

Dietary Linseed Oil Reduces Growth While Differentially Impacting LC-PUFA Synthesis and Accretion into Tissues in Eurasian Perch (*Perca fluviatilis*)

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Abstract The aim of this study was to evaluate the impact of replacing dietary fish oil (FO) with linseed oil (LO) on growth, fatty acid composition and regulation of lipid metabolism in Eurasian perch (*Perca fluviatilis*) juveniles. Fish (17.5 g initial body weight) were fed iso-proteic and isoenergetic diets containing 116 g/kg of lipid for 10 weeks. Fish fed the LO diet displayed lower growth rates and lower levels of DHA in the liver and muscle than fish fed the FO diet, while mortality was not affected by dietary treatment. However, DHA content recorded in the liver and muscle of fish fed the LO diet remained relatively high, despite a weight gain of 134 % and a reduced dietary level of long-chain polyunsaturated fatty acids (LC-PUFA), suggesting endogenous LC-PUFA biosynthesis. This was supported by the higher amounts of pathway intermediates, including 18:4n-3, 20:3n-3, 20:4n-3, 18:3n-6 and 20:3n-6, recorded in the liver of fish fed the LO diet in comparison with those fed the FO diet. However, *fads2* and *elovl5* gene expression and FADS2 enzyme activity were comparable between the two groups. Similarly, the expression of genes involved in eicosanoid synthesis was not modulated by dietary LO. Thus, the present study demonstrated that in fish

fed LO for 10 weeks, growth was reduced but DHA levels in tissues were largely maintained compared to fish fed FO, suggesting a physiologically relevant rate of endogenous LC-PUFA biosynthesis capacity.

Keywords Eurasian perch · FADS2 · LC-PUFA · Fatty acid metabolism · Eicosanoid

Abbreviations

ALA	Alpha-linolenic acid
ALOX5	Arachidonate 5-lipoxygenase
ARA	Arachidonic acid
ATP	Adenosine tri-phosphate
BHT	Butylated hydroxy toluene
cDNA	Complementary deoxyribonucleic acid
DHA	Docosahexaenoic acid
EF1 α	Elongation factor 1 alpha
ELOVL5	Elongation of very long chain 5
EPA	Eicosapentaenoic acid
FA	Fatty acid
FADS	Fatty acid desaturase
FADS2	Fatty acid desaturase 2
FAME	Fatty acid methyl ester
FO	Fish oil
gDNA	Genomic deoxyribonucleic acid
HSI	Hepatosomatic index
IL-1	Interleukin 1
IL-6	Interleukin 6
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acid
LO	Linseed oil
LT4H	Leukotriene a-4 hydrolase
NADH	Nicotinamide adenine dinucleotide
PCR	Polymerase chain reaction
PTGES2	Prostaglandin E synthase 2

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PUFA	Polyunsaturated fatty acid
RNA	Ribonucleic acid
SD	Standard deviation
SGR	Specific growth rate
TLC	Thin layer chromatography
TNF α	Tumor necrosis factor α
UV	Ultra violet
VO	Vegetable oil
WW	Wet weight

Introduction

Cultured fish have traditionally been fed diets based on ingredients of a marine origin containing high levels of n-3 long-chain polyunsaturated fatty acids (LC-PUFA; $\geq$ C20 and $\geq$ 3 double bonds) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) and, to a lesser extent, n-6 LC-PUFA such as arachidonic acid (ARA, 20:4n-6). These LC-PUFA are known to be essential for fish growth, health and development [1]. For example, LC-PUFA of cell membranes can be recruited as precursors for the synthesis of eicosanoids involved in immune function, including the inflammatory response [1]. In human nutrition, LC-PUFA also provide some health benefits by reducing the risk of cardiovascular and neurological disease [2].

Most fish species currently farmed are carnivorous, and they generally have very low capability for endogenous synthesis of LC-PUFA, as they are supplied through the ingestion of prey rich in LC-PUFA bio-accumulated from the phytoplankton primary producers [3]. The high n-3 LC-PUFA concentrations in the flesh of farmed fish are maintained through the use of dietary fish oils rich in LC-PUFA. However, this situation is not sustainable in the long term, as the supply of fishmeal and fish oil derived from marine fish stock is finite and ingredients are limited. In this context, alternatives for replacing fish oil and fish meal in feed for farmed fish have been investigated since the 1990s. Plant oils have been considered the best alternative to dietary fish oil, although vegetable oils, in contrast to fish oil, do not contain LC-PUFA, but can have high proportions of C₁₈ PUFA, with many particularly rich in linoleic acid (LA, 18:2n-6), while a few contain higher levels of α -linolenic acid (ALA, 18:3n-3).

As has been described in vertebrates, the “Sprecher pathway” enables the conversion of PUFA to LC-PUFA via a series of fatty acyl and elongase reactions [4]. Over the last decade, in efforts to better understand the endogenous LC-PUFA synthesis capacity in fish species of aquaculture interest, the functionality and regulation of these enzymes has been investigated. Studies focused on the cloning of fatty acid desaturase (FADS) and elongation of

very long-chain (ELOVL) fatty acid genes and their functional characterization in heterologous systems (e.g., *Saccharomyces cerevisiae*) have demonstrated high genomic heterogeneity among fish species. Two genes encoding distinct FADS2 (Δ 6 desaturase) and FADS1 (Δ 5 desaturase) proteins have been identified in the elasmobranch catshark (*Scyliorhinus canicula*), whereas only *fads2* genes have been found in teleost species [5]. In Atlantic salmon (*Salmo salar*), two *fads2* genes encoding separate Δ 6 and Δ 5 desaturases were identified [6, 7], and a single *fads2* gene encoding a bifunctional Δ 6 Δ 5 FADS protein was identified in zebrafish (*Danio rerio*) [8], while *fads2* genes coding for Δ 6 FADS proteins have been identified in many fish species, including the Atlantic cod (*Gadus morhua*) [9], European sea bass (*Dicentrarchus labrax*) [10], gilt-head sea bream (*Sparus aurata*) [3] and cobia (*Rachycentron canadum*) [11]. Many of the teleost Δ 6 FADS2 proteins also displayed Δ 8 desaturation activity, although activity varied between freshwater/diadromous and marine fish species [12]. An alternative pathway for producing DHA from EPA through Δ 4 desaturation was recently described in the marine herbivorous rabbitfish (*Siganus canaliculatus*) [13, 14], Senegalese sole (*Solea senegalensis*) [14], Mexican whitefish [15] and striped snakehead (*Channa striata*) [16]. The above-mentioned studies revealed differences among fish species in specificity and functionality of the LC-PUFA pathways related to LC-PUFA biosynthesis capacity. Initially, these differences in LC-PUFA biosynthesis capacity were suggested to reflect a dichotomy between freshwater/salmonids and marine fish species based on the relative abundance of these fatty acids in marine and freshwater environments. However, the recent discovery of FADS2 proteins with Δ 4 activity in two marine species, the herbivorous rabbitfish and the Senegalese sole, has confounded this paradigm, suggesting that the trophic level of a species could also influence LC-PUFA biosynthetic capacity.

To test these possible nutritional influences, studies have investigated plant oil sources at different dietary levels in order to evaluate the consequences on fish growth performance and physiology. In anadromous Atlantic salmon [17, 18] and freshwater species including rainbow trout (*Oncorhynchus mykiss*) [19], Arctic charr (*Salvelinus alpinus*) [18], swordtail (*Xiphophorus helleri*) [20], zebrafish [21], African catfish (*Clarias gariepinus*) [22] and common carp (*Cyprinus carpio*) [23], the use of diets with 100 % plant oil did not affect growth performance. Interestingly, similar results on growth performance were observed in the marine species Senegalese sole [24] that was fed a plant oil-based diet. The absence of a reduced growth rate may be associated with activation of endogenous LC-PUFA biosynthesis to compensate for dietary LC-PUFA deficiency in the plant oil-based diets. Indeed, dietary plant oil has been shown to induce LC-PUFA endogenous biosynthesis in salmonids

[7, 9, 25, 26], zebrafish [21, 27], Nile tilapia (*Oreochromis niloticus*) [27] and Senegalese sole [28]. This activation process was characterized by increased desaturase and elongase gene expression and by an increase in desaturation/elongation activity. However, despite the activation of the LC-PUFA biosynthesis pathway, the activities were insufficient to maintain the tissue proportions of LC-PUFA at the same level as in fish fed a fish oil diet [7, 25, 26]. Thus, partial dietary plant oil, up to 75 % of total dietary oil, was found more suitable in these species than the use of 100 % single plant oils.

In contrast, the growth rate of strictly carnivorous fish species such as Atlantic cod (*Gadus morhua*) [18], turbot (*Scophthalmus maximus*) [29], yellowtail king fish (*Seriola lalandi*) [30] and European sea bass (*Dicentrarchus labrax*) [31] was reduced significantly when dietary fish oil was totally replaced with plant oil. In addition, the complete replacement of marine oil with plant oil greatly reduced DHA concentrations in the flesh, reflecting a lower capability for bioconversion of PUFA into LC-PUFA in these fish species than in anadromous and freshwater species. This observation was reinforced by a comparative study between Atlantic salmon and Atlantic cod in which higher absolute values of *fads2* gene expression and FADS2 activity were recorded in the intestine and liver of Atlantic salmon, regardless of dietary treatment [32].

The potential for the biosynthesis of LC-PUFA among freshwater carnivorous fish remains unclear. Researchers have suggested that dietary LC-PUFA from prey may impair biosynthetic capacity in these species [33]. Studies have investigated the bioconversion of ALA and LA in freshwater carnivorous species in Northern pike (*Esox lucius*) [34], Murray cod (*Maccullochella peelii peelii*) [35] and striped snakehead [16], but contradictory results were reported, possibly explained by differences in trophic position. Endogenous LC-PUFA biosynthesis potential has also been investigated in Percidae such as the Eurasian perch (*Perca fluviatilis*) and pikeperch (*Sander lucio-perca*), given the interest in these species for diversification of European inland aquaculture [36]. Young fry of Percidae first feed on algae and zooplankton, and subsequently on insect larvae and small fish, becoming carnivorous at the most mature stages [37]. Thus, LC-PUFA biosynthesis capacities may vary at different life stages. The relatively high concentrations of DHA recorded in the flesh of Eurasian perch after a nutritional challenge with low dietary LC-PUFA levels have suggested the endogenous potential for synthesis of LC-PUFA from PUFA precursors [36, 38]. In some experiments, however, tissue DHA level may be influenced by the low weight gain observed in trials; furthermore, regulation of LC-PUFA biosynthesis in response to dietary plant oil has not been investigated in Eurasian perch.

Therefore, in the present study, we investigated the effect of total replacement of fish oil with linseed oil in the diet of Eurasian perch juveniles for 10 weeks with regard to growth response, fatty acid composition, relative *fads2* and *elovl5* gene expression and FADS2 enzymatic activity, and eicosanoid production.

Materials and Methods

Facilities and Fish

Eurasian perch juveniles were obtained from the Asialor Pisciculture (Nancy, France). After acclimation for 10 days, fish were randomly distributed into four 100-L fiberglass tanks under experimental conditions at a density of 80 fish per tank. During the experiment, the water temperature in the recirculating system was stabilized at 23 °C and subjected to a series of treatments, including bio-filter and UV systems. The oxygen concentration was maintained close to saturation throughout the experiment through the use of a blower system. Nitrite and ammonium concentrations were assessed weekly during the nutritional challenge. Fish with an initial body weight of about 16.5 g were reared under a 12L:12D (light/dark) photoperiod and were hand-fed three times daily, 6 days a week, to apparent satiation for a period of 10 weeks. During the experiment, mortality was recorded daily.

The experimental protocol was approved by the local ethics committee for animal research of the University of Namur (Belgium) (protocol number: UN KE 14/213).

Diets and Feeding Trial

Two isoenergetic and isonitrogenous diets (18.7 MJ/kg; 50 % crude protein, 11.6 % crude lipid) were formulated using two different oil sources (Table 1). The control diet was formulated with cod liver oil (fish oil: FO diet) and was characterized by n-3 high LC-PUFA levels, especially EPA (6.0 % of total fatty acids [FA]) and DHA (7.5 % of total FA) (Table 2). The second diet, prepared with linseed oil (LO diet), was composed of low LC-PUFA content (EPA and DHA, 1 and 2.3 % of total FA) but high C₁₈ PUFA levels (ALA 48.1 % and LNA 14.6 % of total FA) with an n-3/n-6 PUFA ratio close to 3 (Table 2). The diets were formulated to meet the protein and lipid requirements of Eurasian perch juveniles [39, 40].

Sampling Procedures

At the end of the experiment, ten fish were randomly collected from each tank and euthanized with an overdose of 300 mg/ml of MS-222 (aminobenzoic acid). Of these,

Table 1 Formulation (g/kg) and chemical composition (% dry matter) in fish oil (FO) and linseed oil (LO) experimental diets

	FO	LO
Cod fish meal ^a	330	330
Blood meal ^b	80	80
Wheat gluten ^c	80	80
Gelatin ^d	30	30
Starch ^d	200	200
Glucose ^d	25	25
Bacteriologic agar ^d	10	10
Carboxymethylcellulose ^d	50	50
Cellulose ^d	10	10
Cod oil ^e	90	0
Linseed oil ^f	0	90
Vitamin mix ^g	10	10
Mineral mix ^h	65	65
Antioxidant mix ⁱ	10	10
Betaine ^d	10	10
Proximate composition		
Protein	50.0 ± 0.2	50.3 ± 0.1
Lipid	11.6 ± 0.3	11.6 ± 0.2
Moisture	11.0 ± 0.3	10.3 ± 0.4

BHA butylated hydroxyanisole, *BHT* butylated hydroxyl toluene

^a Cod fish meal provided by SNICK Euroingredients NV, Rudder-voorde (Belgium)

^b Actipro hemoglobin; (VEOS NV, Zwevezele Belgium)

^c Roquette Frères, Lestrem (France)

^d Sigma-Aldrich, St. Louis, MO (USA)

^e Mosselman SA, Ghlin (Belgium)

^f Huilerie Emile Noël SAS (France)

^g Vitamin mix was provided by INVE Aquaculture (Breda, Netherlands). Composition of mixture according to Griffin et al. (1994)

^h Mineral mix (g kg⁻¹ of mix) was prepared in the lab, from (CaHPO₄)2H₂O, 727.77; (MgSO₄)7H₂O, 127.50; NaCl, 60.00; KCl, 50.00; (FeSO₄)7H₂O, 25.00; (ZnSO₄)7H₂O, 5.50; (MnSO₄)4H₂O, 2.54; (CuSO₄)5H₂O, 0.78; (CoSO₄)7H₂O, 0.48; (CaIO₃)6H₂O, 0.29; (CrCl₃)6H₂O, 0.13

ⁱ 5 g kg⁻¹ butylated hydroxyanisole (BHA) and 5 g kg⁻¹ butylated hydroxyl toluene (BHT) provided by Fluka, Buchs (Switzerland)

five fish were used to analyze lipid composition in the liver, brain and muscle from the dorsal region. The whole brain tissue and about 1 g of liver and muscle from each fish were stored at -20 °C. From the remaining fish, the brain, liver and intestine were sampled for FADS2 desaturation activity and gene expression assays. More precisely, around 100 mg of liver, 100 mg of anterior intestine and the whole brain were dissected and stored directly in liquid nitrogen and thereafter at -80 °C for gene expression analysis. The remaining parts of liver and intestine were stored in the same way for the FADS2 desaturation activity assay.

Table 2 Fatty acid composition (%mol of total fatty acids) in fish oil (FO) and linseed oil (LO) experimental diets

Fatty acids	FO	LO
14:0	2.7	0.2
16:0	13.6	6.9
17:0	0.2	0.1
18:0	4.2	4.8
20:0	0.2	0.2
Total saturated	20.9	12.2
16:1n-7	4.2	0.3
18:1n-9	27.8	19.3
18:1n-7	3.2	1.1
20:1n-9	2.1	0.0
Total monoenes	37.2	20.7
18:2n-6	17.8	14.7
18:3n-6	0.2	0.0
20:3n-6	0.3	0.0
20:4n-6	0.6	0.2
Total n-6 PUFA	18.9	14.9
18:3n-3	3.4	48.1
18:4n-3	0.8	0.0
20:3n-3	0.2	0.1
20:4n-3	0.0	0.0
20:5n-3	6.7	1.1
22:5n-3	2.9	0.1
22:6n-3	9.0	2.7
Total n-3 PUFA	23.0	52.2

Proximate Analysis

Dietary protein, ash, lipid and fatty acid composition were determined with crude protein content estimated according to the Kjeldahl distillation method after digestion of the sample by sulfuric acid, while the ash content was obtained by combustion of the diets at 550 °C for 12 h. Total lipid was extracted from diets and fish tissues (liver, muscle and brain), essentially according to Folch *et al.* [41]. All frozen tissue samples from the same tank (five fish replicates) were pooled in equivalent proportion and homogenized with a ball mill. Total lipids were extracted from diets and 2 g of muscle and 0.5 g of liver and brain with dichloromethane/methanol (2:1, v/v) containing 0.01 % BHT. Lipid weight was determined gravimetrically after evaporation of solvent under nitrogen gas.

Lipid and Aatty Acid Analysis

Phospholipids and neutral lipids of fish tissues were separated from 50 mg of total lipid prepared as described above. Total lipids were dissolved in 200 µl of chloroform and injected into a Chromafix cartridge (Macherey-Nagel,

Düren, Germany) connected to a glass syringe. The cartridge was washed with 30 ml of chloroform to elute the neutral lipid fraction, followed by 30 ml of methanol to elute the phospholipids. The weight of lipid fractions was determined gravimetrically after evaporation of solvent under nitrogen.

Free fatty acids were extracted after saponification of both lipid classes by incubation at 90 °C for 3 min after the addition of 0.5 ml of 2 M KOH/MeOH. Then, 0.5 ml H₂O, 0.4 ml methanol and 2 ml hexane were added and the mixture centrifuged at 630 g for 10 min. The upper phase was discarded and the fatty acids contained in the lower phase were extracted by the addition of 0.5 ml hydrochloric acid (6 N) and 2 ml hexane. After centrifugation at 630 g for 10 min, the supernatant containing the fatty acids was transferred to a new vial and the solvent evaporated under nitrogen. Fatty acid methyl esters (FAME) were prepared by acid-catalyzed transesterification. One milliliter of methanol containing 2.5 % hydrochloric acid was added to the dried fatty acid samples and incubated for 3 min at 90 °C. After incubation, 1 ml H₂O and 2 ml hexane were added and mixed and centrifuged at 630 g for 10 min, the supernatant collected in a new tube, solvent evaporated under nitrogen, and the FAME resuspended in 0.5 ml hexane. FAME were quantified by gas chromatography (Thermo Scientific™ TRACE™ GC 2000; Thermo Fisher Scientific, Waltham, MA, USA) with a 30 m × 0.32 mm ID column. Hydrogen was used as carrier gas, and temperature programming was as follows: 100 to 130 °C at 5 °C min⁻¹, 130 to 170 °C at 2 °C min⁻¹, 8 min at 170 °C, and then to 200 °C at 2.5 °C min⁻¹. Individual FAME were identified by comparison with known standards (Supelco 37, Sigma-Aldrich, St. Louis, MO, USA).

Desaturation Activity

The desaturation activity assay was performed using microsome preparations of liver and intestine. To this end, frozen tissues from each tank replicate (five fish) were pooled in equal proportions to provide around 4 g and 2.5 g of liver and intestine, respectively. Tissues were chopped on ice and transferred to a glass homogenizer and homogenized in sucrose phosphate buffer solution (0.04 M, pH 7.4) containing 0.25 M sucrose, 0.15 M KCl, 40 mM KF and 1 mM *N*-acetylcysteine. The mixture was centrifuged at 25,000g for 15 min at 4 °C, and the supernatant collected and centrifuged at 105,000g for 60 min. The supernatant was removed and the pellet drained and suspended in 300 µl of phosphate buffer. Microsomal protein concentration was determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA) based on the Bradford dye-binding procedure using bovine serum albumin as standard [42]. To avoid degradation of the microsomal

subcellular fraction, the fatty acid desaturation activity assay in the liver and intestine was performed directly after obtaining the microsomal preparations.

Incubation was performed in a Quickfit conical test tube with 100 µl of microsome solution and 1.1 ml of master mix containing 5 mM MgCl₂, 1.5 mM glutathione, 0.5 mM nicotinamide, 1 mM NADH, 100 µM coenzyme A, 5 mM ATP and 4 µM [1-¹⁴C]18:3n-3 (PerkinElmer, Waltham, MA, USA), in final concentrations. All reagents had been previously dissolved in sucrose phosphate buffer. After incubation for 1 h at 20 °C, the reaction was stopped by the addition of 5 ml of chloroform/methanol (2:1, v/v) containing 0.01 % BHT. Following centrifugation at 1500g, the lower layer was collected and filtered through Whatman Grade 1 filter paper (Sigma-Aldrich) into a new tube. After evaporation of the solvent under nitrogen, 1 ml of toluene and 2.5 ml of 1 % H₂SO₄ in methanol were added. The transmethylation reaction was performed overnight at 50 °C. The next day, 2 ml of 2 % KHCO₃ and 5 ml of isohexane/diethyl ether (1:1, v/v) containing 0.01 % BHT were added, and after centrifugation at 1500 g for 2 min, the upper phase containing FAME was collected in a new tube. To maximize FAME extraction, this procedure was repeated with the addition of 5 ml of isohexane/diethyl ether, but without BHT. The solvent was evaporated under nitrogen and the FAME resuspended in 100 µl of isohexane containing 0.01 % BHT. FAME were separated by argentation thin-layer chromatography (TLC). Briefly, 20 × 20 cm TLC plates (Merck Millipore, Darmstadt, Germany) were prepared by coating with a solution of 2 g silver nitrate in 20 ml acetonitrile. After activating at 110 °C for 30 min, the plate was cooled and FAME applied as 2-cm streaks, and the plates developed in toluene/acetonitrile (95:5, v/v) for 1 h and desiccated in the dark for 30 min to remove the solvent. Plates were incubated in an autorad Amersham Hypercassette (GE Healthcare Life Sciences, Marlborough, MA, USA) with Kodak MR2 film (Sigma-Aldrich, St. Louis, MO, USA) for 7 days. The autoradiography film and TLC plate were aligned to identify the [1-¹⁴C]18:4n-3 desaturation band product on the plate, and the band was scraped into a scintillation fluid tube, 5 ml of scintillation fluid added, and radioactivity determined in a scintillation counter (Tri-Carb 2100TR; Packard BioScience Company, Meriden, CT, USA) and desaturation activity expressed as pmol/h/mg protein.

Gene Expression Analysis

Total RNA from the liver, intestine and brain were extracted individually from each of the five sampled fish in each tank, using 1 ml of EXTRACT-ALL® reagent (Eurobio, Courtaboeuf, France), following the manufacturer's instructions, analyzed by electrophoresis in a 1.2 % agarose gel to check

RNA integrity, and quantified with a NanoDrop 2000 spectrophotometer (Thermo Scientific Waltham, MA, USA). A pool of 20 µg of total RNA corresponding to each tank replicate was formed from 4 µg total RNA of each individual sample, and each pool of total RNA was treated with the RTS DNase™ kit (MO BIO Laboratories, Carlsbad, CA, USA) to avoid gDNA contamination. Three µg of total RNA were reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA). At the end of the reverse transcription reaction, the cDNA was diluted 20 times and kept at −20 °C.

Expression of *fads2*, *elovl5*, leukotriene a-4 hydrolase (*ltah4*), arachidonate 5-lipoxygenase (*alox5*), prostaglandin E synthase 2 (*ptges2*) and the housekeeping elongation factor 1 alpha (*efl1*) and β -actin was investigated by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) using specific primers designed with Primer3 software from Eurasian perch sequences of *fads2* (GenBank accession no. KM924433), *elovl5* (GenBank accession no. KR360724), *ltah4* (GenBank accession no. KR360726), *alox5* (GenBank accession no. KR360727), *ptges2* (GenBank accession no. KR360725), *efl1* (GenBank accession no. KC513785) and β -actin (GenBank accession no. EU664997) (Table 3). The efficiency of each primer set was validated prior to analysis. In this study, the *efl1* and β -actin genes were not regulated by dietary treatment and could be used as reference genes. Amplification of the cDNA samples was performed in triplicate using the iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA). Thermal cycling and fluorescence detection were conducted in a StepOnePlus Real-Time PCR

System (Applied Biosystems, Foster City, CA, USA) under the following conditions: 10 min of initial denaturation at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 60 °C. After each run, amplification of single amplicons was confirmed by analyzing the melt curve, and the PCR products were sequenced. The relative mRNA levels of *fads2*, *elovl5*, *ltah4*, *alox5*, and *ptges2* in each sample were normalized with the geometric mean of *efl1* and β -actin calculated using the relative standard curve method [43].

Statistical Analysis

Statistical analysis was performed using Statistica software (version 8.0; Dell/StatSoft, Tulsa, OK, USA), with results presented as mean $\pm$ SD ($n = 4$). Data were checked for normal distribution and homogeneity of the variances by Pearson and Bartlett tests, respectively, and percentage data were transformed with the arcsine function prior to analysis. The effect of dietary treatment on fatty acid composition was tested using the Student *t* test, while two-way analysis of variance (ANOVA) was performed on gene expression and desaturation activity data. Differences were considered significant at $P < 0.05$.

Results

Fish Growth Performance and Survival

At the end of the nutritional challenge, weight gain reached 180 and 134 % in fish fed FO and LO diets, respectively

Table 3 Primers used for each gene expression analysis by real-time RT-PCR

Gene	Sens	Forward primers (5′–3′)	Annealing temperature (°C)	PCR efficiency (%)
<i>Fads2</i>	Forward	CCACCTGGGTCACATCCTTC	64.0	96.1
	Reverse	GCATGATGGCGCTCAGAAAG	62.0	
<i>Elov15</i>	Forward	CTACGGTCTGTCTAGCCATCC	64.0	93.5
	Reverse	GGGAGTACAGCCATCCCTTG	64.0	
<i>Ltah4</i>	Forward	ACAACCCTCTGACCAACCTG	62.0	94.5
	Reverse	CAGGACGTCCACCTTGTCTT	62.0	
<i>Alox5</i>	Forward	TGACAAGGCTAACGCAACAG	60.0	94.4
	Reverse	GTAGCCTCCACACCCTGTA	64.0	
<i>Ptges2</i>	Forward	GGAGATCAAGTGGTCGGTGT	62.0	102.5
	Reverse	CATGCTTCTCTCCGTGTTGA	60.0	
<i>EF1α</i>	Forward	GGAAATTCGTCGTGGATACG	60.0	91.1
	Reverse	GGGTGGTTCAGGATGATGAC	62.0	
β -actin	Forward	ACCTTCTACAACGAGCTGAGAGTT	50.6	98.2
	Reverse	AGTGGTACGACCAGAGGCATAC	51.6	

Fads2 fatty acid desaturase 2, *elovl5* elongase5, *ltah4* leukotriene A4 hydrolase, *alox5* arachidonate 5-lipoxygenase, *ptges2* prostaglandin E synthase 2, *EF1 α* elongation factor 1 alpha

Table 4 Growth and survival in Eurasian perch juveniles fed fish oil (FO) and linseed oil (LO) diets for 10 weeks

	FO diet	LO diet
Initial weight (g)	17.5 ± 0.2	17.4 ± 0.2
Final weight (g)	47.6 ± 1.2 ^b	40.9 ± 4.5 ^a
Weight gain (%) ¹	180 ± 8 ^b	134 ± 22 ^a
SGR (% day ⁻¹) ²	1.45 ± 0.03 ^b	1.22 ± 0.15 ^a
Mortality (%)	4.7 ± 3.0	5.0 ± 1.0

Mean values in similar row with different superscript letters are significantly different (Student *t* test, *P* < 0.05)

¹ Weight gain (%) = (final weight – initial weight) × initial weight⁻¹ × 100

² Specific growth rate (SGR) = (ln final weight – ln initial weight) × 100 day⁻¹

(Table 4). Fish fed the FO diet had significantly higher final mean weight (47.6 g) than those fed the LO diet (40.9 g) (*P* < 0.05). Mortality over the experimental period did not exceed 5 %, with no significant difference between dietary treatments.

Effects of Diets on the Fatty Acid Composition in Liver, Brain and Muscle

The lipid content of brain and muscle tissue was not affected by dietary treatment, whereas liver lipid content was significantly higher in fish fed the LO diet (14.3 % WW) than those fed FO (12.1 % WW) (Table 5). The relative proportions of neutral and phospholipids was not affected by dietary treatment in any tissue. In all tissues, an LO diet ("diet LO") induced a significant decrease in total saturated and monounsaturated fatty acids in neutral lipids. Similarly, diet LO resulted in a large increase in the proportion of 18:3n-3 (ALA) in both lipid fractions and all tissues compared to that in fish fed the FO diet ("diet FO"). A significant increase in 18:2n-6 was also observed in the phospholipid fraction of liver and muscle. Among the LC-PUFA, diet LO resulted in significantly lower proportions of 20:5n-3 (EPA), 22:6n-3 (DHA) and 20:4n-6 (ARA) in lipid fractions in both liver and muscle. In contrast, there was no significant effect of diet on the percentages of EPA, DHA and ARA recorded in brain phospholipids. Fish fed diet LO displayed significantly higher amounts of intermediate fatty acids such as 18:3n-6, 20:3n-6, 18:4n-3, 20:3n-3 and 20:4n-3 in liver neutral lipid and phospholipid fractions.

Effect of diets on *fads2*, *elovl5*, *ltah4*, *alox5* and *ptges2* gene expression in liver, brain and anterior intestine

The relative gene expression of *fads2* and *elovl5* was significantly higher in the liver compared to the brain and

intestine (Fig. 1a, b). The *ltah4* and *alox5* genes displayed significantly higher expression in the anterior intestine (Fig. 1c, d), whereas a higher *pgh2* gene expression level was observed in brain, followed by the liver (Fig. 1e). However, dietary treatment had no significant effect on the expression of any of the genes investigated in the present study.

Effect of Diets on FADS2 Activity in Liver and Intestine

Liver microsomes displayed significantly higher FADS2 activity than the intestine (Fig. 2). [1-14C]18:3n-3 desaturation activity in the liver was around 1.2 pmol/h/mg protein for fish fed the FO diet and 0.6 pmol/h/mg protein for fish fed LO. However, high variability in values for fish receiving FO meant that there was no significant effect of diet on microsomal FADS2 activity in the liver or intestine.

Discussion

In the present trial, Eurasian perch juveniles fed diet FO displayed better growth performance (SGR = 1.45 % d⁻¹) than that recorded in various previous studies, where SGRs of between 0.26 and 1.11 % d⁻¹ were reported [33, 40, 44], and growth performance was similar to the best performance previously reported in Eurasian perch juveniles, with SGRs of 1.26 % d⁻¹ [38] and 1.40 % d⁻¹ [45]. Thus, in the present study, the use of diets characterized by lower lipid levels than those used in most of the earlier studies did not reduce growth performance. This may be explained by the diet composition and/or the feeding method used in the present study. Indeed, contrary to previous studies in which diets were distributed twice a day or with an automatic feeder, fish in the current study were fed three times per day, although without accurate feed intake, this cannot be confirmed. Differences in genetic pool or domestication level may also account for the growth differences reported among studies.

A comparison of growth between diets showed a significant reduction in growth among fish fed LO (SGR = 1.22 % d⁻¹) in comparison with those fed the FO diet (SGR = 1.45 % d⁻¹). However, fish fed the LO diet displayed higher SGR than in previous trials in which Eurasian perch were fed dietary linseed oil, producing SGRs of between 0.25 and 0.78 % d⁻¹ [33, 40, 44]. In other carnivorous freshwater species, including Murray cod [46] and striped snakehead [47], a significantly lower growth rate was also observed in fish fed 100 % plant oil diets. Similarly, in marine carnivorous species such as gilt-head sea bream [48], European sea bass [49] and Atlantic halibut [50], 100 % VO diets resulted in a significant reduction in growth rates. In contrast, in salmonids [51], marine

Table 5 Total lipid content (%/WW) and fatty acid composition (mol % of total fatty acids) in liver, brain and muscle from fish fed fish oil (FO) and linseed oil (LO) diets

	Liver			Brain			Muscle		
	Total lipid (%/WW)			Total lipid (%/WW)			Total lipid (%/WW)		
	FO	LO	LO 12.1 ± 0.3	FO	LO	LO 14.3 ± 0.3*	FO	LO	LO 0.7 ± 0.3
Lipid fraction (%/lipid)	Neutral lipid			Neutral lipid			Neutral lipid		
	FO	LO	FO	LO	FO	LO	FO	LO	FO
14:0	79.7 ± 2.9	83.9 ± 1.8	20.3 ± 2.9	16.4 ± 1.8	34.0 ± 1.2	35.9 ± 2.4	66.0 ± 1.2	64.1 ± 2.4	35.1 ± 1.1
16:0	3.7 ± 0.1	2.5 ± 0.3*	1.5 ± 0.1	1.1 ± 0.1*	3.3 ± 0.1	2.6 ± 0.2*	1.0 ± 0.1	0.7 ± 0.0*	2.6 ± 0.2*
18:0	24.5 ± 1.1	20.3 ± 1.8*	22.3 ± 0.4	19.9 ± 0.9*	18.8 ± 0.4	16.3 ± 0.6*	19.7 ± 0.2	19.1 ± 0.4	15.7 ± 0.4*
Total satu	2.8 ± 0.2	2.5 ± 0.2	6.5 ± 0.3	6.7 ± 0.3	3.2 ± 0.4	2.2 ± 0.2	7.8 ± 0.3	7.6 ± 0.3	2.2 ± 0.2
rated ^a	30.9 ± 1.1	25.3 ± 2.3*	30.3 ± 0.5	27.7 ± 0.9*	25.3 ± 1.0	21.1 ± 0.6*	28.5 ± 0.8	27.4 ± 0.3	20.5 ± 0.6*
16:1n-7	17.5 ± 1.5	11.6 ± 1.5*	4.8 ± 0.7	3.9 ± 0.9	12.4 ± 1.0	9.2 ± 0.8*	5.8 ± 0.3	5.1 ± 0.1	8.2 ± 0.4*
18:1n-9	32.5 ± 0.6	30.3 ± 1.3	10.5 ± 0.5	11.5 ± 0.8	27.3 ± 0.3	24.0 ± 0.5*	24.9 ± 0.3	25.1 ± 0.1	23.4 ± 0.5*
18:1n-7	2.6 ± 0.2	1.7 ± 0.2*	1.9 ± 0.1	1.4 ± 0.1*	2.9 ± 0.1	2.1 ± 0.1*	2.0 ± 0.0	1.8 ± 0.0*	2.0 ± 0.1*
Total monoe	52.6 ± 1.2	43.6 ± 2.8*	17.2 ± 1.0	16.8 ± 1.8	42.7 ± 1.4	35.3 ± 1.4*	32.7 ± 1.0	32.0 ± 0.6	33.6 ± 0.5*
nes ^b	9.0 ± 1.0	10.7 ± 0.8	4.3 ± 0.5	6.3 ± 0.3*	9.5 ± 0.5	10.6 ± 0.1	2.1 ± 0.2	2.3 ± 0.2	11.3 ± 0.2
18:2n-6	0.3 ± 0.0	0.3 ± 0.0*	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0*
20:3n-6	0.1 ± 0.0	0.1 ± 0.0*	0.5 ± 0.1	0.6 ± 0.1*	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1
20:4n-6	0.1 ± 0.0	0.0 ± 0.0	2.7 ± 0.2	1.4 ± 0.2*	0.7 ± 0.1	0.4 ± 0.0	3.5 ± 0.2	3.2 ± 0.2	0.5 ± 0.0
Total n-6 PUFA	9.5 ± 1.0	11.1 ± 0.9*	7.6 ± 0.5	8.4 ± 0.2*	10.6 ± 2.0	11.4 ± 0.9*	5.8 ± 2.0	5.8 ± 0.9	11.9 ± 0.2*
18:3n-3	1.6 ± 0.8	15.6 ± 3.8*	0.9 ± 0.4	10.3 ± 1.9*	1.8 ± 0.2	16.9 ± 2.2*	0.4 ± 0.1	2.6 ± 0.6*	1.8 ± 0.1
18:4n-3	0.4 ± 0.1	1.4 ± 0.3*	0.2 ± 0.0	0.8 ± 0.1*	0.9 ± 0	1.2 ± 0.0*	0.2 ± 0.0	0.2 ± 0.1	0.8 ± 0.1
20:3n-3	0.1 ± 0.0	0.6 ± 0.1*	0.1 ± 0.1	1.2 ± 0.3*	0.1 ± 0.0	0.4 ± 0.1*	0.0 ± 0.0	0.2 ± 0.0*	0.1 ± 0.0
20:4n-3	0.2 ± 0.0	0.5 ± 0.1*	0.4 ± 0.0	1.5 ± 0.3*	0.6 ± 0.0	0.3 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.2
20:5n-3	0.7 ± 0.2	0.2 ± 0.1*	4.2 ± 0.3	1.7 ± 0.4*	4.7 ± 0.3	3.3 ± 0.1*	4.9 ± 0.2	4.1 ± 0.2	5.2 ± 0.4
22:5n-3	0.4 ± 0.1	0.1 ± 0.0*	0.9 ± 0.1	0.5 ± 0.1*	1.6 ± 0.1	1.0 ± 0.1*	0.4 ± 0.1	0.1 ± 0.0*	1.7 ± 0.1
22:6n-3	3.6 ± 0.2	1.6 ± 0.3*	38.2 ± 1.9	31.2 ± 1.1*	11.6 ± 0.2	9.0 ± 0.3*	27.0 ± 0.7	27.5 ± 1.0	11.8 ± 0.4
Total n-3 PUFA	7.0 ± 1.1	20.0 ± 4.0*	44.9 ± 2.0	47.1 ± 2.4	21.3 ± 3.1	32.2 ± 0.8*	33.0 ± 3.2	34.9 ± 0.9	21.9 ± 1.5

Values are expressed as mean ± SD of four replicates, each originating from a blend of five tissues. In each tissue, asterisks (*) indicate significant differences between diets for each fatty acid from the same lipid fraction (neutral lipid or phospholipid) (Student *t* test, *P* < 0.05)

^a Total saturated (+ 12:0, 15:0, 17:0, 21:0 up to 0.5 %)

^b Total monoenes (+14:1; 20:1n-9 up to 1 %)

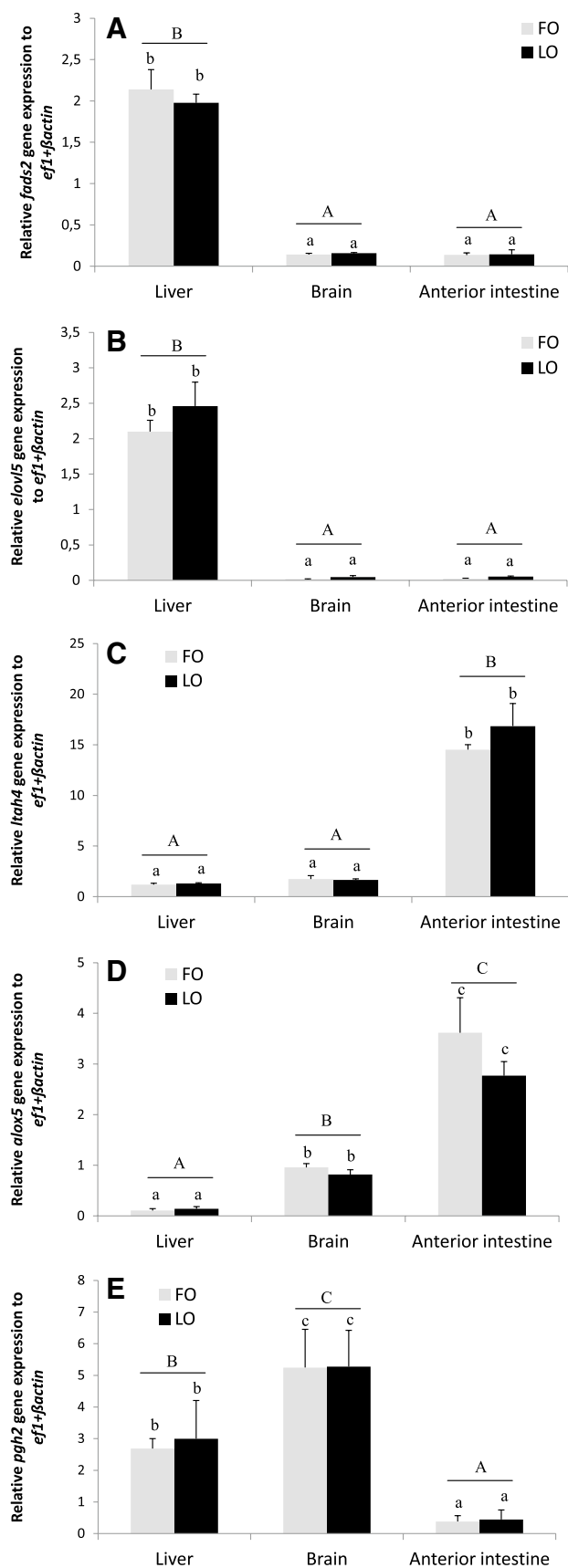


Fig. 1 Relative expression of *fads2* (a), *elovl5* (b), *itah4* (b), *alox5* (b) and *ptges2* (e) genes in liver, brain and anterior intestine from fish fed fish oil (FO) and linseed oil (LO) diets. Relative transcript (mRNA) levels were determined by real-time RT-PCR and normalized by the arithmetic mean of *ef1α* and *β-actin* gene expression. Values are expressed as mean $\pm$ SD of four replicates, each originating from a blend of five tissues. Different letters indicate significant differences (two-way ANOVA, $P < 0.05$)

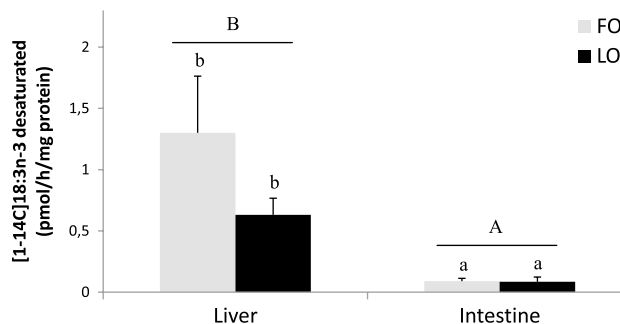


Fig. 2 FADS2 n-3 microsomal desaturase activities in liver and intestine from fish fed fish oil (FO) and linseed oil (LO) diets. Values are expressed as mean $\pm$ SD of four replicates, each originating from a blend of five tissues. Different letters indicate significant differences between dietary treatment (two-way ANOVA, $P < 0.05$)

Senegalese sole [24] and freshwater carnivorous species including pikeperch [52], replacement of 100 % FO with VO was possible without compromising growth. However, growth performance comparisons between experiments must consider the contribution of fish meal to dietary lipid and the final weight gain.

The total lipid content was not affected by the dietary treatment in the brain and muscle, whereas a slight increase of lipid deposition was observed in the liver of fish fed LO. This increase in liver fat content has been attributed to excess dietary lipid, and also to imbalance in the FA composition supplied by plant oils. Indeed, the liver is the primary site for lipid metabolism and is strongly modulated by dietary lipid composition [53]. Moreover, as has been reported in Percidae, including Eurasian perch [44, 45] and pikeperch [52, 54], an increased hepatosomatic index (HSI) and vacuolization of hepatocytes was associated with increased lipid deposition as a consequence of diets rich in plant oil. As previous studies have demonstrated, the increased lipid vacuolization in liver contributes to liver parenchyma degeneration and necrosis [44, 52]. In the present study, histological analysis demonstrated that the increased lipid deposition in the liver of fish fed the LO diet was not associated with increased hepatocyte vacuolization (data not shown). In fact, the relatively low lipid content in the diets appeared to reduce the deleterious effects of plant oil on liver structure and, likely, liver function.

The FA composition of liver, brain and muscle showed significant changes in response to dietary FA. It is well known that fatty acid composition in fish generally reflects dietary fatty acid composition, as reported in other finfish species [55, 56]. Increased ALA (18:3n-3) was observed in all tissues as a consequence of the high concentration of this FA in diet LO, which suggests that ALA is readily incorporated into tissue lipids and stored in lipid pools in Eurasian perch. The accumulation of ALA in tissues of fish fed linseed oil-based diets has also been reported in freshwater Murray cod [57], salmonids [58] and marine species such as sea bream [48] and European sea bass [31]. As suggested by Xu and Kestemont [38], this FA could be a precursor for n-3 LC-PUFA synthesis in Eurasian perch, but could also be catabolized to provide energy for basal maintenance and maturation.

Interestingly, diet LO did not affect the proportions of EPA, DHA and ARA in brain phospholipids, while the proportions of these FA were significantly reduced in liver and muscle. The maintenance of DHA level in the brain suggests the presence of mechanisms for maintaining DHA levels, particularly under limited dietary LC-PUFA conditions. The finding that DHA was primarily incorporated in the brain in fish was reported previously [16]. Accumulation of DHA in the brain is of prime importance for the functionality of the nervous system in fish. For example, Masuda *et al.* [59] demonstrated that DHA in the brain is involved in the myelination of neurocytes and the construction of synapses in yellowtail (*Seriola quinqueradiata*), underscoring the crucial role of this FA in brain functionality. In contrast, decreased proportions of LC-PUFA including EPA, DHA and ARA were recorded in the liver and muscle of fish fed LO. However, there was only a moderate reduction in DHA in the liver and muscle compared to that in fish fed the FO diet, with liver and muscle phospholipid DHA levels reduced by only 18 and 12 %, respectively, in fish fed the LO diet versus the FO diet, despite an almost 70 % reduction of this FA in the LO diet.

The relatively unaffected DHA levels observed in the liver, brain and muscle at the end of the nutritional challenge could reflect some degree of endogenous synthesis. Indeed, as previously suggested in Percidae, including Eurasian perch [38, 44] and pikeperch [52–54], the use of dietary plant oil characterized by low LC-PUFA content may stimulate the endogenous synthesis of EPA and DHA from C₁₈ precursors supplied by plant oils. In the present study, endogenous biosynthesis was supported by the higher amount of 18:3n-3 and 18:2n-6 desaturation and elongation products such as 18:4n-3, 20:3n-3, 20:4n-3, 18:3n-6 and 20:3n-6 observed in the liver of fish fed the LO diet. LC-PUFA biosynthesis capacity in Eurasian perch was previously reported in juveniles fed a linseed oil diet, in which radioactivity was recovered in DHA and

ARA from hepatocytes incubated with [1-14C]18:3n-3 and [1-14C]18:2n-6 [33], thus demonstrating the functionality of the LC-PUFA biosynthetic pathway in Eurasian perch. In the present study, $\Delta 6$ desaturation activity was investigated in the liver and intestine microsomes incubated with [1-14C]18:3n-3, with higher activity detected in the liver than the intestine, although a diet of linseed oil did not increase FADS2 activity in either tissue. This is in contrast to results obtained in isolated hepatocytes of Eurasian perch fed a linseed oil-based diet, who displayed higher LC-PUFA biosynthesis activity than those fed fish oil [33]. The discrepancy between these results could be explained by the different assay methods used (microsome FADS2 assay vs. hepatocyte LC-PUFA assay) in the two studies. Replacing fish oil with vegetable oil was shown to increase LC-PUFA biosynthesis activity in isolated hepatocytes of salmonids and freshwater fish including the Atlantic salmon [17, 26, 51], rainbow trout [25] and Nile tilapia (*Oreochromis niloticus*) [60], and also in marine Senegalese sole [61]. In contrast, dietary vegetable oil did not increase $\Delta 6$ desaturation activity in marine carnivorous species such as Atlantic cod [62] and European sea bass [31]. Interestingly, a comparison of the [1–14]18:3n-3 desaturation activity between a salmonid (Atlantic salmon) and a marine carnivorous fish (Atlantic cod) reinforced this difference between salmonid and strictly marine carnivorous fish. Indeed, Atlantic salmon displayed greater desaturation activity than Atlantic cod, regardless of oil source. In the present study, liver microsomes were prepared from Atlantic salmon juveniles fed a commercial diet rich in LC-PUFA (Neogrower, Le Gouessant), and analyzed in parallel with the microsome samples of Eurasian perch (data not shown). The absolute values of desaturation activity in Atlantic salmon were in the same range (0.8 ± 0.3 pmol h⁻¹ mg protein⁻¹) as values recorded in the liver of Eurasian perch (FO diet: 1.3 ± 0.5 pmol h⁻¹ mg protein⁻¹; LO diet: 0.6 ± 0.1 pmol h⁻¹ mg protein⁻¹), which may reflect relatively high basal activity in Eurasian perch. However, the fatty acid composition data indicated that the $\Delta 6$ desaturation activity was insufficient to maintain tissue proportions of LC-PUFA in fish fed the LO diet at the same level as in fish fed the FO diet.

The lack of increased FADS2 activity in fish fed the LO diet was supported by the *fads2* and *elovl5* gene expression data showing that the LO diet did not increase *fads2* and *elovl5* gene expression in the tissues. This was in contradiction to previous observations reported in other fish species. In salmonids (Atlantic salmon: [63]; rainbow trout [64]), freshwater fish (zebrafish [27], swordtail [20]) and marine fish (sea bass [31], Senegalese sole [28]), the replacement of dietary fish oil with vegetable oils increased the expression of genes involved in LC-PUFA biosynthesis. The lack of increased *fads2* gene expression in perch fed the LO diet

in the present study could be a consequence of the presence of two alternatively spliced *fads2* mRNA in Eurasian perch (Geay *et al.*, submitted), as the primers used to investigate *fads2* gene expression did not discriminate between the two *fads2* transcripts.

Two hypotheses could explain the increased desaturation/elongation activity in fish fed vegetable oil. The biosynthetic pathway may be stimulated by increased substrate C₁₈ PUFA concentration, or low dietary LC-PUFA could increase the activity of the pathway as a result of decreased product inhibition [1, 4]. Indeed, fish need to maintain high LC-PUFA levels in cell membranes, [1] and thus the presence of C₁₈ PUFA in the absence of LC-PUFA may stimulate the production of LC-PUFA in order to preserve the stability of cellular membranes. In the present study, the muscle of fish fed the LO diet displayed higher DHA concentrations (25.7 % of total FA or 52 mol % of total FA) than those of wild Eurasian perch juveniles (21.5 % of total FA) [65]. This suggests that DHA was not deficient in fish fed the LO diet, and thus may explain the lack of stimulation of the LC-PUFA pathway. In contrast, the lack of LC-PUFA activation in fish fed the LO diet could be explained by the fact that the DHA level in fish fed the FO diet was also low in comparison with previous studies. Indeed, the DHA concentration in the liver of fish fed the FO diet (8.9 % of total FA or 14.7 mol % of total FA) was much lower than in fish fed fish oil-based diets in previous experiments, which varied between 16 and 19 % [33, 45, 65]. Moreover, as reported by Henrotte *et al.* [33], in comparison with a fish oil-based diet rich in DHA (20.4 % of total FA), a significant increase in desaturation/elongation activity in the liver was observed not only in fish fed a linseed oil diet, but also in fish fed a blend of sardine oil, safflower oil and Vevodar oil, in which the dietary DHA concentration (5.1 % of total FA) was in the same range as that in the FO diet in the present study (7.5 %). This is supported by the relatively high expression levels of the *fads2* and *elovl5* genes in the liver of fish fed both diets, possibly reflecting stimulation of these genes regardless of dietary condition.

The LO diet affected not only the n-3 LC-PUFA content in the liver and muscle, but also the ARA content. Despite the lower ARA content in tissue phospholipids compared with n-3 LC-PUFA, previous studies have indicated that ARA is the preferred substrate for enzymes involved in eicosanoid pathways in fish, as in mammals [66]. Eicosanoids derived from ARA have proinflammatory and immunoactive functions, whereas eicosanoids derived from n-3 LC-PUFA have anti-inflammatory properties. In the present study, the impact of diet LO on eicosanoid synthesis was investigated in the liver, brain and intestine. Interestingly, *alox5* and *lta4 h* involved in leukotriene synthesis and *ptges2* involved in prostaglandin synthesis displayed

opposite expression patterns among tissues. The highest expression of *lta4 h* and *alox5* genes in the intestine suggests an important role in the inflammatory process in this tissue. Indeed, ALOX5 is an enzyme involved in the biosynthesis of leukotrienes, with ARA-derived leukotriene B₄ (LTB₄) one of the most critical in the inflammatory response. LTB₄ has an important role during the inflammatory process in enhancing the proliferation of T and B cells, and increasing the production of tumor necrosis factor α (TNF α) and interleukins IL-1 and IL-6. However, in the present study, the expression of both genes was unaffected by diet, even in liver tissue in which significantly decreased ARA concentration was recorded. In contrast, the *ptges2* gene was more strongly expressed in the brain than the liver and intestine. As reported in vertebrates [67, 68], prostaglandins have an essential role in neural tissues, and are particularly involved in the modulation of neural transmission, nociception and hypothalamic function [69]. In the current study, *ptges2* gene expression was not affected by dietary oil source, although this is likely explained by the lack of a dietary effect on LC-PUFA concentrations in brain phospholipids.

To summarize, the present study has demonstrated that the use of a full LO diet reduced the growth performance of Eurasian perch juveniles, while maintaining relatively high LC-PUFA content in the brain, liver and muscle. However, molecular analysis showed that genes of LC-PUFA biosynthesis were apparently not activated by the lack of LC-PUFA in diet LO. Similarly, diet LO did not affect the expression of genes involved in eicosanoid synthesis. The absence of dietary effects on gene expression and FADS2 activity could be explained by similar activation of LC-PUFA synthesis by both dietary treatments. Interestingly, the maintenance of high LC-PUFA content in muscle, valuable for consumers, and the higher growth rates recorded in fish fed both dietary treatments compared to previous studies suggests that the present diet composition is suitable for Eurasian perch farming. Future studies using diets with strongly contrasting LC-PUFA content are necessary to provide further insight into the dietary modulation of LC-PUFA biosynthesis capacity in Eurasian perch. In addition, further studies are needed to investigate the health and welfare effects of fish oil replacement with plant sources on the immune system of this species.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interests exist.

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