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ORIGINAL ARTICLE

Effect of Dietary Oils on the Accumulation of Conjugated and Other Polyunsaturated Fatty Acids in Zebrafish Tissues

Apolline Goudmaeker | Anaïs De Simone | Éric Mignolet | Melissa M. Page | Yvan Larondelle | Cathy Debier

Louvain Institute of Biomolecular Science and Technology, UCLouvain, Louvain-la-Neuve, Belgium

Correspondence: Apolline Goudmaeker (apolline.goudmaeker@uclouvain.be)

Received: 19 May 2025 | **Revised:** 30 September 2025 | **Accepted:** 2 October 2025

Funding: The authors received no specific funding for this work.

Keywords: conjugated fatty acids | docosahexaenoic acid | eicosapentaenoic acid | linolenic acid | punicic acid | zebrafish

ABSTRACT

Dietary polyunsaturated fatty acids (PUFA), including conjugated linolenic acids, are increasingly investigated for their potential health benefits. Zebrafish have emerged as a promising in vivo model for studying the metabolism and effects of such bioactive compounds. The present study tested whether zebrafish tissues could be enriched with specific PUFA through dietary modulation. To do so, six-month-old female zebrafish were divided into four groups and fed purified diets over an eight-week period, each diet containing a distinct lipid source: sunflower oil (rich in linoleic acid), linseed oil (rich in alpha-linolenic acid), fish oil (rich in eicosapentaenoic acid and docosahexaenoic acid), and pomegranate seed oil (rich in punicic acid, a conjugated linolenic acid). Body mass, length, and whole-body fatty acid profile were then assessed. An efficient incorporation of linoleic acid, alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, or punicic acid into zebrafish tissues was observed, depending on the dietary source. Linoleic acid and alpha-linolenic acid underwent partial bioconversion into longer and more unsaturated fatty acids. Fish fed with pomegranate seed oil were enriched in conjugated linoleic acids (rumenic acid and 18:2 (trans-9, trans-11)), suggesting the presence of a Δ13 reductase activity in zebrafish. These findings highlight the suitability of zebrafish as an in vivo model for investigating the impacts of specific dietary and metabolically derived PUFA in the context of health and disease.

1 | Introduction

Lipids are essential nutrients that furnish energy, support cell membrane integrity, and regulate gene expression, while also providing hormones, bioactive molecules, and immune system mediators (Tocher 2003; Jayarathne et al. 2017; Nayak et al. 2020; Monroig et al. 2022). Among them, polyunsaturated fatty acids (PUFA) play key regulatory roles. Linoleic acid (LA, 18:2 (9,12)) and α-linolenic acid (ALA; 18:3 (9,12,15)) are essential precursors for omega-6 (n-6) and omega-3 (n-3) long-chain PUFA (LC-PUFA), such as arachidonic acid (ARA, 20:4 (5,8,11,14)), eicosapentaenoic acid (EPA, 20:5 (5,8,11,14,17)), and docosahexaenoic acid (DHA, 22:6 (4,7,10,13,16,19)) (Figure 1) (Monroig et al. 2018, 2022). These compounds modulate inflammation and disease risk, with n-3 PUFA generally exerting anti-inflammatory and cardioprotective effects, while excessive n-6 intake may promote inflammatory conditions (Simopoulos 2008; Calder 2012; Albracht-Schulte et al. 2018; Djuricic and Calder 2021; Dyall et al. 2022).

Beyond classical n-3 and n-6 PUFA, conjugated fatty acids (CFA), positional and geometric PUFA isomers, are gaining attention

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; CFA, conjugated fatty acid(s); CLA, conjugated linoleic acid(s); CLnA, conjugated linolenic acid(s); CVD, cardiovascular diseases; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; DPAn-3, docosapentaenoic acid (n-3); DPAn-6, docosapentaenoic acid (n-6); ELOVL, very-long-chain 3-oxoacyl-CoA synthase (EC 2.3.1.199); EPA, eicosapentaenoic acid; FADS2, acyl-CoA 6-desaturase (EC 1.14.19.3); FO, fish oil; GLA, gamma-linolenic acid; LA, linoleic acid; LO, linseed oil; MUFA, monounsaturated fatty acid(s); OA, oleic acid; PA, palmitic acid; PSO, pomegranate seed oil; PUFA, polyunsaturated fatty acid(s); PunA, punicic acid; RmA, rumenic acid; SDA, stearidonic acid; SFA, saturated fatty acid(s); SO, sunflower oil; STA, stearic acid.

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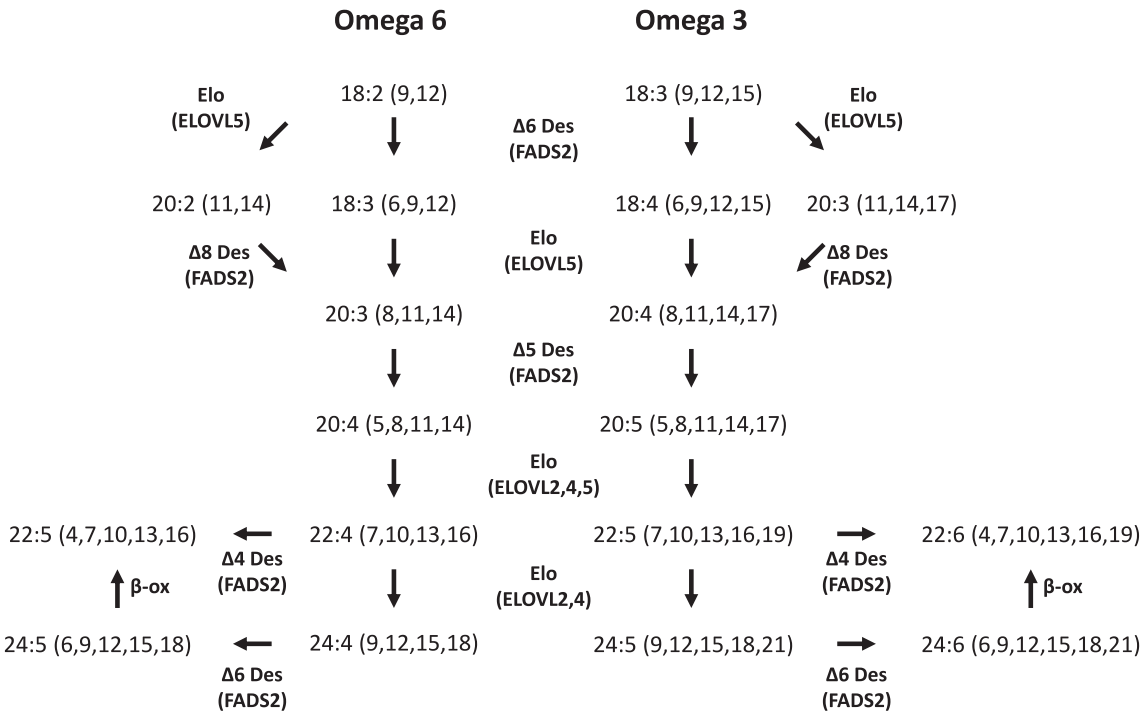


FIGURE 1 | Bioconversion pathways of n-6 and n-3 PUFA in fish. β-ox, β-oxidation; Des, desaturation reaction; Elo, elongation reaction; ELOVL, very-long-chain 3-oxoacyl-CoA synthase; FADS2, acyl-CoA 6-desaturase. Two alternative routes exist in the pathway from C18 PUFA precursors to EPA/ARA, namely the Δ6 and Δ8 pathways. Two pathways also occur for the synthesis of DHA from EPA, the Sprecher pathway and the Δ4 pathway. The Sprecher pathway is widely spread across teleost fish, whereas the Δ4 pathway is only present in some. Adapted from (Monroig et al. 2018, 2022).

for their possible health-promoting effects (Yuan et al. 2014). Conjugated linoleic acids (CLA), naturally found in small amounts in ruminant meat and dairy products, include rumenic acid (RmA, 18:2 (cis-9, trans-11)), the most abundant and primary bioactive isomer, associated with anticarcinogenic, anti-inflammatory, and cardioprotective properties (Ledoux et al. 2005; Blaško et al. 2010; Koba and Yanagita 2014; den Hartigh 2019; Basak and Duttaroy 2020; Watanabe et al. 2020). Another CLA isomer, 18:2 (trans-10, cis-12), commonly found in synthetic supplements, reduces body fat but has also been linked to adverse metabolic outcomes (Park et al. 1999; Riserus 2004; Wang et al. 2006; Onakpoya et al. 2012; Cordobas-Chacon 2019; den Hartigh 2019). Conjugated alpha-linolenic acids (CLnA), positional and geometric isomers of ALA, are naturally present in high amounts in some seed oils, such as pomegranate seed oil (Hennessy et al. 2016). They have been associated with health-promoting effects, including anti-inflammatory and anti-carcinogenic properties, which may potentially be partly mediated through their metabolic conversion into CLA isomers (Schneider et al. 2013; Koba and Yanagita 2014; Yuan et al. 2014; Hennessy et al. 2016; Fontes et al. 2017; Vermonden et al. 2021; Ngo Njembe, Dejonghe, et al. 2021). Despite their promising bioactivity, CLnA remain underexplored, particularly regarding their dietary incorporation and metabolic fate in vertebrate tissues.

Zebrafish (*Danio rerio*) represent a relevant model for studying PUFA metabolism due to their physiological similarity to mammals and responsiveness to dietary lipid modulation (Oka et al. 2010; Zang et al. 2018). While several studies have explored PUFA incorporation in zebrafish (Jaya-Ram et al. 2008; Powell et al. 2015; Araujo et al. 2017; Fowler, Dennis-Cornelius, et al. 2020), none have investigated the tissue-specific accumulation of CFA.

In this article, we compared the effects of four PUFA-enriched diets, containing either linseed oil (LO) rich in ALA, fish oil (FO) rich in EPA and DHA, pomegranate seed oil (PSO) rich in Punicic acid (PunA; 18:3 (cis-9, trans-11, cis-13)), or sunflower oil (SO) rich in LA on body composition and fatty acid profiles of six-month-old zebrafish. The study evaluated (i) the incorporation of specific fatty acids, namely ALA, EPA, DHA, PunA, and LA, into fish tissues across lipid classes, as well as (ii) their bioconversion into other unsaturated compounds. This research serves as a foundation for future studies to investigate how changes in PUFA (n-3, n-6 PUFA, and CFA) body composition affect disease development.

2 | Materials and Methods

2.1 | Zebrafish Husbandry

All animal experiments were performed according to European guidelines and approved by the local Ethics Committee for Animal Research of the UCLouvain, Louvain-la-Neuve, Belgium (Proposal number: 1858703; agreement number: L1220587).

Wild-type zebrafish (*Danio rerio*) of the AB strain were purchased from the European Zebrafish Resource Center (KIT, Eggenstein-Leopoldshafen, Germany) and reared and maintained at 27°C in a recirculating aquarium system (Fleuren and Nooijen B.V., Nederweert, Netherlands) under a 14 h/10 h light/dark cycle with a daily water renewal of 10%. Water quality parameters were monitored and maintained as follows: pH (7.3 ± 0.1), ammonia

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(<0.02 mg/L), nitrite (<0.025 mg/L), nitrate (<2.5 mg/L), oxygen (>7 mg/L). Fish were monitored daily. Prior to the experiment, zebrafish were fed twice a day with caviar (SAFE, Augy, France), which was analyzed in our laboratory. It contained 422.9 μmol total lipids/g of feed wet weight, of which 162.6, 107.7, 71.0, and 81.6 μmol/g of feed were made of SFA, MUFA, n-6 PUFA, and n-3 PUFA, respectively. Palmitic acid (PA; 16:0) and stearic acid (STA; 18:0) were the most abundant SFA with 105.9 and 22.5 μmol/g of feed. Oleic acid (OA; 18:1 (9)) reached 57.0 μmol/g of feed. n-6 PUFA were rich in LA (67.9 μmol/g of feed) and n-3 PUFA in EPA and DHA (37.7 and 28.1 μmol/g of feed, respectively).

2.2 | Experimental Design

Eighty adult female zebrafish of similar weight (6 months post fertilization (mpf), weighing 0.383 ± 0.057 g) were equally divided into four diet groups using simple randomization with a random number generator. The exclusive use of females aimed to limit variability due to known sex-specific differences in lipid metabolism and body composition in zebrafish (Fowler, Williams, et al. 2020). Zebrafish were acclimatized for 1 week prior to the start of the experiment. All diets contained the same amount of total lipids (isolipidic) but differed in their PUFA profiles and were enriched in either (i) sunflower oil (SO; Biotime; Hal, Belgium), (ii) linseed oil (LO; Bio Planète, Bram, France), (iii) menhaden fish oil (FO; Sigma-Aldrich, Saint Louis, Missouri, USA) or (iv) pomegranate seed oil (PSO; Ol'vita; Mysłaków, Poland). Twenty zebrafish were housed per 10 L tank and fed twice daily in a weight-maintaining amount (25 cal per fish per day) for 8 weeks. The feeding order was randomized and investigators were blinded to the diet. At the start of the experiment, four fish were randomly euthanized, lyophilized, and stored individually at −20°C until further analysis. After 4 weeks of experiment, body mass and body length of four random fish per diet were measured. At the end of the trial, zebrafish were fasted for 24 h and euthanized with an overdose of MS-222 (Tricaine methane sulfonate; Sigma-Aldrich; 500 mg/L, pH 7.3). Body mass and length of four random fish per diet were recorded and these fish were lyophilized and stored individually at −20°C until further analysis. All analyses were performed by investigators blinded to the group assignments.

2.3 | Diets

Four isoenergetic, isoproteic, and isolipidic high-fat diets differing in their PUFA profile were formulated. Each diet included a specific oil added to a basal matrix free of fish meal but including casein, gelatin, as well as egg yolk powder to increase palatability. All the experimental diets contained a minimum of 13.1 and 28.0 g/kg of LO and SO, respectively, to meet the minimal n-3 and n-6 PUFA requirements for the fish. The four specific oils (i) SO, (ii) LO, (iii) FO, and (iv) PSO were incorporated at levels of 213.8 g/kg into the diets. The gross composition and fatty acid profile of each diet are detailed in Tables 1 and 2, respectively.

These inclusion levels were set at the maximum lipid incorporation compatible with our purified diet formulation, in order to promote the deposition of dietary fatty acids in whole-body lipids while avoiding excessive β-oxidation. To further promote storage of the targeted dietary fatty acids, digestible carbohydrate content was reduced to limit de novo lipogenesis, while keeping all diets isoenergetic (23 MJ/kg DM), isoproteic (37.6% DM), and isolipidic (34.5% DM). This formulation strategy was based on established zebrafish nutritional requirements (Fowler, Dennis-Cornelius, et al. 2020; Watts and D'Abramo 2021) and, when specific data were unavailable, on NRC (2011) values for carp (*Cyprinus carpio*) for amino acids and minerals, and rainbow trout (*Oncorhynchus mykiss*) for vitamins.

TABLE 1 | Composition (g/kg dry diet) of the four experimental diets (LO, SO, FO, PSO) enriched in different oils (linseed oil, sunflower oil, fish oil, pomegranate seed oil).

Component	LO	SO	FO	PSO
Casein[a]	304.9	304.9	304.9	304.9
Gelatin[a]	58.8	58.8	58.8	58.8
Egg Yolk powder[b]	157.2	157.2	157.2	157.2
L-arginin[a]	7.3	7.3	7.3	7.3
L-threonin[a]	7.3	7.3	7.3	7.3
Dextrin[a]	73.5	73.5	73.5	73.5
Cellulose[a]	51.4	51.4	51.4	51.4
Carboxymethylcellulose[a]	22.0	22.0	22.0	22.0
Agar[a]	7.3	7.3	7.3	7.3
Hydrosoluble vitamin premix[f]	7.1	7.1	7.1	7.1
Liposoluble vitamin premix[g]	0.3	0.3	0.3	0.3
Mineral premix[h]	47.8	47.8	47.8	47.8
Linseed oil[c]	227.0	13.1	13.1	13.1
Sunflower oil[d]	28.0	241.8	28.0	28.0
Fish oil[a]	0.0	0.0	213.8	0.0
Pomegranate seed oil[e]	0.0	0.0	0.0	213.8

[a]Sigma-Aldrich, Saint Louis, Missouri, USA.
[b]Marbaix farm, Chaumont-Gistoux, Belgium.
[c]Bio Planète, Bram, France.
[d]Biotime; Hal, Belgium.
[e]Ol'vita; Mysłaków, Poland.
[f]Hydrosoluble vitamin premix (g/kg premix): ascorbic acid (Vit C) 56.0, thiamin (Vit B1) 6.27, riboflavin (Vit B2) 13.44, pyridoxine (Vit B6) 5.04, calcium pantothenate (Vit B5) 15.79, P-aminobenzoic acid (PABA; Vit H1) 44.79, cyanocobalamin (Vit B12) 0.03, niacin (Vit B3) 33.59, biotin (Vit B8) 0.11, choline chloride 391.94, folic acid (Vit B9) 1.68, inositol 55.99, canthaxanthin (E161g) 11.20, alpha-cellulose 364.01.
[g]Liposoluble vitamin premix (g/kg premix): retinyl acetate (Vit A) 0.13, cholecalciferol (Vit D3) 0.16, menadione (Vit K3) 2.2, butylated hydroxyanisole 1.50, butylated hydroxytoluene 1.50, tocopherol acetate (Vit E) 34.20.
[h]Mineral premix (g/kg premix): $CaHPO_4$ 295.50, $Ca(H_2PO_4).H_2O$ 217.00, $NaHCO_3$ 89.50, $Na_2SeO_3.5H_2O$ 0.01, KCl 100.00, NaCl 172.39, KI 0.20, $MgCl_2$ 63.70, $MgSO_4.7H_2O$ 34.30, $MnSO_4.H_2O$ 2.00, $FeSO_4.7H_2O$ 15.00, $CuSO_4.5H_2O$ 0.40, $ZnSO_4.7H_2O$ 10.00.

2.4 | Fatty Acids Analysis

Total lipids were extracted with chloroform/methanol/water (2:2:1.8, by vol) from 20 mg freeze-dried whole fish homogenates and diets according to the Bligh and Dyer method (Bligh and

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TABLE 2 | Fatty acid composition (μmol/g of feed) of the experimental diets containing different oils (sunflower oil, linseed oil, fish oil, pomegranate seed oil) rich in specific PUFA (i.e., ALA, LA, EPA-DHA, or PunA—in bold).

	Dietary treatments			
	SO	LO	FO	PSO
Fatty acid	μmol/g of feed			
12:0	0.2	0.2	1.4	0.2
14:0	1.6	1.3	68.8	1.3
16:0	125.7	110.5	195.4	109.2
18:0	46.8	41.9	40.8	38.5
20:0	1.7	1.0	1.8	2.7
22:0	4.4	1.0	2.8	1.0
24:0	0.0	0.0	1.4	0.0
Total SFA	180.4	155.9	312.4	152.9
14:1 (9)	0.0	0.0	0.0	0.0
16:1 (9)	4.5	3.9	91.8	4.7
18:1 (9) (OA)	333.1	279.9	214.6	250.3
18:1 (11)	9.7	9.1	24.7	8.7
20:1 (11)	1.8	1.6	5.0	4.9
22:1 (13)	0.0	0.0	1.0	0.0
24:1 (15)	0.0	0.0	1.7	0.0
Total MUFA	349.1	294.5	338.8	268.6
LA 18:2 (9,12)	**586.4**	211.7	114.2	146.5
18:3 (6,9,12)	0.2	0.2	2.3	0.2
20:3 (8,11,14)	0.3	0.3	1.6	0.2
20:4 (5,8,11,14)	5.3	4.6	11.8	6.9
22:4 (7,10,13,16)	0.5	0.4	2.1	0.6
22:5 (4,7,10,13,16)	0.9	0.8	5.2	1.2
Total n-6 PUFA	593.6	218.0	137.2	155.6
ALA 18:3 (9,12,15)	26.3	**427.9**	39.1	27.4
18:4 (6,9,12,15)	0.0	0.0	19.8	0.0
20:3 (11,14,17)	0.0	0.0	1.1	0.0
20:4 (8,11,14,17)	0.0	0.0	7.2	0.0
EPA 20:5 (5,8,11,14,17)	0.0	0.0	**88.7**	0.0
22:5 (7,10,13,16,19)	0.3	0.3	14.9	0.4
DHA 22:6 (4,7,10,13,16,19)	2.1	1.8	**85.6**	2.6
24:5 (9,12,15,18,21)	0.0	0.0	0.4	0.0
24:6 (6,9,12,15,18,21)	0.0	0.0	0.5	0.0
Total n-3 PUFA	28.7	430.0	257.3	30.4
Total PUFA	622.3	648.0	394.5	186.0
n-3/n-6 ratio	0.0	2.0	1.9	0.2
PunA 18:3 (cis-9, trans-11, cis-13)	0.0	0.0	0.0	**563.3**
Other CLnA isomers	0.0	0.0	0.0	88.5
Total CLnA	0.0	0.0	0.0	651.8

(Continues)

TABLE 2 | (Continued)

Dyer 1959). Internal standards were used to monitor potential losses during the process. For fish samples, internal standards included (i) triheptadecanoin (17:0, triacylglycerol) (Larodan, Solna, Sweden), (ii) nonadecanoic acid (19:0, free fatty acid) (Larodan), and (iii) 1,2-dibehenoyl-sn-glycero-3-phosphocho line (22:0, phospholipid) (Avanti Polar Lipids, Birmingham, Alabama, USA). For diet samples, the internal standard used was nonadecanoic acid (Larodan). Total lipid extracts of fish homogenates were separated into three lipid fractions: neutral lipids, phospholipids, and free fatty acids, by solid phase extraction as described by Schneider et al. 2013. Total lipid extracts of diets and each lipid fraction of fish homogenates were dried under nitrogen and methylated as described by Ferain et al. (2016). Resulting fatty acid methyl esters (FAME) were extracted using 2 and 1 mL hexane for diets and fish homogenates, respectively (adapted from Schneider et al. 2013). An injection standard, i.e., methyl-undecanoate (Larodan), was added to all samples. FAMEs were separated by gas chromatography (GC). The chromatograph (GC Trace-1310, ThermoQuest, Milano, Italy) was equipped with a BPX90 capillary column (100 m × 0.25 mm internal diameter, 0.2 μm inner film thickness; SGE, Melbourne, Australia), a TriPlus TP 100 autosampler (Thermo Fischer Scientific, Waltham, Massachusetts, USA), and a flame ionization detector (FID; ThermoQuest) flowed by H_2 (35 mL/min), air (350 mL/min), and N_2 (40 mL/min) at constant temperature (255°C). Samples were injected with H_2 as carrier gas at a constant flow rate (2 mL/min) and pressure (200 kPa). The temperature program started with an increase from 80°C to 175°C at a rate of 25°C/min. After 25 min at 175°C, the temperature increased to 200°C at a rate of 10°C/min. After 20 min at 200°C, the temperature increased to 220°C at 10°C/min. After 5 min at 220°C, the temperature increased to 235°C at 10°C/min. After 15 min at 235°C, the temperature decreased to 80°C at 20°C/min. Resulting chromatograms were analyzed with the ChromQuest software (version 5.0, ThermoFischer Scientific). FAMEs were identified and quantified by comparison of retention time and peak area with an external standard made from known concentrations of 42 FAMEs (Larodan). Additional standards containing either PunA, α-eleostearic acid (α-ESA; 18:3 (cis-9, trans-11, trans-13)), β-eleostearic acid (β-ESA; 18:3 (trans-9, trans-11, trans-13)), and catalpic acid (CA; 18:3 (trans-9, trans-11, trans-13)) methyl esters (Larodan) at known concentrations were used to identify and quantify those fatty acids.

Some lipid extracts were also analyzed by GC–MS (GCMS-QP2020 NX, Shimadzu, Kyoto, Japan) to confirm the identification of the

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TABLE 3 | Body mass, length, and BMI of adult zebrafish after 28 and 56 days of feeding enriched with specific lipid sources (sunflower oil, linseed oil, fish oil, pomegranate seed oil).

	SO		LO		FO		PSO	
Parameters	**Day 28**	**Day 56**	**Day 28**	**Day 56**	**Day 28**	**Day 56**	**Day 28**	**Day 56**
Length (cm)	3.45 ± 0.12	3.50 ± 0.09	3.45 ± 0.05	3.45 ± 0.12	3.53 ± 0.13	3.50 ± 0.07	3.38 ± 0.05	3.63 ± 0.11
Mass (mg)	426 ± 49	468 ± 24	395 ± 17	442 ± 55	442 ± 24	451 ± 35	384 ± 17	499 ± 68
BMI (mg/cm^2)	36 ± 2	38 ± 1	33 ± 1	37 ± 2	36 ± 1	37 ± 2	34 ± 1	37 ± 3

Note: Data are expressed as means ± standard error of mean ($n = 4$).

CLA and CLnA detected. The column and oven temperature program were identical to those used in the GC-FID analysis. Samples were injected with He as the carrier gas at a constant flow rate (3 mL/min). The MS transfer line temperature was 250°C. The parameters used for mass spectrometry analyses were: ionization energy at 70 eV; ion source temperature of 200°C; dwell time of 0.1 s. The system was operated, and the data were evaluated using total ion count for compound identification and processed using the GCMSsolution software (version 4.52, Shimadzu).

2.5 | Statistical Analyses

Statistical analyses were carried out using JMP Pro 16, and graphs were designed using GraphPad Prism version 9.1.2 (GraphPad Software, Boston, Massachusetts, USA). Data normality and variance homogeneity were controlled using the Shapiro–Wilk test, Bartlett's test, or Brown-Forsythe test before performing parametric tests. Data log transformation was used when normality was not confirmed. The experimental diets were compared with each other by one-way ANOVA followed by a Tukey's test or, if non-parametric tests were required, the Kruskal–Wallis test followed by a Dunn's test. Differences from the initiation of the experiment were evaluated by one-way ANOVA followed by Dunnett's test or, if non-parametric tests were required, the Kruskal–Wallis tests followed by a Dunn's test. Type I error (α) was set at 0.05. The results were expressed as mean ± standard error of the mean.

3 | Results

3.1 | Fatty Acid Profiles of Experimental Diets

The experimental diets consisted of a common basal matrix containing casein, gelatin, and egg yolk, with the addition of specific oils. The inclusion of these oils influenced the fatty acid composition of the diets (Table 2).

In the SO diet, LA was the predominant fatty acid, representing 50.9 mol% of the total detected fatty acids. LA was also present in the other diets, ranging from 10.7 mol% to 19.4 mol%. In the LO diet, ALA was the primary fatty acid (38.9 mol%), whereas only trace amounts were detected in the other diets. The PSO diet was characterized by a high content of PunA (41.1 mol%) and up to 13% of other CLnA isomers. It also contained low levels of n-3 PUFA, comparable to those in the SO diet, and reduced levels of n-6 PUFA, similar to the FO diet. In the FO diet, the combined levels of EPA and DHA accounted for 17% of the total detected fatty acids. This diet also had the highest SFA content (30%), mostly PA.

All four diets contained a high proportion of MUFA, OA being the most abundant. Palmitoleic acid (16:1 (9)) was present in relatively high amounts in the FO diet. Although γ-linolenic acid (GLA; 18:3 (6,9,12)), dihomo-γ-linolenic acid (DGLA; 20:3 (8,11,14)), arachidonic acid (ARA; 20:4 (5,8,11,14)), adrenic acid (22:4 (7,10,13,16)), n-6 docosapentaenoic acid (DPAn-6; 22:5 (4,7,10,13,16)), n-3 docosapentaenoic acid (DPAn-3; 22:5 (7,10,13,16,19)), and DHA were absent in plant oils, they were detected in low amounts in the SO, LO, and PSO diets due to the egg yolk powder used in their formulation. The levels of these PUFA were highest in the FO diet, as they were naturally present in the corresponding oil.

3.2 | Zebrafish Growth Parameters

The type of oil in the diets did not significantly affect the size, weight, or BMI of adult zebrafish (Table 3).

3.3 | Zebrafish Fatty Acid Profiles

Neutral lipids constituted 70.1%–77.2% of total zebrafish body lipids, while phospholipids and free fatty acids accounted for 8.4%–13.8% and 10.1%–17.9%, respectively. The total fatty acid concentration in these fractions (phospholipids, free fatty acids, and neutral lipids) remained unchanged across diets and from initial levels (Figure 2e). However, differences emerged when analyzing specific fatty acid classes within each fraction (Figure 2a–d).

3.3.1 | Tissue Incorporation of Saturated Fatty Acids (SFA) and Monounsaturated Fatty Acids (MUFA)

Diet composition did not affect the total SFA and MUFA content in phospholipids or neutral lipids in zebrafish. However, compared to the initial levels, total SFA levels in neutral lipids decreased across all diets. In the free fatty acid fraction, SFA and MUFA levels were higher in fish fed the FO diet than in those fed the PSO diet (Figure 2a,b).

The major SFA in zebrafish tissues were PA (75.9–78.2 mol% of total SFA all fractions combined), STA (10.6–15.8 mol%), and

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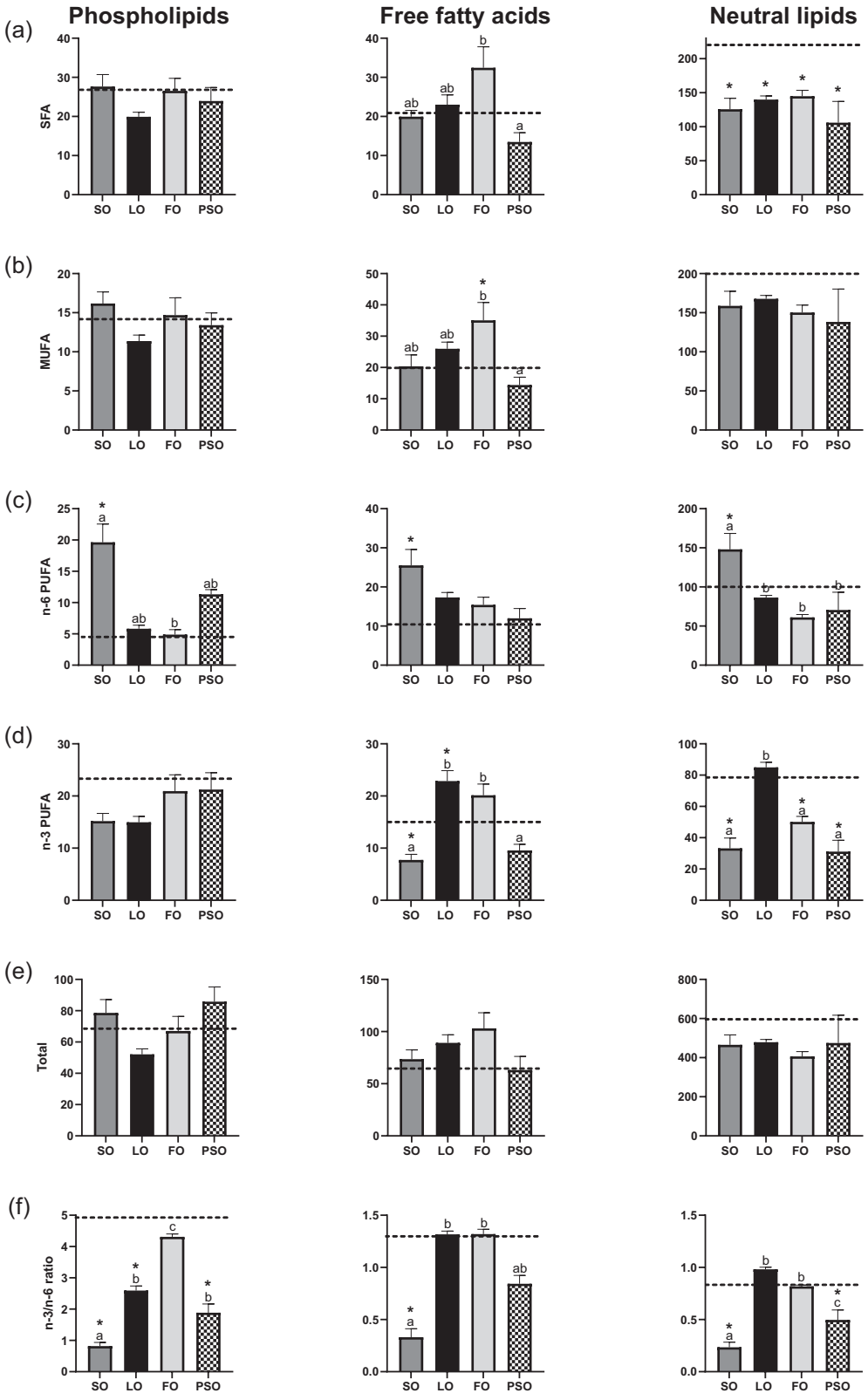


FIGURE 2 | Fatty acid concentrations in lipid classes, i.e., SFA (a), MUFA (b), n-6 PUFA (c), n-3 PUFA (d) (μmol/g freeze dried zebrafish), total fatty acid concentrations (μmol/g freeze dried zebrafish) (e) and n-3/n-6 ratios (f) in zebrafish body phospholipids, free fatty acids, and neutral lipids following 8 weeks of feeding with diets enriched in specific lipid sources (sunflower oil, linseed oil, fish oil, pomegranate seed oil). Data are expressed as mean ± standard error of mean ($n = 4$). The dashed line refers to the level of the different fatty acid classes at the start of the experiment (day 0). Different letters indicate significant differences between diets. Stars indicate significant differences from initial levels ($p < 0.05$).

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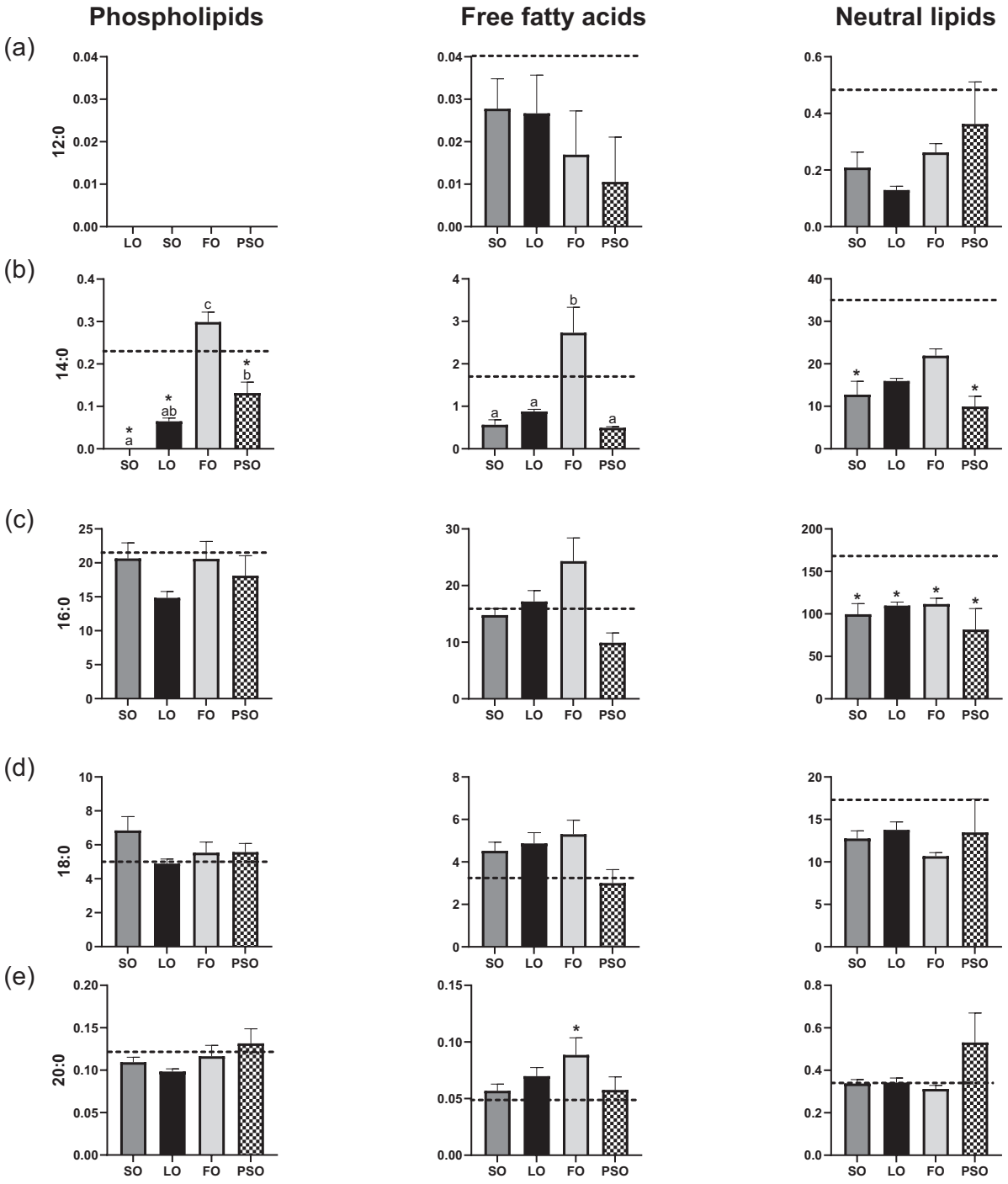


FIGURE 3 | Concentrations of individual SFA, i.e., (a) 12:0, (b) 14:0, (c) 16:0, (d) 18:0, (e) 20:0, (μmol/g freeze dried zebrafish) in zebrafish body phospholipids, neutral lipids, and free fatty acids after 8 weeks of feeding with diets enriched in specific lipid sources (sunflower oil, linseed oil, fish oil, pomegranate seed oil). Refer to the legend of Figure 2 for more details.

myristic acid (14:0; 7.3–11.5 mol%) (Figure 3a–e). Fish fed the FO diet had higher myristic acid concentrations in their phospholipid and free fatty acid fractions compared to other diets. Myristic acid levels in phospholipid and neutral lipid fractions significantly decreased in SO- and PSO-fed fish compared to initial levels. In contrast, this reduction was restricted to phospholipids in LO-fed fish (Figure 3b). Palmitic acid levels significantly declined in neutral lipids across all groups compared to initial levels. However, no diet-induced differences were observed within lipid fractions (Figure 3c). In contrast, STA and arachidic acid (20:0) levels remained stable across dietary treatments and when compared to initial levels. An exception was observed in FO-fed fish, with an increase in free fatty acid-associated arachidic acid (Figure 3e).

Regarding MUFA, OA was the most abundant, followed by palmitoleic acid and cis-vaccenic acid (18:1 (11)), which together accounted for 66.8–78.6 mol%, 7.3–16.8 mol%, and 8.2–11.0 mol% of total MUFA (all fractions combined), respectively. Myristoleic acid (14:1 (9)) and palmitoleic acid concentrations were higher

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in the free fatty acid fraction of FO-fed fish compared to other diets (Figure 4a,b). Palmitoleic acid was also more abundant in the phospholipid and neutral lipid fractions of FO-fed fish (Figure 4b). OA levels were higher in the free fatty acid fraction of FO-fed fish compared to PSO-fed fish (Figure 4c). Other MUFA did not significantly differ between diets. However, several differences were observed when comparing MUFA levels to those at the start of the experiment. Myristoleic acid, cis-vaccenic acid, and nervonic acid (24:1 (15)) concentrations decreased in the neutral lipid fraction across all zebrafish groups (Figure 4a,d,g). Similarly, palmitoleic acid levels were reduced in the neutral lipid fraction of all groups, except those fed the FO diet (Figure 4b). Erucic acid (22:1 (13)) was only detected in the neutral lipid fraction, where its levels decreased in all groups, except those fed the LO diet (Figure 4f). Additionally, concentrations of cis-vaccenic acid in the phospholipid fraction and gadoleic acid (20:1 (11)) in the free fatty acid fraction were significantly lower in SO-, LO-, and PSO-fed fish compared to initial levels (Figure 4d,e). In contrast, nervonic acid levels increased in the phospholipid fraction of all experimental groups, while OA increased in the free fatty acid fraction of LO- and FO-fed zebrafish (Figure 4c,g).

3.3.2 | Tissue Incorporation and Bioconversion of n-6 PUFA

Fish fed the SO diet exhibited higher n-6 PUFA concentrations in neutral lipids compared to those on other diets (Figure 2c). A similar pattern was observed in phospholipids, though the difference was only significant between SO- and FO-fed fish (Figure 2c).

LA was the primary n-6 PUFA in all lipid fractions of SO-fed zebrafish (81.0–89.7 mol% of total n-6 PUFA all fractions combined; Figure 5a). These fish also contained higher levels of LA bioconversion products, such as GLA, DGLA, ARA, and adrenic acid, than those fed the other diets (Figure 5b,d–f). Compared to initial levels, LA and its bioconversion products increased significantly in SO-fed fish. The ARA-to-LA ratio of SO-fed fish was highest in phospholipids, followed by free fatty acids and neutral lipids. Comparable patterns were observed for the other LA bioconversion products (Table 4). These ratios serve as indirect indices of fatty acid bioconversion, as direct measurements of enzymatic activity were not performed. PSO-fed fish had increased DGLA, ARA, and DPAn-6 concentrations in phospholipids compared to initial levels (Figure 5d,e,g). They had higher ARA and DPAn-6 levels than LO-fed fish and higher LA and DGLA levels in phospholipids than FO-fed fish (Figure 5a,b,d,e,g). LO- and FO-fed fish did not accumulate LA or its bioconversion products during the experiment, except for DPAn-6, which was detected in all three fractions of FO-fed fish (Figure 5g).

3.3.3 | Tissue Incorporation and Bioconversion of n-3 PUFA

Total n-3 PUFA concentrations were highest in the neutral lipids of LO-fed fish and free fatty acids of LO- and FO-fed fish, while phospholipids' n-3 PUFA levels remained unchanged across diets. Compared to initial levels, n-3 PUFA concentrations were significantly lower in the neutral lipids of SO-, PSO-, and FO-fed fish, whereas LO-fed fish maintained their initial levels (Figure 2d).

Regarding individual fatty acid, zebrafish fed the LO diet accumulated more ALA (16.4–53.7 mol% of total n-3 PUFA all fractions combined), along with its first bioconversion products—stearidonic acid (SDA; 18:4 (6,9,12,15)), 20:3 (11,14,17), and 20:4 (8,11,14,17)—compared to fish fed the other diets (Figure 6b–d). The ratios of bioconversion products (including EPA and DHA) to ALA were consistently highest in phospholipids compared to free fatty acids and neutral lipids, except for SDA (Table 5). Stearidonic acid levels in LO-fed fish were comparable to those in PSO-fed fish across all lipid fractions, while 20:4 (8,11,14,17) levels in FO-fed fish closely matched those observed in LO-fed fish (Figure 6b,d). EPA concentrations were highest in the phospholipids and free fatty acids of FO-fed fish, with significant differences compared to all other diets. In neutral lipids, EPA levels were significantly elevated in FO-fed fish relative to PSO- and SO-fed fish (Figure 6e). Similarly, DPAn-3 was more abundant in the free fatty acid and neutral lipid fractions of FO-fed fish compared to PSO- and SO-fed fish. However, in the phospholipid fraction, DPAn-3 levels were only significantly higher than in SO-fed fish (Figure 6f). EPA levels declined from initial values in all lipid fractions of fish fed vegetable oils, whereas FO-fed fish maintained their initial EPA levels in phospholipids and free fatty acids but not in neutral lipids (Figure 6e). DHA remained the predominant n-3 PUFA in phospholipids, with its initial levels largely maintained across all diets throughout the experiment. In phospholipids, PSO-fed fish exhibited higher DHA concentrations compared to LO-fed fish, whereas in neutral lipids, their DHA concentrations were lower than those of FO-fed fish. Overall, DHA levels in neutral lipids declined in all zebrafish, with a significant reduction observed in PSO- and SO-fed fish (Figure 7i). Trace amounts of 24:5 (9,12,15,18,21) and 24:6 (6,9,12,15,18,21) were detected in the neutral lipid fraction and across all lipid fractions, respectively (Figure 7g,h).

3.3.4 | n-3/n-6 Ratios

The n-3/n-6 ratio was highest in phospholipids, followed by neutral lipids and free fatty acids. FO- and LO-fed fish exhibited higher ratios than SO-fed fish across all fractions. In phospholipids, FO-fed fish had a higher n-3/n-6 ratio than LO-fed fish. Only FO-fed fish maintained their pre-experimental ratios in all fractions. In both phospholipids and neutral lipids, PSO-fed fish exhibited higher n-3/n-6 ratios compared to SO-fed fish, but lower ratios than those observed in FO-fed fish (Figure 2f).

3.3.5 | Tissue Incorporation and Bioconversion of CLnA

Only zebrafish fed the PSO diet incorporated CLnA and CLA into their body lipids, whereas these CFA were absent in fish fed the other diets. PunA and its isomers (α-ESA, CA, and β-ESA) were detected across all three lipid fractions at levels below 10% of total CFA. Despite being absent from the PSO diet, CLA isomers, including RmA and 18:2 (trans-9,

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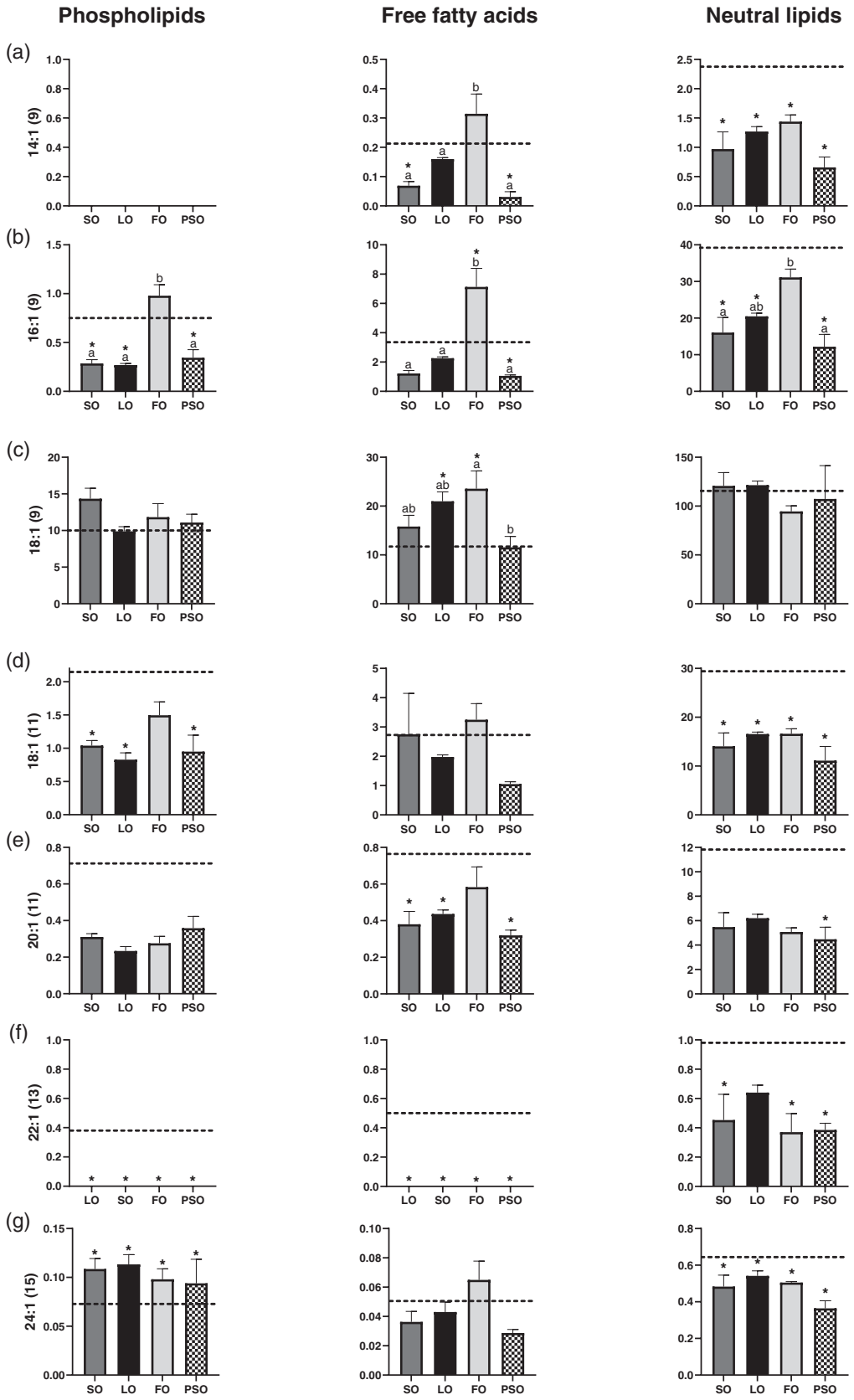


FIGURE 4 | Concentrations of individual MUFA, i.e., (a) 14:1 (9), (b) 16:1 (9), (c) 18:1 (9), (d) 18:1 (11), (e) 20:1 (11), (f) 22:1 (13), (g) 24:1 (15), (μmol/g freeze dried zebrafish) in zebrafish whole body phospholipids, neutral lipids and free fatty acids following 8 weeks of diets enriched in specific lipid sources (sunflower oil, linseed oil, fish oil, pomegranate seed oil). Refer to the legend of Figure 2 for more details.

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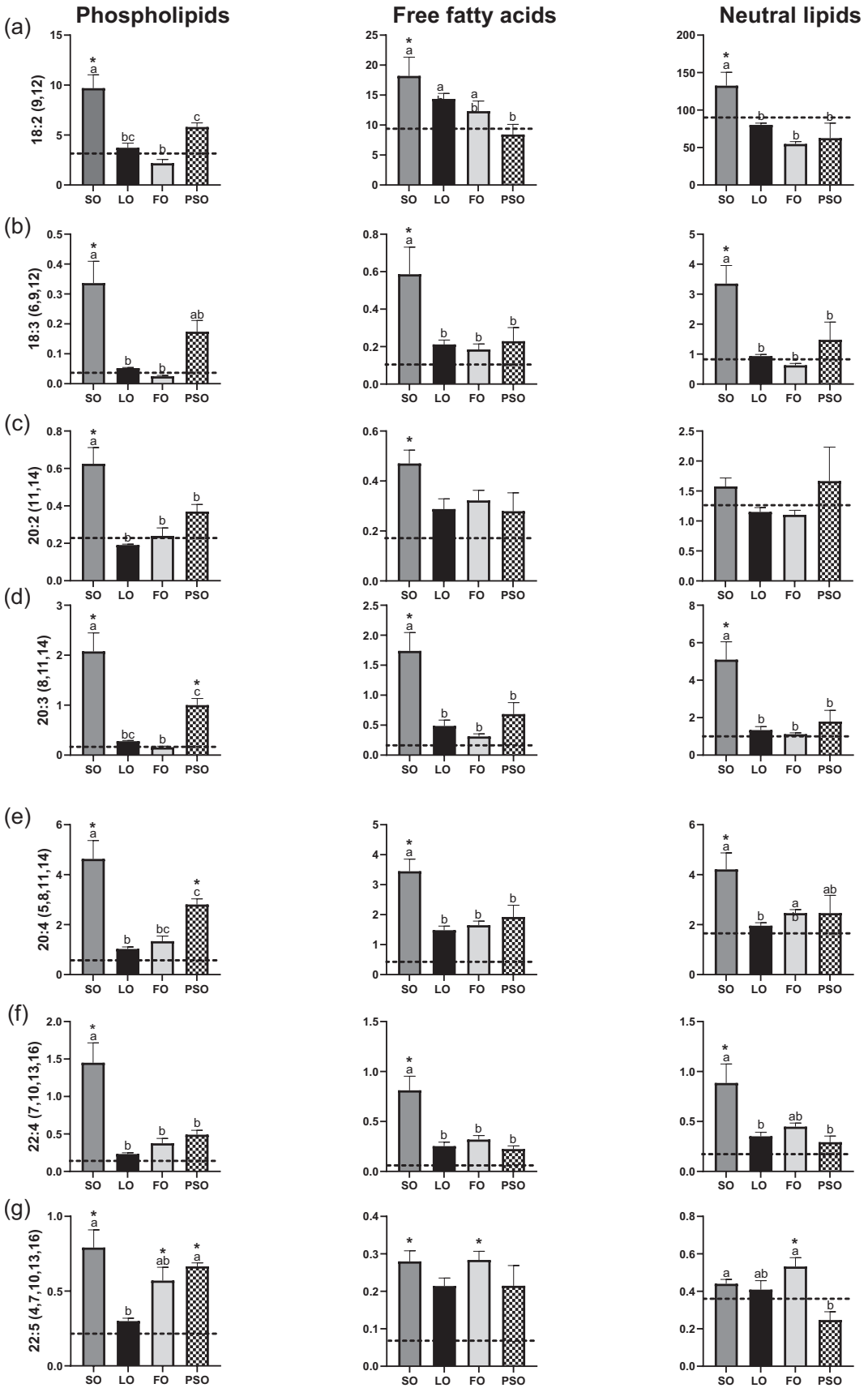


FIGURE 5 | Concentrations of individual n-6 PUFA, i.e., (a) 18:2 (9,12), (b) 18:3 (6,9,12), (c) 20:2 (11,14), (d) 20:3 (8,11,14), (e) 20:4 (5,8,11,14), (f) 22:4 (7,10,13,16), (g) 22:5 (4,7,10,13,16), (μmol/g freeze dried zebrafish) in zebrafish body phospholipids, neutral lipids and free fatty acid following 8 weeks of diets enriched in specific lipid sources (sunflower oil, linseed oil, fish oil, pomegranate seed oil). Refer to the legend of Figure 2 for more details.

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TABLE 4 | Ratios of n-6 bioconversion products to LA (18:2 (9,12)) in zebrafish body phospholipids, neutral lipids and free fatty acid following 8 weeks of diets enriched in sunflower oil.

	SO		
Ratios	**Phospholipids**	**Free fatty acids**	**Neutral lipids**
GLA 18:2 (9,12)/LA	0.033 ± 0.006^{a}	0.031 ± 0.007^{a}	0.025 ± 0.003^{a}
20:2 (11,14)/LA	0.064 ± 0.003^{a}	0.027 ± 0.004^{b}	0.012 ± 0.001^{c}
DGLA 20:3 (8,11,14)/LA	0.210 ± 0.031^{a}	0.097 ± 0.020^{b}	0.038 ± 0.008^{c}
ARA 20:4 (5,8,11,14)/LA	0.473 ± 0.062^{a}	0.196 ± 0.031^{ab}	0.032 ± 0.005^{b}
22:4 (7,10,13,16)/LA	0.147 ± 0.034^{a}	0.046 ± 0.011^{ab}	0.007 ± 0.002^{b}
DPAn-6 22:5 (4,7,10,13,16)/LA	0.081 ± 0.010^{a}	0.016 ± 0.003^{ab}	0.003 ± 0.001^{b}

Note: Data are expressed as mean ± standard error of mean ($n = 4$). Different letters indicate significant differences between lipid fractions ($p < 0.05$).

TABLE 5 | Ratios of n-3 bioconversion products to ALA (18:3 (9,12,15)) in zebrafish body phospholipids, neutral lipids and free fatty acid following 8 weeks of diets enriched in linseed oil.

	LO		
Ratios	**Phospholipids**	**Free fatty acids**	**Neutral lipids**
SDA 18:4 (6,9,12,15)/ALA	0.048 ± 0.009^{a}	0.072 ± 0.006^{b}	0.063 ± 0.008^{ab}
20:3 (11,14,17)/ALA	0.136 ± 0.028^{a}	0.039 ± 0.007^{ab}	0.025 ± 0.002^{b}
20:4 (8,11,14,17)/ALA	0.145 ± 0.034^{a}	0.052 ± 0.009^{ab}	0.041 ± 0.004^{b}
EPA 20:5 (5,8,11,14,17)/ALA	0.833 ± 0.123^{a}	0.302 ± 0.053^{b}	0.176 ± 0.044^{b}
DPAn-3 22:5 (7,10,13,16,19)/ALA	0.374 ± 0.083^{a}	0.113 ± 0.019^{ab}	0.065 ± 0.007^{b}
24:5 (9,12,15,18,21)/ALA	0.018 ± 0.005^{a}	0.008 ± 0.002^{b}	0.008 ± 0.001^{b}
24:6 (6,9,12,15,18,21)/ALA	0.039 ± 0.014^{a}	0.008 ± 0.002^{ab}	0.005 ± 0.001^{b}
DHA 22:6 (4,7,10,13,16,19)/ALA	4.483 ± 0.934^{a}	0.597 ± 0.086^{ab}	0.211 ± 0.018^{b}

Note: Data are expressed as mean ± standard error of mean ($n = 4$). Different letters indicate significant differences between lipid fractions ($p < 0.05$).

trans-11), were present in significantly higher amounts than CLnA (Table 2). RmA and 18:2 (trans-9, trans-11) accounted for 52.3, 52.2, and 46.5 mol% of total CFA in phospholipids, free fatty acids, and neutral lipids, respectively, while 18:2 (trans-9, trans-11) contributed 40.1, 31.4, and 23.9 mol% to these fractions (Figure 8).

4 | Discussion

This study investigated how specific PUFA-enriched diets influence the fatty acid composition of adult zebrafish tissues. Six-month-old zebrafish were fed four different diets, each comprising 34.5% fat and providing 25 cal daily, a moderate caloric intake that aligns with their energy requirements (Pannevis and Earle 1994). While the precise lipid requirements of zebrafish are not fully established, estimates for tropical omnivorous freshwater fish suggest a lipid intake ranging from 10% to 18% (Fowler, Dennis-Cornelius, et al. 2020). Despite the high fat content of the diets, body mass or lipid mass remained unchanged over the 56-day period. This study is the first to examine the effects of various PUFA diets, including a CLnA-rich diet, on the incorporation of specific fatty acids into zebrafish tissues. A clear relationship emerged between dietary and tissue fatty acid composition, particularly for PUFA of interest, reflecting their efficient accumulation across lipid classes (phospholipids, neutral lipids, and free fatty acids).

LA and ALA were the predominant fatty acids in fish fed SO and LO diets, respectively. Zebrafish possess the ability to elongate and desaturate C18 precursors to produce n-6 and n-3 LC-PUFA (Agaba et al. 2004; Monroig et al. 2018). The increase in bioconversion products observed in the present study further supports this capability, especially in SO-fed fish. However, bioconversion efficiency varied across lipid fractions, with ARA-to-LA, EPA-to-ALA, and DHA-to-ALA ratios up to 20 times higher in phospholipids compared to neutral lipids, which account for over 70% of total lipids. Consequently, overall tissue levels of bioconversion remained limited. This observation aligns with prior research on female zebrafish, where fish fed corn oil (rich in LA) exhibited elevated LA concentrations, while ARA levels remained unchanged across diets (containing either corn oil, olive oil, LO, or FO), further highlighting the limited bioconversion capacity (Araujo et al. 2017).

Interestingly, zebrafish fed the LO diet accumulated more 20:3 (11,14,17), suggesting ALA biotransformation into EPA through the delta-8 (Δ8) pathway, as observed in other aquatic

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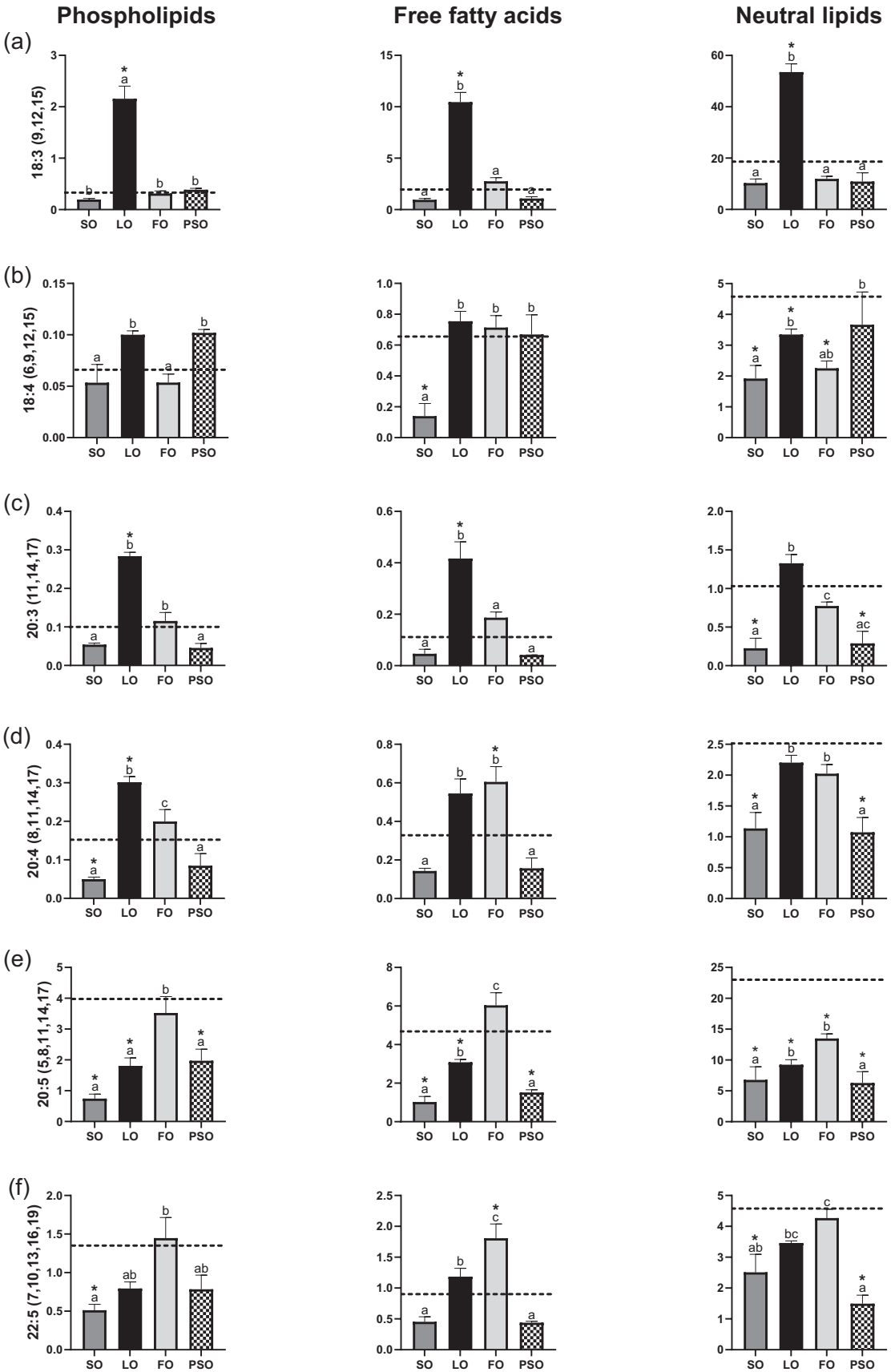


FIGURE 6 | Concentrations of individual n-3 PUFA, i.e., (a) 18:3 (9,12,15), (b) 18:4 (6,9,12,15), (c) 20:3 (11,14,17), (d) 20:4 (8,11,14,17), (e) 20:5 (5,8,11,14,17), (f) 22:5 (7,10,13,16,19), (μmol/g freeze-dried zebrafish) in zebrafish body phospholipids, neutral lipids and free fatty acids following 8 weeks of diets enriched in specific lipid sources (sunflower oil, linseed oil, fish oil, pomegranate seed oil). For more details, refer to the legend of Figure 2.

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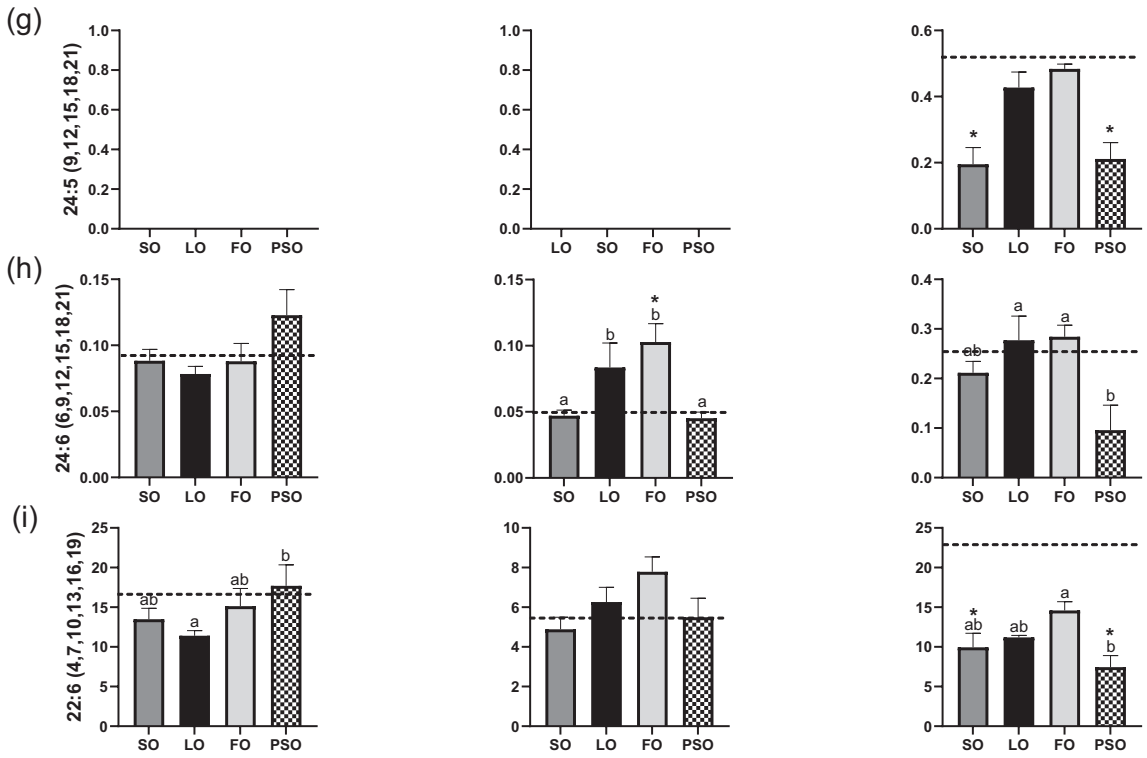


FIGURE 7 | Concentrations of individual n-3 PUFA, i.e., (g) 24:5 (9,12,15,18,21), (h) 24:6 (6,9,12,15,18,21), (i) 22:6 (4,7,10,13,16,19), (μmol/g freeze dried zebrafish) in zebrafish body phospholipids, neutral lipids, and free fatty acids following 8 weeks of diets enriched in specific lipid sources (sunflower oil, linseed oil, fish oil, pomegranate seed oil). For more details, refer to the legend of Figure 2.

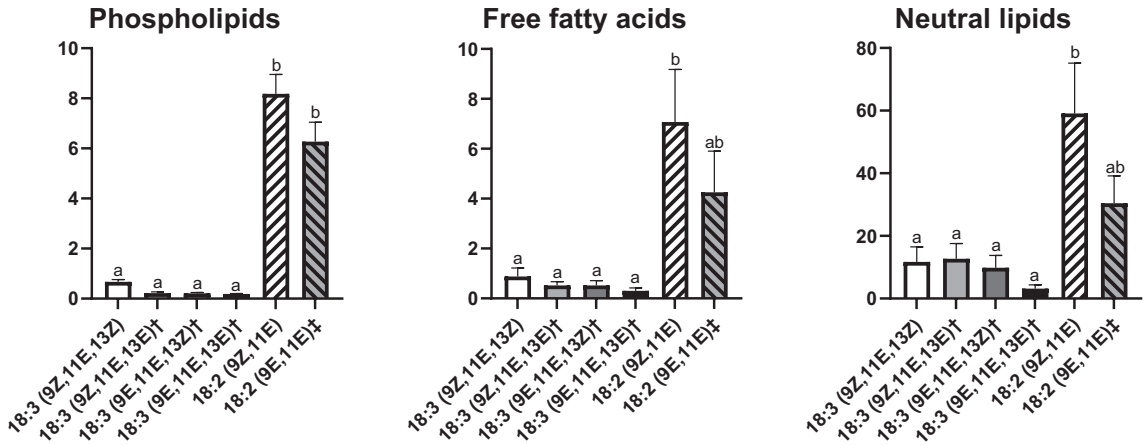


FIGURE 8 | Concentrations of individual conjugated fatty acids, i.e., 18:3 (cis-9, trans-11, cis-13) (PunA), 18:3 (cis-9, trans-11, trans-13)† (α-ESA), 18:3 (trans-9, trans-11, cis-13)† (CA), 18:3 (trans-9, trans-11, trans-13)† (β-ESA), 18:2 (cis-9, trans-11) (RmA), 18:2 (trans-9, trans-11)‡ (μmol/g freeze dried zebrafish) in zebrafish whole body phospholipids, neutral lipids, and free fatty acids following 8 weeks of a diet enriched in pomegranate seed oil. Data are expressed as mean ± standard error of the mean ($n = 4$). †Calculated as punicic acid equivalents. ‡Calculated as rumenic acid equivalents. Letters compare the levels of each conjugated fatty acid, with levels not connected with the same letters being significantly different.

species (Monroig et al. 2022). A recent study supports the existence of this alternative Δ8 pathway in zebrafish, identifying the FADS2 (acyl-CoA 6-desaturase; EC 1.14.19.3) enzyme as trifunctional (delta-5 (Δ5), delta-6 (Δ6), and Δ8 desaturase activity) rather than bifunctional (Δ5 and Δ6 desaturase activity) (Bláhová et al. 2022). However, the presence of EPA and DHA in fish tissues prior to the experiment complicates interpretations regarding bioconversion from n-3 PUFA-rich diets. Notably, EPA levels were lower in LO-fed fish compared to FO-fed fish and pre-experimental fish, reflecting limited ALA bioconversion. These results align with previous research examining the influence of dietary oils differing in their n-3/n-6 PUFA profile on zebrafish fatty acid composition (Jaya-Ram et al. 2008; Araujo et al. 2017). Jaya-Ram and colleagues reported that zebrafish fed with LO instead of squid oil (rich in EPA-DHA) had higher levels of SDA and 20:4 (8,11,14,17) but lower levels of EPA, DPAn-3, and DHA (Jaya-Ram et al. 2008). These data confirm the limited capacity of zebrafish to

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elongate C18 PUFA into LC-PUFA such as ARA, EPA, and DHA, especially when compared to more efficient species like rainbow trout (Ferain et al. 2021).

DHA, which is crucial for fish and mammalian metabolism, showed a notable decline in the neutral lipid fraction after 8 weeks of SO and PSO diets, likely due to the absence of DHA in these diets. On the other hand, DHA levels in phospholipid and free fatty acid fractions remained stable and consistent across all diets. This selective retention of DHA in phospholipids emphasizes its critical role in maintaining membrane structure and metabolism (Tocher 2003; Scharnweber et al. 2021). The n-3/n-6 ratio was highest in LO- and FO-fed fish, intermediate in PSO-fed fish, and lowest in SO-fed zebrafish, reflecting dietary ratios. Comparable trends were reported in common carp, where dietary PUFA profiles influenced fatty acid composition similarly (Zakeri et al. 2019).

Our study did not observe significant changes in body weight or lipid content among PSO-fed zebrafish. Similarly, rainbow trout fingerlings receiving a diet supplemented with 2% PunA exhibited final body weights comparable to controls fed with LO (Yasmin et al. 2004). In contrast, other studies have reported increases in body lipid levels following PSO administration in fish. For instance, juvenile common carp fed a PSO-FO (50–50) blend exhibited higher weight gain and growth rate than those fed either FO or FO-SO (50–50) blend (Zakeri et al. 2019). Similarly, weight gain and growth rates were greater for trout consuming 5–10 g PSO/kg feed relative to controls. However, a higher PSO inclusion (20 g/kg of feed) resulted in reduced weight gain and growth rates (Acar et al. 2018). This discrepancy could be explained either by differences in (i) fish age, as we used mature fish rather than juveniles, (ii) fish species, (iii) PSO content in the diet (≈20% vs. 1.5%–2%), or (iv) feeding regimens (restricted vs. ad libitum feeding or feeding until satiation). Mixed results have also been reported regarding the impact of PSO-enriched diets on lipid metabolism in rodents. While some studies identified reductions in fat mass (Koba et al. 2007; Yuan, Sinclair, et al. 2009; Vroegrijk et al. 2011), others found no significant effect (Arao et al. 2004; Yamasaki et al. 2006; de Melo et al. 2016). The somewhat diverging effects of PSO on body mass in fish versus rodents warrant further investigation. Potential explanations include insufficient stimulation of PPARα expression by dietary PunA in fish, resulting in reduced β-oxidation; differential effects on gut microbiota and digestion; stronger activation of PPARγ expression, promoting lipid accumulation; or the absence of cold-activated thermogenic adipocytes in fish (Aruna et al. 2016; Shen and McIntosh 2016; Acar et al. 2018; Zakeri et al. 2019).

PSO-fed zebrafish accumulated PunA and its isomers across all lipid fractions, with PunA being the most abundant CLnA. Similar patterns were reported in common carp and Nile tilapia (*Oreochromis niloticus*) fed PSO, though isomer distribution varied across studies. Common carp exhibited comparable amounts of PunA, α-ESA, and CA isomers, while Nile tilapia contained mostly PunA with small amounts of its isomers (Schneider et al. 2019; Zakeri et al. 2019). These differences in isomer prevalence are likely attributed to variations in PunA purity across PSO oils, which depend on factors such as pomegranate variety, maturity, and geographical origin (Paul and Radhakrishnan 2020). In addition, differences in fish species, dietary composition, and treatment duration may have further contributed to the observed results.

Remarkably, PSO-fed zebrafish exhibited elevated levels of CLA (RmA and 18:2 (trans-9, trans-11)), despite its absence in the diet, suggesting a formation through CLnA bioconversion. Similar findings were reported in Nile tilapia fed tung oil (rich in α-ESA and β-ESA), which accumulated both CLnA and CLA (Bonafé et al. 2017). The metabolization of α-ESA and PunA into RmA has been reported in various species, including mice, rats, laying hens, and humans (Tsuzuki, Tokuyama, Igarashi, Nakagawa, et al. 2004; Tsuzuki et al. 2006; Yuan, Sinclair, et al. 2009; Yuan, Wahlqvist, et al. 2009; Ngo Njembe, Dejonghe, et al. 2021; Ngo Njembe, Dormal, et al. 2021). In addition, rats receiving catalpa seed oil rich in CA accumulated 18:2 (trans-9, trans-11) in liver and colon mucosa (Suzuki et al. 2006). In vitro studies further highlight this bioconversion process, with PunA and α-ESA converted into RmA, as well as CA and β-ESA transformed into 18:2 (trans-9, trans-11), by Caco-2 cells. These observations point toward a preferential reduction of delta-13 (Δ13) double bonds in the trans configuration (Schneider et al. 2013). This process is likely mediated by an NADPH-dependent reductase enzyme (Tsuzuki, Tokuyama, Igarashi, Nakagawa, et al. 2004; Tsuzuki et al. 2006). Recently, CYP4F13 was identified as the primary enzyme mediating the reduction of α-ESA to RmA (Wu and Tsuduki 2020). However, further investigation is needed to explore its role in the conversion of other CLnA, such as PunA.

The conversion of CLnA into CLA may partly explain some of CLnA's reported biological effects, which overlap with CLA's effects on obesity, diabetes, cancer, and immune response (Schneider et al. 2013). However, CLnA has demonstrated stronger anti-tumor activity than CLA in both in vitro and in vivo studies (Tsuzuki, Tokuyama, Igarashi, and Miyazawa 2004), highlighting the potential intrinsic effect of CLnA and/or a synergistic interaction between both types of CFA.

This study presents several limitations that should be acknowledged. First, the exclusive use of female zebrafish limits extrapolation to males, especially given reported sex-specific differences in lipid metabolism and fatty acid responses in this species (Fowler, Williams, et al. 2020). Second, the precise lipid requirements of zebrafish are not fully established. Our formulation strategy was based on established nutritional requirements for zebrafish, when available (Fowler, Dennis-Cornelius, et al. 2020; Watts and D'Abramo 2021). In cases where species-specific data were lacking, we relied on reference values for related species, namely carp for amino acids and minerals, and rainbow trout for vitamins, as outlined by NRC (2011). Finally, bioconversions were inferred from fatty acid ratios rather than directly quantified. While such indices suggest enzymatic activity, they do not provide definitive evidence of conversion efficiency. Additionally, the presence of pre-existing EPA and DHA in tissues complicates the assessment of their synthesis from dietary C18 PUFA. Future studies using enzymatic assays or isotopic tracers would help confirm these metabolic pathways.

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5 | Conclusion

This study established an approach to enrich adult zebrafish tissues with specific PUFA, including CLnA, through dietary modulation. Our results demonstrate that the FA profiles of zebrafish tissues reflect the composition of dietary lipids and reveal metabolic conversions such as the transformation of CLnA into CLA. These findings provide a robust experimental framework for future studies exploring the impacts of specific dietary and metabolically derived PUFA in zebrafish, particularly in the context of health and disease.

Author Contributions

Apolline Goudmaeker: conceptualization, formal analysis, investigation, methodology, visualization, writing – original draft, writing – review and editing. **Anaïs De Simone:** investigation. **Éric Mignolet:** investigation. **Melissa M. Page:** writing – review and editing. **Yvan Larondelle:** conceptualization, resources, supervision, writing – review and editing. **Cathy Debier:** conceptualization, methodology, resources, supervision, writing – original draft, writing – review and editing.

Ethics Statement

The animal study was approved by the local Ethics Committee for Animal Research of UCLouvain, Louvain-la-Neuve, Belgium (Proposal number: 1858703; agreement number: L1220587). The study was conducted in accordance with the local legislation and institutional requirements.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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