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Effect of α-linolenic acid and DHA intake on lipogenesis and gene expression involved in fatty acid metabolism in growing-finishing pigs

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Abstract

The regulation of lipogenesis mechanisms related to consumption of *n*-3 PUFA is poorly understood. The aim of the present study was to find out whether α-linolenic acid (ALA) or DHA uptake can have an effect on activities and gene expressions of enzymes involved in lipid metabolism in the liver, subcutaneous adipose tissue and *longissimus dorsi* (LD) muscle of growing–finishing pigs. Six groups of ten pigs received one of six experimental diets supplemented with rapeseed oil in the control diet, extruded linseed, microalgae or a mixture of both to implement different levels of ALA and DHA with the same content in total *n*-3. Results were analysed for linear and quadratic effects of DHA intake. The results showed that activities of malic enzyme (ME) and fatty acid synthase (FAS) decreased linearly in the liver with dietary DHA. Although the expression of the genes of these enzymes and their activities were poorly correlated, *ME* and *FAS* expressions also decreased linearly with DHA intake. The intake of DHA down-regulates the expressions of other genes involved in fatty acid (FA) metabolism in some tissues of pigs, such as *fatty acid desaturase 2* and *sterol-regulatory element binding transcription factor 1* in the liver and *2,4-dienoyl CoA reductase 2* in the LD muscle. FA oxidation in the LD muscle and FA synthesis decreased in the liver with increasing amount of dietary DHA, whereas a retroconversion of DHA into EPA seems to be set up in this last tissue.

Key words: Linolenic acid: DHA: Pigs: Lipogenic enzymes

In mammals, malic enzyme (ME) and glucose-6-phosphate dehydrogenase (G6PDH) are the main enzymes involved in supplying $NADPH_2$ for the first step of lipid synthesis[1]. Subsequently, $NADPH_2$ is used by fatty acid synthase (FAS) in order to synthesise palmitic acid from malonyl CoA. However, pigs are unable to synthesise the precursor of long-chain PUFA from palmitic acid because they are deficient in Δ12 and Δ15 desaturases[2]. The precursor of PUFA *n*-3 named α-linolenic acid (ALA) must be provided to animals in their feed in order to synthesise the main fatty acids (FA) of the *n*-3 family. These are EPA and DHA, which are synthesised from a sequence of desaturations with Δ6-desaturase and Δ5-desaturase and elongations with elongases. This latter step essentially takes place in the liver. However, it has been shown that feeding animals with different dietary FA has an effect on the gene expressions and activities of the key enzymes involved, first, in lipogenesis and, second, in *de novo* synthesis of long-chain FA. Indeed, PUFA *n*-6 promotes lipogenic activity in the subcutaneous adipose tissue (SCAT) of pigs[3,4] or rats[5]. Likewise, a diet containing *n*-3 FA increases FAS activity in SCAT, whereas ME activity is decreased in the liver and adipose tissue of pigs[6]. The results of the studies that were conducted on the effect of dietary *n*-3 FA on the gene expressions of lipogenic enzymes are in contrast, even if it was demonstrated that the response is tissue specific. Indeed, Duran-Montge *et al.*[7] found that *FAS* expression decreased in the liver and the SCAT of pigs, respectively, with a supply of fish oil or linseed oil, whereas Meadus *et al.*[8] found that *FAS* expression increased in the liver and muscle with a supply of DHA in the diet. Benitez *et al.*[9] showed that *ME* expression decreased in the liver of pigs when animals were fed PUFA such as C18 : 2*n*-6 and C18 : 3*n*-3, whereas its expression was not modified in the muscle and SCAT. Regarding the expressions of genes encoding for the enzymes involved in long-chain FA synthesis, it was shown that a supply of *n*-3 or *n*-6 FA in the diet decreased mRNA abundance of Δ6-desaturase and Δ5-desaturase in the liver of mammals[10,11] and increased the transcript level of elongation of very long-chain FA 2 (*ELOVL2*) but not *ELOVL5* corresponding to the elongases[12]. In contrast, an *n*-3-deficient diet increased the mRNA levels of Δ6-desaturase and Δ5-desaturase in the liver of rats[13]. Finally, according to the source of the long-chain FA that is ingested by animals, the effect on the gene expressions and activities of enzymes involved in lipid metabolism is different.

Abbreviations: ALA, α-linolenic acid; CON, control diet; DECR2, 2,4-dienoyl CoA reductase 2; ELOVL, elongation of very long chain fatty acid 5; FA, fatty acid; FADS2, fatty acid desaturase 2; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; LD, *longissimus dorsi*; LPL, lipoprotein lipase; ME, malic enzyme; RXRα, retinoid X receptor alpha; SCAT, subcutaneous adipose tissue; SREBP1, sterol-regulatory element binding transcription factor 1.

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The aim of this study was to determine whether PUFA *n*-3 supplementation of the diet has an effect on lipogenic activities and expressions of the genes involved in PUFA synthesis and other lipid metabolism in growing–finishing pigs. In addition, the effect of varying composition of PUFA *n*-3 supplementation was assessed through different ALA:DHA ratios.

Methods

Animals were raised and slaughtered at Saint-Gilles INRA experimental site according to a protocol in accordance with the French legislation, and all procedures were conducted under the responsibility of J. M., approval number 04738.

Animals and diets

In total, thirty castrated male and thirty female cross-breed pigs ((Large white × Landrace) sows × Pietrain boar) with initial body weight (BW) of 64·6 (SEM 5·5) kg were raised from 16 to 24 weeks of age. All animals received feed and water *ad libitum* in individual crates with concrete floor.

Five pigs from each sex were assigned to one of six diets according to their initial BW. All diets contained 4 % of fat and were supplemented with wheat bran to be isoenergetic on a net energy basis[14] (Table 1). Diets followed nutritional recommendations for pigs, so that the amino acid content was not a limiting factor for growth. The FA composition of the diets varied in order to implement different levels of ALA and DHA, which was achieved by the differential inclusion of extruded linseed (EL; Tradilin®, Valorex) or microalgae (MA) (*Schizochytrium* sp. DHA Gold®; DSM). Animals of the first diet, which served as control (CON), did not receive ALA and DHA. Two groups of animals received either 100 % of ALA (diet ALA) or 100 % of DHA (diet DHA). Others three groups of animals were fed mixtures of ALA and DHA (diets 2ALA/1DHA, ALA/DHA, 1ALA/2DHA). According to the experimental design, dietary ALA and DHA contents varied consistently with inclusion level of EL and MA. The ALA concentration decreased in the diets from 29·2 to 2·7 % of total FA, and DHA increased from 0 to 19·9 % of total FA (Table 1). All the diets were supplemented with 80 parts per million vitamin E to prevent PUFA oxidation. Feed consumption per animal was measured twice a week throughout the rearing phase by subtracting feed refusal from distributed feed quantities. Animals were weighed once a week, in the morning and before feed distribution.

Slaughter and carcass measurements

The pigs were fasted for 16 h before slaughter. They were stunned by electronarcosis before slaughter by bleeding at 114·9 (SEM 6·9) kg of BW on average. After evisceration, the liver, heart and kidney were removed from the carcass and weighed. Subsequently, the carcass was split into two parts and weighed in order to obtain total hot weight. The point of the second liver lobe was sampled. The *longissimus dorsi* (LD) and SCAT were sampled at the level of the third last rib on the right side of the carcass. Tissue samples were individually packed and frozen at −20°C until analyses of FA composition. For enzyme activity and PCR analyses, samples were cut into small pieces, snap-frozen in liquid N_2 and stored at −80°C. Carcasses were cooled at 1°C for 24 h and then the pH was measured on LD muscle with a mobile pH-meter (Metrohm 826) at the level of the third last rib. Meat colour (l, a and b parameters) of LD was evaluated using a calibrated colorimeter (CR 300; Konica Minolta). The entire LD muscle and SCAT were collected to estimate their total weight in the carcass.

Chemical analyses

Diets were analysed based on feed samples obtained after manufacturing. The DM of the diets was measured according to AFNOR NF V 18-109 (October 1982) method[15], the amount of crude protein according to NF ISO 16634-1 (December 2008) and Dumas principle[16], and crude energy was measured in an adiabatic calorimeter according to the ISO 9831:1998 method[17] (Table 1).

Lipids were cold-extracted from 10 g of LD, 5 g of feed sample and liver and 1 g of SCAT. The weight of the test sample was determined from previous experiences to be representative of the sample taken from the animal and to obtain at least 15 mg of fat for further assays. Samples of diets and LD were ground twice, especially with a Retsch DR100 equipped with a 1-mm mesh for diets and a Retsch Grindomix GM200 for LD. Samples of diets and tissues were then ground using a Polytron grinder equipped with a spindle (Kinematica) before extraction using chloroform–methanol (2:1, v/v)[18]. FA methyl esters were saponified with methanolic sodium hydroxide solution in the presence of C17 : 0 (margaric acid) as the internal FA standard. Next, they were methylated with boron trifluoride[19]. FA methyl esters were recovered using pentane plus distilled water solution and analysed by GC (Agilent Technologies 7890 GC system). The chromatograph was equipped with a capillary column of 0·25 mm × 30 m polysiloxane polymer filled with dimethylpolysiloxane and 50 % cyanopropylphenyl in stationary phase. Temperature programme was initiated at 150°C and increased by 4°C/min up to 220°C. The temperature was maintained at 220°C for 10·5 min. Injector and flame ionisation detector temperatures were kept constant at 220 and 280°C, respectively. Hydrogen was used as the carrier gas. Retention times and peaks were determined using chromatography software ChemStation Agilent. The identity of the peaks was verified by comparison with the retention time of standard FA methyl esters. Results were expressed as the percentage of total FA in diets and in mg per 100 g of tissue.

Enzyme activities

Depending on the concentrations of FAS, ME and G6PDH enzymes and activities in the tissues, dosages were assessed in 0·4 g of non-crushed backfat or liver and in 1·2 g of non-crushed LD muscle diluted in homogenisation buffer containing 0·25 M-ice-cold sucrose solution, EDTA (1 mM) and DTT (1 mM). Mixtures were ultracentrifuged at 100 000 ***g*** for 1 h at 4°C. The supernatant containing enzymes was aliquoted and stored at −80°C before being analysed. The activity of enzymes was

Table 1. Ingredients and chemical composition of the diets

%	CON	ALA	2ALA/1DHA	ALA/DHA	1ALA/2DHA	DHA
Ingredient composition						
Wheat	25·953	24·581	24·879	25·170	25·454	25·715
Maize	15·849	15·011	15·193	15·371	15·545	15·704
Barley	25·308	23·970	24·261	24·544	24·822	25·076
Wheat bran	5·923	4·977	4·943	4·909	4·876	4·907
Soyabean meal	18·821	17·826	18·042	18·253	18·459	18·648
Rapeseed oil	1·750	–	–	–	–	–
Extruded linseed	–	7·570	5·610	3·700	1·830	–
Microalgae	–	–	0·940	1·850	2·740	3·610
Molasses	2·972	2·815	2·849	2·882	2·915	2·944
Calcium carbonate	1·274	1·207	1·221	1·235	1·249	1·262
Dicalcium phosphate	0·495	0·469	0·475	0·480	0·486	0·491
Salt	0·446	0·422	0·427	0·432	0·437	0·442
L-Lysine-HCl	0·330	0·312	0·316	0·320	0·324	0·327
DL-Methionine	0·042	0·039	0·040	0·040	0·041	0·041
L-Threonine	0·030	0·028	0·028	0·029	0·029	0·029
Vitamins, minerals and additives	0·804	0·772	0·779	0·786	0·793	0·799
Chemical composition						
DM (%)	88·05	88·06	88·32	88·00	87·12	88·34
Gross energy (MJ/kg of DM)	14·40	14·48	14·59	14·48	14·39	14·52
Crude protein (% of DM)	17·77	17·12	16·89	16·13	16·25	16·38
Fat (% of DM)	3·72	3·85	3·79	3·64	3·68	3·50
Fatty acids (% of total fatty acids)						
C16 : 0	13·04	12·67	14·69	16·58	18·63	20·89
C18 : 0	2·28	3·00	2·67	2·27	1·92	1·66
C18 : 1*n*-9*c*	37·08	18·65	16·67	14·75	12·72	11·01
C18 : 2*n*-6*c*	36·64	34·27	32·11	30·96	29·24	27·32
C18 : 3*n*-3	6·60	29·19	22·68	16·13	9·21	2·69
C22 : 6*n*-3	0·00	0·00	5·26	10·07	15·28	19·90
SFA	16·24	16·35	19·10	21·60	24·38	27·50
MUFA	40·41	20·07	18·15	16·09	14·12	12·34
PUFA	43·35	63·57	62·75	62·30	61·50	60·16
n-6	36·64	34·27	34·43	35·42	36·01	36·25
n-3	6·64	29·24	28·27	26·83	25·44	23·83
n-6:*n*-3	5·52	1·17	1·22	1·32	1·42	1·52

CON, control diet; ALA, *α*-linolenic acid; 2ALA/1DHA, diet containing 75 % of EL and 25 % of microalgae (MA); ALA/DHA, diet containing 50 % of EL and 50 % of MA; 1ALA/2DHA, diet containing 25 % of EL and 75 % of MA; DHA, diet containing MA.

determined by spectrometry at 340 nm absorbance[20] using a KoneLab 20i apparatus (Thermo Scientific). Activities were expressed in nanomoles of NADPH formed per minute and per gram of tissue for ME and G6PDH and in nanomoles of NADP formed per minute and per gram of tissue for FAS.

Total RNA extraction and complementary DNA synthesis

For total RNA extraction, samples of frozen SCAT, LD muscle or liver (80–120 mg) were homogenised in TRIZOL® reagent (Invitrogen) using Precellys homogenizer (Ozyme) and treated according to the manufacturer's instructions. Next, RNA was purified using silica-membrane technology (NucleoSpin RNA II kit; Macherey-Nagel) and quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific). The integrity of total RNA was assessed using the Agilent RNA 6000 Nano kit with an Agilent 2100 Bioanalyzer (Agilent Technologies). For later analyses, RNA with RNA Integrity number >5·1; A260:280 ratio >2·0 and A260:230 ratio >1·0 were considered. Complementary DNA (cDNA) was synthetised using the high-capacity RNA-to-cDNA kit (Applied Biosystems) in 20 µl total reaction volume including 1 µg total RNA, 1× RT buffer and 1× RT enzyme mix at 37°C for 60 min. The reaction was terminated at 95°C for 5 min.

Quantitative real-time PCR

The expressions of twenty-seven target genes and three reference genes were measured by PCR in the three tissues (Table 2). Primers were designed from porcine sequences with Primer Express® software 3.0 (Applied Biosystems) (Table 2).

For each primer pair, the amplification efficiency of quantitative real-time PCR (qPCR) reaction was determined using calibration curves generated with six decreasing cDNA concentrations of a pool of samples (10 to $10E^{-03}$ ng RNA). The efficiency was calculated using Data Analysis Gene Expression software (version 1.0.5.6)[21] and values ranged from 61 to 124 %. For all samples, amplification reaction was performed in duplicate in 10 µl with 2 ng of reverse-transcribed RNA, both forward and reverse primers (200 nM) in 1X PCR buffer (Fast SYBRGreen® Mastermix; Applied Biosystem) containing Uracil DNA glycosylase to prevent any DNA contamination in a 7900HT Fast Real-Time PCR System (Appied Biosystems). Thermal cycling conditions were as follows: 50°C during 2 min, 95°C during 20 s, followed by forty cycles of denaturation at 95°C during 3 s and annealing at 60°C for 30 s. Specificity of the amplification products was checked by dissociation curve analysis. No-template and no-reverse transcription controls

Table 2. Primer sequences used for quantitative real-time PCR

Gene names: gene descriptions	Gene ID	Primer sequence (5′–3′)	AL (bp)
PUFA synthesis			
ELOVL5: elongation of very long-chain FA 5	ENSSSCT00000032492	F: TCTATGAGTTAGTGACGGGAGTATGG R: TGCGTGTGCCCTGACAGA	79
FADS2: FA desaturase 2	ENSSSCT00000014289	F: ATGGCATTGAGTACCAGGAAAAG R: CTCAGGGACCCGATGATGTC	64
FADS3: FA desaturase 3	ENSSSCT00000014290	F: GCTGGTCAAGGCGTTGTGT R: CCAGACTTCTTCAGGGACCTAATG	99
FAS: FA synthase	AY183428	F: AGCCTAACTCCTCGCTGCAAT R: TCCTTGGAACCGTCTGTGTTC	196
G6PDH: glucose-6-dehydrogenase	ENSSSCT00000027501	F: GGCAACAGATACAAGAACGTGAAG R: GCAGAAGACGTCCAGGATGAG	66
ME: malic enzyme	X93016	F: TGGTGACTGATGGAGAACGTATTC R: CAGGATGACAGGCAGACATTCTT	96
Peroxysomal oxidation			
ACAA1: acetyl-CoA acyltransferase 1	ENSSSCT00000012317	F: AAGCAGGGCTGACAGTTGATG R: AGAGGGCCTGACTTGCAAAG	69
ACOX1: acyl-CoA oxidase 1	ENSSSCT00000018723	F: GCGTGCCTCCAGACTAGTAGAAAT R: TGGCTCGGACGAGGTCAA	113
DECR2: 2,4-dienoyl CoA reductase 2	ENSSSCT00000008752	F: GGCTGCCACACGGTCATC R: CTTTCTAGCAGCCGTCGACACT	60
EHHADH: enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	ENSSSCT00000024873	F: CCATGCAGAGGCTCAAGTTG R: CCTCTTGCACCAGGAAGGATT	63
SCP2: sterol carrier protein 2	ENSSSCT00000004265	F: TCTTACAATGCTGTCCTACTTCAGATG R: CTGAACAAACACTTCACTAGCCAAA	68
Lipid catabolism			
LPL: lipoprotein lipase	X62984	F: CCCGACGACGCAGATTTC R: GGATGGCTTCCCCAATGTTA	151
Incorporation of PUFA in PL			
GNPAT: glyceronephosphate *O*-acyltransferase	ENSSSCT00000011141	F: GAAGAAATGCTTGGCTGTAAGACA R: TTGGCAATCATTAAAGCTTTGC	67
Transcription factors			
LXRα: nuclear receptor subfamily 1, group H, member 3	ENSSSCT00000014461	F: CTGCAACTAAATGATGCTGAGTTTG R: GCCGGTCTGCAGAGAAGATG	64
LXRβ: nuclear receptor subfamily 1, group H, member 2	ENSSSCT00000003566	F: CATCCACCATTGAGATCATGCT R: CCTTGCTGTAGGTGAAGTCTTTCA	96
PPARα: peroxisome proliferator-activated receptor *α*	ENSSSCT00000000007	F: AAGGTTGCAAGGGCTTCTTTC R: CTTACAGCTCCGATCACATTTGTC	74
RXRα: retinoid X receptor, *α*	DQ279926·1	F: GGAGCTGGTGTCCAAGATGAG R: CCTTGGAGTCCGGGTTGAA	92
SREBP1: sterol-regulatory element-binding protein 1	AF102873	F: CGGACGGCTCACAATGC R: GCAAGACGGCGGATTTATTC	76
PUFA transport			
CD36: FA translocase	NM_001044622·1	F: GCACAGAAAAAGTTGTCTCCAAAAAT R: ATGTACACAGGTTTTCCTTCTTTGC	124
FABP3: FA binding protein 3, adipocyte	AJ416019	F: GCACTTACGAGAAAGAGGCATGA R: GCTGAGTCCAGGAGTAGCCAATT	74
LDLr: LDL receptor	ENSSSCT00000029210	F: AAGCACAGATGCGAAGATATCG R: CCTCGAGGTTCACGCAGATC	73
SCARB1: scavenger receptor class B, member 1	ENSSSCT00000010699	F: TTTTGCCCGTGCATGGA R: GAAACAAGGGTGCATTGAACCT	67
SLC27A1: solute carrier family 27 member 1	ENSSSCT00000015165	F: GCCAGATCGGCGAGTTCTAC R: ACCAACCTTCCCATCCATGTT	71
SLC27A4: solute carrier family 27 member 4	ENSSSCT00000002720	F: CATAAAACAGGGACTTTCAAGCTACA R: TTTCACGACTGCTGGGTCAA	72
VLDLr: VLDL receptor	ENSSSCT00000005763	F: ACCCAGCAATATCAGTTGTAAGCA R: TCAAAGGTGGTGACTTGTCTCAGA	70
Reference genes			
B2M: β-2-microglobulin	DQ178123	F: AAACGGAAAGCCAAATTACC R: ATCCACAGCGTTAGGAGTGA	178
TOP2B: topoisomerase II *β*	ENSSSCG00000011213	F: AACTGGATGATGCTAATGATGCT R: TGGAAAAACTCCGTATCTGTCTC	137

ID, gene identification in Ensembl or NCBI databases; AL, amplification length; FA, fatty acid; PL, phospholipids.

were realised using H_2O or no-reverse RNA as template, respectively.

Two reference genes (topoisomerase (DNA) II β 180 kDa and β-2-microglobulin) were retained as the most stable reference genes for normalisation using geNorm algorithm[22]. For each sample, a normalisation factor (NF) was calculated and used for subsequent normalisation. The normalised expression level (QrN) was calculated according to the following formula

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$QrN = Eff^{-\Delta Ct\ (sample\text{-}calibator)}/NF$, where Eff is the PCR efficiency for each gene calculated from the slope of the calibration curve, Ct the quantification cycle and the calibrator is a pool of samples[23]. The *lipoprotein lipase* (*LPL*) gene was not detected in the liver, whereas the *solute carrier family 27 member 1* (*SLC27A1*A1) and *solute carrier family 27 member 4* (*SLC27A4*) genes were not detected in the SCAT. Finally, we have not managed to identify *ELOVL2* in the three tissues studied.

Statistical analyses

All data were analysed using SAS® 9.4 statistical software. Statistical comparisons were carried out by ANOVA type III (Proc GLM) with diet as the main factor. Results are presented as least squares means by diet. The standard error of the mean and the significance of the test are also presented. Multiple comparisons between groups were applied according to a *t* test. Moreover, for animal performances, enzyme activities and results of gene expressions by RT-qPCR, orthogonal contrasts between diets were calculated to determine the effects of a supplementation with *n*-3 PUFA. Comparisons were made between the CON diet and other diets containing ALA or DHA. The effects of the increasing dose of DHA were measured with a linear contrast according to dietary DHA concentration. Linear and quadratic regression measures between the enzymatic activities or the results of PCR and the total consumption of DHA were also calculated with the DMI in covariate. Finally, correlations between enzyme (FAS, ME and G6PDH) activities and relative gene expressions were calculated as Pearson's coefficient (Proc CORR). *P*-values lower than 0·05 were considered as significant, whereas *P*-values lower than 0·10 were considered as a trend.

Results

Growth performances and measurements at slaughter

The FA composition of the diets did not affect ($P > 0{\cdot}05$) daily feed intake, final BW, average daily gain, feed conversion ratio and SCAT and LD weights (as % of the carcass weight). In contrast, it tended to impact liver weight ($P = 0{\cdot}08$), because the mean was 2·43 % for all the diets, except for the liver of pigs fed the 2ALA/1DHA diet, which weighed 2·70 % of the carcass weight (Table 3). The total fat content of SCAT and LD did not change with the diets. However, the contrasts revealed that the DHA amount of the diets increased the total fat content of the liver from 3·8 % (mean of the CON and ALA diets) to 4·2 % in the diets containing DHA ($P = 0{\cdot}02$).

Fatty acid composition of tissues

The quantities of ALA, EPA, DHA and total *n*-3 FA of the three tissues were affected by the FA composition of the diets ($P < 0{\cdot}01$; Fig. 1). The quantities of EPA, DHA and total *n*-3 FA in the liver increased with a supply of DHA in the diet. On the one hand, EPA increased from 32 to 131 mg/100 g of liver between the CON diet and the diet with only a supply of DHA. In the same way, DHA increased from 49 to 299 mg/100 g of liver between these two diets. The total content of *n*-3 FA

Table 3. Effect of dietary fatty acid composition on growth performance, carcass traits and total fat of subcutaneous adipose tissue (SCAT), liver and *longissimus dorsi* (LD) muscle in growing-finishing pigs (*n* 60)

	CON	ALA	2ALA/1DHA	ALA/DHA	1ALA/2DHA	DHA	SEM	*P**	*P* value contrast *n*-3	*P* value contrast DHA
n	10	10	10	10	10	10				
Initial BW (kg)	65·8	63·7	64·2	64·6	65·2	64·1	0·7	0·97	0·46	0·77
Final BW (kg)	117·6	113·4	112·7	115·0	116·2	114·9	0·9	0·65	0·20	0·35
Average daily gain (kg/d)	1·04	1·00	0·97	1·01	1·03	1·02	0·01	0·65	0·28	0·29
Daily feed intake (kg DM/d)	3·17	3·07	3·05	3·04	3·15	3·14	0·03	0·68	0·40	0·29
Feed conversion ratio (kg/kg)	3·49	3·50	3·52	3·43	3·50	3·52	0·04	0·99	0·95	0·95
Carcass weight (kg)	91·7	89·6	86·7	90·4	90·9	90·1	0·7	0·50	0·28	0·36
Liver weight (% of the carcass)	2·46	2·49	2·70	2·37	2·46	2·36	0·04	0·08	0·87	0·07
SCAT weight (% of the carcass)	3·1	2·8	3·0	3·1	3·1	3·0	0·1	0·78	0·62	0·31
LD weight (% of the carcass)	12·6	12·7	13·0	13·0	13·2	12·9	0·1	0·34	0·14	0·39
Fat content (%)										
Liver	3·9	3·7	4·1	4·2	4·2	4·3	0·1	0·12	0·24	0·02
SCAT	69·5	67·0	64·0	67·8	63·3	69·0	1·3	0·64	0·35	0·75
LD muscle	1·8	1·7	1·9	1·8	1·9	1·5	0·1	0·91	0·88	0·59

CON, control diet; ALA, diet containing extruded linseed (EL); 2ALA/1DHA, diet containing 75 % of EL and 25 % of microalgae (MA); ALA/DHA, diet containing 50 % of EL and 50 % of MA; 1ALA/2DHA, diet containing 25 % of EL and 75 % of MA; DHA, diet containing MA; BW, body weight.
* *P*-value of the GLM procedure.

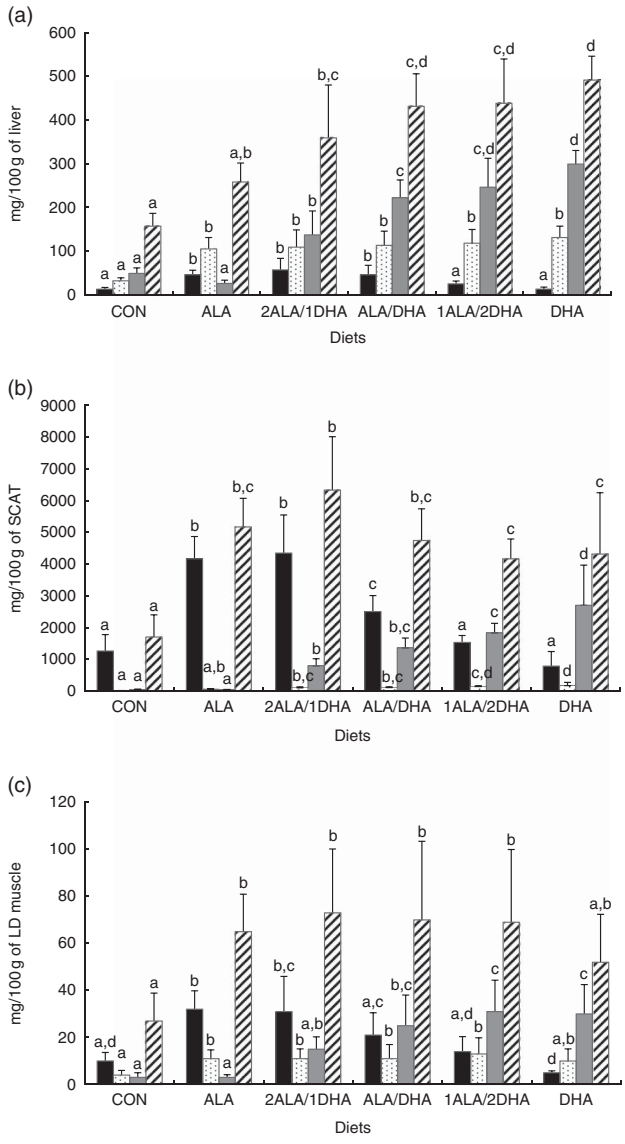

Fig. 1. Effect of dietary fatty acid (FA) composition on *α*-linolenic acid (ALA), EPA, DHA and *n*-3 FA quantities in the liver (a), subcutaneous adipose tissue (SCAT) (b) and *longissimus dorsi* (LD) muscle (c) of growing-finishing pigs. Results are least squares (LS)-mean values and standard deviations represented by vertical bars. Differences between LS means were evaluated using a *t* test. [a,b,c,d] Mean values with unlike letters were significantly different ($P < 0{\cdot}05$). CON, control diet; 2ALA/1DHA, diet containing 75 % of EL and 25 % of microalgae (MA); ALA/DHA, diet containing 50 % of EL and 50 % of MA; 1ALA/2DHA, diet containing 25 % of EL and 75 % of MA; DHA, diet containing MA. ■, ALA; ⊡, EPA; ▩, DHA; ▨, *n*-3 FA.

increased from 157 with the CON diet to 491 mg/100 g of liver with the DHA diet. On the other hand, ALA decreased in this tissue from 46 (ALA diet) to 13 mg/100 g of liver (DHA diet). In the SCAT, EPA and DHA increased, respectively, from 12 to 171 mg/100 g and from 39 to 2701 mg/100 g of tissue between the CON diet and the DHA diet. Quantities of ALA in the SCAT decreased from 4167 in the ALA diet group to 786 mg/100 g of tissue in the DHA diet group. Total *n*-3 PUFA was lower in the SCAT of animals fed the CON diet. The quantity of *n*-3 FA in this tissue increased with a supply of ALA from 1705 mg/100 g in the CON diet to 6334 mg/100 g in the 2ALA/1DHA diet and decreased from 4745 mg/100 g to 4167 mg/100 g with a supply of DHA. As in other tissues, ALA content in the LD increased with the dietary supply of ALA, whereas DHA content in the LD increased with dietary supplementation of DHA. In this tissue, EPA and *n*-3 FA were lower in the CON diet group, respectively, 4 mg/100 g and 27 mg/100 g, compared with other diets. Quantities of EPA and *n*-3 FA seemed to reach a maximum threshold of 66 mg/100 g and 11 mg/100 g, respectively, when ALA or DHA were added to the diets. Finally, in the liver and LD muscle, EPA increased with a supply of PUFA *n*-3 as ALA and DHA compared with the CON diet. In the SCAT, EPA linearly increased with the increasing dose of PUFA *n*-3.

Enzyme activities and gene expressions

Diet composition did not affect activities of the three enzymes (ME, FAS and G6PDH) in the LD and the SCAT ($P > 0{\cdot}05$). A supply of *n*-3 PUFA decreased FAS and G6PDH activity in the liver only, respectively, from 385 to 173 and from 3277 to 2577 nmol/min per g of tissue. In the same way, an increasing dose of DHA decreased FAS and ME activities in the liver from 352 to 173 and 855 to 671 nmol/min per g of tissue, respectively ($P < 0{\cdot}05$). It also tended to decrease the FAS activity in the SCAT from 302 to 201 nmol/min per g ($P = 0{\cdot}07$) (Table 4). The linear regression between enzyme activities and total ingestion of DHA showed that the activity of FAS decreased by 0·18 NADPH/min per g produced in the liver with the supplementary ingestion of 1 g of DHA. In the same way, the activities of G6PDH and ME in the liver decreased, respectively, by 0·45 and 0·17 NADPH/min per g ($P < 0{\cdot}05$) per gram increase of DHA intake (Table 5). It means that the slope of the relationship decreased, respectively, by 17 and 18 % between the activity of ME or FAS in the liver and the intake of DHA, whereas it decreased by 45 % for G6PDH activity.

The results for the gene expressions of *ME, FAS, G6PDH* and the genes whose expressions were modified by the diets are presented in Table 5. The intake of *n*-3 PUFA in the diets decreased the expression level of fatty acid desaturase 2 (*FADS2*) in the liver (*P*-value contrast *n*-3 < 0·01). It also tended to decrease *FAS* expression in the same tissue (*P*-value contrast *n*-3 < 0·1). However, the DHA intake seems to have a stronger impact on gene expression than total *n*-3 PUFA. Indeed, the expression levels of *ME, FAS* and *FADS2* genes decreased in the liver when DHA was introduced in the diet. The expressions of *ME, FAS* and *FADS2* decreased, respectively, from 1·25, 1·32 and 1·99 in the ALA diet to 0·66, 0·48 and 0·55 in the diets with a mixture of ALA and DHA. Expressions of some other genes coding for transcription factors and enzymes implicated in peroxysomal oxidation and incorporation of PUFA in the phospholipids were affected by the FA composition of the diets, such as 2,4-dienoyl CoA reductase 2 (*DECR2*), which linearly decreased in the liver with dietary DHA supply.

The results of the linear regression analysis showed that the expressions of *FADS2, FAS, ME* and sterol-regulatory element binding transcription factor 1 (*SREBP1*) in the liver, respectively, decreased by 0·001, 0·0006, 0·0003 and 0·0005 when 1 supplementary g of DHA was ingested by pigs ($P < 0{\cdot}05$). The *SREBP1* gene in the LD and glyceronephosphate *O*-acyltransferase in

Table 4. Effect of dietary fatty acid composition on FAS, ME and G6PDH activities in the liver, subcutaneous adipose tissue (SCAT) and *longissimus dorsi* (LD) muscle of growing-finishing pigs (*n* 60)‡

		Diet					Effect of DHA intake	
Tissue	Enzyme	CON	ALA	DHA	SEM	*P* value contrast *n*-3	*P* value contrast DHA	Linear regression	Quadratic regression
Liver	FAS	385	352	173	32·79	0·03	<0·01	−0·18***	–
	G6PDH	3277	2898	2577	84·68	<0·01	0·10	−0·45***	–
	ME	780	855	671	35·72	0·23	0·02	−0·17***	–
SCAT	FAS	281	302	201	19·98	0·62	0·07	−0·062†	–
	G6PDH	2160	2265	2027	77·33	0·91	0·11	–	–
	ME	5509	5940	4800	250·50	0·78	0·11	–	–
LD	FAS	2·09	1·51	0·59	0·50	0·44	0·97	–	–
	G6PDH	30	27	32	2·59	0·43	0·62	–	–
	ME	575	521	580	23·39	0·76	0·93	–	–

CON, control diet; ALA, diet containing extruded linseed; DHA, diet containing microalgae; contrast *n*-3, diet CON *v.* other diets; contrast DHA, diets CON and ALA *v.* diets containing a supply of DHA; FAS, fatty acid synthase; G6PDH, glucose-6-dehydrogenase; ME, malic enzyme; –, non-significant.

*** $P < 0·001$.

† $P < 0·1$.

‡ G6PDH and ME are expressed in nanomoles of NADPH; FAS is expressed in nanomoles of NADP per min and gram per tissue; The slope of the linear regression and the quadratic regression conducted between enzyme activities and total DHA intake (g, during 2 weeks).

the SCAT, respectively, decreased by 0·0004 and 0·0001 ($P < 0·05$). In contrast to the enzyme activities, the slope was angled by <1 % for the expression of lipogenesis genes in the liver. However, the results of the quadratic regression showed that the expression of *FADS2* in the liver did not follow a linear decrease but followed an inverse logarithmic curve ($P = 0·004$) (Table 5, Fig. 2). In contrast, *LPL* expression in LD increased by 0·0002 with *n*-3 inclusion in the diet ($P = 0·03$). Finally, the linear regression between DHA intake and the expression of retinoid X receptor *α* (*RXRα*) in the liver as well as *RXRα* and *DECR2* in the LD was not significant, with the *P*-value being higher than 0·05 (Table 5).

Correlations between FAS, ME, G6PDH activities and their gene expressions were low (*ME* and *G6PDH*) or almost non-existent (*FAS*). The correlation between ME activity and its gene expression in the liver was 0·26 ($P = 0·06$), whereas it was 0·27 ($P < 0·05$) for G6PDH and 0·23 ($P = 0·10$) for ME in the SCAT (Table 6).

Discussion

Effect of dietary n-3 PUFA on growth performances and lipid deposition in pigs

We hypothesised that feeding pigs with different dietary levels of ALA and DHA will have an impact on enzyme activities and gene expressions involved in lipid metabolism, especially lipogenesis, so that FA composition of the body compartments will be modified. Unlike classical feeding schemes in farms, pigs were housed individually and fed *ad libitum* in our experiment. This resulted in an average feed intake of 3·1 kg/d, which is 20 % higher than current feeding practices. This feeding method allowed pigs to exhibit the possible effects of ALA and DHA on feed intake and associated lipid metabolism.

We observed that the different levels of PUFA *n*-3 did not change feed intake, growth performances and carcass traits (Table 3), because all diets were formulated to provide the same amount of net energy per kilogram of diet[24]. We have shown that FA composition of the tissues reflects partly the FA composition of the diets, in agreement with Wood *et al.*[25] and Mourot & Hermier[26]. The FA composition of the three tissues (LD, liver and SCAT) varied according to the FA composition of the diets. The total *n*-3 PUFA content of the liver increased linearly when intake of *n*-3 PUFA increased, whereas a maximum threshold was reached in the LD and a decrease was observed in the SCAT. It was demonstrated that lipogenic enzymes activities could be impacted by different dietary levels of energy[27]. However, as our diets were isoenergetic, only PUFA uptake was responsible for the inhibition effect of lipogenic activities in the SCAT.

Effect of dietary n-3 PUFA on the enzyme activities and gene expressions involved in lipogenesis

Pearson's coefficients between lipogenic enzyme activities and associated gene expressions were very low or almost non-existent, despite a similar effect of the supply of DHA in the diets (Table 6). As shown in some studies, enzyme activity is not

Table 5. Relative quantity of gene expression involved in lipids metabolism in the liver, subcutaneous adipose tissue (SCAT) and *longissimus dorsi* (LD) muscle of growing-finishing pigs (arbitrary units)‡

		Diet						Effect of DHA intake	
Tissue	Genes	CON	ALA	DHA	SEM	*P* value contrast *n*-3	*P* value contrast DHA	Linear regression	Quadratic regression
PUFA synthesis									
Liver	*FADS2*	2·09	1·99	0·71	0·23	<0·01	0·01	−0·001***	0·000003***
	FAS	1·17	1·32	0·66	0·16	0·09	0·06	−0·0006*	0·000001†
	G6PDH	1·40	3·05	1·69	0·67	0·99	0·28	–	–
	ME	0·81	1·25	0·75	0·08	0·51	<0·01	−0·0003*	–
SCAT	*FADS2*	2·37	2·23	1·84	0·14	0·38	0·08	−0·0004†	–
	FAS	1·70	1·64	1·51	0·15	0·82	0·72	–	–
	G6PDH	1·21	1·13	0·91	0·09	0·51	0·44	–	–
	ME	1·04	1·48	1·15	0·13	0·14	0·47	–	–
LD	*FADS2*	1·40	1·59	1·46	0·09	0·93	0·55	–	–
	FAS	0·47	0·33	0·39	0·05	0·95	0·93	–	–
	G6PDH	0·79	0·82	0·80	0·04	0·73	0·69	–	–
	ME	0·75	0·76	0·84	0·05	0·80	0·63	–	–
Peroxysomal oxidation									
Liver	*DECR2*	1·09	1·23	0·94	0·13	0·42	0·21	−0·0003†	–
SCAT	*DECR2*	1·94	1·84	1·84	0·10	0·92	0·96	–	–
LD	*DECR2*	1·25	1·74	1·32	0·08	0·10	0·04	–	–
Transcription factors and PUFA transport									
Liver	*RXRα*	1·35	1·81	1·48	0·11	0·08	0·06	–	–
	SREBP1	1·05	1·73	0·87	0·12	0·87	<0·01	−0·0005*	–
SCAT	*LPL*	1·29	1·58	1·36	0·11	0·17	0·39	–	–
	RXRα	1·72	1·84	1·73	0·06	0·81	0·57	–	–
	SREBP1	1·61	1·71	1·47	0·08	0·98	0·38	–	–
LD	*LPL*	0·61	0·63	0·79	0·04	0·05	0·18	0·0002*	−0·0000006*
	RXRα	1·15	1·42	1·15	0·04	0·43	0·07	–	–
	SREBP1	1·44	1·31	0·85	0·08	0·08	0·02	−0·0004**	–
Incorporation of PUFA in PL									
Liver	*GNPAT*	0·95	0·97	0·90	0·04	0·80	0·41	–	–
SCAT	*GNPAT*	1·13	1·05	0·96	0·04	0·21	0·09	−0·0001†	–
LD	*GNPAT*	0·77	0·88	0·83	0·03	0·42	0·51	–	–

CON, control diet; ALA, diet containing extruded linseed; DHA, diet containing microalgae; contrast *n*-3, diet CON *v.* other diets; contrast DHA, diets CON and ALA *v.* diets containing a supply of DHA; *FADS2*, fatty acid desaturase 2; *FAS*, fatty acid synthase; *G6PDH*, glucose-6-dehydrogenase; *ME*, malic enzyme; *DECR2*: 2,4-dienoyl CoA reductase 2; *RXRa*, retinoid X receptor alpha; *SREBP1*, sterol-regulatory element binding transcription factor 1; *LPL*, lipoprotein lipase; PL, phospholipid; *GNPAT*, glyceronephosphate *O*-acyltransferase; –, non-significant.

† $P < 0{\cdot}10$; * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

‡ Slope of the linear regression and the quadratic regression conducted between enzyme activities and total DHA intake (g, during 2 months).

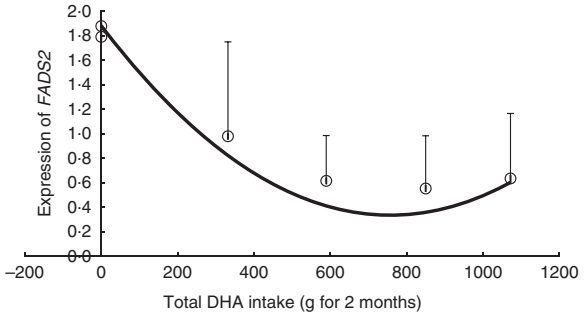

Fig. 2. Expression of the fatty acid desaturase 2 (*FADS2*) gene (arbitrary units) in the liver of growing-finishing pigs according to the six experimental diets. Results are least squares mean values and standard deviations. ○, Quadratic regression between the expression of the *FADS2* gene and the intake of DHA; ——, trend line of the quadratic regression.

bound with its gene expression[28–30]. Our experiment confirmed the difficulty to highlight a relationship between gene expression and the activity of its protein. This could be explained by the fact that our method to determine enzyme activity estimated a potential of activity and not the true activity of the enzyme in alive animals. Moreover, it is difficult to establish good correlations between gene expressions and enzymes activities for two other reasons. First, there exist others steps between both, which are not taken into consideration in the calculations. Second, the results of enzyme activities between the three tissues varied from 16 to 198, whereas the dispersion of gene expression values varied from 30 to 170 according to the CV, suggesting that a higher number of samples would be required to highlight differences between diets.

The experiment was designed to determine the effects of substituting ALA by DHA in the diets of growing-finishing pigs when they contained high levels of *n*-3 PUFA. To account for the direct effects of dietary *n*-3 PUFA on lipid metabolism, we also had a CON diet depleted in *n*-3 PUFA. In agreement with Guillevic *et al.*[6], Kouba *et al.*[31] and Corino *et al.*[32], our study showed that ME, FAS and G6PDH activities in LD muscle of growing-finishing pigs were not affected by diets rich in PUFA *n*-3. In our study and in the study of Guillevic *et al.*[6], G6PDH activity in the SCAT was also not affected by a supply of PUFA *n*-3. In contrast, in our study, we showed that *n*-3 PUFA decreased FAS and G6PDH activities in the liver of pigs, but not ME. The activity of ME decreased linearly in the liver with increasing dose of ALA in the study of Guillevic *et al.*[6]. Enzyme activities in rabbits seem to behave in the same way as in pigs, because ME, FAS and G6PDH activities in the liver decreased with a supply of EL[33]. In contrast, the expressions of the genes involved in lipogenesis were not affected in the three tissues by a supply of *n*-3 PUFA in our experiment, except a trend for *FAS* to decrease in the liver. This trend is supported by the result of Dannenberger *et al.*[34], who showed that *FAS* expression decreased in the liver of pigs with a supply of linseed oil in the diet. According to the experiment conducted on monogastric animals, an increasing dose of *n*-3 PUFA in animal diets seems to decrease the activity of enzymes involved in lipogenesis, suggesting that the longer-chain FA decrease their activities in the liver. About the gene expression, Benitez *et al.*[9] explained that PUFA down-regulate genes related to lipid synthesis contrary to SFA, because of the longer chain of PUFA than SFA.

In our experiment, when we substituted ALA by DHA, we found that FAS and ME activities and their gene expressions in the liver decreased linearly. Moreover, the FAS activity tended to decrease in the SCAT as well with a supply of DHA. According to Clarke[35], PUFA *n*-3 as EPA or DHA from menhaden fish oil decreased the transcription of the *FAS* gene in the liver, confirming that the activity of FAS and its expression in the liver of our pigs decreased because of a supply of DHA in the diets. This could also be explained by the fact that gene expression is impacted by the length of the carbon chain, the number and location of double bonds of the FA[35]. This may explain why DHA, which is a longer FA than ALA, has a deeper impact on enzyme activities and gene expressions.

We showed that the activities of the enzymes and the expressions of the genes were impacted by DHA supply mainly in the liver, whereas the effects in other tissues were limited. The increasing dose of DHA in the diet induced the increase in lipid content in the liver, especially PUFA *n*-3, whereas it decreased in the SCAT and reached a threshold in the LD muscle. In addition, in our experiment, the liver contained up to 13 % of DHA of total fat, whereas the SCAT and LD muscle contained <4 % of total fat as DHA. The hepatic DHA was not exported to others tissues because dietary DHA decreases VLDL secretion by the liver[36]. It was also demonstrated that a supply of DHA could inhibit the maturation of hepatic VLDL of rats[37]. These observations could also explain that the liver was the tissue in which the lipogenic activity and gene expressions were the most influenced by the amounts of *n*-3 FA or DHA in the diets. In contrast, the SCAT was less influenced by the diet composition; however, it is the major site of *de novo* lipogenesis after weaning[38]. Our findings therefore suggest that, at whole-animal level, the effects of dietary DHA on lipogenesis were moderated.

Effect of dietary n-3 PUFA on the gene expressions involved in fatty acid metabolism

In this study, we showed that a DHA supply in the pig diet decreased gene expressions and enzyme activities involved in the hepatic lipogenesis. Other gene expressions involved in long-chain FA synthesis also decreased in tissues with an increasing dose of DHA in the diets, except the *LPL* gene. The *LPL* gene is a target gene of PPARγ and it can hydrolyse the lipoprotein TAG in the blood plasma to release NEFA for muscle uptake[39]. It is directly involved in the deposition of FA in intramuscular fat. The *LPL* gene is also involved in the catabolism of FA in the LD muscle, and its expression in our experiment increased in this tissue. However, contrary to the *n*-3 FA contrast, the contrast with DHA did not reveal a significant difference of the *LPL* gene expression between diets. According to the experiment of Luo *et al.*[40], *LPL* expression increased with PUFA *n*-3 in the muscle of pigs, especially with increasing linseed feeding time, confirming the result of the *n*-3 PUFA contrast. Nevertheless, Wang *et al.*[41] explained that *LPL* expression increases with C18 : 1, but decreases with C16 : 0.

Table 6. Correlations between fatty acid synthase (FAS), malic enzyme (ME) and glucose-6-dehydrogenase (G6PDH) activities and their gene expressions in the subcutaneous adipose tissue (SCAT), *longissimus dorsi* (LD) muscle and liver of growing-finishing pigs

	FAS			ME			G6PDH		
	Liver	SCAT	LD	Liver	SCAT	LD	Liver	SCAT	LD
r	−0·003	0·06	0·12	0·26	0·23	0·13	0·27	−0·03	−0·11
P	0·98	0·66	0·37	0·06	0·10	0·31	0·04	0·81	0·40

r, Pearson's coefficient of correlation.

It seems that the increased *LPL* expression in this experiment could be due to the *n*-3 FA content of the diets or to the amount of C18 : 1 of the LD muscle.

At the level of the cell nucleus, we showed that *SREBP1* expression linearly decreased in the liver with a supply of DHA in the diet. SREBP1 is a transcription factor that binds to the DNA sequence of a sterol-regulatory element. It must be activated by proteolytic cleavage to be able to bind to the nuclear DNA, thereby activating the transcription of lipogenic genes such as *FAS*, acetyl-CoA carboxylase and stearoyl-CoA desaturase. The result regarding *SREBP1* in the pig liver is in accordance with others studies, which showed that mRNA of *SREBP1* decreased in the liver of pigs with supply of a DHA-rich oil extracted from algae[42,43], which could also have an impact on the expressions of its target genes. It was also demonstrated that inactivation of *SREBF1* inhibits the synthesis of HDL and VLDL[44]. We can assume that the liver will secrete less HDL and VLDL containing FA, including PUFA, according to the low transfer of DHA from the liver to the other tissues previously mentioned. We also showed that the dietary DHA decreased *SREBF1* expression in the LD muscle, whereas this was not demonstrated in the *semimembranosus* muscle of fattening pigs receiving fish oil with a high content of DHA[45]. In addition, the *FADS2* gene was also decreased by the dietary FA in our study and is in agreement with other studies[7,45,46]. *FADS2* is a gene encoding Δ6-desaturases, which makes it possible to transform ALA in the endoplasmic reticulum of cells into C18 : 4*n*-3 and then C24 : 5*n*-3 into C24 : 6*n*-3. The C24 : 6*n*-3 FA is finally transformed into DHA by peroxysomal *β*-oxidation. One of these two desaturations or both seem to have been down-regulated by the action of DHA in order to regulate the synthesis of this FA in the liver. However, in our experiment, the amount of EPA increased in the liver with the supply of PUFA *n*-3 in the diets, which did not contain EPA. Two hypotheses could explain this observation. First, only the second Δ6-desaturase involved in PUFA synthesis was impacted by the dietary FA content, allowing the synthesis of EPA and C24 : 5*n*-3, but not C24 : 6*n*-3. Second, EPA can be derived from retroconversion of DHA, when DHA content in the tissue is high, because this possibility was demonstrated in studies on liver of rats[47–48]. In this case, both Δ6-desaturases were impacted by the diets, which could explain that PUFA *n*-6 content in our experiment decreased in the liver of pigs with an increased intake of dietary PUFA *n*-3. Indeed, the long-chain PUFA *n*-6 and *n*-3 competes with the Δ6-desaturase[12,50]. With the decrease in the expression of *FADS2*, we could also expect to see a decrease in *FADS1* and *ELOVL5*. As *FADS1* and *ELOVL5* were not affected by diets, we can assume that the synthesis of C24 : 5 was potentially feasible. Finally, if both Δ6-desaturases were impacted by the diet, the synthesis of C24 : 6*n*-3 would decrease. In this case, it could partly explain that, in the peroxisome, the expressions of the genes involved in PUFA oxidation such as acetyl-CoA acyltransferase 1, acyl-CoA oxidase 1, *DECR2*, enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase, sterol carrier protein 2, *SLC27A1* or *SLC27A4* in the liver of pigs of our study were not increased in order to oxidise C24 : 6*n*-3 into DHA. In contrast, the expression of *RXRα* tended to decrease quadratically in the liver and the LD muscle with increasing dose of dietary DHA. The RXR*α* is a nuclear receptor that mediates the biological effect of retinoid. More precisely, it binds to the PPAR*α* transcription factor and indirectly activates peroxysomal *β*-oxidation of the FA. As the expression of *RXRα* decreased in the LD muscle, this could explain the plateau of PUFA *n*-3 in this tissue. In the same way, even if the decrease was not linear, the intake of DHA inhibits the expression of *DECR2* in the LD muscle, which encodes for a peroxysomal protein involved in the degradation of unsaturated fatty enoyl-CoA esters having Δ4 double bonds such as C24 : 6*n*-3 or DHA. However, there was a plateau of EPA, an increase of DHA and no decrease of PUFA *n*-3 as in the SCAT in this tissue. Its means that the inhibition of *DECR2* suppresses the oxidation of the small amount of dietary DHA introduced in the peroxisome, preventing the retroconversion of this dietary DHA into EPA.

Conclusion

In our experiment, we tested the effect of dietary *n*-3 PUFA from EL (mainly ALA) and MA (mainly DHA) on the activities and the expressions of enzyme involved in lipogenesis in the liver, SCAT and LD muscle of growing–finishing pigs. We also tested their effects on the expressions of genes involved in FA metabolism in the same tissues. The gene expressions and the activities of lipogenic enzymes such as malic enzyme, FA synthase and glucose-6-dehydrogenase in the liver decreased with an increasing dose of DHA in the diets. In contrast, gene expressions and enzyme activities of lipogenesis were unaffected by the dietary FA composition in the LD muscle and the SCAT. In the same way, DHA inhibited the expression of a gene involved in FA synthesis – namely, *SREBP1* – in the liver and in the LD muscle. It also inhibited the expression of the gene encoding for the Δ6-desaturases, that is, *FADS2*, in the liver. Finally, MA decreased the expression of the *DECR2* gene in the LD muscle, which participates in the degradation of unsaturated fatty enoyl-CoA esters having double bonds in the

peroxisome. Thus, the PUFA oxidation in the LD muscle and their synthesis in the liver were limited with the introduction of DHA in the pigs' diet; however, this FA seems to be retroconverted into EPA in this last tissue.

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A. D. T. formulated the research question, designed the study, carried out the study, analysed the data and wrote the article. E. L. assisted in the correction and developed the questions. J. M. designed the study and also assisted in the correction and developed the questions. A. V. was involved in the laboratory assessments and data analysis. All the authors read and approved the final version of the manuscript.

The authors declare that there are no conflicts of interest.

References

1. Lebret B & Mourot J (1998) Caractéristiques et qualité des tissus adipeux chez le porc. Facteurs de variation non génétiques (Characteristics and quality of pig adipose tissues. Influence of rearing factors). *INRA Prod Anim* **11**, 131–143.
2. Lavialle M & Layé S (2010) Acides gras poly-insaturés (omega 3, omega 6) et fonctionnement du système nerveux central (Polyunsaturated fatty acids (omega 3, omega 6) and brain functions). *Innovations Agronomiques* **10**, 25–42.
3. Kouba M & Mourot M (1999) Effect of a high linoleic acid diet on lipogenic enzyme activities and on the composition of the lipid fraction of fat and lean tissues in the pig. *Meat Sci* **52**, 39–45.
4. Mourot J, Peiniau P & Mounier A (1994) Effets de l'acide linoléique alimentaire sur l'activité des enzymes de la lipogenèse dans les tissus adipeux chez le porc (Effect of dietary linoleic acid on lipogenesis in adipose tissue of pigs). *Reprod Nutr Dev* **34**, 213–220.
5. Waterman RA, Romsos DR, Tsai AC, *et al.* (1975) Influence of dietary sunflower oil and tallow on growth, plasma lipids and lipogenesis in rats, pigs and chicks. *Proc Soc Exp Biol Med* **150**, 347–351.
6. Guillevic M, Kouba M & Mourot J (2009) Effect of a linseed diet or a sunflower diet on performances, fatty acid composition, lipogenic enzyme activities and stearoyl-coa-desaturase activity in the pig. *Livestock Sci* **124**, 288–294.
7. Duran-Montge P, Theil PK, Lauridsen C, *et al.* (2009) Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. *Animal* **3**, 535–542.
8. Meadus J, Aalhus J, Uttaro B, *et al.* (2011) DHA reduces triglycerides and stimulates fat metabolism in pigs. *Can J Anim Sci* **91**, 475–521.
9. Benitez R, Nunez Y, Fernandez A, *et al.* (2015) Effects of dietary fat saturation on fatty acid composition and gene transcription in different tissues of iberian pigs. *Meat Sci* **102**, 59–68.
10. Cho HP, Nakamura MT & Clarke SD (1999) Cloning, expression, and fatty acid regulation of the human d-5 desaturase. *J Biochem Chem* **274**, 37335–37339.
11. Cho HP, Nakamura MT & Clarke SD (1999) Cloning, expression, and nutritional regulation of the mammalian d-6 desaturase. *J Biol Chem* **274**, 471–477.
12. Smink W, Gerrits WJ, Gloaguen M, *et al.* (2012) Linoleic and alpha-linolenic acid as precursor and inhibitor for the synthesis of long-chain polyunsaturated fatty acids in liver and brain of growing pigs. *Animal* **6**, 262–270.
13. Igarashi M, Ma K, Chang L, *et al.* (2007) Dietary *n*-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not brain. *J Lipid Res* **48**, 2463–2470.
14. Sauvant D, Perez JM & Tran G (2004) *Tables of Composition and Nutritional Value of Raw Materials for Livestock*. Versailles: INRA.
15. AFNOR (1982) Animal feed. Determination of water content. In *NF V18-109*. Paris: AFNOR.
16. ISO (2008) ISO 16634-1:2008. Food products - determination of total nitrogen content by combustion according to the Dumas principle and calculation of crude protein content. Part 1: Oilseeds and animal feeding stuffs. http://www.iso.org/iso/catalogue_detail.htm?csnumber=46328
17. ISO (1998) ISO 9831:1998. Animal feeding stuffs, animal products, and faeces or urine – determination of gross calorific value – bomb calorimeter method. http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=17702
18. Folch J, Lees M & Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.
19. Morrison WR & Smith LM (1964) Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron fluoride methanol. *J Lipid Res* **5**, 600–608.
20. Bazin R & Ferré P (2001) Assays of lipogenic enzymes. *Methods Mol Biol* **155**, 121–127.
21. Ballester M, Cordon R & Folch JM (2013) Dag expression: high-throughput gene expression analysis of real-time PCR data using standard curves for relative quantification. *PLOS ONE* **8**, e80385.
22. Vandesompele J, De Preter K, Pattyn F, *et al.* (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* **3**, 2–12.
23. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**, 2002–2007.
24. Quiniou N & Noblet J (2012) Effect of the dietary net energy concentration on feed intake and performance of growing-finishing pigs housed individually. *J Anim Sci* **90**, 4362–4372.
25. Wood JD, Enser MB & Macfie HJH (1978) Fatty acid composition of backfat in large white pigs selected for low backfat thickness. *Meat Sci* **2**, 289–296.
26. Mourot J & Hermier D (2001) Lipids in monogastric animal meat. *Reprod Nutr Dev* **41**, 109–118.
27. Bee G, Messikommer R & Gebert S (1999) Dietary fats and energy levels differently affect tissue lipogenic enzyme activity in finishing pigs. *Fett/Lipid* **9**, 336–342.
28. Cherfaoui M (2012) La synthèse et/ou la lipoperoxydation des acides gras polyinsaturés à chaîne longue n-3 sont-elles les étapes limitantes de leur dépôt au niveau musculaire chez le bovin? (Are the synthesis and/or lipid peroxidation of long-chain *n*-3 polyunsaturated fatty acids the limiting steps of their deposition in the muscles of cattle?) PhD Thesis, University of Auvergne.

29. Guillevic M, Ezanno H & Mourot J (2008) La nature des acides gras influence-t-elle l'adipoconversion des préadipocytes en culture primaire chez le porcelet? (Does the nature of fatty acids influences the adipoconversion of preadipocytes in primary culture in piglets?) *Nutr Clin Métab* **22**, S25–S53.
30. Latchman DS (1997) Transcription factors: an overview. *Int J Biochem Cell Biol* **29**, 1305–1312.
31. Kouba M, Enser M, Whittington FM, *et al.* (2003) Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. *J Anim Sci* **81**, 1967–1979.
32. Corino C, Musella M & Mourot J (2008) Influence of extruded linseed on growth, carcass composition, and meat quality of slaughtered pigs at one hundred ten and one hundred sixty kilograms of liveweight. *J Anim Sci* **86**, 1850–1860.
33. Benatmane F, Kouba M, Youyou A, *et al.* (2011) Effect of a linseed diet on lipogenesis, fatty acid composition and stearoyl-coa-desaturase in rabbits. *Animal* **5**, 1993–2000.
34. Dannenberger D, Nuernberg K, Nuernberg G, *et al.* (2014) Impact of dietary protein level and source of polyunsaturated fatty acids on lipid metabolism-related protein expression and fatty acid concentrations in porcine tissues. *J Agric Food Chem* **62**, 12453–12461.
35. Clarke SD (1993) Regulation of fatty acid synthase gene expression: an approach for reducing fat accumulation. *J Anim Sci* **71**, 1957–1965.
36. Gladine C, Roy NC, Rigaudiere JP, *et al.* (2012) Increasing intake of long-chain *n*-3 PUFA enhances lipoperoxidation and modulates hepatic gene expression in a dose-dependent manner. *Br J Nutr* **107**, 1254–1273.
37. Maitin V, Andreo U, Guo L, *et al.* (2014) Docosahexaenoic acid impairs the maturation of very low density lipoproteins in rat hepatic cells. *J Lipid Res* **55**, 75–84.
38. O'Hea EK & Leveille GA (1969) Significance of adipose tissue and liver as sites of fatty acid synthesis in the pig and the efficiency of utilization of various substrates for lipogenensis. *J Nutr* **99**, 338–344.
39. Goldberg IJ (1996) Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J Lipid Res* **37**, 693–707.
40. Luo HF, Wei HK, Huang FR, *et al.* (2009) The effect of linseed on intramuscular fat content and adipogenesis related genes in skeletal muscle of pigs. *Lipids* **44**, 999–1010.
41. Wang W, Xue W, Jin B, *et al.* (2013) Candidate gene expression affects intramuscular fat content and fatty acid composition in pigs. *J Appl Genet* **54**, 113–118.
42. Hsu JM, Wang PH, Liu BH, *et al.* (2004) The effect of dietary docosahexaenoic acid on the expression of porcine lipid metabolism-related genes. *J Anim Sci* **82**, 683–689.
43. Liu BH, Wang YC, Kuo CF, *et al.* (2005) The effects of docosahexaenoic acid oil and soybean oil on the expression of lipid metabolism related mrna in pigs. *AJAS* **18**, 1451–1456.
44. Guesnet P, Alessandri JM, Astorg P, *et al.* (2005) Les rôles physiologiques majeurs exercés par les acides gras polyinsaturés (agpi) (Physiological functions of poly-unsaturated fatty acids). *OCL* **12**, 333–343.
45. Duran-Montge P, Theil PK, Lauridsen C, *et al.* (2009) Fat metabolism is regulated by altered gene expression of lipogenic enzymes and regulatory factors in liver and adipose tissue but not in semimembranosus muscle of pigs during the fattening period. *Animal* **3**, 1580–1590.
46. Mirshekar R, Boldaji F, Dastar B, *et al.* (2015) Longer consumption of flaxseed oil enhances n-3 fatty acid content of chicken meat and expression of fads2 gene. *Eur J Lipid Sci Technol* **117**, 810–819.
47. Schlenk H, Gellerman JL & Sand DM (1967) Retroconversion of polyunsaturated fatty acids *in vivo* by partial degradation and hydrogenation. *Biochem Biophys Acta* **137**, 420–426.
48. Schlenk H, Sand DM & Gellekman JL (1969) Retroconversion of DHA in the rat. *Biochim Biophys Acta* **187**, 201–207.
49. Verdino B, Blank ML, Privett OS, *et al.* (1964) Metabolism of 4,7,10,13,16-docosapentaenoic acid in the essential fatty acid deficient rat. *J Nutr* **83**, 234–238.
50. Guillou H, D'Andrea S, Rioux V, *et al.* (2004) The surprising diversity of delta 6-desaturase substrates. *Biochem Soc Trans* **32**, 86–87.