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.

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 for the first step of lipid synthesis(1). Subsequently, NADPH2 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 or linseed oil, whereas Meadus et al.(8) found that fatty acid desaturase 2 (FAS) expression decreased in the liver and adipose tissue 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.

Abbreviations: ALA, α-linolenic acid; CON, control diet; DECR2, 2,4-dienoyl CoA reductase 2; ELOVL, elongation of very long chain fatty acid; G6PDH, glucose-6-phosphate dehydrogenase; LD, longissimus dorsi; LPL, lipoprotein lipase; ME, malic enzyme; RXRa, 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® 6, 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 assessed through different ALA:DHA ratios.

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 heart, liver 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 lobes 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 N2 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 100000 × 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...
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 per gram of tissue for ME and G6PDH and in nanomoles of NADP formed per minute and per gram of tissue for FAS.

**Table 1. Ingredients and chemical composition of the diets**

<table>
<thead>
<tr>
<th>Ingredient composition</th>
<th>CON (g/kg of DM)</th>
<th>ALA (g/kg of DM)</th>
<th>2ALA/1DHA (g/kg of DM)</th>
<th>ALA/DHA (g/kg of DM)</th>
<th>1ALA/2DHA (g/kg of DM)</th>
<th>DHA (g/kg of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maize 15.849</td>
<td>15.011</td>
<td>15.193</td>
<td>15.371</td>
<td>15.545</td>
<td>15.704</td>
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<tr>
<td></td>
<td>Wheat bran 5.923</td>
<td>4.977</td>
<td>4.943</td>
<td>4.909</td>
<td>4.876</td>
<td>4.907</td>
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<td></td>
<td>Soyabean meal 18.821</td>
<td>17.826</td>
<td>18.062</td>
<td>18.263</td>
<td>18.549</td>
<td>18.648</td>
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<tr>
<td></td>
<td>Rapeseed oil 1.750</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Extruded linseed 7.570</td>
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<td>5.610</td>
<td>3.700</td>
<td>1.830</td>
<td>–</td>
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<tr>
<td></td>
<td>Microalgae –</td>
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<td>0.940</td>
<td>1.850</td>
<td>2.740</td>
<td>3.610</td>
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<td>Molasses 2.972</td>
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<td>2.882</td>
<td>2.915</td>
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<td>Calcium carbonate 1.274</td>
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<td>1.235</td>
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<td>Dicalcium phosphate 0.495</td>
<td>0.469</td>
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<td>0.480</td>
<td>0.486</td>
<td>0.491</td>
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<td>Salt 0.446</td>
<td>0.422</td>
<td>0.427</td>
<td>0.432</td>
<td>0.437</td>
<td>0.442</td>
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<tr>
<td></td>
<td>$\gamma$-Linolenic acid 0.330</td>
<td>0.312</td>
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<td>0.320</td>
<td>0.324</td>
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<td>L-Methionine 0.042</td>
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<tr>
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<td>L-Threonine 0.030</td>
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<tr>
<td></td>
<td>Vitamins, minerals and additives 0.804</td>
<td>0.772</td>
<td>0.779</td>
<td>0.786</td>
<td>0.793</td>
<td>0.799</td>
</tr>
</tbody>
</table>

**Chemical composition**

<table>
<thead>
<tr>
<th>DM (%)</th>
<th>Gross energy (MJ/kg of DM)</th>
<th>Crude protein (% of DM)</th>
<th>Fat (% of DM)</th>
<th>Fatty acids (% of total fatty acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>88.05</td>
<td>14.40</td>
<td>17.77</td>
<td>3.72</td>
<td>C16:0 13.04</td>
</tr>
<tr>
<td>88.06</td>
<td>14.48</td>
<td>17.12</td>
<td>3.85</td>
<td>C18:0 2.28</td>
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<tr>
<td>88.32</td>
<td>14.59</td>
<td>16.89</td>
<td>3.95</td>
<td>C18:1n-9c 37.08</td>
</tr>
<tr>
<td>88.00</td>
<td>14.48</td>
<td>16.13</td>
<td>4.09</td>
<td>C18:2n-6c 36.64</td>
</tr>
<tr>
<td>88.34</td>
<td>14.52</td>
<td>16.38</td>
<td>4.46</td>
<td>C22:6n-3 0.00</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>SFA 16.24</td>
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<td>MUFA 40.41</td>
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<td></td>
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<td>PUFA 43.35</td>
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<td></td>
<td></td>
<td>n-6 36.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n-3 6.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n-6:n-3 5.52</td>
</tr>
</tbody>
</table>

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.

**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 (Applied 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
were realised using H₂O 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

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

<table>
<thead>
<tr>
<th>Gene names: gene descriptions</th>
<th>Gene ID</th>
<th>Primer sequence (5′–3′)</th>
<th>AL (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUFA synthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| ELOVL5: elongation of very long-chain FA 5 | ENSSSCT000000032492 | F: TCTATGAGTTAGTGACGGGAGTAGG  
R: TGGCTGTGCCCCTGAGCAAGA | 79 |
| FADS2: FA desaturase 2        | ENSSSCT000000014289 | F: ATGCAGTTAGTACCAAGGAAAG  
R: CTCAGGACCCAGATGATGCT | 64 |
| FADS3: FA desaturase 3        | ENSSSCT000000014290 | F: GCTGTGCAAAGGCTTGTGT  
R: CCAGACGTTCAGGGACCTATGG | 99 |
| FAS: FA synthase              | AY183428 | F: AGCTCTACTCTTCGCTGCAAT  
R: TCCCTGGAACCTGCTGTTC | 196 |
| G6PDH: glucose-6-dehydrogenase|         |                         |         |
| TOP2B: topoisomerase II β     |         |                         |         |

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

Ct the quantification cycle and the calibrator is a pool of samples. The *lipoprotein lipase (LPL)* gene was not detected in the liver, whereas the *solute carrier family 27 member 1 (SLC27A1)* and *solute carrier family 27 member 4 (SLC27A4)* genes were not detected in the SCAT. Finally, we have not managed to identify *ELOV12* 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 covariance. 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* > 0.05) 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* = 0.08), because the mean was 24.3% for all the diets, except for the liver of pigs fed the 2ALA/1DHA diet, which weighed 27.0% 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* = 0.02).

**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* < 0.01; 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

\[ Qn = \text{Eff} \times \frac{\Delta Ct (sample-calibrator)}{NF}, \]

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. The *lipoprotein lipase (LPL)* gene was not detected in the liver, whereas the *solute carrier family 27 member 1 (SLC27A1)* and *solute carrier family 27 member 4 (SLC27A4)* genes were not detected in the SCAT. Finally, we have not managed to identify *ELOV12* in the three tissues studied.

\[ P \]

\[ \text{Ct} \]

\[ \text{calibrator} \]

\[ \text{NF} \]

\[ \text{Eff} \]

\[ \Delta \]

\[ \text{sample-calibrator} \]

\[ \text{Q} \]

\[ \text{n} \]

\[ \text{ALP} \]

\[ \text{MAG} \]

\[ \text{MAG} \]

\[ \text{SCAT} \]

\[ \text{LD} \]

\[ \text{CON} \]

\[ \text{ALA} \]

\[ \text{2ALA/1DHA} \]

\[ \text{ALA/DHA} \]

\[ \text{1ALA/2DHA} \]

\[ \text{DHA} \]

\[ \text{SEM} \]

\[ \text{BW} \]

\[ \text{gain} \]

\[ \text{ratio} \]

\[ \text{weight} \]

\[ \text{profile} \]

\[ \text{composition} \]

\[ \text{metabolism} \]

\[ \text{tissue} \]

\[ \text{diet} \]

\[ \text{source} \]

\[ \text{enzyme} \]

\[ \text{activity} \]

\[ \text{gene} \]

\[ \text{expression} \]

\[ \text{RT-qPCR} \]

\[ \text{orthogonal} \]

\[ \text{contrast} \]

\[ \text{dose} \]

\[ \text{DHA} \]

\[ \text{concentration} \]

\[ \text{linear} \]

\[ \text{quadratic} \]

\[ \text{regression} \]

\[ \text{enzymatic} \]

\[ \text{activities} \]

\[ \text{results} \]

\[ \text{PCR} \]

\[ \text{activities} \]

\[ \text{relative} \]

\[ \text{experiments} \]

\[ \text{study} \]

\[ \text{system} \]

\[ \text{model} \]

\[ \text{coefficient} \]

\[ \text{Proc CORR} \]

\[ \text{P} \]

\[ \text{values} \]

\[ \text{lower} \]

\[ \text{0.05} \]

\[ \text{considered} \]

\[ \text{significant} \]

\[ \text{P} \]

\[ \text{values} \]

\[ \text{lower} \]

\[ \text{0.10} \]

\text{considered} \}

\text{as} \}

\text{trend} \}

\text{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).}\n
\text{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.}\n
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\text{Pig} & \text{Initial BW (kg)} & \text{Final BW (kg)} & \text{Average daily gain (kg/d)} & \text{Feed conversion ratio (kg/kg)} & \text{Carcass weight (kg)} & \text{Liver weight (% of the carcass)} & \text{SCAT weight (% of the carcass)} & \text{LD weight (% of the carcass)} \\
\hline
\text{CON} & 65.8 & 117.6 & 1.04 & 3.49 & 91.7 & 2.13 & 3.7 & 12.7 \\
\text{ALA} & 65.8 & 117.6 & 1.04 & 3.49 & 91.7 & 2.13 & 3.7 & 12.7 \\
\text{2ALA/1DHA} & 65.8 & 117.6 & 1.04 & 3.49 & 91.7 & 2.13 & 3.7 & 12.7 \\
\text{ALA/DHA} & 65.8 & 117.6 & 1.04 & 3.49 & 91.7 & 2.13 & 3.7 & 12.7 \\
\text{1ALA/2DHA} & 65.8 & 117.6 & 1.04 & 3.49 & 91.7 & 2.13 & 3.7 & 12.7 \\
\text{DHA} & 65.8 & 117.6 & 1.04 & 3.49 & 91.7 & 2.13 & 3.7 & 12.7 \\
\hline
\end{tabular}
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 \( (P > 0.05) \). 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 \( (P < 0.05) \). It also tended to decrease the FAS activity in the SCAT from 302 to 201 nmol/min per g \( (P = 0.07) \) (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 \( (P < 0.05) \) 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 \( (P \text{-value contrast } n-3 < 0.01) \). It also tended to decrease FAS expression in the same tissue \( (P \text{-value contrast } n-3 < 0.01) \). 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 \( (DEGR2) \), 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, 0.0005 and 0.0005 when 1 supplementary g of DHA was ingested by pigs \( (P < 0.05) \). The \( SREBP1 \) gene in the LD and glyceroephosphate O-acyltransferase in
n-3 PUFA effect on pig fatty acid metabolism

Table 4. Effect of dietary n-3 PUFA on growth performances and lipid deposition in pigs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>FAS</th>
<th>ME</th>
<th>G6PDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>36.79</td>
<td>51.17</td>
<td>27.17</td>
</tr>
<tr>
<td>SCAT</td>
<td>32.77</td>
<td>28.10</td>
<td>30.12</td>
</tr>
<tr>
<td>LD</td>
<td>2.160</td>
<td>28.25</td>
<td>30.91</td>
</tr>
<tr>
<td>LD</td>
<td>5.509</td>
<td>20.09</td>
<td>20.91</td>
</tr>
</tbody>
</table>

Discussion

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

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. We have shown that FA composition of the tissues reflects partly the FA composition of the diets, in agreement with Wood et al. and Mourot & Hermier. 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. 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):†

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Genes</th>
<th>Diet</th>
<th>CON</th>
<th>ALA</th>
<th>DHA</th>
<th>SEM</th>
<th>P value contrast n=3</th>
<th>P value contrast DHA</th>
<th>Effect of DHA intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Linear regression</td>
<td>Quadratic regression</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUFA synthesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>FADS2</td>
<td>2.09</td>
<td>1.99</td>
<td>0.71</td>
<td>0.23</td>
<td>&lt;0.01</td>
<td>0.01</td>
<td>-0.001*** 0.000003***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FAS</td>
<td>1.17</td>
<td>1.32</td>
<td>0.66</td>
<td>0.16</td>
<td>0.09</td>
<td>0.06</td>
<td>-0.0006* 0.000001†</td>
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</tr>
<tr>
<td></td>
<td>G6PDH</td>
<td>1.40</td>
<td>3.05</td>
<td>1.69</td>
<td>0.67</td>
<td>0.99</td>
<td>0.28</td>
<td>- -</td>
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<tr>
<td></td>
<td>ME</td>
<td>0.81</td>
<td>1.25</td>
<td>0.75</td>
<td>0.08</td>
<td>0.51</td>
<td>&lt;0.01</td>
<td>-0.0003* -</td>
<td></td>
</tr>
<tr>
<td>SCAT</td>
<td>FADS2</td>
<td>2.37</td>
<td>2.23</td>
<td>1.84</td>
<td>0.14</td>
<td>0.38</td>
<td>0.08</td>
<td>-0.0004† -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FAS</td>
<td>1.70</td>
<td>1.64</td>
<td>1.51</td>
<td>0.15</td>
<td>0.82</td>
<td>0.72</td>
<td>-</td>
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<tr>
<td></td>
<td>G6PDH</td>
<td>1.21</td>
<td>1.13</td>
<td>0.91</td>
<td>0.09</td>
<td>0.51</td>
<td>0.44</td>
<td>-</td>
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<tr>
<td></td>
<td>ME</td>
<td>1.04</td>
<td>1.48</td>
<td>1.15</td>
<td>0.13</td>
<td>0.14</td>
<td>0.47</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>LD</td>
<td>FADS2</td>
<td>1.40</td>
<td>1.59</td>
<td>1.46</td>
<td>0.09</td>
<td>0.93</td>
<td>0.55</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FAS</td>
<td>0.47</td>
<td>0.33</td>
<td>0.39</td>
<td>0.05</td>
<td>0.90</td>
<td>0.93</td>
<td>-</td>
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<tr>
<td></td>
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<td>0.79</td>
<td>0.82</td>
<td>0.80</td>
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<td>0.73</td>
<td>0.69</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>ME</td>
<td>0.75</td>
<td>0.76</td>
<td>0.84</td>
<td>0.05</td>
<td>0.80</td>
<td>0.63</td>
<td>-</td>
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<tr>
<td>Peroxisomal oxidation</td>
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<tr>
<td>Liver</td>
<td>DECR2</td>
<td>1.09</td>
<td>1.23</td>
<td>0.94</td>
<td>0.13</td>
<td>0.42</td>
<td>0.21</td>
<td>-0.0003† -</td>
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<tr>
<td>SCAT</td>
<td>DECR2</td>
<td>1.94</td>
<td>1.84</td>
<td>1.84</td>
<td>0.10</td>
<td>0.92</td>
<td>0.96</td>
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<tr>
<td>LD</td>
<td>DECR2</td>
<td>1.25</td>
<td>1.74</td>
<td>1.32</td>
<td>0.08</td>
<td>0.10</td>
<td>0.04</td>
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<tr>
<td>Transcription factors and PUFA transport</td>
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<tr>
<td>Liver</td>
<td>RXRα</td>
<td>1.35</td>
<td>1.81</td>
<td>1.48</td>
<td>0.11</td>
<td>0.08</td>
<td>0.06</td>
<td>- -</td>
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<tr>
<td></td>
<td>SREBP1</td>
<td>1.05</td>
<td>1.73</td>
<td>0.67</td>
<td>0.12</td>
<td>0.87</td>
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<td>LPL</td>
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<td>1.58</td>
<td>1.36</td>
<td>0.11</td>
<td>0.17</td>
<td>0.39</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RXRα</td>
<td>1.72</td>
<td>1.84</td>
<td>1.73</td>
<td>0.06</td>
<td>0.81</td>
<td>0.57</td>
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<tr>
<td></td>
<td>SREBP1</td>
<td>1.61</td>
<td>1.71</td>
<td>1.47</td>
<td>0.08</td>
<td>0.98</td>
<td>0.38</td>
<td>-0.0002* 0.000006*</td>
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</tr>
<tr>
<td>LD</td>
<td>LPL</td>
<td>0.61</td>
<td>0.63</td>
<td>0.79</td>
<td>0.04</td>
<td>0.05</td>
<td>0.18</td>
<td>-0.0004** -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RXRα</td>
<td>1.15</td>
<td>1.42</td>
<td>1.15</td>
<td>0.04</td>
<td>0.43</td>
<td>0.07</td>
<td>-</td>
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<tr>
<td></td>
<td>SREBP1</td>
<td>1.44</td>
<td>1.31</td>
<td>0.85</td>
<td>0.08</td>
<td>0.08</td>
<td>0.02</td>
<td>-0.0001† -</td>
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<td>Incorporation of PUFA in PL</td>
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<tr>
<td>Liver</td>
<td>GNPAT</td>
<td>0.95</td>
<td>0.97</td>
<td>0.90</td>
<td>0.04</td>
<td>0.80</td>
<td>0.41</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SCAT</td>
<td>GNPAT</td>
<td>1.13</td>
<td>1.05</td>
<td>0.96</td>
<td>0.04</td>
<td>0.21</td>
<td>0.09</td>
<td>-0.0001† -</td>
<td></td>
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<tr>
<td>LD</td>
<td>GNPAT</td>
<td>0.77</td>
<td>0.88</td>
<td>0.83</td>
<td>0.03</td>
<td>0.42</td>
<td>0.51</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

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; RXRα, retinoid X receptor alpha; SREBP1, sterol-regulatory element binding transcription factor 1; LPL, lipoprotein lipase; PL, phospholipid; GNPAT, glyceroneophosphate O-acyltransferase; †, non-significant.

† P<0.10; * P<0.05; ** P<0.01; *** P<0.001.

† Slope of the linear regression and the quadratic regression conducted between enzyme activities and total DHA intake (g, during 2 months).
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.\(^{60}\), Kouba et al.\(^{53}\) and Corino et al.\(^{52}\), 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.\(^{60}\), 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.\(^{60}\). 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\(^{53}\). 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.\(^{54}\), 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.\(^{51}\) 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\(^{37}\). 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\(^{560}\). 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\(^{58}\). 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 PPAR\(\gamma\) 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.
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 triggering 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[42,45], 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 lower transfer of DHA from the liver to the other tissues previously mentioned. We also showed that the dietary DHA decreased SREBP1 expression in the LD muscle, whereas this was not demonstrated in the seminiferous tubules 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 C24:5n-3 into C24:6n-3. The C24:6n-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 PUFA the amount of EPA increased in the liver with the supply of EPA and C24:5 n-3 would decrease. In this case, it could partly explain that in the peroxysome, the expressions of the genes involved in PUFA oxidation such as acetyl-CoA acyltransferase 1, acyl-CoA oxidase 1, DECR2, enoyl-CoA, hydratase/3-hydroxacyl CoA dehydrogenase, sterol carrier protein 2, SLC27A1 or SLC27A4 in the liver of pigs of our study were not increased in order to oxidise C24:6n-3 into DHA. In contrast, the expression of RXRa tended to decrease quadratically in the liver and the LD muscle with increasing dose of dietary DHA. The RXRa is a nuclear receptor that mediates the biological effect of retinoid. More precisely, it binds to the PPARα transcription factor and indirectly activates peroxysomal β-oxidation of the FA. As the expression of RXRa 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 C24:6n-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 peroxysome, preventing the retroconversion of this dietary DHA into EPA.

### 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

<table>
<thead>
<tr>
<th></th>
<th>FAS</th>
<th>ME</th>
<th>G6PDH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver SCAT LD</td>
<td>Liver SCAT LD</td>
<td>Liver SCAT LD</td>
</tr>
<tr>
<td>r</td>
<td>0.98 0.06 0.12</td>
<td>0.26 0.23 0.13</td>
<td>0.27 –0.03 –0.11</td>
</tr>
<tr>
<td>P</td>
<td>0·003 0·66 0·37</td>
<td>0·06 0·10 0·31</td>
<td>0·04 0·81 0·40</td>
</tr>
</tbody>
</table>

r, Pearson’s coefficient of correlation.

**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
28. Cherfaoui M (2012) La synthèse et/ou la lipoperoxidation des acides gras polyinsaturés à chaîne longue n-3 sont-elles les étapes limitantes de leur dépôt au niveau musculaire chez le bœuf? (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.


