



Fish oil replacement with different vegetable oils in *Onychostoma macrolepis*: Effects on fatty acid metabolism based on whole-body fatty acid balance method and genes expression

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Abstract To evaluate the fatty acid (FA) metabolism status and possibility as a DHA source of farmed *Onychostoma macrolepis*, a total of 168 fish (2.03 ± 0.23 g) were fed four diets supplemented with fish oil (FO), linseed oil (LO), soybean oil (SO), and a mixture of LO and SO oil (MO), respectively, for 70 days. Body FA compositions were modified reflecting dietary FAs. Comparing liver and intestine fatty acids with fish fed four diets, the content of ARA in fish fed SO was significantly higher than others ($P < 0.05$), but showed no difference in muscle. The tissue FA profile showed that the FO-fed group successfully deposited DHA, while the LO-fed group converted ALA to DHA effectively, as well as the liver and intestine EPA was notably highest in the FO

group, whereas no difference between the FO and LO group in the muscle. The FA results showed that the DHA contents in the muscle of *Onychostoma macrolepis* are at a medium-high level compared with several other fish species with the highest aquaculture yield. Correspondingly, in the fish fed diet with LO, SO, and MO, the genes of most FA biosynthesis, transportation, and transcriptional regulation factors were increased in the liver and muscle, but no significant difference was observed in the gene expression of *Elovl4b*, *FATP1*, and *FABP10* in the muscle. In addition, the enzyme activity involved in PUFA metabolism was higher in fish fed vegetable oil-based diets, corroborating the results of the gene expression. Increased in vivo elongase and desaturase ($\Delta 5$, $\Delta 6$, and $\Delta 9$) activities were recorded in fish fed fish oil-devoid diets, which resulted in the appearance of products associated with elongase and desaturase activities in fish. Besides, as the specific n-3 PUFA synthesis substrate, the dietary supplementation of ALA not only retains most of the nutrition value but also ensures the muscular texture, such as fiber diameter and density. It is concluded that farmed *O. macrolepis* owns strong n-3 LC-PUFA biosynthetic capacity and high DHA contents so it can be a good DHA source for the population.

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Abbreviations

ALA	α -linolenic acid
ARA	arachidonic acid
BHT	butylated hydroxytoluene
CF	condition factor
DHA	docosahexaenoic acid
EFA	essential fatty acids
<i>Elovl</i>	elongation of very long-chain fatty acid proteins
EPA	eicosapentaenoic acid
<i>Fads</i>	fatty acyl desaturase
FAME	fatty acid methyl esters
FBL	final body length
FBW	final body weight
FCR	feed conversion ratio
FI	feed intake
FLQ	fish lipid quality/flesh lipid quality
HH	hypocholesterolemic/ hypercholesterolemic ratio
HSI	hepatosomatic index
IA	index of atherogenicity
IBL	initial body length
IBW	initial body weight
IT	index of thrombogenicity
IPFI	intra-peritoneal fat body index
LA	linoleic acid
LC-PUFA	long-chain polyunsaturated fatty acid
MUFA	monounsaturated fatty acid
PUFA	polyunsaturated fatty acid
RIL	relative intestine length
RIW	relative intestine weight
SFA	saturated fatty acid
SGR	specific growth rate
SUR	survival rate
VSI	viscerosomatic index
WFAB	whole body fatty acid balance method
WG	weight gain.

Introduction

Omega-3 long chain ($\geq 20C$) polyunsaturated fatty acids (n-3 LC-PUFA), especially DHA and EPA, are essential fatty acids for human beings, which participate in alleviating inflammation, cardiovascular disease, and cancer, and promoting growth and development (Lien et al. 2018; Jalili and Hekmatdoost 2021). Therefore, humans need to consume a certain level of n-3 LC-PUFA, which WHO and FAO recommend the

minimum n-3 LC-PUFA intake of humans is 250 mg/day (Matthew et al. 2013). In life, humans consume 200000 tons of n-3 LC-PUFA annually and 98.5% comes from fish resources (Salem and Eggersdorfer 2015). However, in recent years, natural fishery resources have shown a declining trend, implying that consumers' access to n-3 LC-PUFA in the future will mainly depend on the growing production of cultured fish.

The sources of n-3 LC-PUFA for fish are divided into endogenous synthesis and exogenous intake. It has been widely studied that freshwater and some euryhalin fish can convert C_{18} PUFA into LC-PUFA, so their EFA is 18:2n-6 and 18:3n-3 (Xie et al. 2021; Li et al. 2008). Besides, recent studies have shown that the ability of LC-PUFA de novo synthesis in fish is related to the trophic level itself and may be predicted by, whereby fish occupying a lower trophic level (i.e., the trophic level < 3) can synthesize LC-PUFA from C_{18} PUFA (Jesse and Artur 2020). Otherwise, different dietary oil sources directly affect the fatty acid composition in fish tissues, while whole-body or muscle have high n-3 LC-PUFA content when the fish oil intake in the diet increases (Stefanie et al. 2016; María et al. 2022).

The universal synthesis pathway LC-PUFA in fish is C_{18} PUFA converted to LC-PUFA by a series of biochemistry reactions. Among them, the key desaturation and elongation reactions catalyzed by LC-PUFA biosynthesis mainly include *Fads2*, *Elovl2*, *Elovl4*, *Elovl5*, and *Elovl8*. *Fads2* in fish has $\Delta 6$, $\Delta 4$, $\Delta 5$, $\Delta 8$ Fad multi-function, in which $\Delta 6$ fatty acid desaturase is a very important key enzyme that took part in fish PUFA biosynthesis (Hastings et al. 2001). It is not only involved in LA and α -ALA undergoes dehydrogenation to convert into ARA and EPA, but also participates in dehydrogenation of fatty acids 24:5n-3 to make EPA produce DHA. *Elovl* enables drive carbon chain extension, for example, *Elovl2* is responsible for the elongation of C_{20} , C_{22} PUFA, and a little of C_{18} PUFA (Xu et al. 2020; Kuah et al. 2015; Garrido et al. 2020), while *Elovl5* has a preference for elongating the fatty acid substrates of C_{18} , C_{20} and some euryhaline herbivorous species elongate C_{22} PUFA (Zou et al. 2019; Galindo et al. 2021). Moreover, *Elovl4* generally has two distinct genes in teleosts, *elovl4a* and *elovl4b*, with varying function aims to C_{18} - C_{22} PUFA substrates (Betancor et al. 2019;

Morais et al. 2020). A recently discovered elongation enzyme that may join in LC-PUFA biosynthesis is *elovl8*, *elovl8a*, and *elovl8b* in teleost fish, specifically, which *elovl8a* may be target C18:2n-6 and C18:3n-3 and *elovl8b* may regulate C18:0 elongation (Sun et al. 2021; Wu et al. 2022).

Onychostoma macrolepis is a kind of indigenous economic fish in the Qinba Mountains, China, which inhabits cold water and its trophic level is 2.7 ± 0.3 . Many years ago, due to piscatorial overfishing and the destruction of the ecological environment, the population of wild *O. macrolepis* dramatically decreased and The National Key Protected Wildlife List defines it as a second-class protected animal. Studies have shown that the nutritional value of *O. macrolepis* can reach a muscle fat content of 3.5 to 4.5%, indicating good palatability (Yue Yongsheng et al. 1994). The essential amino acid index (EAAI) in muscles is 65.99%, indicating good amino acid balance and consistent with the human body's needs pattern (Li Zhengwei et al. 2014). In addition, the proportion of fatty acids is reasonable, with an EPA content of 2.89% and a DHA content of 6.20% (Xu Shaogang et al. 2011). This indicates the high nutritional value of the fish and its significant development value. It's worth mentioning that muscular n-3 LC-PUFA contents is 15.66–20.77%, which is at a medium-high level compared with other freshwater fishes (Xiao et al. 2022; Yang et al. 2020; Yang et al. 2021; Luczyńska et al. 2022; Imene et al. 2022; Zhang et al. 2018; Nieminen et al. 2014; Sun et al. 2022; Du et al. 2022). Under the above context, the purpose of this article is to evaluate the biosynthetic characteristics of n-3 LC-PUFA in the liver, intestine, and muscle, and to explore whether the high level of n-3 LC-PUFA in cultured *Onychostoma macrolepis* is owing to their LC-PUFA biosynthesis capacity, or due to the high levels of dietary ALA and n-3 LC-PUFA, together with using the whole-body fatty acid balance method.

Materials and methods

Ethical approval

All experiments were approved by the Northwest A&F University Institutional Animal Care and Use Committee (NWFU-DKXC- 20200602).

Experimental diets

Four types of experimental diets with equal nitrogen (37%) and lipid (9.68%) content were prepared with different dietary lipids. Fish oil, linseed oil, soybean oil, and linseed oil with soybean oil mixture as dietary lipid sources and protein sources were casein and gelatin for these four kinds of diet named FO, LO, SO, and MO, respectively. Table 1 shows the ingredients, proximate compositions, and main fatty acid compositions of experimental diets.

Fish husbandry and sampling

Onychostoma macrolepis were provided by Ankang Fisheries Experimental and Demonstration Station of Northwest A&F University (Shaanxi, China). A total of 168 fish (weight 2.03 ± 0.23 g) were fasted for 24 h and randomly assigned to 12 tanks (215 L, 14 fish/tank). Four groups of experimental diets with three replicates in each group. During the 10-week feeding period, the fish was fed three times a day (08:30, 12:30, and 16:30) which was 3% of the body weight feeding amount, and the feeding amount was calculated regularly. The water temperature of the recirculating aquaculture system is $20.5 \pm 1.5^\circ\text{C}$; moreover, the physical and chemical indicators of water quality are tested periodically and kept at normal levels.

After the end of the feeding trial, the experimental fish were fasted for 24 h and anesthetized with MS222 (60 mg/L), then counted and weighed the fish separately in each bucket (Gou et al. 2020). The anatomical procedure is divided into three parts: first, the liver and muscle of 5 fish were taken on the ice and quickly put into liquid nitrogen and stored at -80°C for subsequent gene expression and western blot detection. Then, the liver, muscle, and intestine tissues of 6 fish were taken for fatty acid detection; meanwhile, the right-side muscles of our fish were removed and stored in 4% paraformaldehyde solution (Biosharp, Hefei, China) for muscle slice analysis, and the remaining fish as whole-fish were stored at -20°C for detecting proximate chemical composition in the end.

Evaluation of the growth performance

Three parameters size increase, feed retention in the body, and feed retention in visceral organs were used to evaluate the growth after feeding with four different

Table 1 Formulation and composition of experimental diets

Components	Dietary treatments			
	FO	LO	SO	MO
Feed ingredients (g kg ⁻¹ , dry matter)				
Casein	357	357	357	357
Gelatin	90	90	90	90
Dextrin	270	270	270	270
Microcrystalline cellulose	125	125	125	125
Carboxymethylcellulose	20	20	20	20
Premix ¹	10	10	10	10
Choline chloride	5	5	5	5
Calcium phosphate primary	20	20	20	20
Cr ₂ O ₃	5	5	5	5
BHT ²	1	1	1	1
Fish oil ³	97	0	0	0
Linseed oil ⁴	0	97	0	48.5
Soybean oil ⁵	0	0	97	48.5
Analyzed proximate composition				
Moisture(%)	9.63	8.77	8.28	8.95
Crude protein(%, N%*6.25)	36.92	37.15	37.03	37.09
Crude lipid (%)	9.62	9.70	9.66	9.67
Ash (%)	5.44	5.36	5.42	5.39
Main Fatty Acid Composition (mg/g)				
C14:0	2.68	0.61	0.58	0.66
C16:0	3.75	1.51	2.40	1.97
C18:0	1.92	2.31	2.27	2.33
C20:0	0.65	0.42	0.59	0.52
C14:1n-5	0.23	ND	ND	ND
C16:1n-7	2.40	ND	0.15	ND
C18:1n-9	6.09	8.63	10.56	9.69
C18:2n-6	1.34	7.28	24.34	13.67
C20:4n-6	0.65	0.39	0.42	0.45
C18:3n-3	1.65	11.61	1.87	7.13
C20:5n-3	3.29	ND	ND	ND
C22:6n-3	8.66	ND	ND	ND
∑SFA	6.32	4.24	5.26	4.82
∑MUFA	8.49	8.63	10.71	9.69
∑n-6 PUFA	1.99	7.67	24.76	14.12
∑n-3 PUFA	13.6	11.61	1.87	7.13

FO fish oil, LO linseed oil, SO soybean oil, MO 50% linseed oil + 50% soybean oil

ND: not detected

¹The premix conclude mineral mix and vitamin mix. The mineral mix contained (g/100 g of the total mineral) the following ingredients: KAl (SO₄)₂, 0.159; CaCO₃, 18.101, Ca(H₂PO₄)₂, 44.601; CoCl, 0.070; MgSO₄, 5.216; MnSO₄·H₂O, 0.070; KCl, 16.553; KI, 0.014; ZnCO₃, 0.192; NaH₂PO₄, 13.605; Na₂SeO₃, 0.006; CuSO₄·5H₂O, 0.075; Ferric citrate, 1.338. The vitamin mix contained (mg/1000 g of diet) the following ingredients: vitamin C, 200; thiamine, 10; riboflavin, 20; vitamin A, 3000IU; vitamin E, 50IU; vitamin D₃, 1500IU; menadione, 10; pyridoxine HCl, 10; cyanocobalamin, 0.02; biotin, 1.0; calcium pantothenate, 40; folic acid, 5; niacin, 20; inositol, 400; choline chloride, 2000; cellulose was used as a carrier

²BHT: Butylated hydroxytoluene. Has the function of preventing oil oxidation (Zhejiang Yi Nuo Biotechnology Co., LTD)

³Fish oil contained 89.27 mg/ g EPA and 235.50 mg/ g DHA

⁴Linseed oil contained 411.89 mg/ g ALA and 194.29 mg/ g LA

⁵Soybean oil contained 479.56 mg/ g LA and 70.05 mg/ g ALA

lipid diets for 10 weeks, including initial and final body length (IBL, FBL), initial and final body weight (IBW, FBW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), survival rate (SUR), condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), intra-peritoneal fat body index (IPFI), relative intestine weight (RIW), and relative intestine length (RIL), were computed with previously reported (Li et al. 2022), to evaluate the growth after feeding with four different lipid diets for 10 weeks.

Proximate composition

According to the procedures of the Association of Official Analytical Chemists (AOAC 2003), the proximate composition of diets and whole-body samples (wet weight, %) was detected. Diets and whole-body samples were dried at 105°C to detect their moisture, the Kjeldahl method to determine crude protein, and then the percentage of crude protein as % N \times 6.25, crude fat was extracted using Soxhlet extraction method and incinerated at 550°C for 6 h using muffle furnace ashing method.

Hematoxylin-eosin (H&E) staining

The fixed muscle samples were washed in water, and then dehydrated in ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100%) two times. According to the standard method, soak the sample in xylene and embed it in paraffin. The embedded paraffin block is cut into a 5 μ M pieces with a slicer (RM2235, Leica, Germany); later, it is installed on the slide and stained with hematoxylin-eosin (H.E). The histological samples were observed and the photos were taken with an upright microscope (Leica Biosystems, Germany). The diameter and density of muscle fibers, as well as the length of the sarcomere, were measured using Image Pro Plus.

Fatty acid profile analysis

Lipid extraction from oil, experimental diets, liver, intestine, muscle, feces, and whole fish was conducted with chloroform-methanol (2:1, v/v) according to the report of Folch et al. (1957). At the same time,

the quantitatively determined content of fatty acids is calculated by adding 1 ml glyceryl triundecanoate (C11:0, 2mg/ml, purity (GC)= 99.06%) in the initial step. Subsequently, fatty acid methyl esters (FAME) were obtained by adding 1ml n-hexane for dissolving lipids first, after that adding 1 ml methanol-KOH (0.4 M, 2.24g KOH dissolved in 100 ml methanol) in the mixture for 2h to methyl esterification adequately. Then, add 2 ml of double distilled water to the organic reagent mentioned above. The liquid in the test tube will be divided into two layers, and the upper layer of liquid will be separated for later analysis, which will be detected by an automatic sampler gas chromatograph (GC, Nexis GC-2030, Shimadzu, Japanese). Refer to previous studies for the detection procedure and setting and compare and identify each fatty acid with the known standard (47015-U, Sigma-Aldrich, Inc., St. Louis, USA) and then muscular nutritional indices were evaluated (Chen and Liu 2020; Xiao et al. 2021).

The whole-body fatty acid balance method

The whole-body fatty acid balance method was originally proposed by Giovanni et al. (2007) and has been gradually modified and improved (Giovanni et al. 2007; Giovanni et al. 2008; Giovanni and David 2009). In order to assess the capacity of fatty acid metabolism, we calculate the elongation, desaturation, and β -oxidation in *Onychostoma macrolepis*. Briefly, the first step for the computation of the overall fatty acid appearance or disappearance, the FA accumulation and FA net intake should be calculated primarily. Among them, FA excretion needs to estimate the apparent fatty acid digestibility individual which a standard formula was used: $ADC_{FA} = 100 - (100 \times (Cr_2O_3 \text{ in diet}) / (Cr_2O_3 \text{ in faeces}) \times (\text{fatty acid in faeces (mg/g DM)}) / (\text{fatty acid in diet (mg/g DM)}))$. Figure 1 shows the detailed calculation steps. The second step is to calculate the fate of each fatty acid undergoing bioconversion, oxidation, or deposition through all known fatty acid bioconversion pathways (SFA+ MUFA, n-6 PUFA, and n-3 PUFA) after converting mg of fatty acid appearance or disappearance to mmol. Eventually, the total activity in different fatty acids pathways of the $\Delta 5$, $\Delta 6$, $\Delta 9$ desaturase and elongase was expressed as $\mu\text{mol g}^{-1}\text{day}^{-1}$.

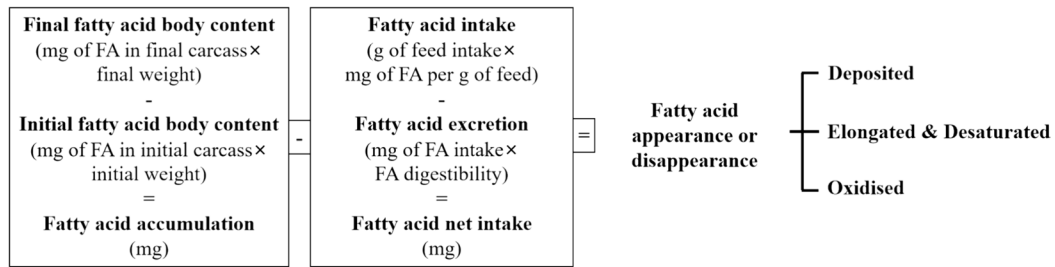


Fig. 1 Schematic representation of the mass balance analysis of the individual fatty acid intake and accumulation. The first step in the whole-body fatty acid balance method

Quantitative real-time PCR (qRT-PCR)

Using RNAiso Plus kit (Takara, Dalian) to extract the total RNA of tissues, and measuring the quality and concentration of total RNA with micro-nucleic acid protein analyzer (SynergyH1, BioTek, USA). Synthesis of cDNA using RNA reverse transcription kit (Takara, Dalian), and the housekeeping gene was *β-actin*. Finally, through real-time fluorescence quantitative PCR (RT qPCR) detection of related gene expression in different tissue samples. The data was $2^{-\Delta\Delta CT}$ method calculation (Sun et al. 2017).

The primer was designed using Primer 5.0 software according to the cDNA sequence of relevant genes in the NCBI database and the primer sequence was synthesized by Xi'an Qingke Biotechnology Co., Ltd. (Xi'an, China). The upstream and downstream primer sequences are shown in Table 2. The method of qRT-PCR was according to the previous study (Bian et al. 2022).

Statistical analysis

All data are expressed by the standard deviation of mean \pm standard error (S.E.), $n=3$. SPSS 22.0 software (SPSS Inc., Chicago, USA) was used for one-way analysis of variance (ANOVA). After the Student's *t*-test, analysis of variance showed significant differences ($P<0.05$) between different groups, with different superscripts. Moreover, using Origin 2019 (Origin Software, CA, USA) drew the graphs. Using SPSS 26.0 (SPSS, IL, USA) software, principal component analysis (PCA) was used to perform covariance matrix tests on the data of fatty acids in tissues which aimed to explain the effects of dietary oil sources on the utilization of fatty acid.

Results

Growth performance, biometric parameter, and proximate composition

The results of growth performance, biometric parameters, and proximate composition (wet weight, %) are shown in Table 3. After quantitative feeding for 70 days, the final body length and final body weight reached levels more than twice the initial weight and initial body length respectively. No significant differences between the four experimental groups were observed in growth performance parameters, such as WG, SGR, SUR, and FCR. However, compared to the FO group, the CF of the LO, SO, and MO groups significantly increased ($P < 0.05$). In the biometric data such as VSI, RIW and RIL of LO are the highest among groups, while RIL was not significant with SO and MO group, RIW is higher than SO group ($P < 0.05$). In the SO group, IPFI is the highest than MO and FO group ($P < 0.05$). Besides, HSI in four dietary groups was not significantly different.

The crude fat content in the MO group was the lowest and not significantly different from the FO group but lower than the LO and SO group ($P < 0.05$). There was no significant difference in moisture, crude protein, and ash content among the groups ($P > 0.05$).

Fatty acid profile in the liver and intestine

The liver and intestine fatty acid profiles are shown in Table 4. The contents of liver ALA and LA in the LO and SO groups were seriously increased respectively, compared to other groups, which showed the same pattern as the main fatty acid profile of

Table 2 Primers used in real-time quantitative PCR

Transcript	Primers	Nucleotide Sequence 5'-3'	Accession Number
FadS2	FadS2-F	TTGTGGAGGATTTCCGAGCC	AIA19310.1
	FadS2-R	AGCCAGGTGCAGGATGAAAA	
Elovl2	Elovl2-F	GGTCTGCCGGTTATCGTCTT	AAH95785.1
	Elovl2-R	ACCACAGTACTTTTGCTACCCT	
Elovl4a	Elovl4a-F	GATCCCAATGAAGTGCGGGT	XP_016391268.1
	Elovl4a-R	TGAACATGGTGCAGTGGTGA	
Elovl4b	Elovl4b-F	CCGGATACAGCTACCTCTGC	KTG39645.1
	Elovl4b-R	CGCTGACGCTATCCTGACTT	
Elovl5	Elovl5-F	TAGTGCCGTATAACCTCGGC	AER39745.1
	Elovl5-R	TCCGCTTGATACGTTGACA	
Elovl8a	Elovl8a-F	CATGGTGTGCCTTTCTGCTT	XP_016394983.1
	Elovl8a-R	TGCCGAGGGAGTTTCAGAGT	
CD36	CD36-F	GTCTGTAGACACGCCAATGT	KM030422.1
	CD36-R	GATGGAGATCATTGCTGCCA	
FATP1	FATP1-F	GGAGCTTGCTGGCTCTTTTA	XM_016265898.1
	FATP1-R	CACGTCCGAACAGACTAACA	
FABP3	FABP3-F	GTCGGTACCTGGAAGCTAGT	NM_010174.2
	FABP3-R	CACACTGCCATGAGTGAGAG	
FABP10	FABP10-F	GAGGAGTTTCTGAGAGCCATCT	EU363801.1
	FABP10-R	CATAGTGGTTCCTCCTACCCT	
SREBP-1	SREBP-1-F	CTCTCCACCTCTCCACAGAA	MG735210.1
	SREBP-1-R	GGTTCTCCATACACGAGCAG	
LXR α	LXR α -F	TGTCCACCCTTTCTACGACT	XM_016488177.1
	LXR α -R	AGGTGATGCTCTCTATCGCT	
β -actin	β -actin-F	TGACCCACACTGTACCCATC	JN254630.1
	β -actin-R	CGGACAATTTCACTCTCGGC	

FadS2, fatty acid desaturase 2; Elovl2, elongation of very long-chain fatty acid 2; Elovl4a, elongation of very long-chain fatty acid 4a; Elovl4b, elongation of very long-chain fatty acid 4b; Elovl5, elongation of very long-chain fatty acid 5; Elovl8b, elongation of very long-chain fatty acid 8b; CD36, fatty acid translocase; FATP1, fatty acid transport protein 1; FABP3, fatty acid-binding protein 3; FABP10, fatty acid-binding protein 10; SREBP-1, sterol regulatory element binding Protein 1; LXR α , liver X receptor α

oil supplemented in feed ($P < 0.05$). The contents of DHA and EPA in the FO group were the highest among the groups, notably, the contents of DHA, EPA, and \sum n-3 LC-PUFA in the LO group reached half of the FO group and significantly higher than that in the SO and MO groups ($P < 0.05$), but there was no significant difference in ARA content between the FO and MO groups ($P > 0.05$). Furthermore, the changing pattern of intestine LA and ALA was similar to the results of the liver. The contents of ARA and EPA in the LO and FO group were modified lower than others ($P < 0.05$). Besides, DHA content in the FO group remained the highest, while that content in the LO group was less than a third of the FO group

and showed no significant difference with the MO group ($P > 0.05$). Taken together, these data indicated that n-3 LC-PUFA can be endogenous synthesis in *Onychostoma macrolepis* and this capacity is stronger than ARA synthesized in SO and MO dietary groups.

The PCA of liver and intestine fatty acids presented serious separation between four groups and the loading plots were also shown (Fig. 2A–B, D–E). The Pearson correlation coefficient between dietary and intestinal fatty acids in the SO and MO groups markedly increased ($P < 0.05$) but no difference in the correlation coefficient between diet and liver was observed (Fig. 2C, F).

Table 3 Effects of dietary oil sources on growth performance and biological parameters in *Onychostoma macrolepis*

Parameters	Dietary treatments			
	FO	LO	SO	MO
IBL (cm)	4.37±0.11	4.35±0.22	4.37±0.17	4.34±0.41
FBL (cm)	7.06±0.86	7.38±0.62	7.33±0.59	7.24±0.72
IBW (g)	2.03±0.23	2.03±0.23	2.02±0.22	2.04±0.22
FBW (g)	6.70±1.98	6.89±1.65	6.78±1.29	6.53±1.18
WG (%)	230.51±6.17	239.88±2.57	219.82±8.14	234.73±3.18
SGR (%/d)	1.71±0.03	1.75±0.01	1.66±0.11	1.73±0.01
FI (g)	122.33±0.12	121.75±1.07	122.37±0.15	121.16±2.32
FCR	1.87±0.04	1.79±0.03	1.94±0.17	1.84±0.03
SUR (%)	97.63±4.10	97.63±4.10	100±0.00	100±0.00
CF	1.64±0.19 ^b	1.72±0.13 ^a	1.74±0.12 ^a	1.75±0.17 ^a
VSI (%)	9.66±1.09 ^b	12.40±0.94 ^a	12.24±1.72 ^a	12.01±1.44 ^a
HSI (%)	2.23±0.77	2.01±0.54	2.05±0.47	2.28±0.54
IPFI (%)	0.93±0.48 ^c	1.53±0.81 ^{ab}	1.65±0.53 ^a	1.13±0.62 ^{bc}
RIW (%)	4.63±1.86 ^c	6.61±1.57 ^a	5.45±1.34 ^{bc}	5.82±1.07 ^{ab}
RIL (%)	263.55±45 ^b	318.17±37.81 ^a	290.90±47.70 ^{ab}	304.55±41.67 ^a
Whole body proximate composition (%)				
Moisture	68.25±0.29	69.99±0.99	69.90±0.85	69.75±1.46
Crude protein	17.08±0.16	17.00±0.93	17.15±0.82	16.77±0.11
Crude lipid	9.84±0.16 ^b	10.81±0.61 ^a	10.78±0.09 ^a	9.67±0.78 ^b
Ash	2.68±0.08	2.62±0.23	2.59±0.82	2.61±0.37

Values are means ±SD of three replicates. Values in the same line with different superscript letters are significantly different ($P < 0.05$)

IBL, initial body length; FBL, final body length; IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; FI, feed intake; FCR, feed conversion ratio; SUR, survival rate; CF, condition factor; VSI, viscerosomatic index; HSI, hepatosomatic index; IPFI, intraperitoneal fat body index; RIW, relative intestine weight; RIL, relative intestine length

Muscular fatty acid profiles, nutritional indices, and histology

Table 5 presents the fatty acid profile of muscle. In terms of PUFA, high contents of LA, ALA, and DHA were detected in fish-fed diets SO, LO, and FO respectively ($P < 0.05$), in which the n-3 LC-PUFA of the LO group reached 82.70% of the FO group and that of the SO group only 27.23% (3.93, 3.25, 1.07 mg/g, respectively), and that of MO group is between LO and SO group. In addition, the ARA content of the SO group was not markedly higher than the LO and MO groups ($P > 0.05$) but was significantly different from the FO group. Meanwhile, the contents of n-3 PUFA synthetic intermediate, C20:3n-3 and C20:5n-3, was significantly higher than SO and MO groups in the LO group, surprisingly, the results of n-6 PUFA synthetic intermediate were still the highest in the LO group. The PCA of muscle fatty acid

presented serious separation among four groups; the loading plots also showed such a pattern (Fig. 2G–H). Although the Pearson correlation coefficient has no difference among groups, the lowest level could be seen in the LO group ($P > 0.05$) (Fig. 2I).

As nutritional indices of muscular fatty acids (Table 5), the index of atherogenicity and index of thrombogenicity showed the lowest results in the LO group ($P < 0.05$). In turn, HH (hypcholesterolemic/hypercholesterolemic ratio) showed the highest results in the LO group ($P < 0.05$). Furthermore, PUFA/SFA was not markedly different between LO and SO group ($P > 0.05$) but was significantly different from others ($P < 0.05$). Two other important indices n-3/n-6PUFA and FLQ (fish lipid quality/flesh lipid quality) both showed a significant trend of gradual decrease between fish-fed diets FO, LO, MO, and SO ($P < 0.05$).

Table 4 Liver and intestine fatty acid profile of *Onychostoma macrolepis* fed the experimental diets (mg/g, wet matter)

Fatty acids	Liver				Intestine			
	FO	LO	SO	MO	FO	LO	SO	MO
C14:0	4.20±1.20 ^a	2.84±1.15 ^b	2.68±1.06 ^b	1.79±0.70 ^b	2.55±0.71 ^a	0.66±0.23 ^b	0.92±0.33 ^b	0.86±0.21 ^b
C16:0	9.69±2.68	9.16±0.86	10.15±3.24	7.56±2.84	6.13±1.55 ^a	3.51±1.03 ^c	5.56±1.38 ^{ab}	4.45±0.47 ^{bc}
C18:0	9.13±2.29	9.87±1.91	8.41±2.92	7.34±1.79	3.43±0.96	2.83±0.58	3.46±1.09	3.52±0.48
C20:0	1.99±0.68 ^a	1.10±0.61 ^b	1.51±0.56 ^{ab}	1.17±0.11 ^b	1.13±0.49	0.63±0.26	0.95±0.45	0.86±0.18
C14:1n-5	1.80±0.76 ^a	0.77±0.02 ^b	0.94±0.29 ^b	0.46±0.08 ^b	0.47±0.18 ^a	0.21±0.09 ^b	0.29±0.02 ^{ab}	0.32±0.10 ^{ab}
C16:1n-7	4.85±1.33	5.90±3.29	5.81±2.84	4.37±1.53	3.45±0.61 ^a	1.29±0.43 ^b	1.70±0.51 ^b	1.32±0.36 ^b
C18:1n-9	25.42±13.38 ^b	45.12±10.61 ^a	37.84±11.71 ^{ab}	34.96±10.45 ^{ab}	11.48±1.79 ^b	13.50±3.73 ^{ab}	16.82±4.32 ^a	12.11±1.38 ^b
C18:2n-6 (LA)	7.09±1.44 ^c	12.36±1.90 ^b	21.40±2.86 ^a	13.20±2.85 ^b	3.55±0.68 ^d	7.29±1.90 ^c	21.97±5.14 ^a	10.71±1.04 ^b
C18:3n-6	1.83±0.82	1.21±0.28	1.31±0.76	1.08±0.26	0.60±0.23 ^b	0.61±0.17 ^b	1.00±0.43 ^a	0.73±0.08 ^{ab}
C20:2n-6	2.34±0.77	2.06±0.50	1.95±0.94	1.75±0.16	0.99±0.30 ^{ab}	0.83±0.27 ^b	1.34±0.50 ^a	1.13±0.14 ^{ab}
C20:3n-6	1.69±0.55	1.68±0.46	1.89±0.92	1.77±0.14	1.21±0.44 ^a	0.68±0.24 ^b	1.15±0.47 ^a	0.94±0.17 ^{ab}
C20:4n-6 (ARA)	2.52±0.91 ^{ab}	1.77±0.34 ^b	3.29±1.07 ^a	2.26±0.32 ^b	1.21±0.50 ^{ab}	0.74±0.24 ^b	1.52±0.65 ^a	1.06±0.11 ^{ab}
C18:3n-3 (ALA)	3.96±0.60 ^c	9.73±0.82 ^a	3.60±1.40 ^c	5.26±1.99 ^b	2.33±0.75 ^c	7.50±1.85 ^a	2.04±0.81 ^c	4.15±0.76 ^b
C20:3n-3	3.12±0.82 ^a	2.22±0.34 ^b	2.04±0.35 ^{bc}	1.47±0.22 ^c	2.04±0.62 ^a	1.49±0.39 ^b	0.77±0.41 ^c	1.17±0.21 ^{bc}
C20:5n-3 (EPA)	2.68±0.45 ^a	1.65±0.22 ^b	0.87±0.05 ^c	1.02±0.09 ^c	1.82±0.70 ^a	0.65±0.18 ^b	0.53±0.10 ^b	0.66±0.09 ^b
C22:6n-3 (DHA)	10.19±1.16 ^a	5.28±0.96 ^b	2.21±0.42 ^d	3.70±0.36 ^c	8.98±1.01 ^a	2.41±0.45 ^b	1.30±0.16 ^c	2.12±0.34 ^b
∑SFA ¹	25.01±3.81 ^a	22.97±2.97 ^b	22.74±5.96 ^{ab}	17.86±5.42 ^b	13.25±3.17 ^a	7.62±1.92 ^c	10.89±2.89 ^{ab}	9.39±0.89 ^{bc}
∑MUFA ²	32.07±13.97 ^b	51.79±13.87 ^a	44.59±13.19 ^{ab}	39.79±11.90 ^{ab}	15.40±2.45 ^{ab}	15.00±4.17 ^{ab}	18.80±4.80 ^a	13.76±1.57 ^b
∑n-6 PUFA ³	13.06±2.51 ^c	18.84±3.14 ^b	29.83±5.50 ^a	20.06±3.07 ^b	7.57±0.86 ^c	10.15±2.80 ^c	26.99±6.09 ^a	14.57±0.79 ^b
∑n-3 PUFA ⁴	18.77±2.45 ^a	18.88±1.79 ^a	7.46±2.47 ^c	11.15±2.21 ^b	15.16±1.99 ^a	12.04±2.67 ^b	4.56±1.06 ^d	7.53±1.48 ^c
∑n-3 LC-PUFA ⁵	12.86±1.38 ^a	6.93±0.98 ^b	3.08±0.46 ^d	4.72±0.41 ^c	10.80±1.45 ^a	3.05±0.61 ^b	1.75±0.26 ^c	2.67±0.55 ^{bc}
DHA/EPA	3.87±0.61 ^a	3.24±0.64 ^a	2.54±0.41 ^b	3.63±0.38 ^a	5.45±1.98 ^a	3.83±0.61 ^b	2.79±0.82 ^c	3.63±0.76 ^b

¹ ∑SFA: sum of saturated fatty acid; ² ∑MUFA: sum of monounsaturated fatty acid; ³ ∑n-6PUFA: sum of n-6 polyunsaturated fatty acids; ⁴ ∑n-3PUFA: sum of n-3 polyunsaturated fatty acids; ⁵ ∑n-3 LC-PUFA: sum of DHA and EPA

Values with different superscript letters in the same row are significantly different ($P < 0.05$)

In this study, there was no significant change in muscle fiber diameter between the LO, SO, and MO groups ($P > 0.05$); however, these were significantly higher than the FO group ($P < 0.05$) (Fig. 3B). Posteriorly, the fiber density was highest in the FO group inversely and gradually reduced in LO, SO, and MO groups ($P < 0.05$) (Fig. 3C). The histology of muscular transverse sections of four groups (magnification: 100 ×) is shown in Fig. 3A.

Gene expression in the liver and muscle

Figure 4 showed the mRNA levels of key enzymatic genes in fatty acid biosynthesis (*FadS2*, *Elovl2*, *Elovl4a*, *Elovl4b*, *Elovl5*, *Elovl8a*), fatty acid transportation (*CD36*, *FATP1*, *FABP3*, *FABP10*), and

transcription factors (*SREBP-1*, *LXRα*) in the liver and muscle. For fatty acid biosynthesis genes, both liver and muscle related genes presented a similar pattern, i.e., the lowest expression in the FO group and the highest expression in the LO group ($P < 0.05$). In particular, the *Elovl2* mRNA levels of the liver in the SO and FO group were higher than those of the LO and MO group ($P < 0.05$), besides, the *Elovl4b* mRNA levels presented about equal levels among the four groups.

With regard to fatty acid transportation related genes, different results are shown in the liver and muscle (Fig. 4). The expression of *CD36* in the liver and muscle, as well as *FATP1* and *FABP10* in the FO group, was the lowest ($P < 0.05$). However, the *FATP1* and *FABP3* mRNA levels showed no

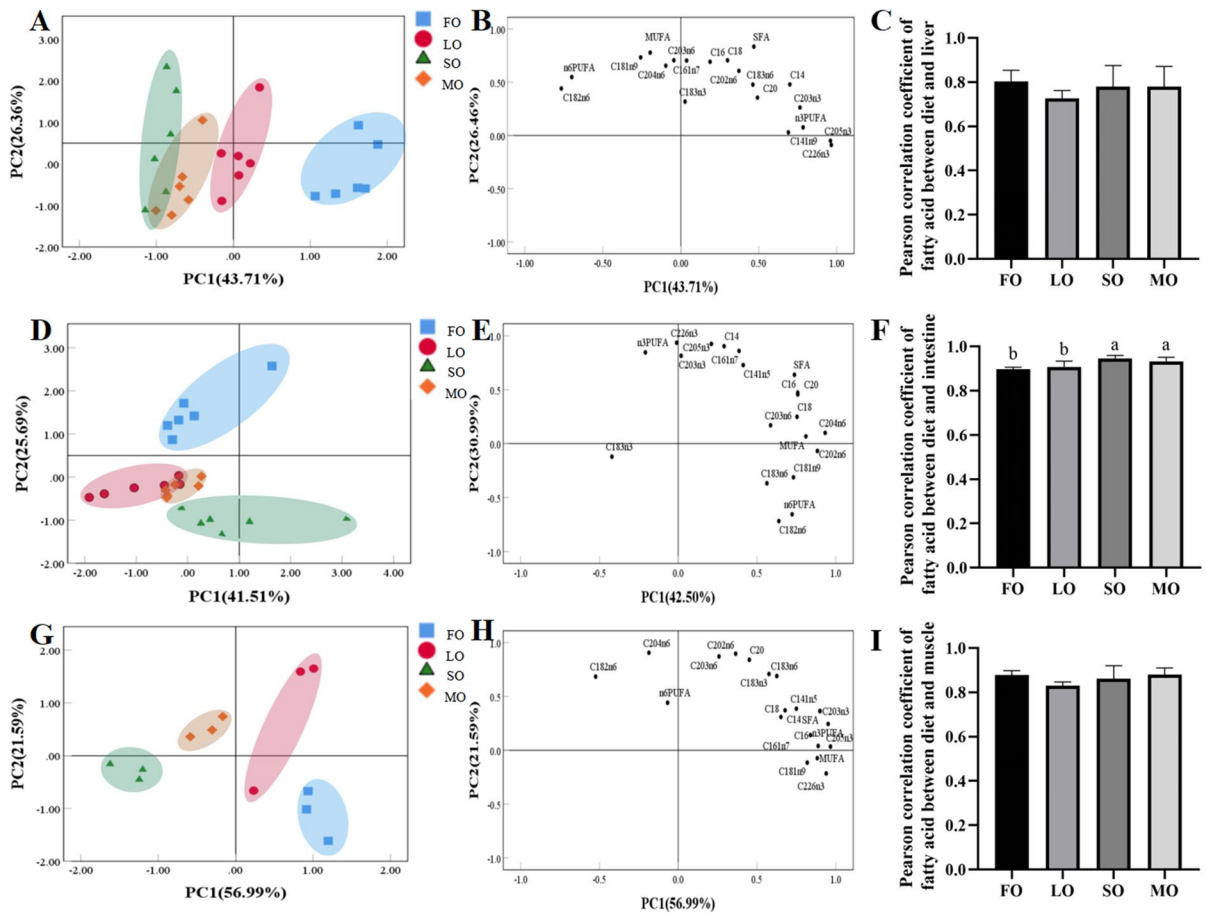


Fig. 2 PCA score plot (A) and loading plot (B) generated based on liver fatty acids composition obtained by analyzing the data and PCA score plot (D) and loading plot (E) generated based on intestine fatty acids composition obtained by analyzing the data. C: Pearson correlation coefficient of fatty acid

between diet and liver. F: Pearson correlation coefficient of fatty acid between diet and intestine. PCA score plot (G) and loading plot (H) generated based on muscle fatty acids composition obtained by analyzing the data. I: Pearson correlation coefficient of fatty acid between diet and muscle

difference among groups. The mRNA expression of transcriptional regulation factors *SREBP-1* and *LXR α* in the liver is the highest in the LO group, followed by SO and MO groups, and the lowest in the FO group ($P < 0.05$). In addition, the mRNA level of these two genes in muscle showed a significant increase in the fish-fed diets LO and MO than FO ($P < 0.05$).

Fatty acid profile of fish whole-body and fatty acid mass balance

The feeding of experimental feed changed the FA distribution of fish to reflect their respective feed FA ratios (Table 6). ALA and DHA were the predominant polyunsaturated fatty acids (PUFA) in the

initial whole-body fatty acids. Dietary linseed oil and soybean oil increased the whole body LA and ALA contents in LO (7.05 ± 0.34 and 7.64 ± 0.51 mg/g), SO (18.04 ± 3.39 mg/g), and MO (12.38 ± 2.06 and 4.56 ± 0.88 mg/g) groups. At the same time, the content of the final product ARA of n-6 was the highest in the SO group ($P < 0.05$). After feeding fish oil feed, the initial EPA content of the whole-fish increased by more than 338% while the content of that in LO and MO groups was equal to the initial level, but there was a 41.18% reduction in initial EPA content after feeding the SO diet. The decreasing order of DHA content throughout the whole body is FO > LO > MO > SO. The content of DHA in the LO group is 2.65 ± 0.31 mg/g, although not as high as

Table 5 Fatty acid profile and nutritional indices of the muscle of *Onychostoma macrolepis* fed with experimental diets (mg/g, wet basis)

Fatty acids	Dietary treatments			
	FO	LO	SO	MO
C12:0	0.21±0.02 ^a	0.17±0.03 ^b	0.15±0.04 ^b	0.22±0.05 ^a
C14:0	0.55±0.11 ^a	0.50±0.13 ^a	0.24±0.03 ^c	0.39±0.06 ^b
C16:0	1.80±0.13 ^a	1.26±0.02 ^b	0.83±0.22 ^c	1.30±0.30 ^b
C18:0	1.71±0.26 ^a	2.00±0.37 ^a	1.05±0.20 ^b	1.69±0.37 ^a
C20:0	0.23±0.05 ^c	0.39±0.13 ^a	0.19±0.03 ^c	0.30±0.02 ^b
C14:1n-5	0.12±0.02 ^a	0.13±0.03 ^a	0.08±0.02 ^b	0.11±0.01 ^a
C16:1n-7	0.93±0.14 ^a	0.84±0.31 ^b	0.26±0.06 ^d	0.47±0.14 ^c
C18:1n-9	4.52±0.80 ^a	3.87±0.15 ^b	2.32±0.99 ^c	3.58±1.06 ^b
C18:2n-6 (LA)	1.62±0.24 ^b	2.68±0.83 ^a	3.03±0.12 ^a	3.08±0.53 ^a
C18:3n-6	0.22±0.02 ^b	0.29±0.08 ^a	0.17±0.03 ^c	0.22±0.04 ^b
C20:2n-6	0.32±0.09 ^b	0.49±0.17 ^a	0.29±0.04 ^b	0.43±0.03 ^a
C20:3n-6	0.30±0.08 ^b	0.42±0.12 ^a	0.30±0.06 ^b	0.39±0.02 ^a
C20:4n-6(ARA)	0.47±0.03 ^b	0.57±0.11 ^a	0.62±0.04 ^a	0.63±0.15 ^a
C18:3n-3(ALA)	0.90±0.14 ^c	2.30±0.47 ^a	0.45±0.07 ^d	1.29±0.06 ^b
C20:3n-3	0.48±0.06 ^b	0.60±0.12 ^a	0.16±0.02 ^d	0.39±0.05 ^c
C20:5n-3(EPA)	0.61±0.06 ^a	0.54±0.12 ^a	0.18±0.03 ^c	0.33±0.03 ^b
C22:6n-3(DHA)	3.33±0.41 ^a	2.71±0.19 ^b	0.89±0.08 ^d	1.58±0.13 ^c
∑SFA ¹	4.50±0.35 ^a	4.33±0.71 ^a	2.48±0.28 ^b	3.89±0.28 ^a
∑MUFA ²	5.58±0.91 ^a	4.83±0.57 ^a	2.67±1.00 ^b	4.16±1.20 ^a
∑n-6 PUFA ³	2.93±0.42 ^b	4.45±1.21 ^a	4.39±0.12 ^a	4.78±0.63 ^a
∑n-3 PUFA ⁴	5.31±0.56 ^a	6.15±0.84 ^a	1.68±0.04 ^c	3.59±0.23 ^b
∑n-3 LC-PUFA ⁵	3.93±0.47 ^a	3.25±0.31 ^b	1.07±0.09 ^c	1.91±0.16 ^d
Nutritional indices of fatty acids				
IA ⁶	0.31±0.03 ^a	0.23±0.02 ^b	0.23±0.02 ^b	0.25±0.02 ^b
IT ⁷	0.18±0.03 ^b	0.15±0.02 ^c	0.24±0.03 ^a	0.21±0.03 ^b
HH ⁸	5.00±0.55 ^c	7.46±0.82 ^a	6.93±0.32 ^b	6.28±0.12 ^b
FLQ ⁹	21.44±1.91 ^a	16.52±0.77 ^b	9.62±1.29 ^d	11.74±1.64 ^c
PUFA/SFA	1.84±0.26 ^c	2.45±0.44 ^a	2.47±0.27 ^a	2.16±0.28 ^b
n-3/n-6 PUFA	1.83±0.28 ^a	1.42±0.22 ^b	0.38±0.02 ^d	0.76±0.09 ^c

¹ ∑SFA: sum of saturated fatty acid; ² ∑MUFA: sum of monounsaturated fatty acid; ³ ∑n-6PUFA: sum of n-6 polyunsaturated fatty acids; ⁴ ∑n-3PUFA: sum of n-3 polyunsaturated fatty acids; ⁵ ∑n-3 LC-PUFA: sum of DHA and EPA; ⁶ IA(Index of atherogenicity)=[C12:0+(4×C14:0)+C16:0]/∑UFA; ⁷ IT(Index of thrombogenicity)=(C14:0+C16:0+C18:0)/[(0.5×∑MUFA)+(0.5×∑n-6PUFA)+(3×∑n-3PUFA)+(n-3/n-6)]; ⁸ HH(Hypocholesterolemic/hypercholesterolemic ratio)=(cis-C18:1+∑PUFA)/(C12:0+C14:0+C16:0); ⁹ FLQ(Fish lipid quality/flesh lipid quality)=100×(C22:6n-3+C20:5n-3)/∑SFA

Values with different superscript letters in the same row are significantly different ($P < 0.05$)

expected, it is still significantly higher than the SO group ($P < 0.05$). On the other hand, the content of intermediate products C18:3n-6 and C20:3n-6 in the n-6 fatty acid synthesis pathway was the highest in the SO group ($P < 0.05$), which C20:2n-6 was no different with LO and MO groups. Likewise, the content of C20:3n-3 in the LO group was significantly higher than that in the SO and MO groups ($P < 0.05$).

In the first step, some indexes were computed (Table 7). Due to the different fatty acid compositions of the three oils, the intake of SFA, MUFA, n-6 PUFA, and n-3 PUFA in the four diets showed significantly higher levels in the FO, SO, SO, and FO groups, respectively ($P < 0.05$). Similarly, the excretion of SFA, MUFA, and n-3 PUFA in the FO group was still the highest, but n-6 PUFA

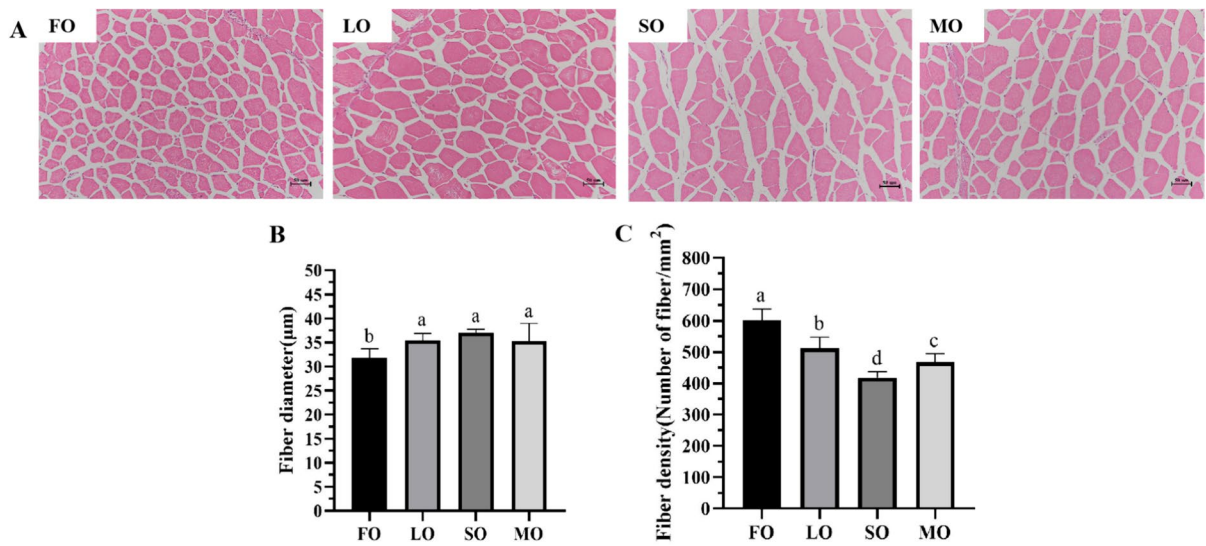


Fig. 3 A: The histology of muscular transverse sections of four groups (magnification: 100 ×); B: muscle fiber diameter; C: The unit area (1 mm²) number of fibers

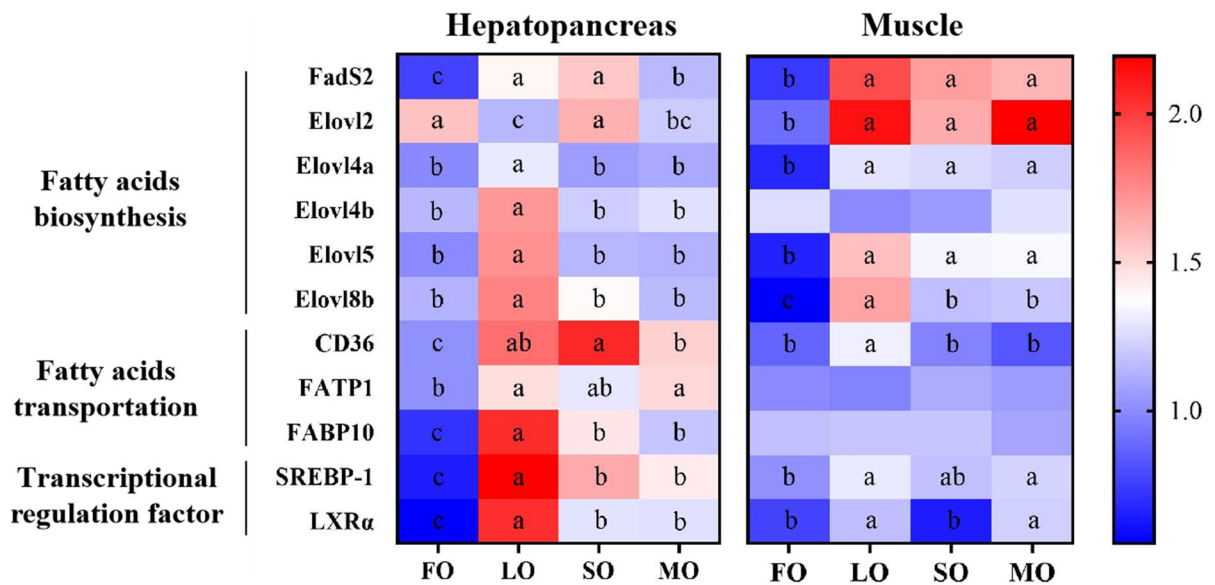


Fig. 4 Heatmap of mRNA expression of genes related fatty acid biosynthesis, transportation and transcriptional regulation factor (red, high concentration; blue, low concentration) ($n=3$). FadS2, fatty acid desaturase 2; Elovl2, elongation of very long-chain fatty acid 2; Elovl4a, elongation of very long-chain fatty acid 4a; Elovl4b, elongation of very long-chain fatty acid 4b;

Elovl5, elongation of very long-chain fatty acid 5; Elovl8b, elongation of very long-chain fatty acid 8b; CD36, fatty acid translocase; FATP1, fatty acid transport protein 1; FABP3, fatty acid-binding protein 3; FABP10, fatty acid-binding protein 10; SREBP-1, sterol regulatory element binding Protein 1; LXRα, liver X receptor α

was not inconsistent with the varying diets ($P > 0.05$). Because of that, the final body accumulation from beginning to end had the same results

that SFA, MUFA, and n-6 PUFA in the SO group and n-3 PUFA in the LO group had the highest content compared to the other three groups. Finally, the

Table 6 Initial and final whole body fatty acid profile after feeding experimental diets for 70 days. (mg/g, wet basis)

Fatty acids	Initial	Final			
		FO	LO	SO	MO
C14:0	0.38	1.58±0.17 ^a	0.48±0.05 ^c	0.82±0.14 ^b	0.70±0.16 ^b
C16:0	1.38	4.53±0.71 ^{ab}	2.86±0.12 ^c	5.13±0.94 ^a	3.96±0.81 ^b
C18:0	2.15	1.90±0.31 ^b	1.90±0.10 ^b	2.41±0.39 ^a	2.19±0.34 ^{ab}
C20:0	0.51	0.37±0.05 ^a	0.23±0.02 ^c	0.34±0.09 ^{ab}	0.30±0.04 ^b
C14:1n-5	0.16	0.19±0.05 ^a	0.08±0.01 ^b	0.12±0.03 ^b	0.11±0.02 ^b
C16:1n-7	0.75	3.14±0.46 ^a	1.32±0.07 ^c	2.29±0.41 ^b	1.96±0.42 ^b
C18:1n-9	3.41	11.54±1.35 ^c	13.94±0.62 ^{bc}	18.04±3.22 ^a	16.43±3.43 ^{ab}
C18:2n-6 (LA)	1.70	3.00±0.50 ^d	7.05±0.34 ^c	18.04±3.39 ^a	12.38±2.06 ^b
C18:3n-6	0.33	0.19±0.06 ^d	0.44±0.02 ^c	0.71±0.12 ^a	0.56±0.11 ^b
C20:2n-6	0.49	0.57±0.32 ^b	0.91±0.06 ^a	0.83±0.20 ^{ab}	0.96±0.28 ^a
C20:3n-6	0.45	0.21±0.04 ^c	0.27±0.02 ^c	0.60±0.10 ^a	0.45±0.07 ^b
C20:4n-6(ARA)	0.75	0.55±0.07 ^c	0.39±0.03 ^d	1.09±0.15 ^a	0.73±0.10 ^b
C18:3n-3(ALA)	0.55	1.78±0.32 ^c	7.64±0.51 ^a	1.81±0.37 ^c	4.56±0.88 ^b
C20:3n-3	0.35	1.06±0.12 ^a	0.96±0.06 ^a	0.29±0.08 ^c	0.64±0.11 ^b
C20:5n-3(EPA)	0.34	1.15±0.10 ^a	0.34±0.05 ^b	0.20±0.06 ^c	0.30±0.05 ^b
C22:6n-3(DHA)	1.44	7.23±0.83 ^a	2.65±0.31 ^b	1.60±0.18 ^c	2.26±0.25 ^b
∑SFA ¹	4.42	8.38±1.15 ^a	5.47±0.23 ^c	8.69±1.47 ^a	7.16±1.30 ^b
∑MUFA ²	4.32	14.76±1.59 ^b	15.34±0.62 ^b	20.44±3.61 ^a	18.49±3.85 ^a
∑n-6 PUFA ³	3.73	4.52±0.91 ^d	9.06±0.42 ^c	21.28±3.88 ^a	13.97±2.18 ^b
∑n-3 PUFA ⁴	2.69	11.21±1.12 ^a	11.58±0.68 ^a	3.63±0.72 ^c	7.76±0.97 ^b
∑n-3 LC-PUFA ⁵	1.78	8.38±0.92 ^a	2.99±0.28 ^b	1.80±0.64 ^c	2.56±0.24 ^b
DHA/EPA	4.25	6.28±0.36	8.01±1.80	7.49±1.25	7.74±1.63

¹ ∑SFA: sum of saturated fatty acid; ² ∑MUFA: sum of monounsaturated fatty acid; ³ ∑n-6PUFA: sum of n-6 polyunsaturated fatty acids; ⁴ ∑n-3PUFA: sum of n-3 polyunsaturated fatty acids; ⁵ ∑n-3 LC-PUFA: sum of DHA and EPA

appearance of SFA and MUFA (expressed in mg per fish) in the SO and MO groups was significantly higher than others ($P < 0.05$), while the disappearance of n-6 and n-3 PUFA was significantly higher in the SO and FO groups ($P < 0.05$).

In addition, Table 8 reported the appearance/disappearance of individual fatty acids during the experimental period. The overwhelming majority of SFA and MUFA had the highest appearance in the SO group, except C20:0, which showed the highest disappearance in the FO group. For n-6 PUFA, ARA had the highest appearance while LA was the fatty acid with the highest disappearance in the SO group ($P < 0.05$). Especially, C18:3n-6 was the uniqueness of appearance in the SO group. In terms of n-3 PUFA, ALA was the fatty acid that disappeared the most in the LO group, and DHA appeared the most along with C20:3n-3 and EPA ($P < 0.05$). Among all

the fatty acids, LA has the highest disappearance rate, while C18:1n-9 has the highest occurrence rate.

Ultimately, the elongase, desaturase, and β -oxidation activity were quantified, all expressed as nmol of product per gram of fish per day (Table 9). The difference between the fatty acid intake of four groups resulted in a significantly higher elongation, $\Delta 6$ desaturation, $\Delta 5$ desaturation, and β -oxidation of n-6 PUFA in the SO group and of n-3 PUFA in the LO group ($P < 0.05$). Moreover, there was no difference in elongation between SFA and MUFA, but the SO and MO groups had $\Delta 9$ desaturation and β -oxidation significantly increased ($P < 0.05$). Overall, total elongation and desaturation were promoted by LO, SO, and MO diet ($P < 0.05$). Specifically, except for $\Delta-9$ desaturation, the $\Delta-6$ and $\Delta-5$ desaturation were the highest among four groups. Feeding SO-based diets led to high oxidation in total apparent.

Table 7 Main fatty acid class balance during a 70-d period in which *Onychostoma macrolepis* were feed four different dietary treatments

	SFA	MUFA	n-6 PUFA	n-3 PUFA
Intake				
FO	78.62±0.09 ^a	81.66±0.10 ^c	24.84±0.03 ^d	144.24±0.16 ^a
LO	42.15±0.36 ^d	75.89±0.66 ^d	74.14±0.64 ^c	100.96±0.87 ^b
SO	51.07±0.06 ^b	93.22±0.11 ^a	224.57±0.26 ^a	16.34±0.02 ^d
MO	47.36±0.92 ^c	84.98±1.65 ^b	130.22±2.52 ^b	61.70±1.20 ^c
Excretion				
FO	14.62±1.06 ^a	5.53±1.28 ^a	7.19±0.18	8.55±1.73 ^a
LO	10.63±0.57 ^b	3.22±0.72 ^b	6.77±1.57	3.75±0.57 ^b
SO	11.96±2.30 ^{ab}	5.03±0.24 ^a	5.17±1.20	1.67±0.51 ^c
MO	12.27±2.06 ^{ab}	5.16±0.98 ^a	6.50±2.29	2.24±0.94 ^{bc}
Initial body content				
FO	8.97±0.07	9.82±0.07	7.56±0.06	5.45±0.04
LO	9.01±0.03	9.86±0.03	7.60±0.02	5.47±0.01
SO	8.96±0.07	9.80±0.07	7.55±0.06	5.44±0.04
MO	8.98±0.05	9.83±0.05	7.57±0.04	5.46±0.03
Final body content				
FO	56.21±6.37 ^a	102.81±10.07 ^b	30.25±6.03 ^d	75.20±6.24 ^a
LO	37.66±1.33 ^b	107.39±2.86 ^b	62.43±2.22 ^c	79.77±5.15 ^a
SO	58.84±8.22 ^a	140.77±17.69 ^a	144.03±22.11 ^a	26.32±3.70 ^c
MO	46.96±10.31 ^{ab}	123.90±31.85 ^{ab}	91.04±13.18 ^b	50.73±7.15 ^b
Accumulation				
FO	47.24±6.30 ^a	92.99±10.00 ^b	22.69±5.98 ^d	69.76±6.22 ^a
LO	28.64±1.35 ^b	97.53±2.89 ^b	54.83±2.24 ^c	74.30±5.13 ^a
SO	49.89±8.17 ^a	130.97±17.64 ^a	136.48±22.07 ^a	20.89±3.67 ^c
MO	37.98±10.33 ^{ab}	114.07±31.87 ^{ab}	83.47±13.19 ^b	45.27±7.16 ^b
Appearance/disappearance				
FO	2.49±0.89 ^c	16.86±3.18 ^c	5.04±2.14 ^a	-65.93±5.67 ^c
LO	13.95±3.23 ^b	24.86±2.92 ^b	-12.54±0.51 ^a	-22.91±5.51 ^b
SO	27.47±8.99 ^a	42.78±17.56 ^a	-82.91±21.96 ^c	6.21±3.85 ^a
MO	22.09±3.13 ^{ab}	34.25±29.71 ^{ab}	-40.26±9.59 ^b	-14.19±5.88 ^b

Data represent the total amount of fatty acid (mg) per fish (mean±S.E.M.;n=3). Different superscript letters within a list denote significant differences among diets as determined by one-way ANOVA with Tukey's comparison test ($P<0.05$)

FO fish oil, LO linseed oil, SO soybean oil, MO 50% linseed oil + 50% soybean oil

Negative and positive values represent disappearance and appearance, respectively

Discussion

In the present research, results were mainly in view of growth performance and proximate composition of whole-fish, fatty acid content among four tissues, transcription and translation of some crucial genes in muscle and liver and put into use the whole-body fatty acid balance method which demonstrated LC-PUFA biosynthetic are possessed in *Onychostoma macrolepis*.

Characteristics of LC-PUFA biosynthetic in the liver, muscle, intestine, and whole body of *Onychostoma macrolepis*

N-3 and n-6 series of fatty acids had different capacities in the LC-PUFA biosynthetic process. As shown in Fig. 5, the net intake of the substrate, LA and ALA, was decreased or increased in steps respectively except for the FO group. In the order of fish-fed diets SO, MO, and LO, ARA content was

Table 8 Main fatty acid appearance/disappearance (mg/fish) during a 70-d period in which *Onychostoma macrolepis* were feed four different dietary treatments

	FO	LO	SO	MO
C14:0	-11.05±0.95 ^c	-0.97±0.37 ^b	1.49±0.51 ^a	0.45±1.34 ^{ab}
C16:0	4.83±1.84 ^c	7.25±0.59 ^b	14.32±5.28 ^a	9.72±5.81 ^b
C18:0	-0.24±1.91 ^{ab}	-2.62±0.72 ^b	1.87±3.46 ^a	-0.59±0.57 ^{ab}
C20:0	-4.18±0.23 ^b	-2.02±0.28 ^a	-3.36±0.91 ^b	-2.38±0.28 ^a
C14:1n-5	-1.05±0.22 ^b	0.24±0.02 ^a	0.48±0.23 ^a	0.40±0.09 ^a
C16:1n-7	-0.04±3.06 ^c	7.57±0.52 ^b	13.43±2.48 ^a	11.32±3.24 ^{ab}
C18:1n-9	19.20±7.48	16.34±3.05	25.41±10.24	20.43±6.34
C18:2n-6 (LA)	7.73±3.96 ^a	-16.61±1.07 ^b	-91.54±18.67 ^c	-38.20±13.42 ^b
C18:3n-6	-0.53±0.28 ^{bc}	-1.40±0.12 ^c	0.91±0.82 ^a	-0.10±0.98 ^{ab}
C20:2n-6	0.47±2.07 ^b	5.21±0.31 ^a	4.65±1.07 ^a	5.30±2.32 ^a
C20:3n-6	-2.12±0.21 ^a	-3.10±0.12 ^b	-1.77±0.74 ^a	-2.17±0.47 ^a
C20:4n-6(ARA)	-0.50±0.17 ^c	0.61±0.20 ^c	4.84±1.14 ^a	2.60±0.35 ^b
C18:3n-3(ALA)	-1.12±2.24 ^a	-47.42±2.72 ^c	-3.53±2.67 ^a	-30.66±5.16 ^b
C20:3n-3	-17.94±0.62 ^d	5.85±0.33 ^a	1.24±0.56 ^c	3.46±0.53 ^b
C20:5n-3(EPA)	-20.85±0.26 ^c	1.64±0.35 ^a	0.62±0.49 ^b	1.25±0.06 ^a
C22:6n-3(DHA)	-25.19±5.12 ^c	15.33±2.13 ^a	7.89±0.56 ^b	11.79±0.46 ^{ab}

Data represent the total amount of fatty acid (mg) per fish (mean±S.E.M. ; n=3). Different superscript letters within a row denote significant differences among diets as determined by one-way ANOVA with Tukey's comparison test ($P<0.05$)

FO fish oil, LO linseed oil, SO soybean oil, MO 50% linseed oil + 50% soybean oil

Negative and positive values represent disappearance and appearance, respectively

gradually decreased in the liver and intestine which may be related to their biosynthetic ability. But in the muscle, no differences were observed with these three groups ($P > 0.05$), which suggested that *O. macrolepis* was not an 'n-6 type' fish like basa catfish (*Pangasius bocourti*) and tilapia (*Oreochromis mossambicus*) who required LA as EFA and had limited ability to utilize LA (Wing and Nicholas 2013; Pornpisanu et al. 2010). On the contrary, muscular DHA content was increased in direct proportion and even close to the control group (fish oil diet, rich in DHA, less in ALA), illustrating that the conversion of ALA has not reached the platform stage, suggesting that the content of dietary ALA could be elevated in practical feed. In addition, the content of liver DHA is also higher than that in other tissues, which indicates that the liver is an important synthetic site for ALA. This finding indicated that the demand for n-3PUFA of *O. macrolepis* is more than that of n-6 PUFA.

Muscle and liver are one of the two most important tissues in the endogenous synthesis process of fish. In this study, different dietary oil sources regulated

the nutritional status of *Fads2*, *Elovl4a*, *Elovl5*, and *Elovl8b* genes in the liver and muscle that showed a widespread feature that the high expression levels of all four genes were detected in fish fed with high ALA level (LO) diet whose fatty acid chain elongation pathway was activated. Additionally, the expression level of the fatty acid transport gene in the liver is higher than that in muscle, proclaiming that the liver has a fatty acid absorption chain of "oil source-cell membrane-membrane protein-fatty acid absorption," which makes it not only a synthetic organ but also can transport FA to other tissues, such as muscle. The capacity of biosynthetic enzymes was calculated by the WFAB method, showing that feeding diets containing linseed oil led to dead-end products with high levels of elongase and desaturase activities in *O. macrolepis*. Overall, the rate-limiting enzyme in the fatty acid biosynthetic pathway was the in vivo $\Delta 6$ desaturase activities (Sun et al. 2022), were 1.5 folds higher in LO fed fish than the value calculated in SO fed fish; on the other hand, elongation in n-3 PUFA was also higher in n-6 PUFA. By contrast, lower elongation and desaturation activities were recorded when

Table 9 Total apparent in vivo fatty acid β -oxidation, desaturation and elongation ($\mu\text{mol g}^{-1} \text{day}^{-1}$) deduced by the whole-body fatty acid balance method

	FO	LO	SO	MO
SFA and MUFA				
Elongation	460.76 \pm 196.86	467.30 \pm 68.61	777.16 \pm 374.83	889.05 \pm 412.09
Δ -9 desaturation	237.85 \pm 109.40 ^b	284.84 \pm 33.71 ^b	470.95 \pm 199.06 ^a	524.22 \pm 217.35 ^a
β -oxidation	141.32 \pm 136.50 ^b	362.01 \pm 44.77 ^b	651.81 \pm 260.71 ^a	634.53 \pm 260.31 ^a
n-6 PUFA				
Elongation	10.06 \pm 17.43 ^c	60.70 \pm 5.00 ^b	100.69 \pm 23.68 ^a	85.38 \pm 18.80 ^b
Δ -6 desaturation	ND	6.41 \pm 2.14 ^c	62.22 \pm 20.09 ^a	32.44 \pm 7.11 ^b
Δ -5 desaturation	ND	6.41 \pm 2.14 ^c	51.64 \pm 12.36 ^a	28.63 \pm 4.58 ^b
β -oxidation	119.77 \pm 24.03 ^b	178.15 \pm 15.49 ^b	967.00 \pm 246.90 ^a	402.56 \pm 212.56 ^b
n-3 PUFA				
Elongation	ND	246.05 \pm 19.09 ^a	104.34 \pm 19.19 ^c	185.74 \pm 3.74 ^b
Δ -6 desaturation	ND	317.12 \pm 38.88 ^a	162.57 \pm 14.14 ^c	254.44 \pm 8.11 ^b
Δ -5 desaturation	ND	167.28 \pm 18.33 ^a	84.59 \pm 8.56 ^c	134.16 \pm 3.96 ^b
β -oxidation	693.33 \pm 71.32 ^a	318.36 \pm 52.23 ^b	ND	199.55 \pm 80.23 ^c
Total				
Elongation	470.82 \pm 211.52 ^b	774.05 \pm 54.42 ^a	982.19 \pm 412.04 ^a	1160.17 \pm 415.73 ^a
Δ -9 desaturation	237.85 \pm 109.40 ^b	284.84 \pm 33.71 ^b	470.95 \pm 199.06 ^a	524.22 \pm 217.35 ^a
Δ -6 desaturation	ND	323.53 \pm 36.80 ^a	224.79 \pm 34.21 ^c	286.89 \pm 11.76 ^b
Δ -5 desaturation	ND	173.69 \pm 6.22 ^a	136.23 \pm 20.02 ^b	162.79 \pm 8.44 ^b
Desaturation	237.85 \pm 109.40 ^b	782.06 \pm 23.72 ^a	831.98 \pm 253.20 ^a	973.90 \pm 226.18 ^a
β -oxidation	954.42 \pm 157.05 ^c	858.52 \pm 90.92 ^c	1618.81 \pm 25.95 ^a	1236.64 \pm 199.48 ^b

Data represent the total amount of fatty acid (mg) per fish (mean \pm S.E.M.; $n=3$). Different superscript letters within a row denote significant differences among diets as determined by one-way ANOVA with Tukey's comparison test ($P<0.05$)

FO fish oil, LO linseed oil, SO soybean oil, MO 50% linseed oil + 50% soybean oil

ND: not detected

rainbow trout (*Oncorhynchus mykiss*), tilapia (*Oreochromis mossambicus*), gilthead seabream (*Sparus aurata*), and higher of those were recorded when Atlantic cod (*Gadus morhua*) and Murray cod (*Maccullochella peelii peelii*) were fed VO-based in comparison to those fed fish oil-based diets (Samuel et al. 2020; Chaiw et al. 2010; Thanuthong et al. 2011; Hixson and Parrish 2014; Giovanni et al. 2006). On balance, these results seemed to suggest that *O. macrolepis* possessed high biological activity of biosynthetic enzymes to synthesize n-3 LC-PUFA.

The muscle of *O. macrolepis* possesses high nutritional value

In recent years, fish have been widely concerned because most of them are rich in DHA and can meet people's nutritional needs. In the present study, notably, muscular DHA content reached 3.33 mg/g and

2.71 mg/g in the FO and LO group, respectively, at a medium-high level compared with muscular DHA content of several other fish species that was the highest aquaculture yield in China. Such as grass carp (*Ctenopharyngodon idella*, 0.14–0.25 mg/g), silver carp (*Hypophthalmichthys molitrix*, 0.2–5.4 mg/g), and bighead carp (*Aristichthys nobilis*, 3.2–3.9 mg/g, cultured in two different patterns), common carp (*Cyprinus carpio* L., 0.83–1.61 mg/g, fed with three algae additive feed) and tilapia fed diets with 1–3% fish oil for 4 weeks was 0.76–0.78 mg/g (Wen et al. 2022; Jing et al. 2020; Radovan et al. 2019; Xie et al. 2022). Moreover, the n-3 LC-PUFA content in the fillet of the *O. macrolepis* by fish oil-fed ranked fifth among the 13 Central European breeds, including intensively cultured, semi-intensively reared, and extensively cultured freshwater fish species (Linhartová et al. 2018). In several economically important marine farmed fish, however, there was 4.56–5.27

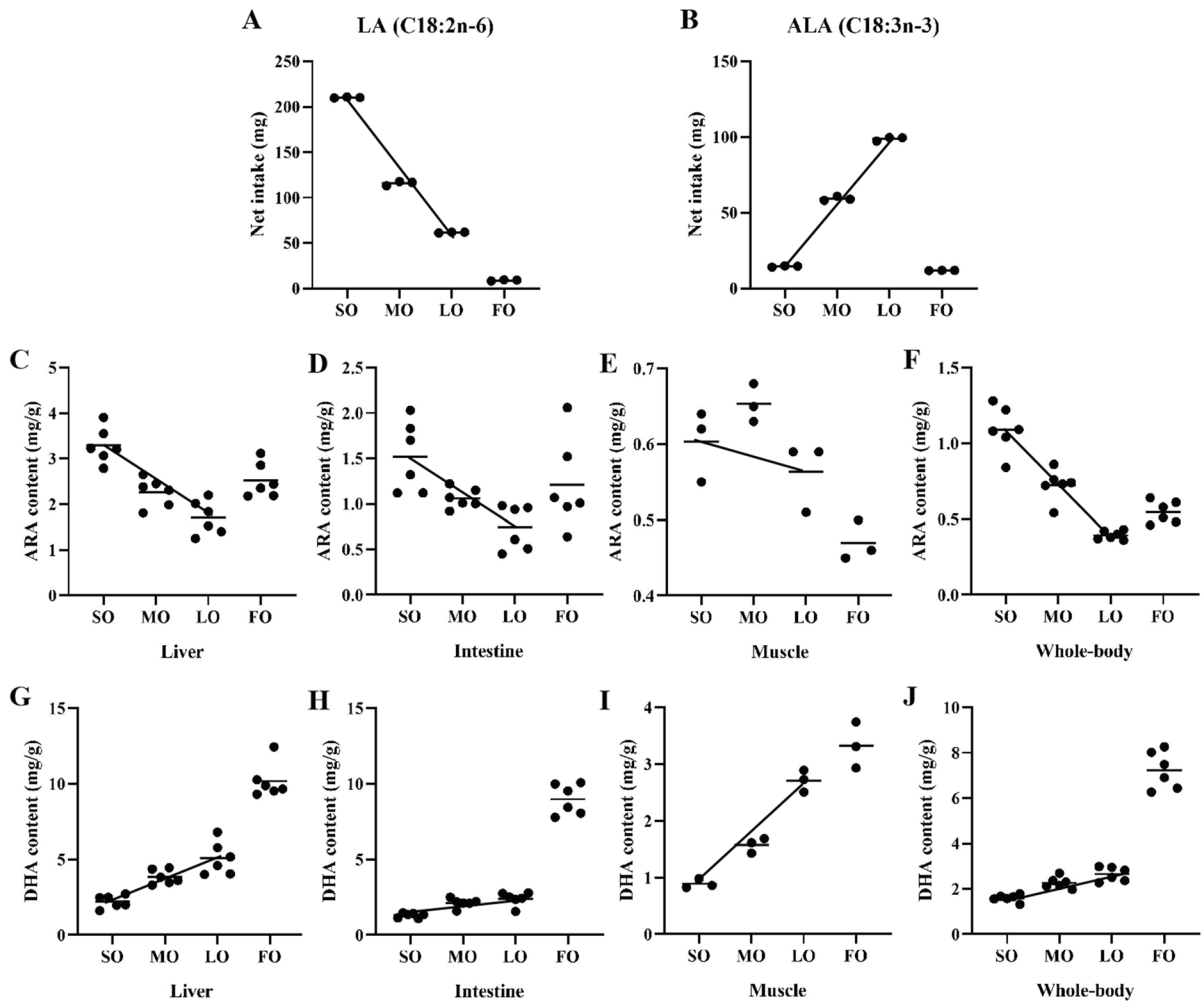


Fig. 5 Scattered point diagram of net intake were represented in LA and ALA (A, B); C–F represented scattered point diagram of ARA content in three tissues and whole-body; G–J

represented scattered point diagram of DHA content in three tissues and whole-body. (Values in the liver, intestine, and whole-body, $n=6$; values in muscle, $n=3$)

mg/g DHA in the muscle of grouper (*Epinephelus coioides*) by dietary fish oil replacement with palm oil, higher muscular DHA content was reported when turbot (*Scophthalmus maximus*, 6.29 ± 0.31 mg/g), tiger puffer (*Takifugu rubripes*, 6.74 ± 0.24 mg/g) and Japanese seabass (*Lateolabrax japonicus*, 8.04 ± 0.08 mg/g) were fed FO-based diet with 12.1 mg/g DHA, while large yellow croaker (*Larimichthys crocea*) only 81.90–150.00 (mg/100g wet weight) of DHA content in muscle from different sources (Qin et al. 2022; Xu et al. 2021; Chen et al. 2022). These results confirmed that n-3 LC-PUFA and DHA were rich in *O. macrolepis* by comparing the absolute content of fatty acids, and *O. macrolepis* could be labeled as a

“n-3 PUFA-rich” and superior aquatic product. “EPA + DHA” was an index recognized worldwide to be applied to evaluate the nutritional value of fish that is recognized worldwide. Because the conversion rate of DHA and EPA from ALA was far from enough for health, EPA and DHA provided by *O. macrolepis* was more direct and effective, which provided a basis for creating high n-3 LC-PUFA varieties.

Using nutritional indicators to evaluate the nutritional value of fatty acids and exploring their potential applications in the prevention and treatment of diseases for human beings, particularly in fatty-acid-rich foods (Chen and Liu 2020). In the current research, two inferior indices IA and IT which are

related to a risk of development of cardiovascular diseases showed the lowest value in the LO group, implying that the products may reduce the levels of LDL-C and total cholesterol in human blood plasma. On the contrary, HH, a superior index that may more accurately reflect the impact of FA components on CVD compared with PUFA/SFA, was significantly higher in the LO group (7.46) than in other three groups, and exceeding the range of 0.87–4.83 reported in previous reports (Chen and Liu 2020). It is noteworthy that the group fed the fish oil had a lower HH and PUFA/SFA ratio, which may be due to the high content of SFA in its PUFA components, and only EPA+DHA. It shows that dietary ALA can effectively improve the health index of muscle fatty acids. Other more direct indices, FLQ, and n-3/n-6 PUFA ratio showed a similar trend that gradually decreased in the fish-fed diets FO, LO, MO, and SO, meeting the requirements of human nutritional recommendation ratios. These nutritional indices data suggest that although FO group *O. macrolepis* had high nutritional value, but might be not beneficial to human health, while the fish fed the LO diet might both improve the health status and nutritional value. One of the key factors determining muscle texture characteristics is muscle structure, including muscle fiber diameter and density. Muscle hardness, elasticity, chewing, and viscosity have been proven to be positively correlated with muscle fiber density (Roy et al. 2012; Periago et al. 2005). In this study, both the FO group that directly provides DHA and the LO group that synthesized and indirectly converts DHA increased the muscular fiber density compared to MO and LO groups and may be conducive to the muscle texture of farmed *O. macrolepis*. Taking together, farmed *O. macrolepis* fed with linseed oil as an oil source could not only retain the high nutritional value close to fish oil-fed fish, but also ensure the health status and texture of muscle, and provide appropriate ALA so that *O. macrolepis* can become a beneficial source of n-3 LC PUFA for human.

Conclusion

In summary, based on the results of growth, tissue fatty acid composition, and the mRNA levels of key enzymatic genes and combined the whole-body fatty

acid balance method, the present study demonstrated that (1) *Onychostoma macrolepis* owns high n-3 LC-PUFA biosynthetic capacity in the liver and muscle, while the intestinal synthesis ability is low; (2) the DHA contents in the muscle of *Onychostoma macrolepis* at a high level; (3) dietary supplementation of ALA retains most of nutrition value and ensures muscular texture. These results prove that *Onychostoma macrolepis* could be a good n-3 LC-PUFA source for the population and provide new insights into creating high n-3 LC-PUFA aquatic products.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval All experiments were approved by the Northwest A&F University Institutional Animal Care and Use Committee (NWAUFU-DKXC-20200602).

Competing interests The authors declare no competing interests.

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