same rat delta 6-desaturase not only acts on 18- but also on 24-carbon fatty acids in very-long-chain polyunsaturated fatty acid biosynthesis. Biochemical Journal 364, 49–55.

De Jong IC, Ekkel ED, van de Burgwal JA, Lambooij E, Korte SM, Ruis MA, Koolhaas JM and Blokhuis HJ 1998. Effects of strawbedding on physiological responses to stressors and behavior in growing pigs. Physiological Behaviour 64, 303–310.

DeLany JP, Windhauser MM, Champagne CM and Bray GA 2000. Differential oxidation of individual dietary fatty acids in humans. American Journal of Clinical Nutrition 72, 905–911.

De la Presa-Owens S, Innis SH and Rioux FM 1998. Addition of triglycerides with arachidonic acid or docosahexaenoic acid to infant formula has tissue- and lipid class-specific effects on fatty acids and hepatic desaturase activities in formula fed piglets. Journal of Nutrition 128, 1376–1384.

DeMar JC Jr, Ma K, Chang L, Bell JM and Rapoport SI 2005. α-Linolenic acid does not contribute appreciably to docosahexaenoic acid within brain phospholipids of adult rats fed a diet enriched in docosahexaenoic acid. Journal of Neurochemistry 94, 1063–1076.

Dobbing J and Sands J 1979. Comparative aspects of the brain growth spurt. Early Human Development 311, 79–83.

Duhaime AC 2006. Large animal models of traumatic injury to the immature brain. Developmental Neuroscience 28, 380–387.

Goyens PLL, Spilker ME, Zock PL, Katan MB and Mensink RP 2006. Conversion of α-linolenic acid in humans is influenced by the absolute amounts of α-linolenic acid and linoleic in the diet and not by their ratio. American Journal of Clinical Nutrition 84, 44–53.

Hrboticky N, MacKinnon MJ and Innis SM 1990. Effect of a vegetable oil formula rich in linoleic acid on tissue fatty acid accretion in the brain, liver, plasma, and erythrocytes of infant piglets. American Journal of Clinical Nutrition 51, 173–182.

Igarashi M, Ma K, Chang L, Bell JM and Rapoport SI 2007a. Dietary n-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not brain. Journal of Lipid Research 48, 2463–2470.

Igarashi M, DeMar JC, Ma K, Chang L, Bell JM and Rapoport SL 2007b. Docosahexaenoic acid synthesis from α-linolenic acid by rat brain is unaffected by dietary n-3 deprivation. Journal of Lipid Research 48, 1150–1158.

Innis SM 1993. The colostrum deprived piglet as a model for study of infant lipid nutrition. Journal of Nutrition 123, 386–390.

Innis SM 2007. Dietary (n-3) fatty acids and brain development. Journal of Nutrition 137, 855–859.

Innis SM and Dyer RA 2002. Brain astrocyte synthesis of docosahexaenoic acid from n-3 fatty acids is limited at the elongation of docosapentaenoic acid. Journal of Lipid Research 43, 1529–1536.

Jan S, Guillou H, D'Andrea S, Daval S, Bouriel M, Rioux V and Legrand P 2004. Myristic acid increases Δ6-desaturase activity in cultured rat hepatocytes. Reproduction Nutrition Development 44, 131–140.

Mantzioris E, James MJ, Gibson RA and Cleland LG 1995. Differences exist in the relationships between dietary linoleic and α-linolenic acids and their respective long-chain metabolites. American Journal of Clinical Nutrition 61, 320–324.

McCann J and Ames BN 2005. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. American Journal of Clinical Nutrition 82, 281–295.

Morais S, Monroig O, Zheng X, Leaver MJ and Tocher DR 2009. Highly unsaturated fatty acid synthesis in Atlantic salmon: characterization of ELOVL5- and ELOVL2-like elongases. Marine Biotechnology (New York) 11, 627–639.

Moughan PJ, Cranwell PD, Darragh AJ and Rowan AM 1991. The domestic pig as a model for studying digestion in humans. In Digestive Physiology in Pigs – Proceedings of the Vth International Symposium on Digestive Physiology in Pigs, 24–26 April 1991 (ed. Huisman J, Den Hartog LA and Verstegen MWA), EAAP Publications no. 80, pp. 389–396. Pudoc, Wageningen, The Netherlands.

National Research Council 1998. Nutrients requirements of swine, 10th edition. National Academy Press, Washington, DC, USA.

Ng KF and Innis SM 2003. Behavioral responses are altered in piglets with decreased frontal cortex docosahexaenoic acid. Journal of Nutrition 133, 3222–3227.

Odutuga AA 1981. Reversal of brain essential fatty acid deficiency in the rat by dietary linoleate, linolenate and arachidonate. International Journal of Biochemistry 13, 1035–1038.

Pond WG, Boleman SL, Fiorotto ML, Ho H, Knabe DA, Merssmann HJ, Savell JW and Su DR 2000. Perinatal ontogeny of brain growth in the domestic pig. Proceedings of the Society for Experimental Biology and Medicine 223, 102–108.

Portolesi R, Powell BC and Gibson RA 2007. Competition between 24:5n-3 and ALA for Δ6 desaturase may limit the accumulation of DHA in HepG2 cell membranes. Journal of Lipid Research 48, 1592–1598.

Portolesi R, Powell BC and Gibson RA 2008. Δ6 desaturase mRNA abundance in HepG2 cells is suppressed by unsaturated fatty acids. Lipids 43, 91–95.

Rioux V, Cathelina D, Beauchamp E, Le Bloc'h J, Pedrono F and Legrand P 2008. Substitution of dietary oleic acid for myristic acid increases the tissue storage of α-linolenic acid and the concentration of docosahexaenoic acid in the brain, red blood cells and plasma in the rat. Animal 2, 636–644.

Rodriguez A, Sarda P, Nessmann C, Boulot P, Leger CL and Descomps B 1998. Δ6- and Δ5-desaturase activities in the human fetal liver: kinetic aspects. Journal of Lipid Research 39, 1825–1832.

Romans JR, Wulf DM, Johnson RC, Libal GW and Costello WJ 1995. Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork: II duration of 15% dietary flaxseed. Journal of Animal Science 73, 1987–1999.

Russo GL 2009. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochemical Pharmacology 77, 937–946.

Sang N and Chen C 2006. Lipid signalling and synaptic plasticity. Neuroscientist 12, 425–434.

Schellingerhout AB 2002. Essential-fatty acid supply of weaning piglets. PhD, Utrecht University.

Smink W, Gerrits WJJ, Hovenier R, Geelen MJ, Lobee HW, Verstegen MW and Beynen AC 2008. Fatty acid digestion and deposition in broiler chickens fed diets containing either native or randomized palm oil. Poultry Science 87, 506–513.

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

Sprecher H 2002. The roles of anabolic and catabolic reactions in the synthesis and recycling of polyunsaturated fatty acids. Prostaglandins Leukotrienes and Essential Fatty Acids 67, 79–83.

Theil PK and Lauridsen C 2007. Interactions between dietary fatty acids and hepatic gene expression in livers of pigs during the weaning period. Livestock Science 108, 26–29.