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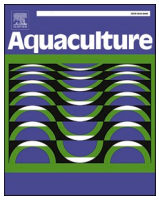

Effective complete replacement of fish oil by linseed oil in diets for thick-lipped grey mullet (*Chelon labrosus*) juveniles reared at three environmental salinities

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ABSTRACT

One of the major challenges for fish oil replacement by alternative oils is the limited capacity of marine fish species to convert 18C fatty acids into long-chain poly unsaturated fatty acids (LC-PUFA). Since salinity markedly affects this conversion capacity, euryhaline species, such as thick-lipped grey mullet (*Chelon labrosus*), may have a good potential to use diets without inclusion of fish oil. To test this hypothesis, thick-lipped grey mullet juveniles were fed two different diets based on fish oil (FO diet) or linseed oil (LO diet) at three different salinities: 46 ppt, 35 ppt, and 16 ppt. At the end of the trial, the biochemical and fatty acid composition of whole-body, liver, and muscle, as well as the relative expression of the genes codifying for the elongase *Elovl5* and the desaturase *Fads2* in liver, were determined. The results showed the good capacity of mullet juveniles to convert 18C fatty acids into LC-PUFA, which was enhanced by the replacement of FO by LO and the reduction of salinity to an isosmotic level (16 ppt). Besides, these juveniles efficiently used diets with complete replacement of FO by LO with growth performance, body composition, and LC-PUFA fatty acid profiles similar to those of fish fed FO, particularly when reared at 16 ppt.

1. Introduction

Together with fish meal (FM), fish oil (FO) is a valuable ingredient in aquafeeds, whose limited availability constrains the further development of the aquaculture industry (Glencross et al., 2023). Although vegetable oils (VO) constitute a more reliable source of lipids (Turchini et al., 2019), they lack the essential long-chain poly unsaturated fatty acids (LC-PUFA) (Montero and Izquierdo, 2010; Oliva-Teles et al., 2015). Nevertheless, VO are abundant in the 18C precursors of these important fatty acids and, therefore, may be effectively used by fish with sufficient desaturase and elongase activities to produce LC-PUFA. Although fish species, particularly marine ones, have a limited ability to synthetize LC-PUFA, desaturase and elongase activities can be modulated by nutritional, epigenetic and environmental factors (Izquierdo et al., 2008; Vagner and Santigosa, 2011; Izquierdo et al., 2015; Torno et al., 2019).

A moderate reduction in the LC-PUFA contents in the diet, together with the increase in their 18C fatty acids precursors, namely the partial or total replacement of FO by different VO, up-regulates the expression of elongases and desaturases involved in LC-PUFA synthesis (Tocher et al., 2001; Izquierdo et al., 2008; Xie et al., 2021). Whereas FO replacement by VO may up-regulate these genes up to a fourfold

Abbreviations: actb, β-actin; ACTH, adrenocorticotropic hormone; ARA, arachidonic acid; BHT, butylated hydroxytoluene; *Elovl5*, fatty acid elongase 5; cDNA, complementary desoxyribonucleic acid; *CPT-I*, carnitine palmitoyl transferase; DW, dry weight; EPA, eicosapentaenoic acid 20:5n-3; *ef1α*, elongation factor-1α; DHA, docosahexaenoic acid 22:6n-3; *Fads2*, *fatty acid desaturase 2*; *FAS*, fatty acid synthetase; FCR, feed conversion ratio; FO, fish oil; FM, fish meal; *G6PD*, glucose-6-phosphate dehydrogenase; HUFA, highly unsaturated fatty acids IGF1 insulin growth factor 1; *L3HOAD*, L-3-hydroxyacyl-CoA dehydrogenase; LC-PUFA, long-chain polyunsaturated fatty acids with more than 18C and >2 double bonds; *LXRα*, liver X Receptor α; LO, linseed oil; mRNA, messenger ribonucleic acid; qPCR, quantitative real-time polymerase chain reaction; rRNA, ribosomal ribonucleic acid; SGR, specific growth ratio; *SREBP-1c*, sterol regulatory binding protein-1c; TSH, thyrotropin; VO, vegetable oil; WW, wet weight.

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increase, excessive reduction in LC-PUFA down-regulated them (Izquierdo et al., 2008), denoting the importance of the balance between products and precursors. Besides, the ratio n-3/n-6 or α-linolenic/linoleic (18:3n-3/18:2n-6) also affects the potential of LC-PUFA biosynthesis, since these fatty acids compete for the same enzymes that regulate these pathways (Sprague et al., 2019). Moreover, the changes in gene expression caused by dietary FO replacement by VO can be transferred to the offspring and last along the whole fish life cycle (Turkmen et al., 2017), implying different epigenetic mechanisms (Turkmen et al., 2019; Naya-Català et al., 2023).

Salinity is one of the more potent environmental factors regulating essential fatty acid requirements and synthesis in fish, in relation to the important role of these fatty acids in osmotic regulation (Izquierdo and Koven, 2010). Thus, whereas individuals of the same species have a higher LC-PUFA content when reared at higher salinities (Izquierdo and Koven, 2010; Dantagnan et al., 2007), they also have a higher requirement for very long chain PUFA such as docosahexaenoic acid (DHA) (Dantagnan et al., 2010). These types of studies have been conducted in both marine and freshwater fish species (Sarker et al., 2011; Xie et al., 2015; You et al., 2019). However, the interaction between dietary lipids and water salinity and their effect on fatty acid metabolism and deposition in fish tissues is not yet well understood and deserves further studies (Xie et al., 2021).

Mullets, including the thick-lipped grey mullet (*Chelon labrosus*), are considered very promising species for sustainable aquaculture diversification due to its worldwide distribution and its omnivore and euryhaline nature (Lebreton et al., 2011). These species have some capacity for LC-PUFA synthesis (Rosas et al., 2019; Galindo et al., 2021) and make a good utilization of dietary VO in replacement of FO (Rosas et al., 2019). Rearing water salinity markedly affects lipid and fatty acid composition of mullet species (Khériji et al., 2003; Loi et al., 2022), including the thick-lipped grey mullet (Imen et al., 2013; Rabeh et al., 2015). In the later species, *Fads2* was characterized to have both Δ6 and Δ8 desaturases activity (Garrido et al., 2019). Although recently one study targeted the partial replacement of dietary FO by VO and reduced water salinity from 35 ppt to 20 ppt (Marrero et al., 2024), until now there are no published studies that evaluate the effect of complete FO replacement combined with salinities found in brackish water (16 ppt) or hypersaline lagoons (46 ppt), natural environments of the thick-lipped grey mullet (Hotos and Vlahos, 1998).

To address this gap of knowledge, thick-lipped grey mullets were fed two diets containing either FO or linseed oil (LO) and tested at three salinities 16 ppt, 35 ppt, 46 ppt, in the range of salinities tolerated by this species (Hotos and Vlahos, 1998).

2. Material and methods

2.1. Experimental fish and rearing conditions

Thick-lipped grey mullet juveniles, captured by an authorized fisherman on the east coast of Gran Canaria (Canary Islands, Spain), were carefully transported to the facilities of GIA-Ecoaqua Institute of the University of Las Palmas de Gran Canaria (Taliarte, Telde, Spain). Fish were acclimated in 500 l tanks provided with an open-flow system and fed to apparent satiation with a commercial extruded feed (R2, Skretting, Burgos, Spain) twice a day until the beginning of the feeding trial.

After acclimatization, 180 mullets (26.74 ± 4.92 g and 13.13 ± 0.89 cm, initial body weight and total length, respectively) were individually weighed and measured and randomly distributed in 18 glass aquaria of 60 l in a recirculating aquaculture system (RAS) (n = 10 fish per aquaria). Photoperiod was natural (12 h of light and 12 h of darkness). Six aquaria were filled with water at 16 ppt (low salinity treatment, LS) mixing natural seawater with fresh spring water, another 6 aquaria were filled with natural seawater at 35 ppt (medium salinity treatment, MS); and the remaining 6 aquaria were filled with water at 46 ppt (high salinity treatment, HS), mixing sea water with natural salt from a local seawater saline. For each salinity, fish were fed the experimental diets (FO vs LO) in triplicates (3 aquaria per combination diet-salinity). The salinity was monitored daily with a refractometer, and the water losses produced by cleaning procedures were renewed weekly from the different reservoirs. Temperature and oxygen were also monitored daily with a digital probe (OxyGuard, Handy Polaris, Acuitec S.L., Guipuzkoa, Spain) (25.43 ± 0.25 °C and 5.71 ± 0.18 mg/l, mean ± SD, respectively). Additionally, ammonia, nitrites, and nitrates were measured weekly with a spectrophotometer (YSI 9500, Xylem Analytics, Yellow Springs, USA), being maintained within the safe levels reported for mullets (Sampaio et al., 2002) and for marine fish in general (Boyd, 2014). Fish were carefully hand-fed with one of the experimental diets until apparent satiation to minimize the loss of feed, twice per day, 6 days per week, for 73 days.

All the procedures were rigorously conducted according to the European Union Directive (2010/63/EU) on animal welfare protection for scientific purposes. The experimental protocol was approved by the Bioethical Committee of the University of Las Palmas de Gran Canaria (OEBA-ULPGC 39/2020).

2.2. Experimental diets

Two isoproteic (42% protein) and isolipidic (13% lipids) diets (Table 1) were formulated containing either FO (FO diet) (Quirós-Pozo et al., 2023b) or LO (LO diet). Replacement of FO by LO reduced by 25% dietary saturated fatty acid contents, by 35% monounsaturated fatty acids and by 19% n-9 fatty acids (Table 2). On the contrary, FO replacement by LO increased n-3 fatty acids by raising 7.5 times the 18:3n-3 contents but reduced by 80% the contents in 18:4n-3, by 69% in 20:3n-3, 89% in 20:4n-3 and by 7- and 4-times 20:5n-3 and 22:6n-3,

Table 1
Experimental diets formula (%) and proximate composition (means± SD).

Ingredients	PERCENT	
	FO	LO
Fish meal[a]	20	20
Blood meal	3	3
Ulva meal[b]	10	10
Rapeseed meal[c]	8	8
Corn meal[d]	6	6
SPC (soy protein concentrate)[e]	20	20
Wheat meal[d]	6	6
Wheat gluten[d]	12	12
Fish oil[a]	9.5	0.0
Lineseed oil[d]	0	9.5
Vit mix[f]	2	2
Min mix[g]	2	2
Ca(H2PO4)2[h]	0.5	0.5
CMC[i]	1	1
Analytical composition		
Protein	41.80 ± 0.30	42.38 ± 0.47
Lipids	13.27 ± 0.16	12.89 ± 0.30
Ash	10.30 ± 0.24	10.38 ± 0.22
Moisture	6.89 ± 0.31	7.39 ± 0.29

[a] Fish meal and fish oil from South America (supplied by Skretting, Spain); [b] Ulva meal (supplied by Puerto Muiños S.L., Spain); [c] Rapeseed 0.0 (supplied by Dibaq, Spain); [d] Flours and oils obtained from local suppliers; [e] Soy protein concentrate (Sopropeche, France); [f] Vitamin premix containing (mg kg-1 or IU/kg of dry feed): tiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, folic acid 10 mg, cyanocobalamin, 0.5 mg, choline chloride 2700 mg, myo-inositol 2000 mg, ascorbic acid 5000 mg, menadione 20 mg, cholecalciferol 2000 IU, etoxyquine 100 mg, retinol acetate 5000 IU. Vitamin E (DL-alpha-tocopherol acetate) 250 mg; [g] Mineral premix containing (g/kg of dry feed): calcium orthophosfate 1.60 g, calcium carbonate 4 g, ferrous sulfate 1.5 g, magnesic sulfate 1.6 g, potassium phosphate 2.8 g, sodium phosphate 1 g, aluminum sulfate 0.0 g, zinc sulfate 0.24 g, copper sulfate 0.20 g, manganese sulfate 0.1 g, potassium iodate 0.0 g; [h] Sigma-Aldrich, Munich, Germany; [i] carboxymethylcellulose (sodium salt, Sigma-Aldrich, Munich, Germany). Abbreviations: FO, fish oil diet; LO, linseed oil diet.

Table 2
Fatty acid profile of diets (expressed as % in the diet).

	FO	LO
14:0	0.36	0.08
15:0	0.04	0.02
16:0	1.91	1.27
16:1n-7	0.55	0.13
18:0	0.54	0.71
18:1n-9	3.78	3.02
18:1n-7	0.43	0.20
18:2n-9	0.01	0.00
18.2n-6	1.85	2.34
18:2n-4	0.02	0.00
18:3n-6	0.02	0.00
18:3n-4	0.02	0.00
18:3n-3	0.54	4.44
18:4n-3	0.11	0.02
20:0	0.04	0.03
20:1n-9	0.04	0.01
20:1n-7	0.38	0.08
20.1n-5	0.03	0.01
20:2n-6	0.08	0.01
20:3n-6	0.03	0.01
20:4n-6	0.08	0.03
20:3n-3	0.03	0.01
20:4n-3	0.08	0.01
20:5n-3	0.55	0.08
22:1n-11	0.28	0.04
22:1n-9	0.06	0.02
22:4n-6	0.02	0.01
22:5n-6	0.03	0.01
22:5n-3	0.19	0.02
22:6n-3	0.93	0.21
Saturated[1]	2.91	2.12
Monoenoics[2]	5.60	3.53
n-3	2.48	4.79
n-6	2.14	2.42
n-9	3.91	3.05
n-3 LC-PUFA	1.78	0.33
ARA/EPA	0.02	0.05
DHA/EPA	0.22	0.36
n-3/n-6	1.16	1.98

Contains: 14:1n-7, 14:1n-5, 15:1n-5, 16: OISO, 16:1n-5, 16:3n-1, 16:4n-3, 16:2n-4, 16:3n-4, 16:2n-6, 16:3n-3, 16:4n-1, 17:0, 18:1n-5, 18:3n-1, 18:4n1, 20:2n-9, and 20:3n-9. [1]Saturated: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0. [2]Monoenoics: 14:1n-7, 14:1n-5, 15:1n-5, 16:1n-7, 16:1n-5, 18:1n-9, 18:1n-7, 18:1n-5, 22:1n-11, 22:1n-9. [3]Total PUFA: 18:2n-9, 18:2n-6, 18:2n-4, 18:3n-6, 18:3n-4, 18:3n-3, 18:3n-1, 18:4n-3, 18:4n-1, 20:2n-9, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:4n-3, 20:5n-3, 22:1n-11, 22:1n-9, 22:4n-6, 22:5n-6, 22:5n-3, 22:5n-6. Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil diet; LC-PUFA, long-chain poly unsaturated fatty acids; LO, linseed oil diet; PUFA, polyunsaturated fatty acids.

respectively. Finally, n-6 fatty acids were also increased in the LO diet in comparison to the FO diet, due to a 30% increase in 18:2n-6 and reductions of 90% in 20:2n-6, 65% in 20:3n-6, 64% in 20:4n-6, 64% in 22:4n-6 and 60% in 22:5n-6 (Table 2). The diets were produced in the Pilot Product and Processing Plant at Ecoaqua Institute. The ingredients were weighed, hand-mixed, and pelletized to the desired size (CL3 CPM, Eriez Magnetics, Caerphilly Mid-Glam, U. K). The corresponding 3 mm size pellets were dried in an air stove at 38 °C for 24 h before being stored at 11 °C until used.

2.3. Growth and feed utilization

At the end of the trial, growth and productive parameters were determined ($n = 3$ aquaria per treatment) according to the following equations:

Weight gain (WG) (%) = 100*(Final weight (g) -Initial weight (g)) / Initial weight (g).

Specific growth rate (SGR) (%/day) = ((lnWf –lnWi)/ days of experiment) x100.

where:

lnWf: Final weight neperian logarithm.

lnWi: Initial weight neperian logarithm.

Feed intake (FI) (%) = (Estimated feed consumption (g) / fish weight average (g)) / days x 100.

Feed conversion ratio (FCR) = Estimated feed consumption (g) / Weight gain (g).

2.4. Biochemical composition

Once sacrificed by immersion in ice-cold water, pools of 3 fish per tank were stored at −80 °C for whole-body proximate and fatty acid composition (9 fish and 3 pools per treatment). The whole-fish samples were homogenized and analysed by a Food Scan™ (FOSS, Hillerød, Denmark) to determine moisture, lipids, and protein contents. Ash content of the whole-body homogenates was determined by incineration in an oven at 600 °C for 24 h (method 942.05, Association of Official Analytical Chemists AOAC, 2005), so the percentage of the remaining inorganic fraction was calculated by the formula: % Ash = (ash weight/ sample weight) x 100.

A total of 4 fish per tank were dissected on ice to obtain samples of muscle and liver and stored at −80 °C until analysed. The proximate composition of diets, as well as muscle and liver samples in pools of 4 fish per tank (12 fish and 3 pools per treatment), was analysed following standard techniques (Association of Official Analytical Chemists AOAC, 2005). To determine crude proteins (Kjeldahl technique, N factor = 6.25; method 2001.11; Association of Official Analytical Chemists AOAC, 2005), homogenized samples were digested in the presence of a catalytic tablet (Panreac, Barcelona, Spain) with 10 ml sulfuric acid at 400 °C for 60 min. Subsequently, the digested material was distilled in 1% boric acid with 20 ml of distilled water and 50 ml of sodium hydroxide (40% weight/volume). Finally, the ammonia released was quantified through titration with hydrochloric acid (HCl) 0.1 M, and crude protein content was determined by the following formula:

% Protein = 100 x ((ml HCl sample–ml HCl blank) x 0.1 × 14.007 × 6.25) / sample weight.

Total lipids were extracted with chloroform/methanol (2:1 *v*/v) (Folch et al., 1957) with 0.01% butylated hydroxytoluene (BHT), homogenizing for 5 min in an Ultraturrax (T25 Digital Ultra-turrax, IKA®, Staufen, Germany). Then, 2 ml of 0.88% KCl were added to the homogenate, which was then centrifuged at 2000 rpm for 5 min. The lower phase with the lipids was filtered and evaporated to dryness under a nitrogen atmosphere. Lipids were weighed, and their percentage was calculated through the formula:

% Lipids = (lipids weight / sample weight) x 100.

Fatty acid compositions of the total lipids extracted (Folch et al., 1957) from diets, whole-body, liver, and muscle, were determined by trans-esterification incubating the lipids in methanol: sulfuric (1%) under conditions of darkness and a nitrogen atmosphere at 50 °C for 16 h (Christie, 1989). After cooling, ultrapure water and hexane: diethyl ether were added to the tubes that were centrifugated at 2000 rpm for 5 min to separate the fatty acid methyl esters in the superior phase. Then, the fatty acids methyl esters were evaporated to dryness under a nitrogen atmosphere, weighed and diluted in hexane at a final concentration of 40 mg/ml. The fatty acids methyl esters obtained were separated by gas liquid chromatography (Agilent 7820 A, Agilent technologies, Shanghai, China) under conditions previously described (Izquierdo et al., 1992), identified by comparison with previously characterized standards, and quantified using a flame ionization detector (Finnigan Focus SG, Thermo Electron Corporation, Milan, Italy). The quantified fatty acids were obtained as a percentage of the total identified fatty acids, and in the case of the whole-body, muscle, and liver were converted to mg/100 g of tissue using the following formula: Fatty acid (FA) in mg/100 g = ((FA in % total FA x Factor)/100) x % total lipid, were the Factor was 0.933–0.143/total lipids (Weihrauch et al., 1977).

2.5. Expression of selected genes

Expression of *Elovl5* and *Fads2* genes in livers from 3 fish per aquaria was determined by quantitative real-time PCR (qPCR). Elongation factor-1α (*ef1α*), β-actin (*actb*), and *18S r-RNA* were tested as potential reference genes to normalize the expression of *Elovl5* and *Fads2*. *18S r-RNA* was selected as the most stable gene according to the "Repeated Pair-Wise Correlation Analysis" (Pfaffl et al., 2004). Total RNA was extracted using TRI reagent (Sigma-Aldrich, Sant Louis, MO, USA) and the extraction kit RNAeasy® MiniKit from Quiagen. 1 μg of RNA of each sample was reverse transcribed to cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad Hercules, California, USA) in 20 μl reaction volume according to the manufacturer's instructions, to be later diluted 1/10 with miliQ water. Real-time PCRs were realized using a l-cycler with an optical module (Bio-Rad Hercules, California, USA) in a final volume of 15 μl containing 7.5 μl of iQTM-SYBER® Green Supermix (Bio-Rad Hercules, California, USA), 5 μl of cDNA, 0.75 μl of each primer (forward and reverse), and 1.0 μl of miliQ water. The conditions for the real-time reactions were adapted from referenced protocols to the conditions of the reagents used in the present study, being resumed in Table 3. All reactions were performed in duplicate for each sample, and blank control reactions were implemented, replacing the cDNA for miliQ water. Relative gene expression was determined by the $2 - \Delta\Delta$ CT method (Livak and Schmittgen, 2001).

2.6. Statistical analysis

The statistical analyses were conducted using the R Project Software for Statistical Computing (v4.2.3; R Core Team 2023). After normality and homoscedasticity tests, the variance analyses were performed using a two-way ANOVA to determine the individual and combined effects of diet and salinity, and the means were compared using the Tukey post-hoc test. Significant differences were considered when $P < 0.05$. Correlations were indicated by performing regression analysis and bivariate correlation determining the significance of the relationship (Deming, 1960; Beh, 2004).

3. Results

3.1. Growth and feed utilization

During the trial, no mortality was recorded due to the experimental diets or salinities and both experimental diets were well accepted by the mullets. Therefore, there were no significant differences in feed intake among fish from the different treatments (Table 4). Neither rearing water salinity affected feed intake. Despite growth parameters were not significantly different among fish of the different experimental treatments, the two-way ANOVA analyses detected that fish reared at the lowest salinity (LS treatments) significantly showed the highest final weight, weight gain and SGR values in comparison to fish reared at the other two salinities. Consequently, these fish also showed significantly lower FCR. Moreover, there was a significant interaction between diet and salinity in FCR, with fish fed diet FO at the lowest salinity (LS) performing better than those fed FO at intermediate salinity (MS) and those fed LO at high salinity (HS).

3.2. Biochemical composition

The proximate composition of mullets whole-body was not significantly affected by either diet or salinity (Table 5). However, the muscle proximate composition (Table 6) showed a significant increase in muscle lipid contents in fish fed the LO diet, particularly when reared at higher salinities (LO-MS, LO-HS), reflecting the interaction between both factors showed by the two-way ANOVA. Muscle ash content was also increased by feeding the LO diet and by rearing the fish at lower salinity (LS, MS vs HS), while muscle protein content was also enhanced

Table 3
Primer sequences and RT-PCR conditions of the different genes analysed.

Gene	Access. Number		Primer Sequence 5'-3'	Initial denaturation (°C) (duration in min)	*Denaturing temperature (°C) (duration in s)	*Anneling T (°C) (duration in s)	*Extension temperature (°C) (duration in s)	*Number of cycles	Amplification efficiency (%)	R_2	Reference
Elovl5	MT019563	F	AGAACGGCTCCTCCCTATCA	95 (3)	95 (15)	58.5 (45)	72 (30)	35	106.9	0.97	Galindo et al., 2021
		R	CAGCATTAGCTAACAGGCTACA								
Fads2	MH293504	F	GTGTCAAGGCTTCGCTGATG	95 (3)	95 (15)	58.5 (30)	72 (30)	35	109.8	0.99	Garrido et al., 2019
		R	AACGTCACTCCTTTCGCATACA								
18S-rRNA		F	CACATCCAAGGAAGGCAGCA	94 (2)	94 (30)	60 (30)	68 (30)	30	102.0	0.99	Raingeard et al., 2006
		R	AAGATACGCTATTGGAGCTG								

Table 4
Growth performance of fish held at 3 different salinities fed FO or LO diets for 73 days (10 fish/tank, 30 fish/treatment,90 fish/diet, 60 fish/salinity).

	Diet		Salinity			Two way Anova (*P* value)			Treatment means					
	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
Initial weight (g)	27.27 ± 1.94	27.68 ± 1.61	27.89 ± 2.73	27.69 ± 0.35	26.85 ± 1.41	ns	ns	ns	28.20 ± 3.23	27.42 ± 0.28	26.20 ± 1.23	27.58 ± 2.81	27.97 ± 0.06	27.49 ± 1.50
Final weight (g)	40.91 ± 6.84	41.39 ± 7.14	47.50^{a} ± 6.82	39.45^{b} ± 4.65	36.49^{b} ± 3.26	ns	<0.01	ns	48.33 ± 4.54	37.18 ± 4.84	37.20 ± 4.39	46.67 ± 9.68	41.73 ± 3.90	35.78 ± 2.42
Weight gain (%)	49.70 ± 19.98	49.43 ± 22.80	70.34^{a} ± 17.50	42.44^{b} ± 16.32	35.92^{b} ± 9.83	ns	<0.01	ns	71.69 ± 4.86	35.68 ± 18.30	41.72 ± 11.10	68.98 ± 27.14	49.20 ± 13.91	30.12 ± 4.17
SGR (%/day)	0.54 ± 0.18	0.54 ± 0.20	0.72^{a} ± 0.15	0.48^{b} ± 0.16	0.42^{b} ± 0.10	ns	<0.01	ns	0.74 ± 0.04	0.41 ± 0.18	0.47 ± 0.11	0.71 ± 0.23	0.54 ± 0.13	0.36 ± 0.04
Feed intake (% BW)	2.21 ± 0.03	2.18 ± 0.20	2.32 ± 0.12	2.16 ± 0.07	2.11 ± 0.11	ns	ns	ns	2.23 ± 0.20	2.20 ± 0.24	2.18 ± 0.12	2.41 ± 0.67	2.11 ± 0.35	2.03 ± 0.01
FCR	3.37 ± 1.02	3.22 ± 0.74	2.48^{b} ± 0.26	3.64^{a} ± 0.97	3.78^{a} ± 0.40	ns	<0.01	<0.05	2.29^{a} ± 0.05	4.32^{b} ± 1.15	3.49^{ab} ± 0.74	2.66^{ab} ± 0.43	2.95^{ab} ± 0.23	4.06^{b} ± 0.53

Values in a row followed by different superscripts indicate significant differences ($P < 0.05$). Abbreviations: FO, fish oil diet; LO, linseed oil diet; LS, low salinity; HS, high salinity; MS, medium salinity; BW, whole-body weight.

Table 5
Proximate whole-body composition (% WW) of fish held at 3 different salinities and fed FO or LO diets for 73 days (3 fish pools/tank, 9 fish pools/treatment, 27 fish pools/diet, 18 fish pools/salinity).

		Diet		Salinity			Two way ANOVA (P value)			Treatment means					
	Initial	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
Moisture	63.56 ± 0.35	68.38 ± 2.31	68.06 ± 1.28	67.23 ± 2.49	68.61 ± 1.65	68.68 ± 1.34	ns	ns	ns	67.04 ± 3.38	69.00 ± 1.78	69.10 ± 1.63	67.51 ± 1.31	68.21 ± 1.77	68.27 ± 1.15
Lipids	16.13 ± 0,67	10.80 ± 2.59	9.92 ± 1.36	11.82 ± 2.83	10.22 ± 1.87	9.36 ± 0.85	ns	ns	ns	12.39 ± 3.85	10.08 ± 2.46	9.94 ± 0.62	10.97 ± 0.07	10.37 ± 1.63	8.78 ± 0.64
Protein	15.45 ± 0.11	12.97 ± 2.18	12.46 ± 1.85	12.71 ± 2.24	12.72 ± 2.27	12.76 ± 1.85	ns	ns	ns	11.93 ± 1.40	14.27 ± 2.24	12.71 ± 2.77	13.88 ± 3.40	11.16 ± 0.79	12.82 ± 0.93
Ash	3.56 ± 0.21	5.23 ± 0.59	5.31 ± 0.72	4.72 ± 0.63	5.35 ± 0.54	5.64 ± 0.44	ns	ns	ns	4.83 ± 0.53	5.12 ± 0.51	5.74 ± 0.46	4.55 ± 0.96	5.58 ± 0.57	5.53 ± 0.50

Values in a row without different superscripts indicate the absence of significant differences ($P \geq 0.05$). Abbreviations: FO, fish oil diet; LO, linseed oil diet; LS, low salinity; HS, high salinity; MS, medium salinity; WW, wet weight basis.

Table 6
Proximate muscle composition (% WW) of fish held at 3 different salinities and fed FO or LO diets for 73 days (3 fish pools/tank, 9 fish pools/treatment,27 fish pools/diet, 18 fish pools/salinity).

		Diet		Salinity			Two way ANOVA (P value)			Treatment means					
	Initial	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
Moisture	74.90 ± 0.75	76.16 ± 2.05	75.08 ± 0.84	74.88 ± 0.79	75.94 ± 1.87	76.19 ± 1.51	ns	ns	ns	74.88 ± 0.29	76.74 ± 1.98	77.22 ± 1.76	75 ± 1.12	74.76 ± 0.2	75.49 ± 0.84
Lipids	4.55 ± 0.50	2.61^{a} ± 0.61	3.15^{b} ± 0.59	3.33 ± 0.26	2.84 ± 0.94	2.79 ± 0.49	<0.01	ns	<0.01	3.34 ± 0.12	2.01 ± 0.17	2.37 ± 0.14	2.80 ± 0.28	3.56 ± 0.46	3.10 ± 0.41
Protein	17.34 ± 0.24	20.83 ± 0.67	20.43 ± 0.9	21.33^{1} ± 21.33	20.61^{12} ± 0.66	19.94^{2} ± 19.94	ns	<0.01	ns	21.28 ± 0.39	20.66 ± 0.75	20.35 ± 0.19	21.21 ± 0.48	20.61 ± 0.45	19.55 ± 0.48
Ash	1.22 ± 0.10	1.45^{a} ± 0.43	1.98^{b} ± 0.47	1.81^{1} ± 1.81	1.62^{1} ± 0.52	1.47^{2} ± 1.47	<0.01	<0.05	ns	1.81 ± 0.13	1.19 ± 0.21	1.45 ± 0.19	2.27 ± 0.43	2.10 ± 0.12	1.60 ± 0.22

Values in a row with different superscripts indicate the presence of significant differences ($P < 0.05$). Abbreviations: FO, fish oil diet; LO, linseed oil diet; LS, low salinity; HS, high salinity; MS, medium salinity; WW, wet weight basis.

at lower salinity (LS vs HS). In addition, there was a significant interaction between salinity and diet in liver lipids (Table 7), with a 42% reduction of fish fed LO diet in comparison with those fed FO diet at the lowest salinity and a 36% increase at the medium salinity (LO-MS). Finally, ash content was reduced in the liver of fish fed the LO diet.

As denoted by the two-way ANOVA analysis, dietary replacement of FO by LO led to a 16% reduction in saturated fatty acids in whole-body of mullet, by the significant reduction in 14:0, 15:0, 16:0 and 20:0 (Table 8). Besides, monounsaturated fatty acids were also reduced by 21% in fish fed LO in comparison to those fed FO, by the reduction in 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 20:1n-7, 22:1n-11 and 22:1n-9. However, n-6 fatty acids, particularly 20:2n-6, 20:3n-6, 20:4n-6 and 22:4n-6, were only slightly reduced by 4% in fish fed the LO diet, n-3 fatty acids were increased, not only by the increase in 18:3n-3, but also by a 22% increase in 20:3n-3. On the contrary, there was no reduction of 18:4n-3, 20:5n-3 and 22:6n-3, despite the very low contents of these PUFA in the LO diet (Table 2). The two-way ANOVA analysis also showed that the increase in salinity from LS to HS led to an 18% reduction in saturated fatty acids, particularly 14:0, 15:0 and 16:0, in mullet whole-body, together with a 15% reduction in monounsaturated and n-9 fatty acids, such as 16:1n-7, 18:1n-9 and 18:1n-7. Fatty acids of the n-6 family, such as 18:2n-6, were also reduced (14%) in whole-body of mullet when salinity was increased. Moreover, increase in salinity reduced in a higher extend (21%) n-3 fatty acids, particularly 18:4n-3

Table 7
Proximate liver composition (% WW) of fish held at 3 different salinities and fed FO or LO diets for 73 days (3 fish pools/tank, 9 fish pools/treatment,27 fish pools/diet, 18 fish pools/salinity).

	Diet		Salinity			Two way ANOVA (P value)			Treatment means					
	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
Moisture	64.46 ± 2.47	65.62 ± 2.47	66.61 ± 2.52	65.78 ± 1.36	62.74 ± 1.28	ns	ns	ns	61.83 ± 5.69	66.74 ± 0.85	64.83 ± 2.89	63.65 ± 2.65	64.82 ± 4.11	68.39 ± 1.09
Lipids	13.55 ± 3.68	11.86 ± 1.94	14.02 ± 5.26	12.45 ± 2.24	11.66 ± 0.57	ns	ns	<0.05	17.74 ± 2.74	10.86 ± 1.36	12.06 ± 3.02	10.30 ± 2.72	14.03 ± 4.48	11.26 ± 2.70
Ash	1.27^{a} ± 0.27	0.85^{b} ± 0.10	0.88 ± 0.2	1.04 ± 0.25	1.04 ± 0.43	<0.05	ns	ns	1.03 ± 0.22	1.22 ± 0.40	1.56 ± 0.38	0.74 ± 0.37	0.86 ± 0.20	0.95 ± 0.41

Values in a row with different superscripts indicate the presence of significant differences ($P < 0.05$). Abbreviations: FO, fish oil diet; LO, linseed oil diet; LS, low salinity; HS, high salinity; MS, medium salinity; WW, wet weight basis.

(25%), 20:4n-3 (24%), 20:5n-3 (24%), and 22:5n-3 (26%) and 22:6n-3 (26%), all of them products of elongation and desaturation of 18:3n-3. Finally, the two-way ANOVA denoted the interaction between diet and salinity, and, therefore, the combined replacement of FO by LO and the increase in salinity further reduced saturated fatty acids by 30%, namely 16:0, and monounsaturated and n-9 fatty acids, mostly 18:1n-9 (31% reduction). Besides, the reduction in n-3 fatty acids, particularly in 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3, caused by salinity was stronger when fish were fed the FO diet; whereas in n-6 fatty acids, particularly 18:2n-6, such reduction was stronger when fish was fed LO.

In muscle (Table 9), feeding the LO diet increased saturated fatty acids by 9%, due to the increase in 16:0, 18:0, 20:0, and monounsaturated (5%) and n-9 fatty acids, such as 18:1n-9. On the contrary, in these fish fed LO, there was a 33% increase in n-3 fatty acids in muscle, due to the increase in 18:3n-3 together with a 63% increase in 20:3n-3, a product of elongation of the former fatty acid. Similarly, muscle of fish fed LO showed a 22% higher content in n-6 fatty acids, due primarily to the increase in 18:2n-6 (30%). However, the ratio n-3/n-6 in muscle of fish fed the LO diet was still higher than those fed the FO diet. Regarding other PUFA, there were only 16% reductions in n-3 LC-PUFA, as well as 20:4n-3, 22:6n-3, 22:5n-3, 20:5n-3 and 20:4n-3, despite strong reduction of these fatty acids in the LO diet (>80%, Table 2). The two-way ANOVA analysis only denoted very mild, although significant variations by salinity in saturated and monounsaturated fatty acids, such as 16:0 (3%) and 18:1n-9 (4%), respectively. However, n-3 and n-6 fatty acids were not significantly affected by salinity. Nevertheless, there was a significant interaction between diet and salinity denoted by the two-way ANOVA, and thus, whereas in fish fed FO the increase in salinity tended to reduce saturated and monounsaturated fatty acids, in fish fed LO salinity elevated the contents in these fatty acids. Similarly, 18:4n-3, 20:4n-3 and 20:5n-3 were reduced (5%) by salinity in fish fed FO but were increased (3%) by salinity in fish fed LO.

In liver (Table 10), feeding LO diet reduced by 16% monounsaturated fatty acids, such as 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 20:1n-7 and 22:1n-11. Besides, feeding LO increased by 6% 18:2n-6 and reduced by 40% 20:2n-6, by 33% 20:3n-6, by 28% 20:4n-6 and by 25% 22:4n-6 in liver. Moreover, feeding LO increased by 33% n-3 fatty acids, due to the increase by 4 times in 18:3n-3, but also increased by 110% 20:3n-3. On the contrary, 20:4n-3, 20:5n-3 and 22:5n-3 were significantly reduced by 38%, 23% and 36%, respectively, whereas 22:6n-3 was not affected. Besides, the ratios ARA/EPA, DHA/EPA and n-3/n-6 were increased by feeding LO. The increase in salinity reduced by 16% saturated fatty acids, such as 14:0, 15:0, 16:0, as well as monounsaturated (21%) and n-9 (21%) fatty acids, such as 16:1n-7, 18:1n-9, 18:1n-7; 20:1n-9, 22:1n-11, 22:1n-9. Moreover, 17% of 20:3n-6 contents in liver were reduced by increased salinity. Finally, there was a combined effect of diet and salinity, with a 33% reduction in saturated fatty acids caused in livers of fish fed FO by the increase in salinity, particularly, 14:0, 15:0, 16:0, 20:0, and a 5% reduction in monounsaturated and n-9 fatty acids, mostly 18:1n-9. None of these fatty acids were affect in fish fed LO. Furthermore, when fish were fed FO the increase in salinity reduced by 25% n-6 fatty acids, particularly 18:2n-6, as well as 18:3n-6 and 20:3n-6, products of elongation and desaturation; whereas in fish fed LO, only 18:2n-6 was reduced.

3.3. Expression of selected genes

Regarding the liver gene expression of *Elovl5* and *Fads2*, there were no statistical differences between the fish fed the two diets evaluated (Fig. 1). The increase in salinity significantly downregulated gene expression of *Elovl5* (Fig. 2), whereas salinity did not affect the expression of *Fads2*.

4. Discussion

4.1. Effect of dietary replacement of FO by LO

High FO replacement by VO, namely insufficient dietary levels of essential fatty acids, markedly reduces growth in most marine fish species, such as European seabass (*Dicentrarchus labrax*), black seabream (*Acanthopagrus schlegeli*) or several marine tropical carnivore species (Montero et al., 2005; Peng et al., 2008; Alhazzaa et al., 2019). In the present study, juveniles of thick-lipped grey mullet, an omnivorous and euryhaline species with worldwide distribution, were found to accept very well diets completely lacking FO, keeping a good growth, and denoting its great prospective for a more sustainable aquaculture. Thus, complete replacement of FO by LO in diets for the juvenile thick-lipped grey mullet, did not negatively affect feed intake, growth parameters or feed utilization. These results agree well with the good growth found in other fish species such as red tilapia (*Oreochromis mossambicus*♀ × *O. niloticus*♂) or gilthead seabream (*Sparus aurata*), when FO is completely replaced by VO (Yu et al., 2021; Carvalho et al., 2022). In gilthead seabream, despite the diet did not contain any FO, it had other sources of LC-PUFA derived from algae that allowed to cover the essential fatty acid requirements of this species (Carvalho et al., 2022). In the present study, similarly to the results obtained in red tilapia (Yu et al., 2021), thick-lipped grey mullet juveniles showed a certain ability to desaturase and elongate 18C fatty acids precursors of LC-PUFA that allowed to maintain the levels of these important fatty acids in fish tissues. This fact highlights the potential of thick-lipped grey mullet to efficiently utilize diets with low LC-PUFA content, which is of great interest to rear this species with more sustainable feeds.

Following dietary patterns, FO replacement by LO duplicated 18:3n-3 contents in whole-body of thick-lipped grey mullet and reduced saturated and monounsaturated fatty acids, in agreement with other fish species (Izquierdo et al., 2001b; Grigorakis, 2017). However, despite the strong dietary reduction on LC-PUFA in LO diet (down to 80% of the levels in FO diet), whole-body profiles of LO fed mullets showed that 20:3n-3 levels were increased and 18:4n-3, 20:5n-3 and 22:6n-3 maintained at the same level than in whole-body of fish fed FO. Being 20:3n-3 and 18:4n-3 products of elongation and desaturation from 18:3n-3, their relatively high contents in mullet fed the LO diet denote a LC-PUFA

Table 8
Whole-body fatty acid composition (g FAMES/100 g whole-body DW) of fish held at 3 different salinities and fed FO or LO diets for 73 days (3 fish pools/tank, 9 fish pools/treatment,27 fish pools/diet, 18 fish pools/salinity).

	Initial	Lipid source		Salinity			Two-way ANOVA (P value)			Treatment means					
	values	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
14:0	1.28	0.89 ± 0.07	0.65 ± 0.08	0.86^{2} ± 0.15	0.77^{12} ± 0.14	0.71^{1} ± 0.14	<0.01	<0.05	ns	0.96 ± 0.06	0.88 ± 0.05	0.83 ± 0.04	0.71 ± 0.04	0.66 ± 0.1	0.59 ± 0.07
15:0	0.14	0.11 ± 0.01	0.08 ± 0.01	0.10^{2} ± 0.02	0.10^{2} ± 0.01	0.09^{1} ± 0.02	<0.01	<0.01	ns	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.09 ± 0.00	0.08 ± 0.01	0.07 ± 0.01
16:0	6.77	5.23 ± 0.39	4.35 ± 0.53	5.42^{3} ± 0.37	4.75^{2} ± 0.4	4.38^{1} ± 0.66	<0.01	<0.01	<0.05	5.66^{b} ± 0.20	5.05^{a} ± 0.36	4.97^{ad} ± 0.11	5.04^{b} ± 0.06	4.46^{d} ± 0.04	3.79^{c} ± 0.16
16:1n-7	2.26	1.72 ± 0.17	1.21 ± 0.15	1.67^{2} ± 0.33	1.46^{12} ± 0.26	1.34^{1} ± 0.27	<0.01	<0.01	ns	1.90 ± 0.12	1.66 ± 0.12	1.58 ± 0.02	1.32 ± 0.11	1.25 ± 0.19	1.09 ± 0.06
18:0	0.95	0.95 ± 0.06	0.95 ± 0.12	1.01 ± 0.09	0.94 ± 0.10	0.90 ± 0.07	ns	ns	ns	0.96 ± 0.08	0.92 ± 0.08	0.96 ± 0.05	1.08 ± 0.06	0.96 ± 0.13	0.85 ± 0.03
18:1n-9	11.5	9.46 ± 0.56	7.95 ± 0.81	9.57^{3} ± 0.77	8.67^{2} ± 0.38	8.16^{1} ± 1.28	<0.01	<0.01	<0.01	10.11^{b} ± 0.27	8.97^{a} ± 0.28	9.31^{a} ± 0.27	8.76^{ad} ± 0.17	8.37^{d} ± 0.07	7.00^{c} ± 0.06
18:1n-7	1.33	1.16 ± 0.10	0.83 ± 0.08	1.12^{2} ± 0.22	0.98^{12} ± 0.17	0.93^{1} ± 0.17	<0.01	<0.05	ns	1.27 ± 0.03	1.12 ± 0.02	1.07 ± 0.1	0.88 ± 0.07	0.84 ± 0.12	0.78 ± 0.02
18:2n-9	0.09	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.05 ± 0.01	ns	ns	ns	0.07 ± 0.02	0.07 ± 0.03	0.06 ± 0.01	0.09 ± 0.01	0.07 ± 0.02	0.05 ± 0.01
18:2n-6	5.45	4.18 ± 0.23	4.10 ± 0.42	4.47^{3} ± 0.13	4.16^{2} ± 0.19	3.85^{1} ± 0.27	ns	<0.01	<0.01	4.44^{a} ± 0.17	4.02^{bc} ± 0.14	4.08^{ab} ± 0.11	4.52^{ac} ± 0.02	4.29^{ac} ± 0.15	3.63^{d} ± 0.13
18:2n-4	0.07	0.06 ± 0.00	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	<0.01	ns	ns	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	0.03 ± 0.00
18:3n-6	0.08	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	ns	ns	ns	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.01	0.04 ± 0.00
18:3n-4	0.06	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	<0.01	ns	ns	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
18:3n-3	1.39	1.04 ± 0.10	3.17 ± 0.93	2.13 ± 1.49	2.14 ± 1.53	1.86 ± 0.92	<0.01	ns	ns	1.09 ± 0.01	0.97 ± 0.05	1.07 ± 0.17	3.70 ± 0.86	3.32 ± 1.32	2.66 ± 0.44
18:4n-3	0.4	0.21 ± 0.04	0.21 ± 0.02	0.24^{2} ± 0.04	0.21^{12} ± 0.02	0.18^{1} ± 0.02	ns	<0.01	<0.01	0.26^{b} ± 0.02	0.20^{ac} ± 0.01	0.18^{a} ± 0.03	0.20^{ac} ± 0.00	0.23^{c1} ± 0.01	0.19^{ac} ± 0.00
20:0	0.1	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	<0.01	ns	ns	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.08 ± 0.00	0.08 ± 0.01	0.07 ± 0.01
20:1n-9	0.1	0.10 ± 0.01	0.07 ± 0.01	0.09 ± 0.03	0.08 ± 0.02	0.08 ± 0.02	<0.01	ns	ns	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.00	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
20:1n-7	1.01	0.95 ± 0.07	0.62 ± 0.09	0.83 ± 0.22	0.78 ± 0.18	0.77 ± 0.20	<0.01	ns	ns	0.98 ± 0.08	0.92 ± 0.09	0.95 ± 0.06	0.60 ± 0.07	0.64 ± 0.14	0.60 ± 0.08
20:1n-5	0.12	0.12 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	<0.01	ns	ns	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.00
20:2n-6	0.24	0.21 ± 0.02	0.15 ± 0.02	0.19 ± 0.04	0.18 ± 0.03	0.18 ± 0.04	<0.01	ns	ns	0.22 ± 0.02	0.21 ± 0.02	0.21 ± 0.01	0.15 ± 0.01	0.16 ± 0.03	0.14 ± 0.01
20:3n-6	0.06	0.05 ± 0.00	0.03 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	<0.01	ns	ns	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.0
20:4n-6	0.23	0.18 ± 0.02	0.14 ± 0.02	0.17 ± 0.04	0.16 ± 0.03	0.14 ± 0.02	<0.01	ns	ns	0.20 ± 0.02	0.17 ± 0.02	0.16 ± 0.01	0.14 ± 0.01	0.14 ± 0.03	0.13 ± 0.01
20:3n-3	0.11	0.09 ± 0.01	0.11 ± 0.02	0.10 ± 0.01	0.10 ± 0.02	0.09 ± 0.01	<0.01	ns	ns	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.12 ± 0.02	0.12 ± 0.02	0.10 ± 0.01
20:4n-3	0.23	0.16 ± 0.03	0.12 ± 0.02	0.16^{2} ± 0.05	0.14^{12} ± 0.02	0.12^{1} ± 0.02	<0.01	<0.05	<0.05	0.19^{b} ± 0.01	0.15^{ab} ± 0.01	0.14^{a} ± 0.02	0.11^{a} ± 0.01	0.13^{a} ± 0.03	0.11^{a} ± 0.01
20:5n-3	1.93	0.97 ± 0.22	0.89 ± 0.14	1.07^{2} ± 0.24	0.94^{12} ± 0.14	0.81^{1} ± 0.11	ns	<0.05	<0.05	1.23^{b} ± 0.08	0.90^{ab} ± 0.09	0.78^{a} ± 0.16	0.82^{a} ± 0.09	0.98^{ab} ± 0.2	0.84^{a} ± 0.03
22:1n-11	0.44	0.41 ± 0.04	0.25 ± 0.04	0.35 ± 0.1	0.32 ± 0.09	0.34 ± 0.09	<0.01	ns	ns	0.42 ± 0.03	0.38 ± 0.05	0.41 ± 0.04	0.24 ± 0.03	0.26 ± 0.07	0.26 ± 0.04
22:1n-9	0.12	0.12 ± 0.01	0.08 ± 0.01	0.10 ± 0.03	0.10 ± 0.02	0.10 ± 0.03	<0.01	ns	ns	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	0.08 ± 0.01
22:4n-6	0.04	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	<0.01	ns	ns	0.04 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00
22:5n-6	0.1	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	ns	ns	ns	0.09 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.07 ± 0.02	0.06 ± 0.00
22:5n-3	0.68	0.45 ± 0.11	0.38 ± 0.08	0.49^{2} ± 0.13	0.42^{12} ± 0.08	0.36^{1} ± 0.05	<0.05	<0.05	<0.05	0.58^{b} ± 0.02	0.42^{ab} ± 0.05	0.36^{a} ± 0.08	0.35^{a} ± 0.06	0.41^{ab} ± 0.12	0.36^{a} ± 0.03
22:6n-3	2.96	1.60 ± 0.45	1.54 ± 0.28	1.83^{2} ± 0.41	1.58^{12} ± 0.33	1.35^{1} ± 0.26	ns	<0.05	<0.05	2.11^{b} ± 0.11	1.45^{ab} ± 0.19	1.24^{a} ± 0.36	1.39^{ab} ± 0.12	1.71^{ab} ± 0.43	1.45^{ab} ± 0.10
Saturated	9.36	7.33 ± 0.45	6.17 ± 0.70	7.55^{3} ± 0.46	6.71^{2} ± 0.49	6.22^{1} ± 0.88	<0.01	<0.01	<0.05	7.87^{b} ± 0.17	7.12^{a} ± 0.32	7.01^{a} ± 0.16	7.06^{a} ± 0.06	6.3^{d} ± 0.03	5.43^{c} ± 0.24

(*continued on next page*)

Table 8 (*continued*)

	Initial	Lipid source		Salinity			Two-way ANOVA (P value)			Treatment means					
	values	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
Monoenoics	17.1	14.17 ± 0.82	11.19 ± 1.04	13.97^{3} ± 1.69	12.61^{2} ± 1.06	11.92^{1} ± 2.10	<0.01	<0.01	<0.01	15.19^{b} ± 0.23	13.52^{a} ± 0.23	13.81^{a} ± 0.48	12.15^{d} ± 0.47	11.70^{d} ± 0.54	10.03^{c} ± 0.27
n-3	7.86	4.62 ± 0.89	6.48 ± 0.71	6.11^{2} ± 0.68	5.62^{2} ± 1.55	4.85^{1} ± 1.12	<0.01	<0.01	<0.05	5.67^{b} ± 0.19	4.26^{a} ± 0.37	3.93^{a} ± 0.64	6.76^{bc} ± 0.59	6.99^{c} ± 0.53	5.78^{bc} ± 0.36
n-6	6.3	4.86 ± 0.29	4.63 ± 0.43	5.13^{3} ± 0.17	4.76^{2} ± 0.17	4.42^{1} ± 0.34	<0.01	<0.01	<0.01	5.19^{b} ± 0.21	4.68^{a} ± 0.21	4.71^{a} ± 0.10	5.05^{d} ± 0.04	4.85^{c} ± 0.08	4.12^{c} ± 0.14
n-9	11.9	9.80 ± 0.58	8.20 ± 0.83	9.89^{3} ± 0.81	8.96^{2} ± 0.41	8.43^{1} ± 1.34	<0.01	<0.01	<0.01	10.46^{c} ± 0.28	9.29^{b} ± 0.28	9.64^{ab} ± 0.3	9.03^{be} ± 0.18	8.62^{e} ± 0.07	7.22^{d} ± 0.06
n-3 LC-PUFA	5.91	3.28 ± 0.81	3.03 ± 0.51	3.64 ± 0.81	3.18 ± 0.56	2.74 ± 0.42	ns	<0.05	<0.05	4.21^{b} ± 0.18	3.01^{ab} ± 0.34	2.61^{a} ± 0.61	2.78^{ab} ± 0.27	3.35^{ab} ± 0.76	2.86^{a} ± 0.17
ARA/EPA	0.12	0.19 ± 0.03	0.15 ± 0.01	0.16 ± 0.02	0.17 ± 0.02	0.18 ± 0.04	<0.01	ns	ns	0.16 ± 0.02^{ab}	0.19 ± 0.01^{ab}	0.21 ± 0.04^{a}	0.17 ± 0.01^{ab}	0.15 ± 0.00^{b}	0.15 ± 0.00^{b}
DHA/EPA	1.53	1.63 ± 0.11	1.73 ± 0.07	1.72 ± 0.07	1.67 ± 0.09	1.65 ± 0.14	ns	ns	ns	1.72 ± 0.09	1.62 ± 0.06	1.57 ± 0.14	1.71 ± 0.04	1.73 ± 0.09	1.74 ± 0.08
n-3/n-6	1.25	0.95 ± 0.14	1.40 ± 0.09	1.19 ± 0.16	1.18 ± 0.3	1.12 ± 0.33	<0.01	ns	<0.05	1.10 ± 0.07^{ac}	0.91 ± 0.04^{a}	0.84 ± 0.15^{a}	1.34 ± 0.13^{c}	1.44 ± 0.10^{bc}	1.40 ± 0.07^{bc}

Data expressed as means+SD. Contains: 14:1n-7, 14:1n-5, 15:1n-5, 16:0ISO, 16:1n-5, 16:3n-1, 16:4n-3, 16:2n-4, 16:3n-4, 16:2n-6, 16:3n-3, 16:4n-1, 17:0, 18:1n-5, 18:3n-1, 18:4n1, 20:2n-9, and 20:3n-9. [1]Saturated: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0. [2]Monoenoics: 14:1n-7, 14:1n-5, 15:1n-5, 16:1n-7, 16:1n-5, 18:1n-9, 18:1n-7, 18:1n-5, 22:1n-11, 22:1n-9. [3]Total PUFA: 18:2n-9, 18:2n-6, 18:2n-4, 18:3n-6, 18:3n-4, 18:3n-3, 18:3n-1, 18:4n-3, 18:4n-1, 20:2n-9, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:4n-3, 20:5n-3, 22:1n-11, 22:1n-9, 22:4n-6, 22:5n-6, 22:5n-3, 22:5n-6. Values in a row with different lowercase letters denote interactions diet x salinity ($P < 0.05$), while different numbers denote significant differences between culture salinities ($P < 0.05$). Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; ns, not significant; PUFA, polyunsaturated fatty acids; FO, fish oil diet; LO, linseed oil diet; LS, low salinity; HS, high salinity; MS, medium salinity; FAMES, fatty acid methyl esters; DW, dry weight basis.

synthesis activity that allowed grey mullet juveniles to sustain the levels of these important fatty acids in whole-body while keeping good growth rates. Additionally, despite replacement of FO by LO lead to a huge dietary increase in ARA/EPA and DHA/EPA ratios, both ratios were very similar between fish fed FO or LO diets, denoting a compensatory mechanism to preserve physiological LC-PUFA's ratios, previously described in other marine species (Montero and Izquierdo, 2010; Benítez-Dorta et al., 2013).

Dietary replacement of FO by LO also increased by 17% the lipid contents in muscle, by the incorporation of 18:2n-6 and 18:3n-3 (with a 30% and 33% increase in relation to muscle of fish fed FO). These results denote a specific incorporation of these fatty acids into muscle, a phospholipid rich tissue, when their dietary levels are high and those of LC-PUFA are low, in agreement with the high affinity for these fatty acids of the enzymes involved in phospholipids and lipoprotein synthesis pathways (Caballero et al., 2006a, 2006b). Increase in muscle lipid contents in fish fed diets where FM and FO were replaced by plant ingredients has also been found in other marine fish species, such as turbot (*Psetta maxima*) or Senegalese sole (*Solea senegalensis*) (Grigorakis, 2017). In the pacu (*Piaractus mesopotamicus*), the 7% increase in muscle lipids was related to the increase in 18:2n-6 and 18:3n-3 (Gonçalves et al., 2021), which is in agreement with the present study's results. Besides, other intermediate fatty acids such as 20:3n-3, 20:3n-6, and 22:4n-6, products of desaturation and elongation of 18:3n-3 or 18:2n-6, were significantly increased in muscle of mullet fed the LO diet in comparison to those fed FO, further supporting the increased LC-PUFA synthesis activity suggested by whole-body fatty acids composition.

Fatty acid profiles of livers from fish fed the LO diet followed a similar pattern than whole-body and muscle, with significant increases in 18:4n-3 and 20:3n-3, direct products of desaturation and elongation from 18:3n-3, and constant 22:6n-3 levels, in comparison to fish fed the FO diet. This fact supports the important role of liver in LC-PUFA biosynthesis and denoted the potential of grey mullet to synthesize n-3 LC-PUFA when these fatty acids are limited in the diet, but their precursors are provided. These results agree well with the tendency for a higher expression of both *Elovl5* and *Fads2* found in liver of mullet fed LO diet, which were enough to maintain sufficient LC-PUFA tissue levels and growth. Besides, they further support the potential of thick-lipped grey mullet to synthesize LC-PUFA from their precursors suggested by previous studies (Rabeh et al., 2015), and the promoting effect of FO replacement by VO on LC-PUFA production (Izquierdo et al., 2005, 2008; Eroldoğan et al., 2012). In contrast with the other tissues, the DHA/EPA ratio was higher in the liver of fish fed LO, highlighting the preferential conservation of DHA in comparison to EPA in this species, as described for other species (Izquierdo et al., 2001a; Fernández-Palacios et al., 2011; Quirós-Pozo et al., 2023a).

The results of the present study also suggested that thick-lipped grey mullet has low requirements for n-3 LC-PUFA, since a reduction in dietary n-3 LC-PUFA from 1.78% in FO diet to 0.33% in LO diet did not significantly reduce growth, increase liver lipid contents, reduce 20:5n-3 or 22:6n-3, or cause any of the other essential fatty acid deficiency symptoms observed in fish (Izquierdo et al., 2005). These n-3 LC-PUFA dietary levels are lower than those described for other mullets' juveniles like *Mugil liza* (Rosas et al., 2019) or other marine fish species with a lower ability to synthetize LC-PUFA such as gilthead seabream (Izquierdo and Koven, 2010), meagre (*Argyrosomus regius*) (Carvalho et al., 2018; Pfalzgraff et al., 2023) or European seabass (Skalli and Robin, 2004).

4.2. Effect of rearing salinity

Regardless of the diet fed in the present study, thick-lipped grey mullet juveniles reared at 16 ppt showed significantly better growth and feed utilization than when they were reared at 35 ppt or 46 ppt. These results agree well with the improved growth in flathead grey mullet

Table 9
Muscle fatty acid composition (g of FAMES/100 g of muscle in DW), for the different experimental groups ($n = 12$ per treatment) at the end of the experiment.

	Initial	Lipid source		Salinity			Two-way ANOVA (P value)			Treatment means					
	values	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
14:0	0.42	0.21 ± 0.05	0.21 ± 0.05	0.22 ± 0.06	0.19 ± 0.04	0.22 ± 0.04	ns	ns	<0.05	0.26^{b} ± 0.03	0.15^{a} ± 0.02	0.21^{ab} ± 0.03	0.18^{ab} ± 0.06	0.22^{ab} ± 0.02	0.24^{ab} ± 0.04
15:0	0.05	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	ns	ns	<0.01	0.04^{b} ± 0.00	0.02^{ab} ± 0.00	0.03^{a} ± 0.00	0.02^{a} ± 0.01	0.03^{ab} ± 0.00	0.03^{ab} ± 0.00
16:0	2.79	1.65 ± 0.38	1.78 ± 0.22	1.82^{2} ± 0.32	1.60^{1} ± 0.41	1.73^{12} ± 0.11	<0.05	<0.01	<0.01	2.09^{c} ± 0.10	1.23^{b} ± 0.06	1.64^{a} ± 0.05	1.55^{a} ± 0.19	1.97^{c} ± 0.07	1.81^{ac} ± 0.08
16:1n-7	0.8	0.45 ± 0.10	0.42 ± 0.08	0.46 ± 0.14	0.40 ± 0.07	0.45 ± 0.04	ns	ns	<0.01	0.57^{a} ± 0.03	0.35^{b} ± 0.05	0.44^{ab} ± 0.03	0.35^{b} ± 0.1	0.45^{ab} ± 0.03	0.46^{ab} ± 0.06
18:0	0.45	0.37 ± 0.07	0.47 ± 0.04	0.43 ± 0.03	0.41 ± 0.12	0.43 ± 0.05	<0.01	ns	<0.01	0.42^{b} ± 0.04	0.30^{a} ± 0.05	0.38^{a} ± 0.04	0.44^{b} ± 0.02	0.51^{b} ± 0.02	0.47^{b} ± 0.03
18:1n-9	4.8	2.78 ± 0.59	3.11 ± 0.39	3.06^{2} ± 0.46	2.83^{1} ± 0.76	2.94^{1} ± 0.26	<0.01	<0.01	<0.01	3.48^{c} ± 0.03	2.14^{b} ± 0.07	2.72^{a} ± 0.06	2.65^{a} ± 0.06	3.52^{c} ± 0.05	3.15^{a} ± 0.14
18:1n-7	0.56	0.31 ± 0.07	0.3 ± 0.06	0.31 ± 0.09	0.28 ± 0.06	0.32 ± 0.03	ns	ns	<0.01	0.39^{b} ± 0.01	0.23^{bc} ± 0.02	0.30^{a} ± 0.01	0.24^{c} ± 0.03	0.34^{a} ± 0.01	0.33^{a} ± 0.03
18:2n-9	0.04	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	<0.01	ns	<0.01	0.03^{ab} ± 0.01	0.01^{a} ± 0.00	0.02^{ab} ± 0.00	0.03^{ab} ± 0.01	0.04^{ab} ± 0.01	0.03^{b} ± 0.01
18:2n-6	1.96	1.25 ± 0.24	1.63 ± 0.22	1.47 ± 0.08	1.44 ± 0.50	1.41 ± 0.19	<0.01	ns	<0.01	1.52^{cd} ± 0.04	0.99^{b} ± 0.04	1.24^{a} ± 0.03	1.42^{b} ± 0.08	1.90^{e} ± 0.05	1.58^{c} ± 0.05
18:2n-4	0.03	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	<0.01	ns	<0.01	0.02^{a} ± 0.00	0.01^{b} ± 0.00	0.02^{ab} ± 0.00	0.01^{b} ± 0.00	0.01^{b} ± 0.00	0.02^{b} ± 0.00
18:3n-6	0.02	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	<0.01	<0.05	<0.01	0.02^{b} ± 0.00	0.01^{b} ± 0.00	0.02^{a} ± 0.00	0.02^{a} ± 0.00	0.02^{b} ± 0.00	0.02^{ab} ± 0.00
18:3n-4	0.02	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	ns	ns	ns	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
18:3n-3	0.49	0.31 ± 0.08	1.25 ± 0.39	0.76 ± 0.46	0.93 ± 0.76	0.65 ± 0.46	<0.01	ns	ns	0.39 ± 0.05	0.23 ± 0.04	0.29 ± 0.02	1.12 ± 0.35	1.62 ± 0.16	1.01 ± 0.36
18:4n-3	0.13	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	ns	ns	<0.01	0.08^{b} ± 0.01	0.05^{a} ± 0.01	0.06^{ab} ± 0.01	0.06^{ab} ± 0.01	0.07^{ab} ± 0.01	0.07^{ab} ± 0.01
20:0	0.05	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	<0.01	ns	<0.01	0.03^{b} ± 0.00	0.02^{a} ± 0.00	0.03^{ab} ± 0.00	0.03^{a} ± 0.00	0.03^{b} ± 0.00	0.03^{b} ± 0.00
20:1n-9	0.05	0.02 ± 0.01	0.02 ± 0.00	0.02^{12} ± 0.01	0.02^{2} ± 0.00	0.03^{1} ± 0.00	ns	<0.05	<0.01	0.03^{b} ± 0.00	0.02^{a} ± 0.00	0.02^{ab} ± 0.00	0.02^{a} ± 0.00	0.02^{ab} ± 0.00	0.03^{b} ± 0.00
20:1n-7	0.45	0.25 ± 0.04	0.22 ± 0.04	0.24 ± 0.07	0.22 ± 0.02	0.26 ± 0.03	ns	ns	<0.01	0.30^{b} ± 0.02	0.20^{ac} ± 0.01	0.25^{ab} ± 0.00	0.18^{c} ± 0.00	0.23^{ac} ± 0.01	0.26^{ab} ± 0.05
20:1n-5	0.05	0.03 ± 0.01	0.03 ± 0.01	0.03^{12} ± 0.01	0.03^{2} ± 0.00	0.04^{1} ± 0.00	ns	<0.05	<0.01	0.04^{b} ± 0.00	0.03^{a} ± 0.00	0.03^{ab} ± 0.00	0.02^{c} ± 0.00	0.03^{a} ± 0.00	0.04^{b} ± 0.01
20:2n-6	0.02	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.00	ns	ns	<0.01	0.08^{b} ± 0.01	0.06^{a} ± 0.00	0.07^{abc} ± 0.00	0.06^{ac} ± 0.00	0.07^{c} ± 0.00	0.07^{b} ± 0.01
20:3n-6	0.11	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	<0.01	ns	<0.01	0.03^{b} ± 0.00	0.02^{a} ± 0.00	0.02^{ab} ± 0.00	0.01^{a} ± 0.00	0.02^{a} ± 0.00	0.02^{a} ± 0.00
20:4n-6	0.13	0.12 ± 0.02	0.10 ± 0.01	0.11 ± 0.02	0.10 ± 0.01	0.11 ± 0.02	<0.01	ns	<0.05	0.13^{b} ± 0.01	0.10^{a} ± 0.02	0.12^{ab} ± 0.02	0.09^{a} ± 0.01	0.10^{a} ± 0.00	0.10^{ab} ± 0.01
20:3n-3	0.05	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	<0.01	ns	ns	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
20:4n-3	0.09	0.05 ± 0.01	0.04 ± 0.01	0.05^{2} ± 0.01	0.04^{1} ± 0.00	0.05^{12} ± 0.01	<0.01	<0.05	<0.01	0.06^{b} ± 0.00	0.04^{ac} ± 0.00	0.05^{ab} ± 0.00	0.04^{c} ± 0.00	0.04^{bc} ± 0.00	0.05^{bc} ± 0.01
20:5n-3	0.8	0.44 ± 0.07	0.37 ± 0.06	0.42 ± 0.10	0.37 ± 0.03	0.44 ± 0.06	<0.01	ns	<0.05	0.50^{b} ± 0.03	0.36^{a} ± 0.02	0.47^{ab} ± 0.02	0.34^{a} ± 0.08	0.37^{ab} ± 0.05	0.40^{ab} ± 0.06

(*continued on next page*)

Table 9 (*continued*)

	Initial	Lipid source		Salinity			Two-way ANOVA (P value)			Treatment means					
	values	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
22:1n-11	0.19	0.09 ± 0.02	0.08 ± 0.02	0.09 ± 0.03	0.08 ± 0.01	0.10 ± 0.02	ns	ns	<0.01	0.11^{b} ± 0.01	0.07^{a} ± 0.01	0.09^{abc} ± 0.00	0.06^{c} ± 0.00	0.08^{abc} ± 0.00	0.10^{ab} ± 0.03
22:1n-9	0.06	0.03 ± 0.01	0.03 ± 0.00	0.03^{12} ± 0.01	0.03^{2} ± 0.00	0.03^{1} ± 0.00	ns	<0.05	<0.01	0.04^{b} ± 0.00	0.03^{a} ± 0.00	0.03^{abc} ± 0.00	0.03^{a} ± 0.00	0.03^{abc} ± 0.00	0.04^{c} ± 0.00
22:4n-6	0.02	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	<0.05	ns	<0.01	0.02^{b} ± 0.00	0.01^{a} ± 0.00	0.01^{ab} ± 0.00	0.01^{a} ± 0.00	0.01^{ab} ± 0.00	0.01^{ab} ± 0.00
22:5n-6	0.06	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	ns	ns	ns	0.05 ± 0.00	0.04 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.01
22:5n-3	0.3	0.19 ± 0.02	0.16 ± 0.03	0.19 ± 0.04	0.16 ± 0.02	0.19 ± 0.03	<0.05	ns	ns	0.22 ± 0.01	0.17 ± 0.01	0.2 ± 0.00	0.16 ± 0.02	0.16 ± 0.02	0.18 ± 0.04
22:6n-3	1.46	0.98 ± 0.12	0.83 ± 0.13	0.95 ± 0.10	0.82 ± 0.13	0.95 ± 0.17	<0.05	ns	ns	1.02 ± 0.07	0.86 ± 0.10	1.08 ± 0.03	0.88 ± 0.08	0.78 ± 0.16	0.83 ± 0.16
Saturated	3.79	2.31 ± 0.49	2.54 ± 0.29	2.54^{2} ± 0.39	2.26^{1} ± 0.57	2.46^{12} ± 0.17	<0.01	<0.01	<0.01	2.86^{c} ± 0.11	1.75^{b} ± 0.05	2.31^{a} ± 0.05	2.22^{ac} ± 0.26	2.78^{d} ± 0.09	2.60^{ac} ± 0.09
Monoenoics	7.02	4.01 ± 0.83	4.26 ± 0.54	4.29^{1} ± 0.78	3.93^{2} ± 0.91	4.19^{1} ± 0.33	<0.01	<0.01	<0.01	5.01^{c} ± 0.05	3.1^{b} ± 0.10	3.93^{a} ± 0.09	3.58^{e} ± 0.05	4.76^{ce} ± 0.08	4.45^{d} ± 0.24
n-3	3.37	2.11 ± 0.27	2.81 ± 0.32	2.51 ± 0.25	2.45 ± 0.76	2.42 ± 0.29	<0.01	ns	<0.01	2.34^{b} ± 0.07	1.77^{a} ± 0.11	2.22^{a} ± 0.06	2.68^{cd} ± 0.25	3.14^{d} ± 0.16	2.61^{c} ± 0.29
n-6	2.39	1.59 ± 0.28	1.94 ± 0.23	1.80 ± 0.13	1.74 ± 0.52	1.74 ± 0.17	<0.01	ns	<0.01	1.90^{c} ± 0.07	1.26^{b} ± 0.02	1.59^{a} ± 0.05	1.70^{a} ± 0.07	2.21^{d} ± 0.04	1.89^{b} ± 0.04
n-9	4.97	2.87 ± 0.6	3.21 ± 0.40	3.16 ± 0.47^{3}	2.92 ± 0.78^{2}	3.04 ± 0.26^{1}	<0.01	<0.01	<0.01	3.59^{c} ± 0.03	2.20^{b} ± 0.06	2.82^{a} ± 0.07	2.73^{a} ± 0.07	3.63^{c} ± 0.04	3.26^{d} ± 0.14
n-3 LC-PUFA	2.7	1.70 ± 0.21	1.46 ± 0.19	1.65 ± 0.23	1.43 ± 0.17	1.66 ± 0.25	<0.05	ns	ns	1.83 ± 0.10	1.45 ± 0.14	1.83 ± 0.05	1.47 ± 0.16	1.42 ± 0.23	1.50 ± 0.26
ARA/EPA	0.16	0.27 ± 0.03	0.27 ± 0.03	0.27 ± 0.03	0.27 ± 0.03	0.26 ± 0.03	ns	ns	ns	0.27 ± 0.03	0.27 ± 0.03	0.26 ± 0.02	0.28 ± 0.04	0.27 ± 0.03	0.26 ± 0.03
DHA/EPA	1.83	2.23 ± 0.17	2.27 ± 0.37	2.33 ± 0.41	2.24 ± 0.22	2.18 ± 0.2	ns	ns	<0.05	2.05 ± 0.06	2.37 ± 0.14	2.28 ± 0.05	2.62 ± 0.40	2.10 ± 0.20	2.08 ± 0.26
n-3/n-6	1.41	1.34 ± 0.10	1.46 ± 0.12	1.40 ± 0.20	1.41 ± 0.08	1.39 ± 0.08	<0.05	ns	<0.01	1.23^{a} ± 0.06	1.40^{ab} ± 0.09	1.40^{ab} ± 0.01	1.57^{b} ± 0.09	1.42^{ab} ± 0.08	1.38^{ab} ± 0.13

Data expressed as means+ SD. Contains: 14:1n-7, 14:1n-5, 15:1n-5, 16:OISO, 16:1n-5, 16:3n-1, 16:4n-3, 16:2n-4, 16:3n-4, 16:2n-6, 16:3n-3, 16:4n-1, 17:0, 18:1n-5, 18:3n-1, 18:4n1, 20:2n-9, and 20:3n-9. [1]Saturated: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0. [2]Monoenoics: 14:1n-7, 14:1n-5, 15:1n-5, 16:1n-7, 16:1n-5, 18:1n-9, 18:1n-7, 18:1n-5, 22:1n-11, 22:1n-9. [3]Total PUFA: 18:2n-9, 18:2n-6, 18:2n-4, 18:3n-6, 18:3n-4, 18:3n-3, 18:3n-1, 18:4n-3, 18:4n-1, 20:2n-9, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:4n-3, 20:5n-3, 22:1n-11, 22:1n-9, 22:4n-6, 22:5n-6, 22:5n-3, 22:5n-6. Values in a row with different lowercase letters denote interactions diet x salinity ($P < 0.05$), while different numbers denote significant differences between culture salinities ($P < 0.05$). Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; ns, not significant; PUFA, polyunsaturated fatty acids; FO, fish oil diet; LO, linseed oil diet; LS, low salinity; HS, high salinity; MS, medium salinity; FAMES, fatty acid methyl esters; DW, dry weight basis.

(*Mugil cephalus*) juveniles reared in brackish water (Barman et al., 2005; Olukolajo and Omolara, 2013). In comparison to marine or hypersaline waters, the growth improvement (over 42% higher SGR) in grey mullet reared in brackish water, a close to isosmotic medium to fish, can be partly explained by the lower energetic cost necessary to maintain the osmotic regulation, which accounts for over 10% of the total fish energy budget (Bœuf and Payan, 2001). In agreement, in the present study the increase in rearing salinity from 16 ppt to 35 or 46 ppt led to significant reductions in saturated and monounsaturated fatty acids in all fish tissues, particularly 16:0 and 18:1n-9, main substrates fatty acids for beta-oxidation in fish (Froyland et al., 2000; Izquierdo et al., 2001b; Morais et al., 2006). The reduction in these fatty acids suggested a higher energetic demand in fish reared at non isosmotic mediums (35 or 46 ppt), since osmotic regulation uses lipid as an energy source (Brichon et al., 1980). These results agree with the reduction in lipid contents in whole-body of Nile tilapia reared in increased salinity (Gan et al., 2016). In contrast, rearing flathead grey mullet at extreme salinities (0 and 36 ppt) does not affect lipid contents or fatty acid profiles (Loi et al., 2022). This lack of effect must be related to the comparison between two non-isotonic salinities in flathead grey mullet, whereas in the present study an isotonic salinity was compare with two extreme salinities. Similarly, extreme salinity (55 ppt) also reduces digestive efficiency in thick-lipped grey mullet juveniles (Pujante et al., 2018). Moreover, salinity is a determining environmental factor in fish that directly affects receptors to regulate growth, affecting many hormones involved in both osmoregulation and growth regulation including growth hormone, IGF1, prolactin, calcitonin, ACTH, cortisol, gonadotropins, estrogens, TSH or thyroid hormones (Bœuf and Payan, 2001). For instance, plasma prolactin levels in coho salmon (*Oncorhynchus kisutch*) are markedly reduced in fish reared in seawater in comparison to freshwater (Young et al., 1989). Higher plasma prolactin levels in fish reared in freshwater induces the release of the parathyroid hormone related peptide that

Table 10
Liver fatty acid composition (g of FAMES/100 g of liver DW), for the different experimental groups (n = 12 per treatment) at the end of the experiment.

	Lipid source		Salinity			Two-way ANOVA (P value)			Treatment means					
	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
14:0	0.61 ± 0.16	0.48 ± 0.08	0.63 ± 0.23	0.49 ± 0.03	0.54 ± 0.11	<0.05	ns	<0.05	0.78^{b} ± 0.1	0.47^{a} ± 0.05	0.58^{ab} ± 0.14	0.45^{a} ± 0.12	0.50^{a} ± 0.02	0.50^{a} ± 0.05
15:0	0.08 ± 0.02	0.07 ± 0.01	0.09 ± 0.03	0.07 ± 0.01	0.08 ± 0.01	<0.01	ns	<0.01	0.10^{b} ± 0.01	0.07^{a} ± 0	0.08^{ab} ± 0.01	0.06^{a} ± 0.01	0.06^{a} ± 0	0.08^{a} ± 0.01
16:0	5.84 ± 1.45	5.6 ± 0.85	6.60 ± 1.44	5.43 ± 1.1	5.3 ± 0.67	ns	ns	<0.05	7.48^{b} ± 0.38	4.65^{a} ± 0.86	5.39^{ab} ± 1.02	5.18^{ab} ± 1.07	6.20^{a} ± 0.69	5.22^{a} ± 0.22
16:1n-7	1.65 ± 0.44	1.10 ± 0.06	1.73^{2} ± 0.59	1.20^{1} ± 0.09	1.30^{12} ± 0.34	<0.01	<0.01	<0.01	2.15^{b} ± 0.11	1.26^{a} ± 0.09	1.53^{a} ± 0.35	1.09^{a} ± 0.07	1.15^{a} ± 0.05	1.07^{a} ± 0.02
18:0	1.23 ± 0.17	1.49 ± 0.2	1.37 ± 0.20	1.29 ± 0.22	1.38 ± 0.27	<0.05	ns	ns	1.26 ± 0.07	1.25 ± 0.24	1.17 ± 0.21	1.35 ± 0.36	1.34 ± 0.24	1.60 ± 0.02
18:1n-9	10.39 ± 2.62	9.68 ± 1.25	11.62^{2} ± 3.00	9.56^{1} ± 1.50	9.25^{1} ± 0.85	ns	<0.01	<0.01	13.68^{c} ± 0.70	8.26^{b} ± 0.69	9.22^{ab} ± 1.24	8.74^{ab} ± 1.29	10.86^{a} ± 0.24	9.28^{ab} ± 0.5
18:1n-7	1.43 ± 0.31	1.05 ± 0.06	1.50^{2} ± 0.46	1.12^{1} ± 0.09	1.17^{1} ± 0.12	<0.01	<0.01	<0.01	1.83^{b} ± 0.08	1.19^{ac} ± 0.06	1.27^{a} ± 0.05	0.99^{ac} ± 0.03	1.06^{c} ± 0.06	1.07^{ac} ± 0.07
18:2n-9	0.19 ± 0.09	0.36 ± 0.17	0.32 ± 0.15	0.28 ± 0.22	0.22 ± 0.09	<0.05	ns	ns	0.28 ± 0.00	0.11 ± 0.04	0.18 ± 0.10	0.35 ± 0.20	0.45 ± 0.19	0.27 ± 0.05
18:2n-6	4.05 ± 0.77	4.29 ± 0.59	4.47 ± 0.73	3.86 ± 0.49	4.21 ± 0.78	ns	ns	<0.01	4.95^{b} ± 0.09	3.62^{a} ± 0.41	3.57^{a} ± 0.56	3.93^{ac} ± 0.52	4.11^{ac} ± 0.50	4.84^{bc} ± 0.04
18:2n-4	0.06 ± 0.01	0.03 ± 0.01	0.05 ± 0.03	0.04 ± 0.02	0.04 ± 0.01	<0.01	ns	ns	0.07 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
18:3n-6	0.1 ± 0.03	0.15 ± 0.03	0.13 ± 0.01	0.12 ± 0.06	0.11 ± 0.03	<0.01	ns	<0.01	0.13^{b} ± 0.01	0.07^{a} ± 0.01	0.08^{ab} ± 0.02	0.14^{abc} ± 0.03	0.17^{c} ± 0.03	0.13^{c} ± 0.01
18:3n-4	0.05 ± 0.01	0.03 ± 0.00	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	<0.01	ns	<0.01	0.06^{b} ± 0.00	0.04^{a} ± 0.00	0.04^{a} ± 0.00	0.02^{c} ± 0.00	0.03^{ac} ± 0.00	0.03^{c} ± 0.00
18:3n-3	0.87 ± 0.22	3.52 ± 0.64	1.82 ± 1.05	2.24 ± 1.65	2.24 ± 1.70	<0.01	ns	ns	1.10 ± 0.09	0.78 ± 0.23	0.73 ± 0.13	3.31 ± 0.88	3.70 ± 0.57	3.75 ± 0.58
18:4n-3	0.17 ± 0.03	0.23 ± 0.04	0.2 ± 0.02	0.2 ± 0.06	0.19 ± 0.05	<0.01	ns	<0.01	0.21^{b} ± 0.01	0.15^{a} ± 0.03	0.15^{a} ± 0.01	0.21^{d} ± 0.06	0.26^{c} ± 0.01	0.24^{c} ± 0.03
20:0	0.08 ± 0.01	0.08 ± 0.01	0.08^{2} ± 0.01	0.08^{1} ± 0.01	0.08^{12} ± 0.01	<0.05	<0.05	<0.05	0.09^{ab} ± 0.00	0.07^{a} ± 0.01	0.07^{a} ± 0.01	0.07^{ab} ± 0.02	0.08^{ab} ± 0.00	0.09^{b} ± 0.00
20:1n-9	0.14 ± 0.04	0.06 ± 0.01	0.13^{2} ± 0.07	0.09^{1} ± 0.03	0.09^{1} ± 0.02	<0.01	<0.01	<0.01	0.18^{b} ± 0.01	0.12^{a} ± 0.01	0.11^{a} ± 0.01	0.05^{c} ± 0.01	0.06^{c} ± 0.01	0.07^{c} ± 0.01
20:1n-7	0.97 ± 0.19	0.58 ± 0.12	0.9 ± 0.42	0.71 ± 0.17	0.77 ± 0.13	<0.01	ns	<0.01	1.20^{b} ± 0.12	0.83^{ac} ± 0.09	0.87^{ad} ± 0.05	0.45^{d} ± 0.04	0.58^{c} ± 0.11	0.67^{ac} ± 0.10
20:1n-5	0.18 ± 0.04	0.11 ± 0.02	0.17 ± 0.08	0.14 ± 0.02	0.14 ± 0.04	<0.01	ns	<0.01	0.23^{b} ± 0.03	0.16^{ac} ± 0.01	0.17^{ab} ± 0.02	0.09^{bc} ± 0.00	0.12^{ac} ± 0.01	0.11^{ac} ± 0.02
20:2n-6	0.27 ± 0.05	0.19 ± 0.05	0.25 ± 0.09	0.22 ± 0.05	0.24 ± 0.05	<0.01	ns	ns	0.31 ± 0.03	0.26 ± 0.03	0.25 ± 0.07	0.16 ± 0.04	0.18 ± 0.02	0.23 ± 0.04
20:3n-6	0.06 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	<0.01	<0.05	<0.01	0.07^{b} ± 0.00	0.06^{a} ± 0.01	0.06^{a} ± 0.00	0.03^{c} ± 0.01	0.03c ± 0.00	0.05^{ac} ± 0.00
20:4n-6	0.42 ± 0.08	0.30 ± 0.11	0.39 ± 0.12	0.35 ± 0.13	0.36 ± 0.10	<0.05	ns	ns	0.41 ± 0.06	0.46 ± 0.03	0.38 ± 0.14	0.31 ± 0.18	0.24 ± 0.05	0.33 ± 0.05
20:3n-3	0.10 ± 0.02	0.21 ± 0.04	0.13 ± 0.02	0.15 ± 0.07	0.16 ± 0.08	<0.01	ns	<0.01	0.12^{a} ± 0.01	0.09^{a} ± 0.02	0.09^{a} ± 0.03	0.18^{ac} ± 0.05	0.22^{b} ± 0.02	0.23^{b} ± 0.02
20:4n-3	0.16 ± 0.04	0.10 ± 0.03	0.15 ± 0.07	0.12 ± 0.04	0.13 ± 0.02	<0.01	ns	<0.01	0.20^{a} ± 0.02	0.15^{ab} ± 0.03	0.14^{abc} ± 0.03	0.07^{c} ± 0.01	0.09^{c} ± 0.02	0.13^{b} ± 0.01
20:5n-3	1.11 ± 0.19	0.68 ± 0.25	0.96 ± 0.4	0.82 ± 0.35	0.95 ± 0.18	<0.01	ns	ns	1.21 ± 0.17	1.12 ± 0.14	1.00 ± 0.25	0.55 ± 0.28	0.51 ± 0.12	0.89 ± 0.1
22:1n-11	0.47 ± 0.11	0.19 ± 0.07	0.43^{2} ± 0.25	0.29^{1} ± 0.15	0.32^{12} ± 0.09	<0.01	<0.05	<0.01	0.41^{a} ± 0.07	0.39^{a} ± 0.03	0.16^{d} ± 0.06	0.17^{d} ± 0.08	0.24^{c} ± 0.04	0.24^{c} ± 0.04
22:1n-9	0.16 ± 0.04	0.13 ± 0.03	0.17^{2} ± 0.05	0.13^{1} ± 0.01	0.13^{12} ± 0.02	ns	<0.01	<0.05	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.04	0.12 ± 0.01	0.13 ± 0.03	0.13 ± 0.03
22:4n-6	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	<0.01	ns	ns	0.05 ± 0	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
22:5n-6	0.08 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.02	ns	ns	ns	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.00
22:5n-3	0.55 ± 0.14	0.35 ± 0.13	0.46 ± 0.2	0.42 ± 0.21	0.49 ± 0.12	<0.01	ns	ns	0.59 ± 0.08	0.57 ± 0.2	0.5 ± 0.17	0.25 ± 0.08	0.28 ± 0.09	0.47 ± 0.09
22:6n-3	2.94 ± 0.78	2.83 ± 1.29	2.96 ± 1.34	2.58 ± 0.84	3.14 ± 0.99	ns	ns	ns	2.84 ± 0.6	3.25 ± 0.62	2.73 ± 1.22	2.67 ± 1.96	1.91 ± 0.13	3.55 ± 0.68
Saturated	7.88 ± 1.63	7.75 ± 0.83	8.82 ± 1.51	7.39 ± 1.26	7.43 ± 0.66	ns	<0.05	<0.05	9.76^{b} ± 0.48	6.56^{a} ± 1.01	7.33^{a} ± 1	7.13^{ab} ± 1.08	8.22^{ab} ± 0.94	7.52^{ab} ± 0.25
Monoenoics	15.53 ± 3.72	13.03 ± 1.26	16.81^{2} ± 4.84	13.36^{1} ± 1.0	13.30^{1} ± 1.13	<0.01	<0.01	<0.01	20.26^{b} ± 0.91	12.49^{a} ± 0.65	13.84^{a} ± 1.51	11.81^{a} ± 1.16	14.23^{a} ± 0.22	12.76^{a} ± 0.22
n-3	5.97 ± 1.27	7.95 ± 1.49	6.74 ± 1.42	6.59 ± 1.01	7.36 ± 2.45	<0.01	ns	ns	6.35 ± 0.92	6.17 ± 1.26	5.40 ± 1.81	7.28 ± 1.62	7.00 ± 0.66	9.32 ± 0.38
n-6	5.11 ± 0.87	5.17 ± 0.68	5.5 ± 0.93	4.8 ± 0.41	5.17 ± 0.86	ns	ns	<0.01	6.08^{b} ± 0.17	4.69^{ab} ± 0.47	4.54^{a} ± 0.8	4.77^{ab} ± 0.7	4.90^{ab} ± 0.42	5.8^{ab} ± 0.12
n-9	10.93 ± 2.79	10.29 ± 1.35	12.31 ± 3.11	10.11 ± 1.66	9.76 ± 0.91	ns	<0.01	<0.01	14.43^{b} ± 0.73	8.67^{a} ± 0.7	9.71^{ac} ± 1.35	9.31^{ac} ± 1.42	11.55^{c} ± 0.40	9.8^{ac} ± 0.50
n-3 LC-PUFA	4.87 ± 1.11	4.16 ± 1.64	4.66 ± 1.68	4.09 ± 1.36	4.87 ± 1.29	ns	ns	ns	4.96 ± 0.83	5.18 ± 1	4.46 ± 1.68	3.73 ± 2.31	3.0 ± 0.16	5.28 ± 0.91

(*continued on next page*)

Table 10 (*continued*)

	Lipid source		Salinity			Two-way ANOVA (P value)			Treatment means					
	FO	LO	LS	MS	HS	Diet	Salinity	DxS	FO-LS	FO-MS	FO-HS	LO-LS	LO-MS	LO-HS
ARA/EPA	0.38 ± 0.06	0.47 ± 0.14	0.45 ± 0.14	0.45 ± 0.13	0.37 ± 0.04	<0.05	ns	ns	0.15 ± 0.01	0.12 ± 0.02	0.12 ± 0.02	0.18 ± 0.02	0.17 ± 0.07	0.13 ± 0.01
DHA/EPA	2.62 ± 0.40	4.12 ± 0.84	3.44 ± 1.53	3.37 ± 0.84	3.3 ± 0.83	<0.01	ns	ns	1.01 ± 0.08	0.87 ± 0.06	0.83 ± 0.18	1.43 ± 0.32	1.36 ± 0.35	1.42 ± 0.12
n-3/n-6	1.17 ± 0.18	1.52 ± 0.11	1.28 ± 0.31	1.37 ± 0.12	1.39 ± 0.27	<0.01	ns	ns	0.45^{a} ± 0.05	0.40^{abc} ± 0.05	0.37^{a} ± 0.06	0.48^{c} ± 0.05	0.50^{c} ± 0.00	0.58^{bc} ± 0.01

Data expressed as means+ SD. Contains: 14:1n-7, 14:1n-5, 15:1n-5, 16:OISO, 16:1n-5, 16:3n-1, 16:4n-3, 16:2n-4, 16:3n-4, 16:2n-6, 16:3n-3, 16:4n-1, 17:0, 18:1n-5, 18:3n-1, 18:4n1, 20:2n-9, and 20:3n-9. [1]Saturated: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0. [2]Monoenoics: 14:1n-7, 14:1n-5, 15:1n-5, 16:1n-7, 16:1n-5, 18:1n-9, 18:1n-7, 18:1n-5, 22:1n-11, 22:1n-9. [3]Total PUFA: 18:2n-9, 18:2n-6, 18:2n-4, 18:3n-6, 18:3n-4, 18:3n-3, 18:3n-1, 18:4n-3, 18:4n-1, 20:2n-9, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:4n-3, 20:5n-3, 22:1n-11, 22:1n-9, 22:4n-6, 22:5n-6, 22:5n-3, 22:5n-6. Values in a row with different lowercase letters denote interactions diet x salinity ($P < 0.05$), while different numbers denote significant differences between culture salinities ($P < 0.05$). Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; ns, not significant; PUFA, polyunsaturated fatty acids; FO, fish oil diet; LO, linseed oil diet; LS, low salinity; HS, high salinity; MS, medium salinity. FAMES, fatty acid methyl esters;DW, dry weight basis.

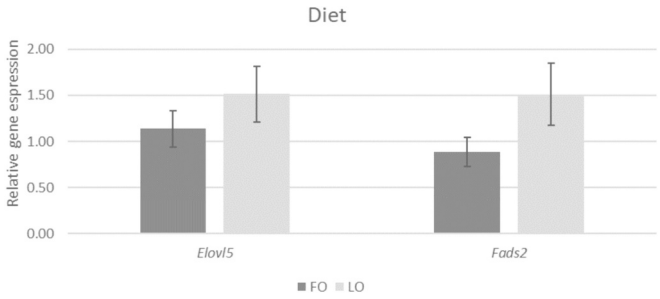

Fig. 1. Liver relative gene expression of *Elovl5* and *Fads2* for the fish fed the different diets at the end of the experiment ($n = 27$ per dietary treatment). Data are presented as normalized expression ratios ± standard error. Columns without superscripts indicate the absence of significant differences ($P \geq 0.05$). Abbreviations: FO, fish oil diet; LO, linseed oil diet.

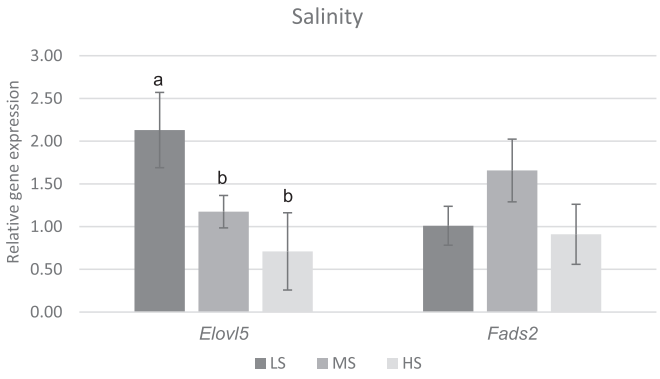

Fig. 2. Liver relative gene expression of *Elovl5* and *Fads2* for the fish reared under the different salinities at the end of the experiment ($n = 18$ per rearing salinity). Data are presented as normalized expression ratios ± standard error. Columns with different superscripts indicate the presence of significant differences ($P < 0.05$). Abbreviations: LS, low salinity; MS, medium salinity; HS, high salinity water.

enhances bone resorption.These results agree well with the reduction in ash content found in shyrbot (*Barbus grypos*) reared at lower salinities (Jalali et al., 2013). However, muscle ashes, related to mineral ions contents with calcium being required for the optimum functioning of muscle contraction, were higher in mullets reared at 16 ppt.

Thick-lipped grey mullet juveniles reared at 16 ppt also showed the highest contents in n-3 PUFA, 20:4n-3, 20:5n-3, and 22:5n-3 and 22:6n-3 in whole-body, and, particularly, 18:4n-3 in whole-body and 20:3n-6 in liver, products of desaturation and elongation from 18:3n-3 and 18:2n-6. On the contrary, the contents in n-3 PUFA were reduced with the increase in salinity, in agreement with the lower levels of 20:5n-3 and 22:6n-3 found in flathead grey mullet reared at 35 ppt and the down-regulation of *Fads2* and *Elovl5* found in thick-lipped grey mullet reared at 35 ppt instead of 20 ppt (Khériji et al., 2003; Marrero et al., 2024). Similarly, LC-PUFA biosynthesis and higher expression of *Δ4 Fads2, Δ6 Fads2* is found in rabbitfish (*Siganus canaliculatus*) reared at 10 ppt than in fish reared at 32 ppt (Xie et al., 2015). Moreover, in the present study, the content in each of these n-3 PUFA in whole-body followed significant negative lineal regressions with the salinity levels (R^2 = 0.98–0.99), denoting a proportional inhibition of n-3 PUFA biosynthesis. In agreement, expression of *Elovl5* in liver was also significantly downregulated by the increase in salinity, following a negative lineal regression ($R^2 = 0.99$). Since specific n-3 PUFA contents in cell membranes are required at different environmental conditions for the adequate fluidity and functioning of biomembranes, the biosynthesis of these fatty acids should be closely modulated by those environmental factors, particularly salinity. For instance, fatty acid profiles markedly differ between freshwater and marine environments (Borlongan and Benitez, 1992; Dantagnan et al., 2007) and accordingly essential fatty acid requirements of organisms differ when living in different salinity environments (Izquierdo and Koven, 2010; Dantagnan et al., 2010). Moreover, migration of fish between these environments requires a series of physiological adaptations, including changes in the membrane fatty acid profiles, not only to cope with osmoregulation, but also with food availability and their different fatty acid profiles. Indeed, n-3 LC-PUFA contents in gill are essential to regulate ion control in the sturgeon (*Acipenser naccarii*) adaptation to seawater (Martínez-Álvarez et al., 2005), and in salmonids this adaptation involves the nutritional and environmental modulation of highly unsaturated fatty acids (HUFA) biosynthesis (Zheng et al., 2005). As discussed above, environmental salinity couples with different receptors, which in turn modulate production or release of several hormones related with osmoregulation and growth, among them gonadotropins and estrogens (Bœuf and Payan, 2001). Estradiol plays an important role in maintaining osmoregulation mechanisms in teleosts (Carrera et al., 2007), and their plasma levels increase in fish reared at low and intermediate salinities (Su et al., 2019; Iffat et al., 2023). Similarly, estradiol levels increase in migrating female chum salmon (*Oncorhynchus keta*) when fish are transferred to freshwater (Choi et al., 2014). In mammals, estrogen injections induces the up regulation of fatty acid elongases and desaturases in the PUFA synthesis pathways (Harris et al., 2021). Besides, progesterone, modulated by gonadotropins, up-regulates *Fads2*, *Fads1*, *Elovl5* and *Elovl2* mRNA expression in mammals' hepatocytes, increasing the conversion of 18:3n-3 to 20:5n-3, 22:5n-3 and 22:6n-3 (Sibbons et al., 2014). Such up-regulation of hepatic *Elovl5* expression seems to be mediated by liver X Receptor α (LXRα) and sterol regulatory binding protein-1c (SREBP-1c) (Qin et al., 2009). In red tilapia, increased salinity

downregulates the expression of LXR (Yu et al., 2021), supporting our hypothesis of the regulation of LC-PUFA synthesis by rearing salinity in fish being mediated by steroid hormones.

4.3. Interaction between diet and rearing salinity

Combined replacement of FO by LO together with increased salinity further reduced 16:0 and 18:1n-9 in whole-body, suggesting an additional reduction in lipogenesis or rise in lipolysis. Replacement of FO by VO inhibits lipogenesis and promotes lipolysis in European seabass (Torrecillas et al., 2017) and in gilthead seabream, where the activity of the hepatic lipogenic enzymes glucose-6-phosphate dehydrogenase (*G6PD*) and fatty acid synthetase (*FAS*) was inhibited and that of carnitine palmitoyltransferase (*CPT—I*) and L-3-hydroxyacyl-CoA dehydrogenase (*L3HOAD*) were stimulated (Menoyo et al., 2004). Therefore, the combined effect of increase salinity and FO replacement promoted a higher utilization of fatty acids as energy source in grey mullets in the present study. However, there was an interaction in the utilization of fatty acids among salinity and dietary lipid source. Thus, in whole-body and muscle, when fish were fed FO increase in salinity reduced n-3 PUFA in a higher extend than in fish fed LO, whereas in fish fed LO, salinity increased n-6 fatty acids in liver, particularly 18:2n-6. These results denote the inhibition of desaturase and elongase activities by increased salinity when fish was fed FO diets, but when fish was fed the LO diet the inhibiting effect of salinity was milder, since this diet promoted LC-PUFA synthesis. In agreement with these findings, *Fads2* expression is up regulated in red seabream (*Pagrus major*) fed a VO diet and reared at 15 ppm (Sarker et al., 2011). Therefore, the effect of salinity on LC-PUFA synthesis was different depending on the diet fed.

5. Conclusion

The present study has shown that thick-lipped grey mullet juveniles have a good capacity to convert 18C fatty acids into LC-PUFA that is enhanced by the replacement of FO by LO and the reduction of salinity to an isosmotic level (16 ppt). Besides, these juveniles efficiently used diets with complete replacement of FO by LO with growth performance, body composition, and LC-PUFA fatty acid profiles similar to those of fish fed FO, particularly when reared at 16 ppt. Although more studies are needed to evaluate this potential with larger fish in the long term, overall, the present results suggest the feasibility of feeding this species with diets without FO but with sufficient contents of 18:3n-3 and 18:2n-6, optimizing the performance when fish are reared in brackish water (16 ppt).

CRediT authorship contribution statement

Raquel Quirós-Pozo: Investigation, Conceptualization, Writing – original draft. **Javier Roo:** Conceptualization, Writing – review & editing. **Marisol Izquierdo:** Conceptualization, Writing – review & editing. **William Koven:** Conceptualization. **Sara Ramírez-Bolaños:** Investigation. **Anais Ventura-Castellano:** Investigation. **Antonio Serradell:** Formal analysis, Data curation. **Lidia Robaina:** Supervision, Project administration, Funding acquisition, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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