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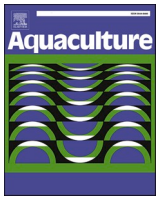

Essential fatty acid requirement of juvenile meagre (*Argyrosomus regius*)

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ABSTRACT

Meagre (*Argyrosomus regius*) is a fast-growing marine teleost and a promising candidate for the diversification of the Mediterranean aquaculture industry. Information on specific dietary requirements is necessary to improve the competitiveness of meagre farming. While the essential fatty acids (EFA) requirement of meagre fingerlings was previously estimated, knowledge regarding the EFA requirement of on-growing meagre juveniles is still lacking. To assess the EFA requirement of juvenile meagre, six isolipidic, isoproteic, semi-purified diets with different levels of n-3 long-chain polyunsaturated fatty acid (n-3 LC-PUFA) contents 0.0%, 0.3%, 0.6%, 0.9%, 1.2%, and 1.5% of diet dry matter (DM) were formulated and fed three times daily to triplicate groups of juvenile meagres of 35.6 ± 0.3 g for twelve weeks. Growth rates, whole body composition and fatty acid profile, liver lipid content as well as fatty acid profiles were assessed. In addition, the incidence of hepatic steatosis and granulomatosis was evaluated. Juvenile meagre fed the 0.0% n-3 LC-PUFA diet showed significantly lower growth and final body weight compared to all other treatments. There was an increase in growth of meagre with increasing dietary n-3 LC-PUFA levels and weight gain plateaued at levels above 0.9% n-3 LC-PUFA. Occurrence of granulomas examined in the liver supported the data on growth as the hepatic granulomatosis incidence in the groups fed <0.9% n-3 LC-PUFA were greater compared to fish fed diets containing higher levels of n-3 LC-PUFA. The findings of this study showed that the dietary n-3 LC-PUFA requirement of juvenile meagre for optimal growth and liver health status lays between 0.7 and 0.8% of the dietary dry matter.

1. Introduction

Fish production in the Mediterranean has been dominated by two fish species, European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). For the last 20 years, the increase in variety of cultured Mediterranean fish species has been considered as an opportunity to expand the market and improve the competitiveness and sustainability of the region's aquaculture sector (Basurco and Abellan, 1999; Couto et al., 2016). One promising candidate ascribed for the diversification of Mediterranean aquaculture, has been the meagre (*Argyrosomus regius*), acknowledged for its fast growth, high flesh quality, and economic value (Monfort, 2010). Indeed, easy adaptation to captivity, hardiness, good processing yield and nutritional values have enabled a fourfold increase in production volumes in the European Union from 2010 to 2019 (EUMOFA, 2022). Even though meagre has proven to fulfil its role as 'diversifier', limited knowledge on the nutritional requirements of this still emerging species might constrain the full exploitation of meagres' production potential. While the crude protein and lipid requirements of meagre for optimal growth rates have been estimated to be around 50% and 17%, respectively (Chatzifotis et al., 2012; Chatzifotis et al., 2010), knowledge about the requirements of essential fatty acids (EFA) is still scarce. Carvalho et al. (2018) determined the n-3 long chain polyunsaturated fatty acid (LC-PUFA) requirements of meagre fingerlings in a growth trial from 3 to 10 g body weight using fishmeal and fish oil based practical diets and suggested a requirement of 2.1% of diet dry matter (DM). The dietary n-3 LC-PUFA requirement of on-growing red drum juveniles (*Sciaenops ocellatus*), another fish of the Sciaenidae family was reported to be 0.5% of diet DM (Lochmann and Gatlin, 1993). To our knowledge, no information about the EFA requirements of on-growing meagre juveniles is yet available.

As occurs in higher vertebrates, PUFA are essential in fish diets with varying requirements among species (Tocher, 2003). While C_{18} PUFA

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satisfy the essential fatty acid requirements of fresh water fish, most marine teleosts require dietary n-3 LC-PUFA, such as docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) due to their insufficient activity in key enzymes required for their endogenous biosynthesis, specifically the Δ5 or Δ6 desaturases and elongases (Monroig et al., 2018; Tocher, 2010). n-3 LC-PUFA are important biological molecules, as they are involved in numerous metabolic pathways such as membrane structure and function, eicosanoids and energy production and control of lipid homeostasis (Tocher, 2003). High levels of these EFA are present in fish oil, which has been used as the main dietary lipid source to provide sufficient n-3 LC-PUFA for farmed fish. The abundant use of fish oil, which is mainly originating from dedicated marine fisheries is considered an unsustainable solution and a limit for continuing growth of the aquaculture industry (Tacon et al., 2022). Alternatives to fish oil are already increasingly used as lipid sources in aqua feeds, mainly vegetable oils, which are usually rich in C_{18} PUFA but lacking n-3 LC-PUFA (Kaushik and Troel, 2010; Turchini et al., 2022; Turchini et al., 2018). To allow a feed formulation that satisfies the dietary n-3 LC-PUFA requirements of marine teleosts while utilizing fish oil in an optimal manner, studies on EFA requirements at different developmental stages of fish are necessary. The present study aimed to investigate the dietary n-3 LC-PUFA requirements of on-growing meagre juveniles.

2. Material and methods

The growth study was performed according to the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes, at the Ecoaqua Institute of University of Las Palmas de Gran Canaria (ULPGC, Telde, Las Palmas, Canary Islands, Spain).

2.1. Fish and culture conditions

Meagre juveniles for the trial were obtained from broodstock-induced spawning at the Grupo de Investigación en Acuicultura (GIA) facilities of the EcoAqua Institute of ULPGC. After anaesthetization with clove oil (4 ml/ 100 l), fish for the trial were individually weighted, measured and randomly distributed in triplicates of fifteen fish (average initial body weight 35.6 ± 0.3 g) per tank among eighteen round fiberglass tanks of dark grey color and 500 l volume. Each tank was supplied with natural seawater (37 ‰) from the Atlantic Ocean, at a flow rate of 350 l per hour, aerated by air stones. Dissolved oxygen concentrations ranged from 6.9 mg/ l to 7.7 mg/ l with an average concentration of 7.3 ± 0.4 mg/ l. The water temperature was on average 20.5 ± 0.3 °C. Fish were exposed to a natural photoperiod of approximately 13 h light – 11 h dark. Nets covered the tanks to prevent fish from escaping. Fish were fed ad libitum seven days a week, three times per day: at 8:30 a.m., at 12:45 p.m., and at 17:00 p.m. Each feeding was followed by collection of uneaten pellets, which were dried and weighted to correct feed intake.

2.2. Experimental diets

Six experimental semi-purified isonitrogenous (50% crude protein (N x 6.25)) and isolipidic (17.5% fat) diets were formulated using graded levels of fish oil (0, 20, 40, 60, 80 and 100% of the added oils). These inclusion levels resulted in n-3 LC-PUFA contents of 0, 0.3, 0.6, 0.9, 1.2, and 1.5% of diet dry matter (DM). The plant oil fraction was a mixture of linseed oil and rapeseed oil (1:1), which was gradually replaced by fish oil in the different diets. The dietary protein fraction was achieved without fishmeal using a variable blend composed of soy protein concentrate, casein and casein hydrolysate. The ingredient composition of the diets is reported in Table 1. Ingredients were weighed, carefully mixed and pelletized dry without steam using a laboratory pelleting machine (California Pellet mill, CPM, 2HP mod 8.3, USA) through a 3 mm diameter die. The pelleted diets were dried at 40 °C for 24 h in a ventilated oven and afterwards stored in a dark and refrigerated chamber at a temperature of 10 °C until use. A sample of each diet was taken for subsequent biochemical analysis and was analyzed for moisture, ash and protein (N X 6.25) contents according to Association of Official Analytical Chemists (AOAC) (2000). The moisture content was determined by drying samples at 105 °C until reaching constant weight. Lipids were extracted after homogenizing (T25 Digital Ultra Turrax, IKA, Germany) with chloroform/methanol (2:1 *v*/v) (Folch et al., 1957) and measured gravimetrically to calculate the total lipid content. Fatty acid methyl esters (FAMES) were obtained by transmethylation of total lipids as suggested by Christie (1989). Separation and identification of the fatty acids was realized with gas liquid chromatography (GC) (Finnigan Focus SG, Thermo Electron Corporation, Milan, Italy) under the conditions described by Izquierdo et al. (1990), quantified by a flame ionization detector and identified by comparison with previously characterized standards. The calculation of dietary fatty acid levels did not account for around 11% of non-saponifiable lipids in the diets and, therefore, all the values of n-3 LC-PUFA contents in DM are slightly overestimated. The proximate composition of the diets is presented in Table 1 and the respective fatty acid profiles are shown in Table 2.

Table 1
Ingredient composition and proximate composition of the experimental diets.

	Dietary n-3 LC-PUFA level (% DM)					
	0.0%	0.3%	0.6%	0.9%	1.2%	1.5%
Ingredients (g/100 g)						
Casein	20.0	20.0	20.0	20.0	20.0	20.0
Casein hydrolysate	5.0	5.0	5.0	5.0	5.0	5.0
Gelatin	5.8	5.8	5.8	5.8	5.8	5.8
Soy protein concentrate	33.3	33.3	33.3	33.3	33.3	33.3
DL-Methionine	0.4	0.4	0.4	0.4	0.4	0.4
L-Threonine	0.02	0.02	0.02	0.02	0.02	0.02
Dextrine	11.2	11.2	11.2	11.2	11.2	11.2
Soy Lecithin	2.0	2.0	2.0	2.0	2.0	2.0
Fish oil [#]	0.0	3.0	6.0	9.0	12.0	15.0
Vegetable oil [$]	15.0	12.0	9.0	6.0	3.0	0.0
$CaHPO_4.H_2O$	2.2	2.2	2.2	2.2	2.2	2.2
Mineral premix [*]	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix [†]	2.0	2.0	2.0	2.0	2.0	2.0
Taurine	0.3	0.3	0.3	0.3	0.3	0.3
Betaine	0.3	0.3	0.3	0.3	0.3	0.3
Glucosamine	0.4	0.4	0.4	0.4	0.4	0.4
Ethoxyquin	0.1	0.1	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0	100.0	100.0
Proximate composition						
Crude protein, % DM	51.6	51.7	51.9	51.6	51.7	52.2
Crude lipid, % DM	17.7	17.9	17.6	17.8	17.9	17.9
Ash, % DM	6.0	5.9	6.1	6.0	6.0	6.0
Moisture, %	7.9	7.5	7.6	7.6	8.3	8.1

[#] Herring (*Clupea harengus*) oil.
[$] Mix of linseed oil and rapeseed oil (1:1).
[*] Supplied the following (g/kg mix): calcium hydrogen phosphate 500, calcium carbonate (40% Ca) 215, sodium chloride 40, ferrous sulphate (21% Fe) 20, manganese sulphate 3, zinc sulphate 4, copper sulphate 3, cobalt (II) chloride (25% Co) 0.02, potassium iodine 0.04, sodium selenite 0.03, sodium fluoride 1, magnesium hydroxide (60% Mg) 124 and potassium chloride 90.
[†] Supplied the following (g/kg mix, except as noted): retinyl acetate 1, DL-cholecalciferol 2.5, DL-α tocopheryl acetate 5, menadione sodium bisulphite 1, ascorbic acid 20, thiamin 0.1, riboflavin 0.4, pyridoxine 0.3, vitamin B12 10 mg, nicotinic acid 1, pantothenic acid 2, folic acid 0.1, biotin 10 mg, choline chloride 200, inositol 30.

2.3. Sample collection

All fish were individually weighed and measured (total length in cm) at the start and the end of the experiment and bulk weighted in the middle of the trial, after a fasting period of 24 h. At the beginning of the experiment, a pooled sample of ten fish from the initial stock was stored at −80 °C for subsequent whole-body chemical and fatty acid

Table 2
Fatty acid composition (% of total fatty acids) of the experimental diets.

Fatty acid	Dietary n-3 LC-PUFA level (% DM)					
	0.0%	0.3%	0.6%	0.9%	1.2%	1.5%
14:0	0.12	0.48	0.86	1.27	1.63	2.02
14:1n-5	0.00	0.01	0.03	0.04	0.05	0.07
15:0	0.03	0.06	0.09	0.13	0.16	0.20
16:0ISO	0.00	0.01	0.02	0.02	0.03	0.03
16:0	5.58	6.50	7.64	8.85	9.88	10.81
16:1n-7	0.14	0.62	1.08	1.57	2.03	2.53
16:1n-5	0.01	0.02	0.03	0.05	0.06	0.09
16:2n-4	0.00	0.04	0.07	0.10	0.14	0.17
17:0	0.05	0.02	0.05	0.07	0.09	0.12
16:3n-4	0.00	0.07	0.09	0.11	0.13	0.16
16:3n-3	0.00	0.02	0.03	0.04	0.05	0.06
16:4n-3	0.00	0.03	0.06	0.08	0.10	0.13
18:0	2.56	2.52	2.54	2.67	2.61	2.60
18:1n-9	32.89	32.21	31.74	32.44	30.91	30.60
18:1n-7	1.66	1.76	1.97	2.22	2.32	2.47
18:1n-5	0.02	0.04	0.05	0.07	0.09	0.11
18:2n-9	0.00	0.01	0.01	0.02	0.03	0.04
18:2n-6	19.22	18.09	17.74	16.75	16.44	15.11
18:2n-4	0.00	0.02	0.05	0.06	0.09	0.11
18:3n-6	0.00	0.02	0.05	0.07	0.11	0.14
18:3n-4	0.01	0.03	0.05	0.07	0.09	0.12
18:3n-3	26.59	22.34	17.45	11.61	8.66	4.02
18:4n-3	0.01	0.13	0.25	0.39	0.51	0.65
18:4n-1	–	0.02	0.04	0.06	0.08	0.10
20:0	0.31	0.30	0.29	0.29	0.27	0.24
20:1n-9	0.02	0.09	0.17	0.24	0.32	0.40
20:1n-7	0.56	1.01	1.46	1.95	2.37	2.87
20:1n-5	0.01	0.04	0.06	0.08	0.11	0.13
20:2n-9	0.00	0.02	0.03	0.05	0.06	0.07
20:2n-6	0.04	0.17	0.30	0.44	0.57	0.72
20:3n-9	0.01	0.01	0.01	0.01	0.01	0.02
20:3n-6	0.01	–	0.09	0.14	0.18	0.22
20:4n-6	0.01	0.07	0.13	0.19	0.25	0.32
20:3n-3	0.03	0.07	0.12	0.17	0.22	0.28
20:4n-3	0.00	0.11	0.22	0.34	0.43	0.59
20:5n-3	0.02	0.51	0.95	1.45	1.91	2.46
22:1n-11	0.02	0.40	0.79	1.19	1.58	2.01
22:1n-9	0.08	0.15	0.21	0.28	0.35	0.42
22:4n-6	0.01	0.02	0.03	0.03	0.05	0.06
22:5n-6	0.00	0.02	0.04	0.06	0.08	0.10
22:5n-3	0.01	0.19	0.37	0.56	0.74	0.95
22:6n-3	0.03	0.93	1.68	2.55	3.38	4.32
Σ SFA	8.65	9.89	11.47	13.29	14.67	16.01
Σ MUFA	35.40	36.36	37.62	40.16	40.21	41.73
Σ PUFA (n-3 + n-6)	45.98	42.67	39.42	34.73	33.52	29.95
Σ LC-PUFA (n-3 + n-6)	0.11	1.93	3.62	5.47	7.23	9.30
Σ n-3	26.69	24.33	21.12	17.17	15.99	13.46
Σ n-6	19.29	18.39	18.38	17.67	17.67	16.68
Σ n-9	32.99	32.48	32.17	33.04	31.68	31.55
Σ n-3 PUFA	26.69	24.28	21.04	17.06	15.84	13.27
Σ n-6 PUFA	19.29	18.39	18.38	17.67	17.67	16.68
Σ n-3 LC-PUFA	0.09	1.81	3.34	5.06	6.67	8.59
Σ n-6 LC-PUFA	0.02	0.11	0.28	0.41	0.55	0.70
Σ DHA + EPA	0.05	1.44	2.63	4.00	5.28	6.78
Σ DHA + EPA + ARA	0.06	1.51	2.75	4.19	5.53	7.10
18:1n-9/n-3 LC-PUFA	358.62	17.74	9.51	6.41	4.63	3.56
n-3/n-6	1.38	1.32	1.15	0.97	0.91	0.81
EPA/ARA	3.49	7.59	7.55	7.62	7.62	7.74
DHA/EPA	1.49	1.80	1.77	1.77	1.77	1.76

Σ SFA (saturated fatty acids) include 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0).
Σ MUFA (monounsaturated fatty acids) include 14:1, 15:1, 16:1, 17:1, 18:1, 20:1, 22:1).
Σ PUFA (n-3 + n-6) (n-3 + n-6 polyunsaturated fatty acids) include n-3 and n-6 of 16:2, 16:3, 16:4, 18:2, 18:3, 18:4, 20:2, 20:3, 20:4, 20:5, 22:5, 22:6).
Σ LC-PUFA (n-3 + n-6) (n-3 + n-6 long chain polyunsaturated fatty acids) include n-3 and n-6 of 20:3, 20:4, 20:5, 22:4, 22:5, 22:6).
Σ n-3 include 16:3n-3, 16:4n-3, 18:3n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3.
Σ n-6 include 16:2n-6, 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.
Σ n-9 include 18:1n-9, 18:2n-9, 20:1n-9, 20:2n-9, 20:3n-9, 22:1n-9.
Σ n-3 PUFA include 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3.
Σ n-6 PUFA include 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.
Σ n-3 LC-PUFA include 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3.
Σ n-6 LC-PUFA include 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.

composition analyses. At the end of the trial, fish were euthanized with excess of a clove oil solution with ethanol (50/50). Four fish from each tank were sampled for whole-body composition analysis resulting in twelve fish per treatment, analyzed in triplicates. In addition, three fish per tank were dissected for liver samples to examine liver lipid and moisture content and liver total lipid fatty acid composition. Fish and liver samples were stored at −80 °C until biochemical analyses. The liver and viscera of the dissected fish were weighed to calculate hepatosomatic and viscerosomatic indices. For histological analysis of the liver, three more fish were sampled per tank.

2.4. Analytical methods for fish samples

All fish samples were kept at −80 °C until analysis. Prior to biochemical analysis, fish for the whole-body composition were cut into pieces and ground to attain one pooled sample of five fish from each tank. The same method was applied for one sample of 10 fish at the beginning of the trial. Moisture, lipid and protein content for the whole-body composition were determined by near-infrared spectroscopy (FOSS FoodScan Analyser, FOSS Analytical AB, Sweden). The extraction of lipids for fatty acid analysis of whole body and liver tissues was carried out following the Folch et al. (1957) method with chloroform-methanol (2:1 v/v) and fatty acids were prepared as described for the diets by transmethylation of total lipids as suggested by Christie (1989). Liver lipid content was evaluated by gravimetric determination, and moisture content was determined by drying samples at 105 °C until reaching constant weight (Association of Official Analytical Chemists (AOAC), 2000).

2.5. Histological analysis

Three liver samples per tank ($n = 9$ per treatment) were fixed in 10% buffered formaldehyde for histological analysis and dehydrated through graded alcohol, then xylene, and finally embedded in paraffin. The paraffin blocks were consecutively cut at 4 μm with a Leica microtome (Mod. Jung Autocut 2055; Leica, Nussloch, Germany) and stained with haematoxylin and eosin (H&E) (Martoja and Martoja-Pierson, 1970). Sections were immersed twice for 2 min in xylol, twice for 2 min in 100% ethanol, 2 min in 70% ethanol, thrice for 2 min in distilled water, 13 min in haematoxylin, then rinsed under tap water for 30 s, dipped five times into acid ethanol, and rinsed again under tap water for 30 s. Sections were then immersed five times in ammonia water, rinsed under tap water for 5 min, immersed in 96% ethanol for 1 min, transferred to eosin for 6 min, immersed twice for 2 min in 96% ethanol, twice for 2 min in 100% ethanol, and then thrice for 2 min in 100% xylol. For mounting, a drop of xylol based DPX was placed on each slide using a glass cover slip. Sections were examined under a light microscope (BX51TF, Olympus, Tokyo, Japan) with an objective lens working distance of 0.45 mm and a numerical aperture of 0.65. A four-grade score was used to assess stained sections of liver for cytoplasmic lipid vacuolization: 0, not observed; 1, few; 2, medium; 3, severe. In addition, the number of granulomas in each liver sample was counted to evaluate the presence and incidence of hepatic granulomatosis.

2.6. Calculations and statistical analysis

The following formulae were used for calculating growth and feed utilization parameters: Specific growth rate, SGR (% day^{-1}): (ln final mean weight − ln initial mean weight)/number of days×100; Thermal growth coefficient, TGC: (final weight$^{1/3}$ − initial weight$^{1/3}$)/

(temperature × days); Feed intake, FI (g feed fish^{-1} day^{-1}): feed intake (g)/days of experiment/number of fish; Feed conversion ratio, FCR: feed intake (g) / whole body weight gain (g); Feed efficiency. FE: wet weight gain (g) / dry feed intake (g); Daily growth index, DGI: 100 x ((final body weight)$^{1/3}$ - (initial body weight)$^{1/3}$)/days; Condition factor, K: body weight/total length^3 × 100.

All data were tested for normality of distribution and homogeneity of variance (Zar, 1999). Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons when significant differences were observed among groups ($P < 0.05$). When data did not display normal distribution and homogeneity of variance, a non-parametric analysis and multiple-range test (Kruskal-Wallis) was applied followed by a multiple comparison of means. Analyses were performed using the SPSS 15.0 (IBM Corp., New York, USA) statistical package. Significant differences were considered when $P \leq 0.05$. The required dietary n-3 LC-PUFA level was estimated by broken-line regression analysis of the thermal growth coefficient (TGC) using GraphPad Prism version 8.0.0 (GraphPad Software, San Diego, California USA) (Robbins et al., 1979). The description of the model used is as follows: $Y_1 = a_1 + b_1 * X$; Y at $X_0 = b_1 * X_0 + a_1$; $Y_2 = Y$ at $X_0 + b_2 * (X - X_0)$; Y = if ($X < X_0$, Y_1, Y_2), where Y = response criteria (TGC); X = dietary n-3 LC-PUFA level (% DM); a_1 = intercept on y-axis for X = 0; b_1 = slope of the first line (Y_1, ascending segment); b_2 = slope of second line (Y_2, plateau segment; =0); X_0 = breakpoint X value (respective FA requirement value). All results are presented as means ± standard deviation (SD).

3. Results

3.1. Growth performance and feed utilization

After 84 days of feeding the respective diets, fish growth was significantly affected by the dietary n-3 LC-PUFA levels. Diets with low-levels of n-3 LC-PUFA led to significantly reduced feed intake, growth rates and feed efficiency in fish fed the 0.0% n-3 LC-PUFA diets but there was no mortality over the 12 weeks. At the end of the trial, average body weight showed a threefold increase or higher for all dietary treatments except the 0.0% n-3 LC-PUFA group, which only doubled in weight. The three groups with the highest n-3 LC-PUFA levels (0.9, 1.2, and 1.5%) had a more than fourfold increase in body weight without any significant differences in any growth-related parameters. Accordingly, growth rates were not significantly different between these dietary treatments, while diets containing less n-3 LC-PUFA caused decreased growth rates and below 0.6% n-3 LC-PUFA content also lower feed efficiency. The condition factor was not affected by n-3 LC-PUFA contents of the diets. Data on growth performance and feed utilization of all groups are summarized in Table 3.

The broken line regression model of growth rates (TGC) against n-3 LC-PUFA levels was used to estimate the dietary n-3 LC-PUFA requirements (Fig. 1). The results showed that meagre juveniles with an initial weight of 35 g require a dietary n-3 LC-PUFA content of 0.76% (DM) for optimal growth rates. Using other growth parameters, such as DGI or SGR against the dietary n-3 LC-PUFA levels for the broken line regression model (not shown) yielded results with a maximum difference of 3% from the TGC value. The model fitted very well for all growth parameters against the dietary n-3 LC-PUFA levels (all R^2 values >0.95).

3.2. Whole body composition, organ indices and fatty acid composition

Data on the whole body composition of the different groups of fish is presented in Table 4. Compared to fish at the beginning of the study, lipid content of the fish after the feeding trial was higher while ash content was lower. Protein, lipid, and ash contents were not affected by dietary n-3 LC-PUFA levels but whole body lipid contents of fish fed diets containing 0.9% n-3 LC-PUFA content or above were significantly higher compared to the 0.0% n-3 LC-PUFA treatment. The

Table 3
Growth performance and feed utilization of meagre fed different n-3 LC-PUFA levels for 84 days.

	Dietary n-3 LC-PUFA level (% DM)					
	0.0%	0.3%	0.6%	0.9%	1.2%	1.5%
Initial weight (g)	35.7 ± 3.3	35.4 ± 3.5	35.5 ± 3.7	35.7 ± 4.0	35.7 ± 3.5	35.4 ± 3.6
Initial length (cm)	15.6 ± 0.1	15.6 ± 0.1	15.6 ± 0.2	15.6 ± 0.1	15.5 ± 0.1	15.6 ± 0.0
Final weight (g)	73.2^{a} ± 6.7	106.0^{b} ± 12.6	129.3^{c} ± 6.4	151.9^{d} ± 13.3	153.3^{d} ± 12.4	160.2^{d} ± 23.3
Final length (cm)	19.1^{a} ± 0.5	21.2^{a,b} ± 0.7	22.6^{bc} ± 0.7	23.9^{c} ± 0.8	24.3^{c} ± 0.8	24.3^{c} ± 1.0
K	1.05 ± 0.01	1.11 ± 0.04	1.11 ± 0.04	1.11 ± 0.05	1.07 ± 0.01	1.11 ± 0.03
DFI (g)	0.63^{a} ± 0.17	0.90^{ab} ± 0.08	0.91^{ab} ± 0.09	1.09^{b} ± 0.11	1.06^{b} ± 0.11	1.17^{b} ± 0.13
FCR	1.39^{a} ± 0.21	1.09^{a} ± 0.11	0.82^{ab} ± 0.03	0.79^{b} ± 0.00	0.76^{b} ± 0.07	0.79^{b} ± 0.05
FE	0.79 ^{a} ± 0.13	1.00^{a} ± 0.10	1.32^{b} ± 0.05	1.37^{b} ± 0.04	1.44^{b} ± 0.01	1.39^{b} ± 0.13
SGR (% d^{-1})	0.85^{a} ± 0.11	1.30^{b} ± 0.15	1.54^{bc} ± 0.05	1.72^{c} ± 0.12	1.74^{c} ± 0.09	1.80^{c} ± 0.17
DGI (%)	1.06^{a} ± 0.15	1.72^{b} ± 0.23	2.10^{bc} ± 0.09	2.42^{c} ± 0.20	2.45^{c} ± 0.16	2.56^{c} ± 0.31
TGC	0.51^{a} ± 0.07	0.84^{b} ± 0.11	1.03^{bc} ± 0.04	1.18^{c} ± 0.10	1.19^{c} ± 0.08	1.24^{c} ± 0.15

Values (mean ± SD, $n = 3$) with different superscript letters in the same row are significantly different ($P < 0.05$).

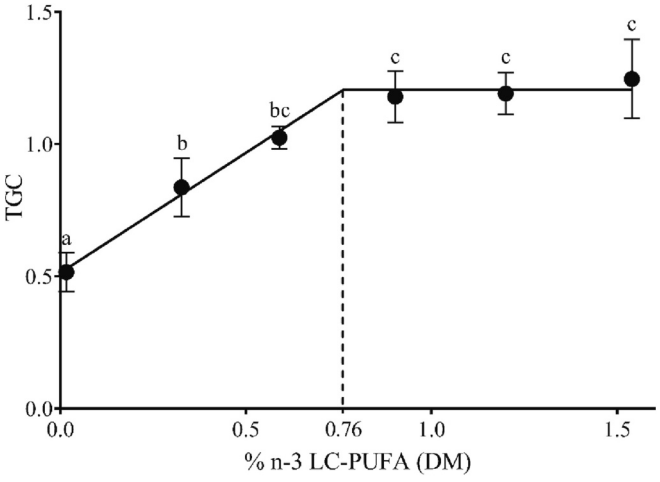

Fig. 1. Broken-line regression model fitting dietary n-3 LC-PUFA levels to TGC of meagre juveniles fed the experimental diets for 84 days (mean ± SD, n=3); the dashed line indicates the requirement for dietary n-3 LC-PUFA (% of DM) ($X_0 = 0.76$; $a_1 = 0.51$; $b_1 = 0$ 0.90); $R^2 > 0.95$). Different letters indicate that treatments are significantly different ($P < 0.05$).

hepatosomatic index (HSI) was significantly affected by the dietary treatments with the highest values in fish fed the 0.3% n-3 LC-PUFA diets. The viscerosomatic index (VSI) was also the highest in the 0.3% n-3 LC-PUFA group, resulting in significant differences compared to the fish fed n-3 LC-PUFA contents of 0.9 to 1.5%.

After 12 weeks of feeding, the fatty acid composition of the fish showed pronounced changes compared to the initial values, overall reflecting the fatty acid compositions of the diets (Table 5). Saturated fatty acid levels were higher in fish fed diets with high dietary n-3 LC-PUFA contents, whereas the opposite was found for n-9 fatty acids. Compared to the initial sample, 18:1n-9 (OLA), 18:2n-6 (LA) and 18:3n-

Table 4
Whole body composition (% fresh weight), HSI, and VSI of juvenile meagre fed the experimental diets for 84 days.

	initial	Dietary n-3 LC-PUFA level (% DM)					
		0.0%	0.3%	0.6%	0.9%	1.2%	1.5%
Moisture (%)	75.6	76.3 ± 0.4	76.6 ± 0.9	75.7 ± 1.2	75.9 ± 0.6	75.6 ± 1.8	76.5 ± 1.9
Protein (%)	13.3	13.4 ± 0.2	13.6 ± 0.9	15.1 ± 0.3	15.3 ± 2.1	16.1 ± 1.9	15.9 ± 2.5
Lipid (%)	1.4	1.8^{a} ± 0.3	3.2^{ab} ± 0.6	5.8^{bc} ± 0.9	6.7^{bc} ± 1.8	7.0^{c} ± 0.3	6.9^{bc} ± 2.4
Ash (%)	2.9	2.9 ± 0.1	2.6 ± 0.4	2.7 ± 0.4	2.5 ± 0.1	2.5 ± 0.3	2.4 ± 0.5
HSI (%)		1.41^{a} ± 0.19	2.34^{b} ± 0.11	1.92^{bc} ± 0.10	1.86^{c} ± 0.12	1.61^{ac} ± 0.15	1.77^{ac} ± 0.16
VSI (%)		3.65^{ab} ± 0.37	4.30^{b} ± 0.18	3.84^{ab} ± 0.01	3.55^{a} ± 0.24	3.29^{a} ± 0.25	3.35^{a} ± 0.14

HSI: hepatosomatic index; VSI: viscerosomatic index. Values (mean ± SD, n = 3) with different superscript letters in the same row are significantly different ($P < 0.05$).

Table 5
Fatty acid composition (% of total identified fatty acids) of whole-body total lipids.

Fatty acid	initial	Dietary n-3 LC-PUFA level (% DM)					
		0.0%	0.3%	0.6%	0.9%	1.2%	1.5%
14:0	3.15	0.96 ± 0.06	0.82 ± 0.05	0.85 ± 0.12	1.14 ± 0.10	1.55 ± 0.14	1.73 ± 0.04
15:0	0.37	0.14 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.21 ± 0.01	0.23 ± 0.01
16:0ISO	0.06	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
16:0	15.84	9.31 ± 0.06	9.95 ± 0.49	10.81 ± 0.47	12.03 ± 0.22	13.08 ± 0.45	14.23 ± 0.35
16:1n-7	4.73	1.60 ± 0.16	1.66 ± 0.17	1.94 ± 0.09	2.47 ± 0.16	2.95 ± 0.24	3.42 ± 0.20
16:1n-5	0.16	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.09 ± 0.00	0.10 ± 0.00
16:2n-4	0.34	0.07 ± 0.01	0.06 ± 0.00	0.06 ± 0.01	0.10 ± 0.02	0.15 ± 0.02	0.16 ± 0.03
17:0	0.21	0.08 ± 0.00	0.05 ± 0.00	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
16:3n-4	0.22	0.13 ± 0.00	0.12 ± 0.00	0.14 ± 0.01	0.15 ± 0.00	0.17 ± 0.00	0.20 ± 0.00
16:3n-3	0.13	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.06 ± 0.01	0.07 ± 0.00
16:3n-1	0.38	0.25 ± 0.01	0.15 ± 0.03	0.12 ± 0.00	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.01
16:4n-3	0.23	0.19 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.01
18:0	4.46	5.65 ± 0.13	5.27 ± 0.32	4.96 ± 0.21	4.53 ± 0.16	4.21 ± 0.33	4.25 ± 0.15
18:1n-9	26.55	37.20^{a} ± 0.92	35.84^{abc} ± 0.50	36.28^{ab} ± 0.09	36.47^{a} ± 0.48	34.68^{bc} ± 0.59	34.37^{c} ± 0.39
18:1n-7	3.16	2.26 ± 0.03	2.18 ± 0.04	2.33 ± 0.06	2.49 ± 0.06	2.72 ± 0.06	2.86 ± 0.04
18:1n-5	0.16	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.11 ± 0.00	0.13 ± 0.00
18:2n-9	0.08	0.29^{a} ± 0.05	0.16^{b} ± 0.03	0.13^{c} ± 0.01	0.13^{c} ± 0.01	0.10^{c} ± 0.01	0.12^{c} ± 0.02
18:2n-6	11.10	19.23^{a} ± 0.33	18.92^{a} ± 0.49	18.52^{ab} ± 0.42	17.61^{bc} ± 0.22	16.91^{cd} ± 0.44	16.22^{d} ± 0.34
18:2n-4	0.15	0.04 ± 0.01	0.04 ± 0.00	0.06 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.11 ± 0.01
18:3n-6	0.13	0.31 ± 0.05	0.22 ± 0.04	0.18 ± 0.00	0.19 ± 0.01	0.19 ± 0.01	0.22 ± 0.01
18:3n-4	0.17	0.04 ± 0.02	0.05 ± 0.00	0.06 ± 0.01	0.08 ± 0.00	0.10 ± 0.02	0.12 ± 0.01
18:3n-3	2.24	14.34^{a} ± 0.45	16.20^{b} ± 0.51	14.14^{a} ± 0.88	10.01^{c} ± 0.17	7.35^{d} ± 0.21	3.71^{e} ± 0.09
18:4n-3	0.73	0.24 ± 0.04	0.23 ± 0.03	0.21 ± 0.01	0.27 ± 0.03	0.35 ± 0.05	0.40 ± 0.02
18:4n-1	0.09	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.06 ± 0.01	0.07 ± 0.00
20:0	0.35	0.29 ± 0.02	0.26 ± 0.02	0.27 ± 0.02	0.27 ± 0.01	0.27 ± 0.01	0.26 ± 0.00
20:1n-9	0.45	0.10 ± 0.01	0.12 ± 0.00	0.18 ± 0.01	0.26 ± 0.01	0.34 ± 0.01	0.41 ± 0.00
20:1n-7	3.88	1.73 ± 0.02	1.65 ± 0.02	1.95 ± 0.08	2.31 ± 0.05	2.78 ± 0.07	3.13 ± 0.03
20:1n-5	0.22	0.08 ± 0.00	0.07 ± 0.00	0.08 ± 0.01	0.10 ± 0.00	0.13 ± 0.00	0.15 ± 0.00
20:2n-9	0.12	0.09 ± 0.03	0.07 ± 0.01	0.07 ± 0.00	0.10 ± 0.00	0.11 ± 0.01	0.13 ± 0.02
20:2n-6	0.63	0.34 ± 0.02	0.38 ± 0.02	0.47 ± 0.00	0.57 ± 0.01	0.68 ± 0.01	0.80 ± 0.01
20:3n-6	0.16	0.10 ± 0.02	0.11 ± 0.01	0.13 ± 0.00	0.18 ± 0.01	0.22 ± 0.01	0.26 ± 0.00
20:4n-6	0.88	0.23^{ab} ± 0.02	0.20^{a} ± 0.03	$0.21^{a.b}$ ± 0.01	0.27^{b} ± 0.01	0.34^{c} ± 0.03	0.40^{c} ± 0.03
20:3n-3	0.22	0.30 ± 0.01	0.37 ± 0.02	0.33 ± 0.02	0.30 ± 0.00	0.30 ± 0.01	0.30 ± 0.00
20:4n-3	0.48	0.17^{a} ± 0.07	0.18^{a} ± 0.01	0.24^{a} ± 0.01	0.36^{b} ± 0.01	0.47^{c} ± 0.02	0.58^{d} ± 0.02
20:5n-3	4.06	0.70^{a} ± 0.23	0.74^{a} ± 0.07	0.78^{ab} ± 0.03	1.20^{bc} ± 0.15	1.63^{cd} ± 0.23	1.91^{d} ± 0.12
22:1n-11	3.37	0.69 ± 0.05	0.57 ± 0.01	0.73 ± 0.11	1.04 ± 0.10	1.47 ± 0.10	1.70 ± 0.04
22:1n-9	0.71	0.40 ± 0.00	0.37 ± 0.02	0.39 ± 0.01	0.43 ± 0.01	0.50 ± 0.01	0.55 ± 0.00
22:4n-6	0.12	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.09 ± 0.00
22:5n-6	0.22	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.00	0.08 ± 0.00	0.11 ± 0.01	0.13 ± 0.01
22:5n-3	1.33	0.29^{a} ± 0.06	0.36^{ab} ± 0.03	0.47^{b} ± 0.01	0.73c ± 0.02	0.94^{d} ± 0.10	1.14^{e} ± 0.04
22:6n-3	7.68	1.79^{a} ± 0.42	1.99^{a} ± 0.22	2.25^{ab} ± 0.06	3.25^{bc} ± 0.07	4.10^{cd} ± 0.54	4.94^{d} ± 0.30
Σ SFA	24.44	16.45^{a} ± 0.11	16.50^{a} ± 0.61	17.08^{ab} ± 0.84	18.19^{bc} ± 0.20	19.40^{cd} ± 0.80	20.80^{d} ± 0.45
Σ MUFA	43.55	44.21^{a} ± 0.74	42.62^{a} ± 0.64	44.05^{ab} ± 0.49	45.77^{bc} ± 0.41	45.86^{c} ± 0.67	46.91^{c} ± 0.37
n-3	17.09	18.05^{a} ± 0.72	20.24^{b} ± 0.81	18.56^{ab} ± 0.94	16.25^{ac} ± 0.30	15.28^{c} ± 1.06	13.11^{d} ± 0.57
n-6	13.25	20.32^{a} ± 0.23	19.92^{ab} ± 0.52	19.62^{abc} ± 0.41	18.99^{bcd} ± 0.25	18.56^{cd} ± 0.48	18.15^{d} ± 0.37
n-9	27.93	38.09^{a} ± 0.87	36.57^{ab} ± 0.52	37.06^{ab} ± 0.10	37.40^{a} ± 0.49	35.75^{b} ± 0.59	35.58^{b} ± 0.40
n-3 LC-PUFA	13.76	3.25^{a} ± 0.78	3.64^{a} ± 0.29	4.08^{a} ± 0.07	5.84^{b} ± 0.24	7.45^{c} ± 0.83	8.86^{c} ± 0.47
n-6 LC-PUFA	2.02	0.43^{a} ± 0.04	0.40^{a} ± 0.04	0.45^{a} ± 0.01	0.60^{b} ± 0.01	0.74^{c} ± 0.05	0.87^{d} ± 0.04
(n-3 + n-6) LC-PUFA	15.77	3.69^{a} ± 0.82	4.04^{a} ± 0.33	4.53^{a} ± 0.06	6.43^{b} ± 0.24	8.19^{c} ± 0.88	9.74^{c} ± 0.51
18:1n-9/n-3 LC-PUFA	1.93	11.43^{a} ± 2.79	9.85^{a} ± 0.90	$8.90^{a,b}$ ± 0.18	$6.25^{b,c}$ ± 0.34	4.66^{c} ± 0.62	3.88^{c} ± 0.25
n-3/n-6	1.29	0.89^{ab} ± 0.04	1.02^{c} ± 0.03	0.95^{bc} ± 0.03	0.86^{ab} ± 0.02	0.82^{a} ± 0.04	0.72^{d} ± 0.03
EPA/ARA	4.60	3.03^{a} ± 0.70	3.80^{ab} ± 0.69	3.76^{ab} ± 0.04	4.45^{ab} ± 0.49	4.78^{b} ± 0.57	4.78^{b} ± 0.22
DHA/EPA	1.89	2.57 ± 0.26	2.68 ± 0.37	2.88 ± 0.20	2.70 ± 0.28	2.51 ± 0.28	2.59 ± 0.04
n-3 PUFA	16.74	17.83^{a} ± 0.72	20.07^{b} ± 0.80	18.42^{ab} ± 0.95	16.12^{ac} ± 0.30	15.15^{c} ± 1.05	12.97^{d} ± 0.57
n-6 PUFA	13.24	20.31^{a} ± 0.23	19.92^{ab} ± 0.52	19.62^{abc} ± 0.41	18.96^{bcd} ± 0.24	18.52^{cd} ± 0.51	18.11^{d} ± 0.37
(n-3 + n-6) PUFA	29.98	38.14^{a} ± 0.70	39.99^{a} ± 1.25	38.04^{ab} ± 1.36	35.08^{bc} ± 0.35	33.67^{cd} ± 1.50	31.07^{d} ± 0.84

Values (mean ± SD, $n = 3$) with different superscript letters in the same row are significantly different ($P < 0.05$).

3 (αLNA), the major dietary fatty acid components were observed in higher contents, in particular in the fish fed diets containing high vegetable oil contents. αLNA, a precursor of EPA and DHA gradually increased from the 1.5% n-3 LC-PUFA diet to the 0.3% n-3 LC-PUFA diet according to the vegetable oil added and induced significant differences between treatments but in the 0.0% n-3 LC-PUFA treatment the content of αLNA was significantly lower compared to the 0.3% n-3 LC-PUFA group. In contrast, saturated fatty acids as well as fatty acids of marine origin such as DHA, EPA, and other n-3 LC-PUFA decreased compared to initial fish. According to the level of fish oil in the various diets, there was an increase of n-3 LC-PUFA from the 0.0% n-3 LC-PUFA to the 1.5% n-3 LC-PUFA treatment. However, within each treatment compared to initial fish, a decrease of n-3 LC-PUFA and an increase of 18:1n-9 and 18:2n-6 occurred. In all treatments, the DHA/EPA ratio after the feeding trial was between 2.5 and 2.9 – higher than in initial fish – with no significant differences between the treatments.

3.3. Liver lipid content and fatty acid composition

Liver total lipid content was affected by the dietary treatments (Table 6). The diets containing 0.0% n-3 LC-PUFA caused significantly reduced liver lipid contents compared to all other treatments. The liver fatty acid composition (Table 6) showed similar trends as observed in the whole body lipids, reflecting the dietary fatty acid levels with increasing n-3 LC-PUFA levels corresponding to the dietary treatments. Contrary to the whole body lipids, the DHA:EPA ratio was significantly affected by dietary fatty acid compositions with a higher ratio in the group fed essential acid deficient diets. Saturated fatty acid content was

Table 6
Liver total lipid content and fatty acid composition (% of total identified fatty acids).

	Dietary n-3 LC-PUFA level (% DM)					
	0.0%	0.3%	0.6%	0.9%	1.2%	1.5%
Liver total lipid content (%)	$21.74^{a} \pm 2.03$	$34.6^{b} \pm 2.24$	$33.94^{b} \pm 2.49$	$33.1^{b} \pm 1.99$	$30.84^{b} \pm 1.59$	$31.78^{b} \pm 2.70$
Fatty acid						
14:0	0.48 ± 0.01	0.41 ± 0.04	0.64 ± 0.01	0.80 ± 0.01	0.85 ± 0.00	1.10 ± 0.13
15:0	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.00	0.13 ± 0.01	0.15 ± 0.00	0.17 ± 0.02
16:0	6.63 ± 0.30	8.46 ± 0.88	13.59 ± 0.93	12.83 ± 0.60	14.07 ± 0.04	16.23 ± 0.36
16:1n-7	1.63 ± 0.24	2.01 ± 0.34	3.71 ± 0.52	3.81 ± 0.42	4.36 ± 0.00	5.10 ± 0.29
16:1n-5	0.04 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.09 ± 0.00	0.11 ± 0.00	0.13 ± 0.00
16:2n-4	0.00 ± 0.00	0.02 ± 0.02	0.01 ± 0.02	0.04 ± 0.00	0.06 ± 0.00	0.08 ± 0.01
17:0	0.07 ± 0.01	0.07 ± 0.06	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
16:3n-4	0.14 ± 0.01	0.10 ± 0.07	0.15 ± 0.00	0.20 ± 0.01	0.22 ± 0.00	0.23 ± 0.01
16:3n-3	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.07 ± 0.00
18:0	7.81 ± 0.58	8.48 ± 1.08	7.90 ± 0.74	6.41 ± 0.25	6.57 ± 0.31	6.70 ± 0.72
18:1n-9	$43.64^{a} \pm 0.51$	$40.69^{b} \pm 0.41$	$38.95^{c} \pm 0.42$	$40.38^{b} \pm 0.34$	$38.55^{c} \pm 0.17$	$37.19^{d} \pm 0.23$
18:1n-7	2.18 ± 0.06	2.25 ± 0.07	2.43 ± 0.08	2.72 ± 0.01	2.88 ± 0.03	3.05 ± 0.00
18:1n-5	0.04 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.11 ± 0.02	0.12 ± 0.00	0.14 ± 0.00
18:2n-9	$1.24^{a} \pm 0.20$	$0.56^{b} \pm 0.09$	$0.53^{b} \pm 0.02$	$0.38^{c} \pm 0.06$	$0.36^{c} \pm 0.02$	$0.36^{c} \pm 0.03$
18:2n-6	$19.05^{a} \pm 0.42$	$17.39^{ab} \pm 1.13$	$14.94^{c} \pm 0.23$	$15.67^{bc} \pm 0.72$	$14.78^{c} \pm 0.31$	$13.77^{c} \pm 0.90$
18:2n-4	0.01 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.11 ± 0.00
18:3n-6	1.39 ± 0.15	0.71 ± 0.03	0.60 ± 0.02	0.47 ± 0.05	0.48 ± 0.00	0.47 ± 0.01
18:3n-4	0.03 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.08 ± 0.01	0.11 ± 0.00	0.12 ± 0.01
18:3n-3	$11.26^{ab} \pm 0.76$	$12.67^{a} \pm 1.00$	$9.75^{b} \pm 0.12$	$7.29^{c} \pm 0.32$	$5.67^{c} \pm 0.35$	$2.73^{d} \pm 0.14$
18:4n-3	0.65 ± 0.01	0.40 ± 0.04	0.39 ± 0.03	0.27 ± 0.03	0.28 ± 0.01	0.28 ± 0.02
20:0	0.20 ± 0.01	0.21 ± 0.02	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.20 ± 0.00
20:1n-9	0.03 ± 0.01	0.09 ± 0.01	0.15 ± 0.00	0.24 ± 0.01	0.31 ± 0.01	0.38 ± 0.02
20:1n-7	1.32 ± 0.02	1.87 ± 0.14	2.12 ± 0.03	2.62 ± 0.08	2.92 ± 0.10	3.28 ± 0.09
20:1n-5	0.05 ± 0.00	0.07 ± 0.01	0.08 ± 0.00	0.11 ± 0.00	0.13 ± 0.01	0.15 ± 0.00
20:2n-9	0.17 ± 0.06	0.17 ± 0.04	0.16 ± 0.01	0.15 ± 0.03	0.19 ± 0.02	0.20 ± 0.02
20:2n-6	0.30 ± 0.02	0.50 ± 0.04	0.43 ± 0.01	0.60 ± 0.02	0.72 ± 0.02	0.79 ± 0.03
20:3n-6	0.14 ± 0.05	0.18 ± 0.03	0.13 ± 0.00	0.16 ± 0.01	0.22 ± 0.01	0.25 ± 0.01
20:4n-6	$0.03^{a} \pm 0.01$	$0.06^{a} \pm 0.02$	$0.07^{a} \pm 0.00$	$0.12^{b} \pm 0.01$	$0.17^{c} \pm 0.01$	$0.20^{c} \pm 0.01$
20:3n-3	$0.33^{abc} \pm 0.03$	$0.56^{d} \pm 0.03$	$0.36^{b} \pm 0.00$	$0.33^{bc} \pm 0.01$	$0.34^{bc} \pm 0.00$	$0.29^{c} \pm 0.00$
20:4n-3	$0.14^{a} \pm 0.05$	$0.22^{ab} \pm 0.03$	$0.19^{ab} \pm 0.00$	$0.25^{b} \pm 0.00$	$0.35^{c} \pm 0.01$	$0.43^{c} \pm 0.02$
20:5n-3	$0.04^{a} \pm 0.01$	$0.17^{b} \pm 0.02$	$0.25^{c} \pm 0.01$	$0.43^{d} \pm 0.02$	$0.68^{e} \pm 0.02$	$0.89^{f} \pm 0.03$
22:1n-11	0.03 ± 0.00	0.19 ± 0.02	0.40 ± 0.02	0.70 ± 0.02	0.86 ± 0.04	1.05 ± 0.06
22:1n-9	0.34 ± 0.03	0.47 ± 0.08	0.50 ± 0.01	0.58 ± 0.02	0.63 ± 0.03	0.70 ± 0.02
22:4n-6	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
22:5n-6	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
22:5n-3	$0.02^{a} \pm 0.00$	$0.13^{b} \pm 0.02$	$0.16^{b} \pm 0.01$	$0.34^{c} \pm 0.05$	$0.55^{d} \pm 0.02$	$0.75^{e} \pm 0.03$
22:6n-3	$0.35^{a} \pm 0.10$	$0.51^{a} \pm 0.08$	$0.62^{a} \pm 0.00$	$1.09^{b} \pm 0.16$	$1.66^{c} \pm 0.01$	$2.08^{d} \pm 0.12$
Σ SFA	$15.27^{a} \pm 0.39$	$17.69^{a} \pm 1.49$	$22.50^{bc} \pm 0.16$	$20.45^{b} \pm 0.83$	$21.92^{bc} \pm 0.29$	$24.50^{c} \pm 0.91$
Σ MUFA	$49.33^{a} \pm 0.80$	$47.79^{b} \pm 0.56$	$48.53^{ab} \pm 0.13$	$51.43^{c} \pm 0.33$	$50.96^{c} \pm 0.34$	$51.24^{c} \pm 0.11$
n-3	$12.84^{a} \pm 0.78$	$14.68^{b} \pm 0.88$	$11.76^{ac} \pm 0.12$	$10.07^{cd} \pm 0.48$	$9.60^{d} \pm 0.36$	$7.52^{e} \pm 0.15$
n-6	$20.94^{a} \pm 0.28$	$18.89^{ab} \pm 1.08$	$16.21^{c} \pm 0.23$	$17.08^{bc} \pm 0.70$	$16.45^{c} \pm 0.29$	$15.57^{c} \pm 0.94$
n-9	$45.42^{a} \pm 0.77$	$41.99^{b} \pm 0.39$	$40.29^{c} \pm 0.39$	$41.74^{b} \pm 0.31$	$40.06^{c} \pm 0.24$	$38.62^{d} \pm 0.25$
n-3 LC-PUFA	$0.88^{a} \pm 0.04$	$1.58^{b} \pm 0.14$	$1.58^{b} \pm 0.02$	$2.45^{c} \pm 0.23$	$3.58^{d} \pm 0.00$	$4.44^{e} \pm 0.18$
n-6 LC-PUFA	$0.21^{a} \pm 0.07$	$0.28^{b} \pm 0.09$	$0.24^{ab} \pm 0.02$	$0.35^{c} \pm 0.04$	$0.48^{d} \pm 0.02$	$0.55^{d} \pm 0.04$
(n-3 + n-6) LC-PUFA	$1.09^{a} \pm 0.06$	$1.86^{b} \pm 0.24$	$1.81^{b} \pm 0.04$	$2.80^{c} \pm 0.26$	$4.07^{d} \pm 0.01$	$4.99^{e} \pm 0.14$
18:1n-9/n-3 LC-PUFA	$49.45^{a} \pm 2.78$	$25.75^{b} \pm 2.23$	$24.72^{b} \pm 0.08$	$16.50^{c} \pm 1.51$	$10.76^{d} \pm 0.05$	$8.38^{d} \pm 0.39$
n-3/n-6	$0.61^{a} \pm 0.03$	$0.78^{b} \pm 0.04$	$0.73^{b} \pm 0.00$	$0.59^{a} \pm 0.01$	$0.58^{a} \pm 0.01$	$0.48^{c} \pm 0.03$
EPA/ARA	$1.13^{a} \pm 0.16$	$2.81^{b} \pm 0.50$	$3.49^{bc} \pm 0.16$	$3.67^{bc} \pm 0.32$	$3.95^{bc} \pm 0.10$	$4.48^{c} \pm 0.48$
DHA/EPA	$9.25^{a} \pm 2.11$	$3.06^{b} \pm 0.28$	$2.51^{b} \pm 0.14$	$2.50^{b} \pm 0.27$	$2.45^{b} \pm 0.04$	$2.32^{b} \pm 0.10$
n-3 PUFA	$12.79^{a} \pm 0.77$	$14.65^{b} \pm 0.87$	$11.72^{a} \pm 0.12$	$10.01^{cd} \pm 0.48$	$9.53^{d} \pm 0.36$	$7.45^{e} \pm 0.15$
n-6 PUFA	$20.94^{a} \pm 0.28$	$18.89^{ab} \pm 1.08$	$16.21^{c} \pm 0.23$	$17.08^{bc} \pm 0.70$	$16.45^{c} \pm 0.29$	$15.57^{c} \pm 0.94$
(n-3 + n-6) PUFA	$33.73^{a} \pm 1.00$	$33.55^{a} \pm 1.73$	$27.93^{b} \pm 0.34$	$27.10^{b} \pm 1.14$	$25.98^{bc} \pm 0.66$	$23.02^{c} \pm 0.93$

Values (mean ± SD, $n = 3$) with different superscript letters in the same row are significantly different ($P < 0.05$).

lowest in the two groups with the least n-3 LC-PUFA contents. In these groups, n-6 and n-9 fatty acid levels were higher than in other treatments, specifically in the 0.0% n-3 LC-PUFA group, where 18:1n-9 and 18:2n-9 contents were significantly greater than in all other treatments. Likewise, LA was reduced with higher inclusion of dietary n-3 LC-PUFA. Alpha linolenic acid (18:3n-3, αLNA) content was also decreasing with higher n-3 LC-PUFA contents in the diets but as observed in the fatty acid composition of the whole body lipids – contrary to the dietary inclusion levels – the αLNA content was higher in livers of the 0.3% compared to those of the 0.0% n-3 LC-PUFA treatment.

3.4. Liver histomorphology

Necrotic tissue was not observed upon the histological examination of hepatic tissue in meagre fed the different diets. However, hepatic steatosis with cytoplasmic vacuolization was observed in all groups, and the degree of cytoplasmic vacuolization was relatively high throughout the treatments. The results of the examinations are summarized in Table 7, with respective figures for each treatment in Fig. 2. The highest degree of cytoplasmic vacuolization was observed in the hepatocytes of fish fed the 0.3% n-3 LC-PUFA diets, showing large hepatocytes with a displacement of cell nuclei (Fig. 2B). Granulomas were observed in all treatments, however with significantly higher incidence in meagre fed dietary n-3 LC-PUFA levels of 0.6% or less. (See Fig. 3.)

4. Discussion

Meagre is one of the promising candidates for Mediterranean aquaculture and its production is steadily increasing over the recent years (FEAP, 2021). Even so, whereas energy, protein, and lipid requirements of this species have been investigated (Chatzifotis et al., 2012; Jauralde et al., 2021), limited information on the essential fatty acid requirements is available, which is crucial for a competitive production and optimal growth. While the essential fatty acid requirement for meagre fingerlings was previously reported (Carvalho et al., 2018), to our knowledge, information on EFA requirements of on-growing juveniles for this species is lacking. The present study showed that meagre juveniles with an initial weight of 35 g require a dietary n-3 LC-PUFA content between 0.7 and 0.8% (DM) for optimal growth rates. The requirement was evaluated by broken line regression of growth rates (TGC) of the fish against n-3 LC-PUFA levels. The broken line regression model is well established for requirement studies and has been applied to determine nutrient requirements of fish and marine invertebrates (e.g. Antony Jesu Prabhu et al., 2013; Carvalho et al., 2018; Lee et al., 2003; Lozano et al., 2017; Richard et al., 2010; Skalli and Robin, 2004).

For requirement studies, acceptance of the diets and good growth rates are important factors, and such were observed within this study obtained with semi-purified diets devoid of fishmeal with linearly increasing levels of EFA in the diets. The observed growth rates (2.4–2.5 for DGI, 1.7–1.8 for SGR, and 1.2 for TGC) combined with feed conversion ratios below 0.8 of fish fed diets containing 0.9% n-3 LC-PUFA or higher demonstrated the great potential of this species for commercial production in agreement with previous recent studies. Similar results for growth rates and feed efficiency for this species were reported by Lozano et al. (2017) with a SGR of 1.5 and a FCR below 0.8 for juveniles with an initial weight of 60 g. Carvalho et al. (2018) reported the same TGC (1.2) in meagre fingerlings of 3 g as observed in the present study. With 3 °C higher rearing temperatures than in the current study and a high dietary fish meal content, even higher growth rates (DGI of 3.6) of meagre juveniles with an initial weight of 35 g could be obtained (Couto et al., 2016), ascertaining the growth potential of this species under farming conditions. In the current study, even feeding diets devoid of n-3 LC-PUFA resulted in a doubling of the initial body weight after 84 days without any mortality and with a FCR of 1.4. In another fish species of the Sciaenidae family, the red drum (*Sciaenops ocellatus*), feeding of essential fatty acid deficient diets to juveniles of similar size for six weeks caused fin erosion, a "shock syndrome", and increased mortality (Lochmann and Gatlin, 1993). Such typical signs of essential fatty acid deficiencies were not observed in the present study, demonstrating that for a marine species, meagre can tolerate very low levels of n-3 LC-PUFA in the diets. In accordance, the requirement for n-3 LC-PUFA between 0.7 and 0.8% diet DM found in this study is lower than many of the other marine teleost species studied. For gilthead seabream (*Sparus aurata*) juveniles, Ibeas et al. (1996), who tested three different EFA levels (0.2, 1.1, and 1.5% of diet DM), reported a dietary n-3 LC-PUFA requirement of 1.0% DM and accordingly, juveniles of this species showed limited growth rates at 0.6% DHA + EPA DM compared to 0.9% (Kalogeropoulos et al., 1992). For juvenile striped jack (*Longirostris delicatissimus*), a fast-growing species with high swimming activity, the n-3 LC-PUFA requirement was stated at 1.7% DM in a study testing five dietary EFA levels from 0.7 up to 3.5% (Watanabe et al., 1989). Lee et al. (2003) feeding semi-purified diets with six different EFA levels and using breakpoint analysis reported a requirement for the flatfish starry flounder (*Paralichthys stellatus*) of 0.9% DM n-3 LC-PUFA. For juveniles of the bastard halibut (*Paralichthys olivaceus*), another flatfish of the same family and genus, Takeuchi (1997) reported a dietary n-3 LC-PUFA requirement between 1.1 and 1.4%. Available data on the n-3 LC-PUFA requirement of red sea bream (*Pagrus major*) juveniles are reported to vary between 1.2 and 2.7% n-3 LC-PUFA in the diet with higher requirements in diets containing higher lipid contents (Takeuchi et al., 1992a). Takeuchi et al. (1992b) also reported a high n-3 LC-PUFA requirement (2.1%) for yellowtail juveniles (*Seriola quinqueradiata*). For juveniles of a fast growing grouper species (*Epinephelus malabaricus*), Lin and Shiau (2007) found a dietary n-3 LC-PUFA requirement of 1.0% of the diet examined during a growth trial of eight weeks.

Similar dietary n-3 LC-PUFA requirements as found for meagre in the current study were reported for turbot (*Scophtalamus maximus*) juveniles with a dietary n-3 LC-PUFA requirement of 0.8% (Gatesoupe et al., 1977). Also, European seabass (*Dicentrachus labrax*) had a similar requirement of 0.7% n-3 LC-PUFA, which was assessed using graded dietary EFA levels from 0.2 to 1.9% and breakpoint analysis (Skalli and Robin, 2004). However, other authors proposed a higher dietary n-3 LC-PUFA requirement of 1.0% for the same species (Coutteau et al., 1996; Torrecillas et al., 2017). One marine species with a fairly low dietary n-3 LC-PUFA requirement (0.5% of the diet) is the red drum (*Sciaenops ocellatus*) (Lochmann and Gatlin, 1993), which together with data presented herein suggests that juveniles of the Sciaenidae family might tolerate lower essential fatty acid levels in their diets than most marine teleost species. Interestingly, the recently reported n-3 LC-PUFA requirement of meagre fingerlings grown from 3 to 10 g body weight (2.1% of diet DM) (Carvalho et al., 2018) was considerably higher compared to that of the larger fish in the present study. This divergence between the requirements of different sized fish of the same species, which was similarly shown in other marine teleosts (Izquierdo, 2005), emphasizes the importance of conducting requirement studies throughout the life cycle of fish. For a better comparison of the former EFA requirement trial in meagre fingerlings by Carvalho et al. (2018) with the current study in on-growing meagre, we have plotted the relative weight gain as percentage of the maximum in each study against

Table 7
Histomorphological evaluation of hepatic tissue of juvenile meagre fed the experimental diets for 84 days.

	Dietary n-3 LC-PUFA level (% DM)					
	0.0%	0.3%	0.6%	0.9%	1.2%	1.5%
Steatosis	$1.9^{a} \pm 0.3$	$2.7^{b} \pm 0.2$	$2.3^{ab} \pm 0.1$	$2.1^{a} \pm 0.2$	$2.2^{a} \pm 0.1$	$2.0^{a} \pm 0.1$
Granulomas	$2.7^{a} \pm 0.3$	$3.2^{a} \pm 0.3$	$2.0^{a} \pm 0.3$	$0.1^{b} \pm 0.2$	$0.4^{b} \pm 0.5$	$0.3^{b} \pm 0.5$

Values (mean ± SD, n = 3) with different superscript letters in the same row are significantly different ($P < 0.05$).

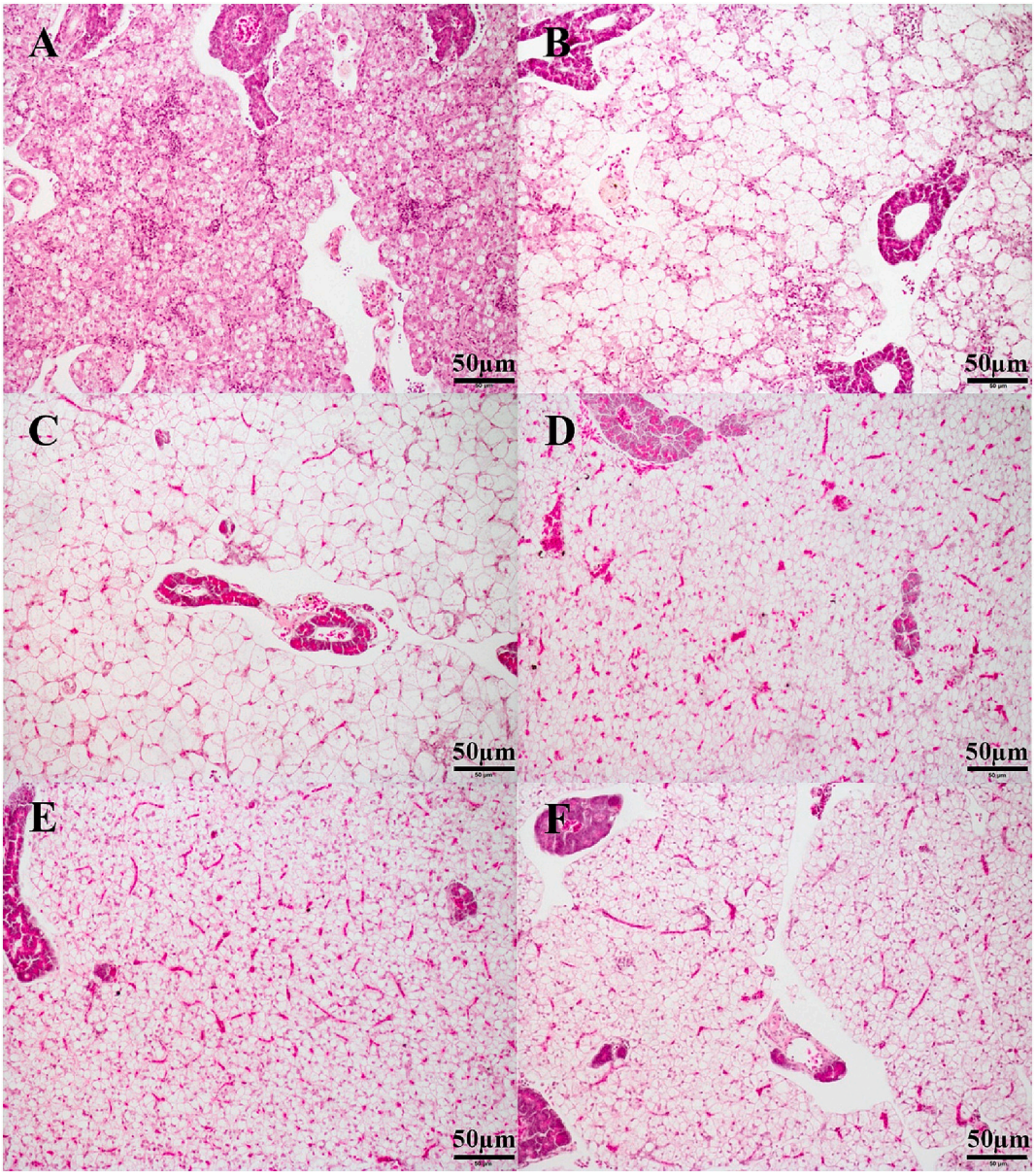

Fig. 2. Liver sections of meagre fed different n-3 LC-PUFA levels for 84 days, stained with H & E: A: 0.0%; B: 0.3%; C: 0.6%; D: 0.9%; E: 1.2%; F: 1.5%. Bars 50 µm.

the dietary inclusion levels of n-3 LC-PUFA (Fig. 4). In the study by Carvalho et al. (2018), which reported a dietary requirement of 2.1% n-3 LC-PUFA, practical diets containing 15% fish meal were fed to the meagre fingerlings for 30 days. Due to the inclusion of fish meal, the diets with the lowest EFA content, which did not contain any fish oil, had already a n-3 LC-PUFA level of 0.8%. Feeding meagre fingerlings graded dietary n-3 LC-PUFA levels from 0.8 to 3.6% of diet DM for 30 days did not cause significant differences among any groups in weight gain or growth indicators, conceivably because the dietary EFA levels were not low enough. The diets used by Carvalho et al. (2018) contained 16.5% DM lipids and had a DHA/EPA ratio of 1.1, whereas the diets fed to the meagre juveniles in the present study had a lipid content of 18% DM and a DHA/EPA ratio of 1.8. These differences in dietary lipid levels, fatty acid profiles and in fish size can explain the variability observed in data on total n-3 LC-PUFA requirements (Tocher, 2010).

The EFA requirement level estimated based on maximal growth performance as the response criterion is also supported by observations on the hepatic health status of meagre, analyzed based on the presence of granulomas in the livers. While the incidence of granulomas was high in the livers of fish fed diets containing 0, 0.3, and 0.6% n-3 LC-PUFA, there were very few granulomas found in the livers of the 0.9, 1.2 and 1.5% n-3 LC-PUFA treatments. This finding suggests a possible link between dietary EFA deficiency and hepatic granulomas in juvenile meagre as previously proposed in even younger meagre (Carvalho et al., 2019). The occurrence of hepatic granulomas of juvenile meagre may be associated with a reduced secretion of anti-inflammatory n-3 LC-PUFA-derived eicosanoids by immune system cells, ultimately compromising the anti-inflammatory defense of the fish (Carvalho et al., 2019). Hepatic steatosis as observed in the present work has also been reported in various studies on meagre nutrition (e.g. Ribeiro et al., 2015; Fountoulaki et al., 2017; Lozano et al., 2017; Carvalho et al., 2019). While it has been proposed that steatotic alterations might be caused by EFA deficiencies or shifting the dietary lipid sources from fish to plant based alternatives (Montero et al., 2001; Caballero et al., 2002, 2004), even in meagre fed fishmeal and fish oil based diets, increasing lipid deposition in the liver was described (Ribeiro et al., 2015). In our study, hepatic steatosis was lowest in the fish fed diets lacking n-3 LC-PUFA, which also showed lowest liver lipid levels, HSI, and whole-body lipid levels.

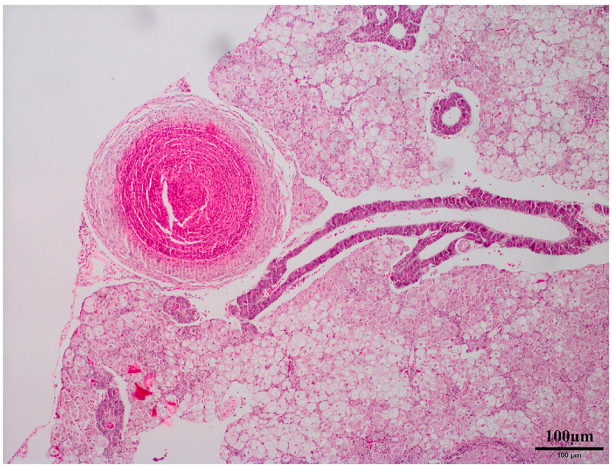

Fig. 3. Granuloma observed in the liver of a fish fed the diet containing 0.0% n-3 LC-PUFA. Bar 100 µm.

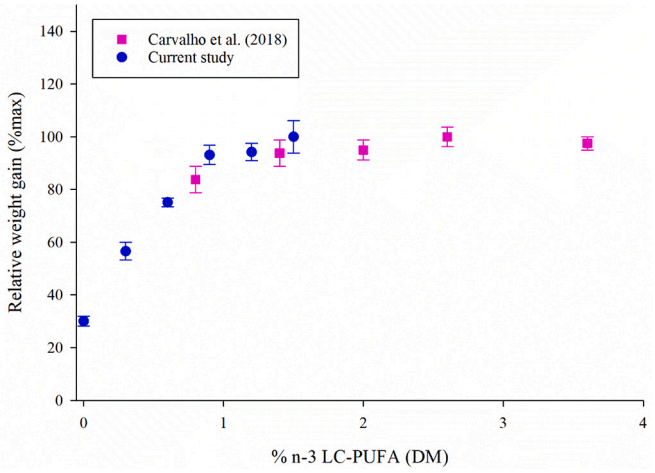

Fig. 4. Relative weight gain of fish from this trial (blue) and the EFA requirement trial from Carvalho et al. (2018) (magenta) (%max weight gain of fish in each trial). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Therefore, other causes than the EFA deficiency are conceivably responsible for the increased lipid accumulation in the meagre livers. Fat accumulation in fish livers is a common morphological alteration when the dietary lipid amounts exceed the capacity of hepatocytes to oxidize fatty acids causing excessive triglyceride deposition of in vacuoles (Spisni et al., 1998). Fountoulaki et al. (2017) observed an increasing occurrence of steatotic alterations in hepatocytes when the lipid content of the diets increased from 15 to 20%, leading to the assumption, that the lipid vacuolization in the present study observed in all treatments was due to a slightly higher than optimal fat content of 18% of diet DM.

As reported in several studies in a variety of fish species, such as *Salmo salar* (Bell et al., 2003), *Psetta maxima* (Regost et al., 2003), *Dicentrachus labrax* (Izquierdo et al., 2003), *Sparus aurata* (Izquierdo et al., 2005), and *Solea senegalensis* (Borges et al., 2014), the fatty acid profile of the fish changed in accordance with different fatty acid profiles of the diets and overall reflected the dietary fatty acid compositions. However, as previously reported for Atlantic salmon (*Salmo salar*) post-smolts fed graded levels of n-3 LC-PUFA (Bou et al., 2017), not all of the fatty acids found in the whole body and liver of fish were in line with the dietary treatments. Limited data are available about the effects of graded dietary EFA levels on the fatty acid composition of marine fish tissues from long-term growth trials, where fish at least triple their weight (Mock et al., 2020). Shorter trials may risk concealing the actual impact of the administered dietary treatment due to a partial 'dilution' of the pre-trial fatty acid composition (Jobling, 2003). In the present study, the level of 18:3n-3 in the 0% n-3 LC-PUFA diet was very high due to the high vegetable oil level in this diet. Nevertheless, in the whole fish as well as in the liver, the proportion of αLNA of the sum of identified fatty acids was lower compared to the 0.3% n-3 LC-PUFA treatment. The whole-body 20:3n-3% of the sum of identified fatty acids of the 0% n-3 LC-PUFA group did not result in significant differences compared to the other treatments, even though the 20:3n-3 levels in their diets were much lower, containing only 0.03% DM. This finding leads to the suggestion that meagre is capable of elongating 18:3n-3 to 20:3n-3. The responsible enzyme for this elongation step is elongase 5 (Elovl5), and in line with our results, Carvalho et al. (2018) reported that the relative expression of *elovl5* gene was upregulated in meagre fed the lowest n-3 LC-PUFA level in their study. Additionally, Monroig et al. (2013) showed that meagre possess at least one elongase (Elovl5) capable of elongating C18 and C20 PUFA. 18:3n-6, a desaturated product of 18:2n-6, was found in highest levels in fish fed diets containing the lowest levels of 18:3n-6 (0% n-3 LC-PUFA treatment). This result suggests that meagre also has some desaturase 6 (Δ6) activity to desaturate C18 PUFA. Both results are in agreement with findings of Lochmann and Gatlin (1993) in red drum exhibiting some limited ability to elongate and desaturate linoleic acid (18:2n-6) and linolenic acid (18:3n-3). Such desaturation capabilities are supported by the higher relative gene expression of *fads2* in meagre fed low n-3 LC-PUFA contents compared to high n-3 PUFA contents and a functional characterization of meagre Fads2, which revealed that the enzyme has Δ6-desaturase activity (Carvalho et al., 2018; Monroig et al., 2013). Nevertheless, the results obtained in this study are in agreement with previous findings and suggest only a limited capability to desaturate and elongate C18 and C20 precursors of EFA in meagre, which is not sufficient to feed diets deficient in n-3 LC-PUFA as the fish showed reduced growth rates and liver health when the dietary n-3 LC-PUFA content was 0.6% or below. Hence, diets have to contain at least above 0.7–0.8% n-3 LC-PUFA to meet the EFA requirements of on-growing meagre juveniles for ensuring good growth performance, feed efficiency and liver health status.

5. Conclusion

In conclusion, the findings of the present study emphasize the potential for meagre farming due to not only the high growth rates and feed efficiency but also the relatively low amount of n-3 LC-PUFA required to obtain those growth rates. With an n-3 LC-PUFA requirement between 0.7 and 0.8% of diet DM for optimal growth and liver health status, diets for on-growing meagre can be developed relying less on fish oil compared to other marine fish species, making it a potentially more sustainable candidate for the diversification of Mediterranean aquaculture. The essential fatty acid requirement suggested in the current study should be considered with diets containing 18% lipid (DM) under optimal rearing conditions.

CRediT authorship contribution statement

Tilo Pfalzgraff: Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Pedro Borges:** Conceptualization, Writing – review & editing. **Lidia Robaina:** Conceptualization, Funding acquisition, Writing – review & editing, Supervision. **Sadasivam Kaushik:** Conceptualization, Resources, Writing – review & editing, Supervision. **Marisol Izquierdo:** Resources, Funding acquisition, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Tilo Pfalzgraff reports financial support was provided by European Union.

Data availability

Data will be made available on request.

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