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Research Article

Oils Combination with Microalgal Products as a Strategy for Increasing the N-3 Long-Chain Polyunsaturated Fatty Acid Content in Fish Oil-Free Diets for Meagre (*Argyrosomus regius*)

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Diversification of species and ingredients is essential for further developing aquaculture production. The present study aimed to evaluate the potential of a blend of microalgae oils and poultry oil as total replacers of fish oil in aquafeeds, a fast-growing species for aquaculture diversification, that was shown to require high dietary n-3 LC-PUFA levels. For that purpose, meagre juveniles (2.74 ± 0.01 g initial body weight) were fed one of the four experimental diets for 30 days. A control diet (FO diet) was based on 5% fish oil and 7% of rapeseed oil, whereas in the other three diets, fish oil was totally replaced by either poultry oil only (PO diet), or blending poultry oil and one of two commercial algal oils (ED and DD diets). Growth performance, tissue composition, and the expression of genes related to fatty acid biosynthesis, antioxidant defense system, and heat shock proteins were determined. Diets with blends of poultry and microalgae oils supported good growth and feed utilization of meagre. Besides, these diets maintained high DHA tissue contents and good fillet lipid quality indices, like in those fish fed the control diet containing fish oil. Furthermore, the meagre fed ED diet showed the highest expression of *gpx*, in agreement with the highest dietary polyunsaturated fatty acid content. These results demonstrated the good potential of blending these two commercial microalgae oils with poultry oil to completely replace fish oil cost-effectevily in diets for meagre. In contrast, PO as the sole replacer of fish oil was not able to support fish growth performance and tissue contents of n-3 LC-PUFA, leading to an increased thrombogenic index in fish fillets. Additionally, the low dietay n-3 LC-PUFA content of PO diet upregulated the relative expression of*fads2* while downregulating *gpx* compared to microalgal diets.

1. Introduction

The limited availability and the increased costs of fish meals (FM) and oils (FO), considered the "gold" feedstuffs for farmed fish, have stimulated the search for novel and more sustainable alternative raw materials, to the tradditional marine ones. [1]. When it comes to FO replacemement, most of the research conducted in the last decades has focused on alternative terrestrial plant or animal oils, due to their competitive price and availability. These feedstuffs are frequently included in fish diets nowadays, making current aquafeeds more responsible than they were before. However, these oils lack n-3 long-chain polyunsaturated fatty acids (LC-PUFA), including docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), which have important biological functions in fish as well as

in humans [2]. Furthermore, n-3 LC-PUFA are essential (EFA) for marine fish species, which have a limited capacity to biosynthesize them through endogenous pathways, and, thus, need to be supplied through the diet [3]. Therefore, whereas the replacement of FM has been successfully achieved [4], the total replacement of FO in modern fish diets, with low contents of FM, faces more challenges. Indeed, high dietary inclusion of plant or animal oils in marine fish diets can reduce fish performance or negatively affect fish health, when EFA requirements of the target species are not met by other sources [5]. In addition, feeding low n-3 LC-PUFA diets also decrease the content of these FA in fish fillets [6], reducing the nutritional value of the final product for the consumer, as well as the beneficial effects associated with fish consumption for human nutrition, for instance in the prevention of human coronary and neural disorders [7, 8].

Thus, given the importance of n-3 LC-PUFA for vertebrates, recent studies have focused on the search for novel lipid sources containing n-3 LC-PUFA that should not only meet fish requirements and maximize productive parameters, but also guarantee aquaculture sustainability and produce fish of high nutritional quality for human consumption. Among the several novel sources that have been showing a good potential for replacing FO, for example oils from microorganisms, krill, or genetically modified plants [9–13], microalgae products are being more and more exploited. Microalgae stands out for their simple nutritional requirements, their higher content in EPA and/or DHA, and their lower footprint, using less land and freshwater to be cultivated compared with other conventional raw materials (terrestrial plants for example) [14, 15]. Some of the most studied microalgae are-*Crypthecodinium*, *Thraustochytrium*, and *Schizochytrium*, that are known to present high DHA contents [16, 17], whereas others like *Phaeodactylum* or *Nannochloropsis* are rich in EPA [18, 19], and, therefore, could be effective EFA sources for fish and potentially replace FO [19]. However, despite their high nutritional potential, the use of microalgae as lipid sources in aquafeeds has been limited by the still high costs of their production, which limits the availability of commercial products in the market and their inclusion in high dietary levels in feeds [14, 20]. Consequently, they have been used in aquafeeds mostly in highly valuable fish species or during early developmental stages or specific phases of the grow-out cycle, when higher DHA and EPA levels are required. Furthermore, commercial microalgal products have been mostly used as biomass, which can present some digestibility problems due to algal cell-wall, but recently, the use of extracted oils have been showing to present advantages to algal biomasses, avoinding digestibility issues and facilitating their combination with lower-cost conventional oils like vegetable or animal fats. Therefore, the use of microalgae oils allow the optimization of the fatty acid profiles of the feed and provide higher flexibility for feeds formulators. For this reason, microalgae oils are being studied and pointed out as a promising lipid source, not only for fish larvae but also for juveniles or adult fish feeds. Indeed, blending microalgae oils with poultry oil effectively replaced FO in practical diets for gilthead sea bream, while maintained growth performance, the utilization of the dietary fatty acids, and the nutritional quality of fish fillets for human consumption [21]. Thus, blends of these oils could also be interesting in farmed species with different nutritional requirements and different nutrient utilization capacity. This is particularly important in emerging species that allow the diversification of the European farmed fish market like meagre (*Argyrosomus regius*), which is a fast-growing species that requires a high dietary content of n-3 LC-PUFA in juvenile stage for maximing growth performance and feed utilization (2% on dry matter dietary basis, with an EPA/DHA proportion of 0.9) [22], a requirement that is considerable higher than those of other marine species with lower growth rates.

Therefore, the general objective of this study was to determine if the blend of microalgae oils with lower-cost lipid sources (poultry and vegetable oils) could constitute an effective lipid source for meagre juveniles with the potential to totally replace the FO in modern aquaculture feeds. The effects of practical combinations with the tested lipid sources were assessed on fish growth, nutrient utilization, tissue fatty acid deposition, lipid quality of flesh, and the expression of some genes, mostly related with the fatty acid biosynthesis, fish antioxidant defense system, and the heat shock proteins, known to play an important role in fish health.

2. Materials and Methods

2.1. Experimental Diets. Four experimental diets were formulated with 15% of fish meal and similar levels of protein, lipids, and energy. The control diet was based on 5% fish oil and 7% of rapeseed oil (FO diet), whereas in the other experimental diets, the fish oil was totally replaced by either blending poultry oil with one of two commercial microalgal oils extracted from the *Schizochytrium* sp. (ED and DD diets), or poultry oil only (PO diet) (Table 1). The two novel algal oils were commercial oils: one containing both EPA and DHA (Veramaris algal oil, Veramaris, The Netherlands; used in ED diet) and the other containing mainly DHA and n-6 DPA (DHA Natur Oil, Archer Daniels Midland, USA; used in DD diet). The dietary inclusions of the microalgae oils were based on the objective of formulating novel diets with similar n-3 LC-PUFA levels of the FO diet (in % of dry weight), although the diets differed in their specific EPA and DHA contents as well as in their EPA/ARA and EPA/DHA ratios (Table 2). Feeds were produced by Skretting ARC Feed Technology Plant (Stavanger, Norway) and were analyzed at ECOAQUA Institute facilities (Canary Islands, Spain). Diets formulation, proximate composition and fatty acid profile are detailed in Tables 1 and 2, respectively.

2.2. Experimental Fish and Feeding Trial Conditions. Meagre juveniles (*Argyrosomus regius*), with an initial body weight of 2.74 ± 0.01 g (mean ± SE) were stocked in 12 tanks of

Table 1: Formulation and proximate composition of the experimental diets.

Ingredients (%)	Diets			
	FO	ED	DD	PO
Fishmeal[a]	15.00	15.00	15.00	15.00
Wheat[a]	12.30	12.43	12.13	11.43
Corn gluten[a]	6.58	6.12	6.20	10.00
Hi-pro soya[a]	5.00	5.00	5.00	5.00
Wheat gluten[a]	17.71	18.02	17.92	15.38
Soya protein concentrate[a]	25.00	25.00	25.00	25.00
Faba beans[a]	5.00	5.00	5.00	5.00
Fish oil[a]	5.28	—	—	—
Rapeseed oil[a]	7.92	7.60	5.69	7.29
Veramaris algal oil[b]	0.00	2.46	0.00	0.00
DHA Natur oil[c]	0.00	0.00	3.67	0.00
Poultry oil[f]	0.00	3.16	3.92	5.70
Vitamin premix[d]	0.10	0.10	0.10	0.10
Mineral premix[e]	0.10	0.10	0.10	0.10
Microalgae:PO	—	1 : 1.3	1 : 1.1	—
Proximate analysis (% dry weight)				
Crude protein	49.31	48.35	48.06	50.18
Crude lipids	18.19	17.53	18.23	17.50
Neutral lipids (% total lipids)	89.32	89.91	90.15	91.96
Polar lipids (% total lipids)	10.68	10.09	9.85	8.04
Moisture	6.60	8.23	7.85	8.28
Ash	4.66	4.51	4.70	4.64
Energy ($MJ\,kg^{-1}$)	22.52	22.06	22.23	22.14
Protein/energy ($g\,MJ^{-1}$)	21.9	21.92	21.62	22.66
Starch (theoretical value)	10.03	10.04	10.18	9.87
Fiber (theoretical value)	2.53	2.53	2.53	2.54

[a]Skretting AS (Norway). [b]Veramaris algal oil (Veramaris, the Netherlands). [c]DHA Natur oil (ADM Animal Nutrition, USA). [d,e]Include vitamins and minerals; Trouw Nutrition, Boxmeer, the Netherlands, proprietary composition Skretting ARC. [f]Poultry oil: Sonac, B.V., the Netherlands.

500 L, at a density of $0.6\,kg\,m^{-3}$ (55 fish per tank). The experimental tanks were filled with seawater ($37\,mg\,L^{-1}$) at an open flow of $500\,L\,h^{-1}$, maintaining the dissolved oxygen over $8\,mg\,L^{-1}$, constant temperature ($23.6 \pm 0.3^{\circ}C$) and natural photoperiod (12 : 12 h light/dark). Fish were fed the experimental diets for 30 days, until apparent satiety, 3 times a day, from Monday to Saturday. Each experimental diet was randomly assigned to an experimental tank and tested in triplicate (i.e., each diet was assayed in 3 different tanks; $n = 3$). Feed delivery was estimated daily, and uneaten pellets were collected after 30 min of each meal, 24 h dried in the oven, and then weighed to estimate feed intake (FI, g feed $fish^{-1}$ day^{-1}).

2.3. Sampling Procedures. Fish weight and length were assessed in all individuals at the starting point and after the feeding period. Prior to each sampling, fish were fasted for 24 h. Productive parameters were calculated using the following equations:

$$\text{Specific growth rate}\left(SGR, \%day^{-1}\right) = 100 * (\ln\ BW_1 - \ln\ BW_0)/\text{n}^{\circ}\ \text{days of the trial}, \quad (1)$$

where BW_0 and BW_1 corresponded to fish body weight (g) at the beginning and at the end of the trial, respectively.

Allometric exponents corresponded to the slope (b) of the potential regression between the wet weight (W) and the total length (TL) of all fish in each tank ($W = a * TL^b$) at the end of the experiment, where a is the allometric coefficient (interception of the equation with Y):

$$\begin{aligned}&\text{Thermal growth coefficient(TGC)} = \left(BW_1{}^{1/3} - BW_0{}^{1/3}\right)/(\text{Temperature} * \text{days}),\\ &\text{Biological feed conversion ratio}\left(FCR_b\right) = \text{Feed delivered}(t_1 - t_0)/(\text{Biomass}\ t_1 - (\text{Biomass}\ t_{0+}\text{Biomass}_{\text{harvested}} + \text{Biomass}_{\text{lost}})),\end{aligned} \quad (2)$$

where t_0 and t_1 corresponded to the beginning and end of the trial, respectively, and $\text{Biomass}_{\text{harvested}}$ is the total fish biomass that was harvested during the trial for samples collection or other purpose, whereas $\text{Biomass}_{\text{lost}}$ was the total fish biomass lost if fish deaths were recorded during the trial.

$$\begin{aligned}&\text{Efficiency of protein retention (\%of protein intake)} = 100 * (BW_1(g) * \text{protein in whole} - \text{body}_1\,(\%)) - \text{initial weight}(g) * \text{protein in whole} - \text{body}_0\,(\%)/\text{FI(g)} * \text{dietary protein}(\%),\\ &\text{Efficiency of lipid retention(\%of lipid intake)} = \text{Efficiency of protein retention (\%of lipid intake)} = 100 * (BW_1(\text{g}) * \text{lipid in whole} - \text{body}_1(\%)) - \text{initial weight(g)} * \text{lipid in whole} - \text{body}_0(\%)/\text{FI(g)} * \text{dietary lipid}(\%),\\ &\text{Efficiency of energy retention (\%of energy intake)} = \text{Efficiency of energy retention (\%of energy intake)} = 100 * (BW_1(\text{g}) * \text{energy in whole} - \text{body}_1\,(\%)) - \text{initial weight (g)} * \text{energy in whole} - \text{body}_0\,(\%)/\text{FI(g)} * \text{dietary energy}\,(\%).\end{aligned} \quad (3)$$

In addition, lipid quality indices for human nutrition [23] were estimated in fish fillets based on FA profile, as

Table 2: Fatty acid composition of the experimental diets (% total identified FA).

Fatty acid (% total identified FA)	Diet			
	FO	ED	DD	PO
14:0	2.05	0.73	1.33	0.72
14:1n-7	0.40	0.59	0.34	0.08
14:1n-5	0.37	0.52	0.35	0.03
15:0	0.45	0.41	0.35	0.10
15:1n-5	0.29	0.42	0.36	0.03
16:0ISO	0.37	0.49	0.40	0.03
16:0	9.72	8.80	10.51	11.59
16:1n-7	2.51	1.24	1.42	1.90
16:1n-5	0.31	0.64	0.38	0.06
16:2n-4	0.44	0.59	0.52	0.07
17:0	0.49	n.d	0.41	0.06
16:3n-4	0.28	0.68	0.35	0.11
16:3n-3	0.31	0.65	0.49	0.04
16:3n-1	0.28	0.42	0.46	0.05
16:4n-3	0.54	0.55	0.44	0.13
18:0	2.72	2.48	2.70	3.40
18:1n-9	30.01	28.16	29.26	39.78
18:1n-7	2.68	1.94	1.73	2.38
18:1n-5	0.37	0.47	0.47	0.07
18:2n-9	0.41	0.49	0.39	0.00
18:2n-6	15.74	15.52	16.36	23.16
18:2n-4	0.32	0.62	0.41	0.03
18:3n-6	0.23	0.94	0.52	0.08
18:3n-4	0.34	0.53	0.49	0.10
18:3n-3	5.02	4.30	3.61	5.29
18:3n-1	0.29	0.47	0.46	0.00
18:4n-3	0.94	0.57	0.53	0.39
20:0	0.68	0.73	0.50	0.52
20:1n-9	0.37	0.41	0.43	0.18
20:1n-7	2.33	2.20	1.91	1.96
20:1n-5	0.47	0.47	0.41	0.17
20:2n-9	0.30	0.56	0.35	0.05
20:2n-6	0.32	1.10	0.56	0.27
20:3n-9	0.28	0.00	0.00	0.18
20:3n-6	0.32	0.66	0.58	0.11
20:4n-6	0.59	0.80	0.48	0.32
20:3n-3	0.29	0.60	0.46	0.10
20:4n-3	0.44	0.72	0.64	0.11
20:5n-3	5.30	3.33	1.54	1.24
22:1n-11	2.37	2.26	1.56	1.94
22:1n-9	0.56	0.56	0.58	0.39
22:4n-6	0.36	1.08	1.32	0.15
22:5n-6	0.47	1.11	3.05	0.13
22:5n-3	1.31	1.04	1.09	0.31
22:6n-3	5.36	8.72	9.04	2.11
EPA + DHA	10.66	12.05	10.58	3.35
EPA/ARA	8.91	4.18	3.22	3.88
EPA/DHA	0.99	0.38	0.17	0.59
SFA	16.11	13.15	15.80	16.39
MUFA	43.02	39.89	39.20	48.96
n-9	31.93	30.18	31.01	40.58
n-6	17.79	21.21	22.88	24.29
n-6 LC-PUFA	2.06	4.75	5.99	0.98
n-3	19.51	20.49	17.84	9.72
n-3 LC-PUFA	12.70	14.41	12.77	3.87
n-3 LC-PUFA (%DW)	2.34	2.75	2.39	0.74
n-6/n-3	0.91	1.04	1.28	2.50

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; LC-PUFA: long-chain saturated fatty acids; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ARA: arachidonic acid. [1]n-6 LC-PUFA: 20: 2n-6; 20: 3n-6; 20: 4n-6; 22: 4n-6; 22: 5n-6. [2]n-3 LC-PUFA: 20: 3n-3; 20: 4n-3; 20:5n-3; 22: 5n-3; 22:6n-3; n.d: not detected.

follows:

$$\begin{aligned}
&\text{Hypocholesterolemic fatty acids (h)} \\
&\quad = 18:0 + \Sigma\,\text{MUFA} + \Sigma\,\text{PUFA}, \\
&\text{Hypercholesterolemic fatty acids (H)} \\
&\quad = 12:0 + 14:0 + 16:0, \\
&\text{Thrombogenic index (TI)} \\
&\quad = (14:0 + 16:0 + 18:0)/(0.5 * \Sigma\,18:1) \\
&\qquad + (0.5 * \Sigma\,\text{MUFA}) + (0.5 * \Sigma\,\text{n} - 6\,\text{PUFA}) \\
&\qquad + (3 * \Sigma\,\text{n} - 3\,\text{PUFA}) \\
&\qquad + (\Sigma\,\text{n} - 3\,\text{PUFA}/\Sigma\,\text{n} - 6\,\text{PUFA}), \\
&\text{Atherogenic index (AI)} = (12:0 + 4 * 14:0 + 16:0) \\
&\qquad /(\Sigma\,\text{MUFA} + \Sigma\,\text{n} - 3\,\text{PUFA} + \Sigma\,\text{n} - 6\,\text{PUFA}), \\
&\text{Peroxidation index (PI)} = 0.025 \times (\Sigma\,\text{monoenoic fatty acids}) \\
&\qquad + 1 \times (\Sigma\,\text{dienoic fatty acids}) + 2 \\
&\qquad \times (\Sigma\,\text{trienoic fatty acids}) + 4 \\
&\qquad \times (\Sigma\,\text{tetraenoic fatty acids}) + 6 \\
&\qquad \times (\Sigma\,\text{pentaenoic fatty acids}) + 8 \\
&\qquad \times (\Sigma\,\text{hexaenoic fatty acids}).
\end{aligned} \tag{4}$$

At the end of the experiment, fish euthanasia was carried out with an excess of anesthesic (clove oil), and samples of fish whole-body, liver, and muscle from 6 fish per tank were collected and pooled (per tissue and per tank) for analysis of proximal composition and fatty acid profile. Additionally,livers from 5 fish per tank were also collected and conserved in RNAlater (Sigma-Aldrich, Madrid, Spain) at −80°C until gene expressionanalysis. Samples were frozen at −80°C until analysis.

2.4. Biochemical Analysis. Samples pools as well as diets were homogenized for analyzing proximate composition. Protein content was determined from the total nitrogen content of each sample, according to the Kjeldahl method [24]. Briefly, approximately 200 mg samples were digested with 10 ml sulphuric acid at 400°C for 60 min in presence of a catalytic tablet with 1.5% CuSO4· 5H2O and 2% of selenium (Panreac, Barcelona, Spain) to convert organic nitrogen in ammonia. After acid digestion, it follows a distillation with 20 ml of distilled water and 50 ml of sodium hydroxide (40% w/v) to ammonia separation, which is then quantified by titration with hydrochloric acid (HCl) 0.1 M to calculate nitrogen content of the sample. Ash contents were determined by incineration in a muffle furnace at 600°C overnight and moisture was determined by drying each sample in a temperature-controlled oven at 110°C for 24 h [24]. Total lipids were extracted by adding a mixture of chloroform/methanol (2 : 1 v/v) with 0.01% butylated hydroxytoluene (BHT) to samples (0.2 g approximately), followed by homogenization with an ultra-turrax (T25 Digital Ultra-turrax, IKA®, Germany). Lipid separation was carried out by a gravimetric method, followed by filtration using anhydrous sodium sulphate, and then evaporated to dryness under a nitrogen atmosphere for lipid content quantification [25]. Fatty acid methyl esters (FAMES) were obtained though acid media transmethylation, by adding toluene with BHT 0.1% and methanol:sulphuric (1%) to total lipids and 16-h incubation at 50°C under nitrogen atmosphere and darkness [26]. FAMES were then recovered by centrifugation (2500 rpm; 5 minutes) with hexane: diethyl ether (1 : 1) and filtered, diluted in hexane, and evaporated until dryness under nitrogen atmosphere. FAMES were then separated by gas-liquid chromatography with helium at a constant pressure of 100 KPa (Supelcowax 10, Superconductores) [27], quantified by a flame ionization detector at 250°C (Finnigan Focus SG, Thermo electron Corporation, Milan, Italy), and then identified by comparison with previously characterized standards. The quantifications of each fatty acid is expressed in percentage of the total identified FA.

The conversion of selected fatty acids to mg/g edible portion of fish fillet (100 g) was done according to Weihrauch et al. [28], applying a conversion factor, that was calculated for each tank based on the total lipid content of the fillet using the formula: Factor = 0.933 − 0.143/TL. After calculating the conversion factor for each tank based on the total lipid content of fish muscle, the selected FA and groups were converted to mg/g of edible portion by applying the respective conversion factor using the following equation: FA in mg/g edible portion = (FA in%total FA ∗ Factor) ∗ %total lipid in muscle.

2.5. Gene Expression. Total RNA was extracted from livers using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Briefly, samples were homogenized with the TissueLyzer-II (Qiagen) with TRI Reagent (Sigma-Aldrich, Sant Louis, MO, USA) and centrifuged with chloroform at 12000 g for 15 min, at 4°C. The RNA phase was mixed with 75% ethanol and transferred into an RNeasy spin column, using RW1 and RPE buffer (Qiagen) to purify RNA bonded to a membrane obtaining purified RNA which was then eluted with 25 μL of RNase-free water. The NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and Gel Red™ staining (Biotium Inc., Hayward, CA) on 1.4% agarose electrophoresis gel were used to determine the quantity and integrity of RNA, respectively. Complementary DNA (cDNA) was synthetized using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions using an iCycler thermal cycler (Bio-Rad).

Liver *mRNA* levels of *fatty acyl desaturase 2* (*fads2*), *fatty acyl elongase 5* (*elovl5*), *heat shock proteins 70* and *90* (*hsp70* and *hsp90*), *glutathione peroxidase* (*gpx*), and *superoxide dismutase* (*sod*) were determined by RT- PCR in an iQ5 Multicolour Real-Time PCR detection system (Bio-Rad). *Elongation factor 1 alpha (ef1a)* was used as a housekeeping gene. Primer sequences are detailed in Table 3. RT-PCR conditions used were the following: a first step of 3 min 30 s at 95°C followed by 40 cycles of 15 s at 95°C, 30 s at annealing temperature (Table 3), 30 s at 72°C, 1 min at 95°C, and a final 81 cycles of 10s from 55°C to 95°C. Reactions were carried out with a final volume of 15 μl, with 7.5 μl of Brillant SYBR Green QPCR Master Mix (Bio-Rad, Hercules, CA, USA), 0.6 μl of each primer (10 mM), 5 μl of cDNA at 1 : 10 dilution, and 1.3 μl of MiliQ water. Milli-Q water also replaced cDNA in blank control reactions. Each run ended with an analysis of the melting curve leading to a melting peak specific for the amplified target DNA.

2.6. Statistical Analysis. Each tank was considered a statistical replicate ($n = 3$ for each treatment). Normal distribution and homogeneity of variances of the dataset were assessed with Shapiro–Wilk and Levene's tests, respectively. A one-way analysis of variance (ANOVA) was applied to the data and means were compared with Tukey's multiple range test [29], with data transformation to normalize the data when necessary [30]. If data normalization was not possible, the data were analyzed using the nonparametric Kruskal-Wallis test. A principal component analysis (PCA) was performed with the overall performance and bological parameters anayzed to obtain a more integrated interpretation of the main effects produced by the different diets in fish. The first two components that explained more than 50% of the total variance among the different diets were selected and graphed. Confidence levels were established at 95% ($P < 0.05$) and when appropriate, the data were also subjected to best-fit regressions (linear, exponential, or logarithmic) or correlation matrixes (Pearson's coefficients), which were further checked for significance. All statistical analyses were done with SPSS 21.0 or Prism9 software packages.

2.7. Ethical Statement. All protocols involving animals in this experiment were strictly conducted according to the European Union Directive (2010/63/EU) and Spanish legislation (RD 1201/2005) on the protection of animals for scientific purposes, at ECOAQUA-UI from University of Las Palmas de Gran Canaria (Canary Islands, Spain). All procedures were approved by the Bioethical Committee of the

Table 3: Sequences of primers used for running RT-PCR analysis of *fads2* and *elovl5* gene expression in meagre livers.

Gene	Primer sequence (5′-3′)	Temperature (°C)
ef1a	F: 5′- GGTGCTGGACAAACTGAAGG-3′ R: 5′- GAACTCACCAACACCAGCAG -3′	59
fads2	F: 5′-TGACTGGGTGACAATGCAGT-3′ R: 5′-TGGTGCTAACTTTGTGCCCT-3′	60.5
elovl5	F: 5′-CATCACACAGTTACAGCTGGTC-3′ R: 5′-GAATTGTGTGCACGGTTTCT-3′	60.5
hsp70	F: 5′- AACGTTCAGGACTTGCTGCT-3′ R: 5′- CCCTTCGTAGACCTGGATGA-3′	56.9
hsp90	F: 5′-AAAAGGCCGAGAAGGAAGAG-3′ R: 5′-GGCTTGGTCTTGTTCAGCTC-3′	61
gpx	F: 5′-AAGCAGTTTGCCGAGTCCTA-3′ R: 5′-GCTGGTCTTTCAGCCACTTC-3′	57
sod	F: 5′-GGCCCTCACTTCAATCCCTA-3′ R: 5′- TCCTTTTCCCAGATCGTCGG-3′	59

fads2: fatty acyl desaturase 2; elovl5: fatty acyl elongase 5; hsp70/hsp90: heat shock proteins 70 and 90; gpx: glutathione peroxidase; sod: superoxide dismutase.

Table 4: Growth performance of meagre juveniles fed the experimental diets for 30 days.

	Diets			
	FO	ED	DD	PO
Performance parameters				
Total length (cm)	8.77 ± 0.12	8.59 ± 0.09	8.76 ± 0.03	7.82 ± 0.07 ∗
Body weight (g)	9.28 ± 0.29^{a}	9.18 ± 0.16^{a}	9.12 ± 0.32^{a}	6.73 ± 0.10^{b}
Weight gain (g)	6.55 ± 0.28	6.43 ± 0.17	6.38 ± 0.33	4.00 ± 0.10
SGR (% day^{-1})	3.90 ± 0.05	3.94 ± 0.07	3.96 ± 0.13	2.95 ± 0.04 ∗
TGC	1.00 ± 0.04	0.97 ± 0.02	0.98 ± 0.04	0.71 ± 0.01 ∗
Allometric exponent	2.85 ± 0.12	3.09 ± 0.05 ∗	2.88 ± 0.08	2.74 ± 0.04
FCR	0.67 ± 0.05^{b}	0.71 ± 0.02^{b}	0.75 ± 0.04^{b}	1.03 ± 0.07^{a}
Feed intake (g feed fish^{-1} day^{-1})	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.14 ± 0.01
Nutrient retention efficiencies				
Efficiency of protein retention (% of protein intake)	45.05 ± 3.52^{a}	43.91 ± 2.67^{a}	42.99 ± 2.52^{a}	29.39 ± 2.61^{b}
Efficiency of lipid retention (% of lipid intake)	38.17 ± 4.46^{a}	31.82 ± 4.20^{a}	24.93 ± 7.24^{ab}	15.82 ± 1.90^{b}
Efficiency of energy retention (% of energy intake)	37.23 ± 3.42^{a}	34.59 ± 3.11^{a}	32.06 ± 0.86^{a}	21.66 ± 1.78^{b}

Superscripts with lowercase letters indicate significant differences with $P < 0.05$. ∗Denotes a tendency ($P < 0.09$).

University of Las Palmas de Gran Canaria (reference OEBA_ULPGC_23/2019).

3. Results

3.1. Growth Performance and Feed Utilization. After 30 days of feeding, meagre juveniles triplicated their weight (Table 4). Fish fed the diets FO, ED, and DD showed similar growth performance (total length, body weight, WG, SGR, and TGC) that was significantly ($P < 0.05$) or, at least, tendentially ($P < 0.09$) higher than meagre fed PO (Table 4). Indeed, according to the PCA analysis, SGR was one of the variables that most explained the variability between the treatments (Figures 1(a) and 1(b)), separating particularly the fish fed ED due to its highest SGR from those fed PO (Figures 1(a) and 1(b)). Furthermore, fish fed ED diet also presented a tendency to show the highest allometric exponent, particularly when compared with those fed PO

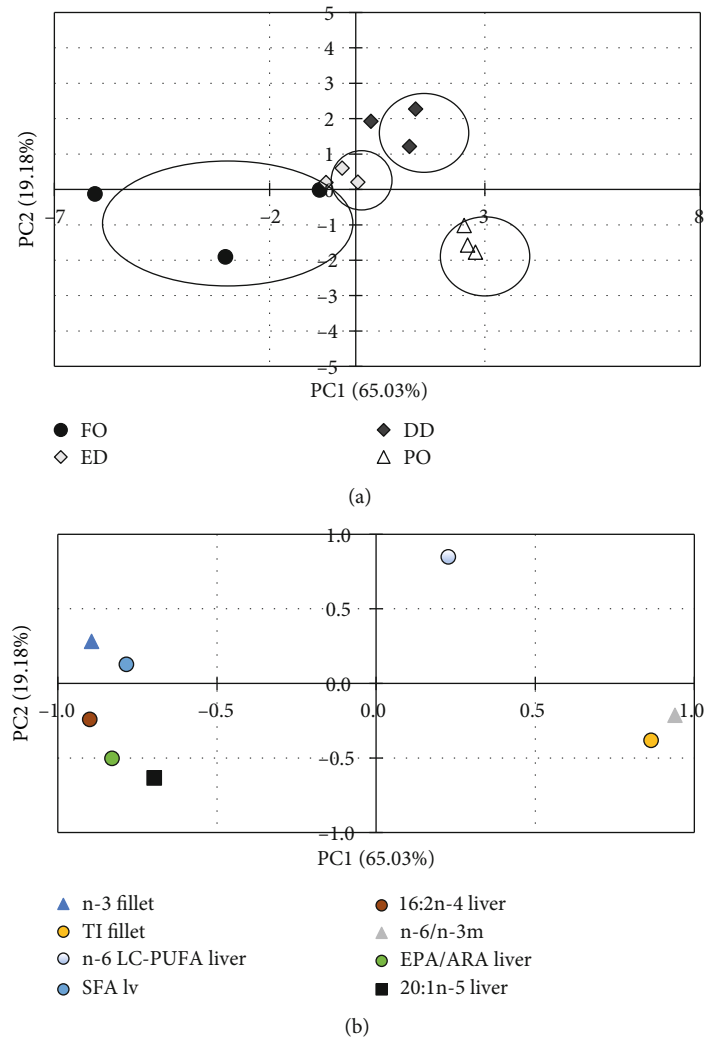

Figure 1: Principal component analysis (PCA) of overall performance, gene expression, and tissue composition of meagre juveniles fed the experimental diets for 30 days. (a) Plot of the scores of the cases (three replicates for each treatment: FO, DD, ED, and PO). (b) Projection of the variables in the principal components 1 and 2 (factor loadings).

($P < 0.09$). FCR was also lower in ($P < 0.05$) in meagre fed FO, ED or DD diets compared to those fed PO, despite the similar feed intake among all (Table 4). This was also reflected in similar way in the efficiency of protein and energy retention ($P < 0.05$; Table 4). Interestingly, meagre fed DD diet presented an efficiency of lipid retention with intermediate values between fish fed FO or ED diets, and those fed PO (Table 4).

3.2. Tissue Biochemical and Fatty Acid Composition. The different dietary oil combinations unaffected the proximate composition of meagre whole body and muscle. However, fish fed PO presented slightly lower protein and lipid contents in whole body and higher hepatic lipids and lower hepatic water content, when compared to those fed FO, ED, and/or DD diets (Table 5).

Regarding fish tissue fatty acid composition, 93% of the identified FA consisted of the SFA 16:0 and 18:0; the MUFA 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-7, and 22:1n-11; and the PUFAs 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, and 22:6n-3 in all tissues (Tables 6, 7, and 8). In general, tissue FA composition reflected the FA composition of the respective diet. For instance, meagre fed DD diet showed the highest content of n-6 DPA in all tissues ($P < 0.05$; Tables 6, 7, and 8). In contrast, fish fed PO diet presented the highest 18:2n-6 and n-6/n-3 ratio in muscle and liver ($P < 0.05$), as well as the same tendency for whole body (Tables 6, 7, and 8). Indeed, muscle n-6/n-3, along with other FA, including the total n-6 PUFA in muscle, and the liver contents in 16:2n-4, 16:1n-5, 20:0, and 20:1n-5 were the FA that most drove the variability among fish fed the different diets, according to the PCA analysis (Figures 1(a) and 1(b)). EPA was the highest in livers and fillets of fish fed FO diet, followed by those fed ED diets, and the lowest in fish fed DD or PO diets ($P < 0.05$; Tables 7 and 8), with the whole body following the same tendency (Table 6). In contrast, DHA was the highest in livers of meagre fed ED and DD diets, particularly when compared with those fed PO ($P < 0.05$; Table 8). A similar tendency was also observed in whole body and muscle (Tables 6 and 7). Therefore, when

Table 5: Whole-body, muscle and liver biochemical composition (% ww) of meagre juveniles fed the experimental diets for 30 days.

	Diets			
	FO	ED	DD	PO
Whole body				
Protein	14.09 ± 0.36	14.10 ± 0.31	14.46 ± 0.03	13.84 ± 0.22
Lipid	3.94 ± 0.12	3.49 ± 0.31	3.22 ± 0.84	2.64 ± 0.14
Ash	1.22 ± 0.03	1.26 ± 0.02	1.35 ± 0.10	1.32 ± 0.01
Water	79.78 ± 0.41	79.98 ± 0.87	79.71 ± 0.69	81.44 ± 0.14
Muscle				
Protein	18.76 ± 0.06	18.56 ± 0.11	18.62 ± 0.12	18.66 ± 0.03
Lipid	1.69 ± 0.09	1.64 ± 0.13	1.67 ± 0.09	1.52 ± 0.10
Ash	1.18 ± 0.15	1.24 ± 0.17	1.20 ± 0.17	1.06 ± 0.18
Water	78.38 ± 0.10	78.72 ± 0.36	78.51 ± 0.09	78.72 ± 0.23
Liver				
Lipid	9.20 ± 1.46	9.37 ± 1.12	8.52 ± 0.87	17.75 ± 3.03*
Water	73.12 ± 1.65	73.12 ± 0.68	73.53 ± 0.23	67.71 ± 2.08*

*Denotes a tendency ($P < 0.09$).

calculating FA contents in mg/g of the edible portion of fillet, DHA, EPA + DHA, and the total n-3 LC-PUFA were higher in meagre fed ED and DD diets than in those fed PO, but those diets leading to lower EPA contents compared to FO ($P < 0.05$; Table 9). DD diet also led to the highest n-6 DPA and n-6 LC-PUFA in mg/g edible portion of fillet ($P < 0.05$; Table 9). Flesh lipid quality indices like AI or PI, were unaffected by the dietary treatment, PO led to a significant increase of fillet TI compared with FO, ED and DD diets ($P < 0.05$; Table 7). Interestingly, TI was significantly and positively correlated with 18 : 0 and 18:2n-6 contents of fish muscle, while negatively correlated with 20:5n-3 and 22:6n-3 contents ($P < 0.05$; Pearson's coefficient).

Nevertheless, for some FA, a contrary trend to the dietary pattern was noted. For instance, the contents of 18:2n-9, 20:2n-9, 18:3n-6, and 20:3n-6, all products from FA desaturation pathways, were the highest ($P < 0.05$) in all tissues of meagre fed PO, contrary to the lowest dietary contents of these FA (Tables 6, 7, and 8). The similar trend was also observed for some elongation products, 20:1n-5, 20:1n-9, 22:1n-9, 20:2n-6, and 22:4n-6, being higher ($P < 0.05$) in fish fed PO than in those fed FO, ED, or DD diets, despite the lowest concentrations of these FA in PO diet (Tables 6, 7, and 8). Hepatic ARA was also the highest in the livers of meagre fed DD diet, despite the second lowest level of this FA in that diet ($P < 0.05$).

3.3. Gene Expression. Meagre fed ED diet presented higher ($P < 0.05$) hepatic *gpx* mRNA levels than those fed PO (Figure 2). In contrast, PO diet induced an upregulation of hepatic *fads2* relative expression compared with FO or DD diets ($P < 0.1$; Figure 2). Indeed, *gpx* and *fads2* relative expressions were two of the most explaining variables that contributed for the varibiality between fish fed these two treatments, according to the PCA Figures 1(a) and 1(b). Furthermore, *gpx* relative expression was positively correlated with the hepatic EPA contents, whereas *fads2* relative expression was negatively correlated with the dietary n-3 LC-PUFA while positively correlated with the dietary 18:1n-9, dietary and hepatic 18:2n-6 contents, and dietary n-6/n-3 ratio ($P < 0.05$; Pearson's coefficient). Expressions of *sod* and *elovl5* were not affected by the different treatments (Figure 2). Similarly, no significant differences were detected in the expression of *hsp90* and *hsp70* among fish fed the different diets, but fish fed PO showed slightly lower expression values for both genes compared to those fed the other diets (Figure 2). Indeed, *hsp90* relative expression levels were positively correlated with the dietary EPA/ARA contents ($P < 0.05$; Pearson's coefficient), whereas *hsp70* relative expression levels were positively correlated with the hepatic ARA content ($P < 0.05$; Pearson's coefficient).

3.4. Principal Component Analysis with Overall Variables. The PCA results showed a good separation of groups fed different diets, with PC1 explaining 84.21% of the variance among the different treatments (Figure 1(a)). The variability among those groups were mostly driven by SGR, some specific fatty acids in the liver, fatty acid ratios in muscle and liver, as well as fillet TI, and the relative gene expression levels of some antioxidant enzymes (Figure 1(b)). Consequently, meagre fed PO were clearly separated from meagre fed the other diets by their highest n-6/n-3 and TI in fillet, their highest hepatic *fads2* expression, and their lowest SGR (Figures 1(a) and 1(b)), whereas those fed FO, ED, and DD diets were generally more close among them.

4. Discussion

Aquaculture diversification is an effective strategy to meet the global need of sustainability of the industry, by increasing the number of farmed species as well as the ingredients used in aquafeeds. In this context, meagre is one of the most promising species for diversifying aquaculture production [31], whereas microalgae oils are one of the top potential

Table 6: Whole-body fatty acid composition (% total identified fatty acids) of meagre juveniles fed the experimental diets for 30 days.

	Diets			
	FO	ED	DD	PO
14 : 0	1.22 ± 0.41	0.20 ± 0.05	0.58 ± 0.17	0.27 ± 0.13
15 : 0	0.22 ± 0.05	0.09 ± 0.01	0.12 ± 0.02	0.08 ± 0.03
16 : 0	13.13 ± 1.80	10.45 ± 0.94	11.92 ± 1.33	9.62 ± 1.73
16:1n-7	3.11 ± 0.36^{a}	1.50 ± 0.08^{b}	1.85 ± 0.14^{ab}	1.88 ± 0.47^{ab}
16:2n-4	0.23 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.02
17 : 0	0.16 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.01
16:3n-4	0.18 ± 0.01^{a}	0.12 ± 0.01^{b}	0.12 ± 0.01^{b}	0.15 ± 0.01^{ab}
16:3n-3	0.10 ± 0.01^{a}	0.04 ± 0.00^{b}	0.05 ± 0.00^{b}	0.07 ± 0.01^{ab}
16:3n-1	0.32 ± 0.02	0.31 ± 0.04	0.31 ± 0.03	0.57 ± 0.06
16:4n-3	0.14 ± 0.02^{b}	0.16 ± 0.01^{b}	0.17 ± 0.01^{b}	0.27 ± 0.02^{a}
18 : 0	4.92 ± 0.32	4.91 ± 0.46	4.38 ± 0.50	7.45 ± 1.07
18:1n-9	35.48 ± 1.16	38.33 ± 1.09	34.17 ± 0.84	37.50 ± 2.18
18:1n-7	2.69 ± 0.19	2.71 ± 0.13	2.37 ± 0.17	3.14 ± 0.21
18:1n-5	0.10 ± 0.00^{ab}	0.08 ± 0.01^{b}	0.09 ± 0.00^{b}	0.11 ± 0.01^{a}
18:2n-9	0.05 ± 0.00^{a}	0.04 ± 0.00^{ab}	0.03 ± 0.01^{b}	0.05 ± 0.01^{a}
18:2n-6	16.77 ± 0.62^{b}	19.94 ± 0.60^{a}	18.73 ± 0.75^{ab}	19.52 ± 0.67^{a}
18:2n-4	0.14 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.08 ± 0.01
18:3n-6	0.11 ± 0.01	0.07 ± 0.01	0.13 ± 0.03	0.08 ± 0.02
18:3n-3	3.32 ± 0.36	3.56 ± 0.38	2.97 ± 0.34	2.39 ± 0.26
18:4n-3	0.47 ± 0.10	0.23 ± 0.04	0.32 ± 0.05	0.24 ± 0.05
20 : 0	0.42 ± 0.02	0.45 ± 0.03	0.36 ± 0.04	0.45 ± 0.09
20:1n-9	0.20 ± 0.01	0.20 ± 0.01	0.23 ± 0.02	0.30 ± 0.05
20:1n-7	2.19 ± 0.07	2.20 ± 0.08	2.15 ± 0.14	2.70 ± 0.39
20:1n-5	0.15 ± 0.00^{a}	0.09 ± 0.00^{bc}	0.08 ± 0.00^{c}	0.13 ± 0.02^{ab}
20:2n-9	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	0.07 ± 0.01
20:2n-6	0.30 ± 0.01^{b}	0.34 ± 0.01^{b}	0.32 ± 0.02^{b}	0.51 ± 0.05^{a}
20:3n-9	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.00
20:3n-6	0.08 ± 0.02^{ab}	0.08 ± 0.00^{b}	0.14 ± 0.00^{a}	0.11 ± 0.01^{ab}
20:4n-6	0.78 ± 0.19	0.66 ± 0.05	0.80 ± 0.05	0.75 ± 0.07
20:3n-3	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.09 ± 0.00
20:4n-3	0.26 ± 0.05	0.17 ± 0.02	0.23 ± 0.03	0.16 ± 0.01
20:5n-3	3.35 ± 0.93	2.33 ± 0.49	1.68 ± 0.27	1.96 ± 0.33
22:1n-11	1.46 ± 0.07	1.44 ± 0.04	1.73 ± 0.15	1.79 ± 0.26
22:1n-9	0.54 ± 0.03	0.53 ± 0.01	0.51 ± 0.05	0.78 ± 0.10
22:4n-6	0.26 ± 0.15	0.18 ± 0.05	0.17 ± 0.03	0.18 ± 0.04
22:5n-6	0.32 ± 0.07^{b}	0.34 ± 0.03^{b}	2.97 ± 0.36^{a}	0.26 ± 0.03^{b}
22:5n-3	1.26 ± 0.35	0.76 ± 0.07	0.57 ± 0.05	0.64 ± 0.12
22:6n-3	4.94 ± 1.65	7.01 ± 1.36	9.18 ± 1.66	5.25 ± 0.92
EPA + DHA	8.28 ± 2.59	9.34 ± 1.85	10.86 ± 1.93	7.21 ± 1.24
EPA/ARA	4.62 ± 1.24	3.62 ± 0.86	2.08 ± 0.23	2.58 ± 0.29
EPA/DHA	0.71 ± 0.05^{a}	0.33 ± 0.01^{b}	0.18 ± 0.01^{c}	0.38 ± 0.02^{b}

Table 6: Continued.

	Diets			
	FO	ED	DD	PO
SFA	20.10 ± 2.57	16.16 ± 1.43	17.44 ± 2.03	17.95 ± 0.98
MUFA	46.07 ± 1.45	47.13 ± 1.40	43.25 ± 1.49	48.41 ± 2.67
n-9	36.33 ± 1.15	39.13 ± 1.11	34.99 ± 0.91	38.69 ± 2.30
n-6	18.64 ± 0.62[b]	21.61 ± 0.55[ab]	23.26 ± 1.16[a]	21.42 ± 0.61[ab]
n-6 LC-PUFA	1.75 ± 0.36	1.60 ± 0.09	4.39 ± 0.40	1.81 ± 0.12
n-3	13.91 ± 3.26	14.35 ± 2.32	15.27 ± 2.36	11.08 ± 1.63
n-3 LC-PUFA	9.88 ± 2.80	10.35 ± 1.92	11.75 ± 1.97	8.11 ± 1.34
n-6/n-3	1.46 ± 0.27	1.57 ± 0.21	1.59 ± 0.20	2.02 ± 0.30*
H	14.35 ± 2.20	10.65 ± 0.97	12.50 ± 1.49	9.89 ± 1.84
H	84.82 ± 2.26	88.74 ± 1.00	86.93 ± 1.55	89.50 ± 1.81
h/H	6.32 ± 1.32	8.51 ± 0.95	7.18 ± 0.94	9.90 ± 2.30
AI	0.23 ± 0.05	0.14 ± 0.01	0.18 ± 0.03	0.13 ± 0.03
TI	0.21 ± 0.04	0.16 ± 0.02	0.18 ± 0.03	0.19 ± 0.01
PI	104.29 ± 22.26	112.69 ± 15.38	139.80 ± 18.95	94.38 ± 11.16

Superscripts with lowercase letters indicate significant differences with $P < 0.05$. *Denotes a tendency ($P < 0.09$). The following FA were detected at less than 0.05% and therefore were removed from the table: 14:1n-7, 14:1n-5, 15:1n-5, 16:1n-5, 16:2n-6, 16:4n-1, 18:3n-4, 18:3n-1, and 18:4n-1.

ingredients for totally replacing FO and for achieving aquafeeds sustainability, maximizing fish production, and guaranteeing a safe and a high nutritional quality product for consumers [1, 32]. In the present study, the combinations of commercial microalgal oils (Veramaris algal oil and DHA Natur oil) with poultry oil were effective in supporting the growth, feed utilization, and nutrient retention of meagre juveniles, in a similar way of a traditional diet with FO, achieving 100% of FO replacement in the current context of the modern and practical fish diets. These results indicate that the dietary incorporation of these two microalgae oils is capable of meeting the meagre relatively high n-3 LC-PUFA requirements [22], as recently demonstrated also for gilthead sea bream (*Sparus aurata*) [21, 33]. Being a new species for aquaculture, the meagre n-3 LC-PUFA requirement for maximum growth performance was recently estimated at 2.1% of the dry weight of the diet [22], a dietary level that was indeed covered by the diets containing FO, Veramaris algal oil, and DHA Natur oil of the present study. However, despite both microalgal oils have a high DHA content, the largest difference between them was in their EPA content, which was more than double in the diet containing Veramaris algal oil than in the diet with DHA Natur oil. Therefore, these differences in the dietary EPA of both oils could, at least, partially explain the best lipid retention efficiency that was observed in fish fed the ED diet compared with those fed DD diet, like what was suggested in sea bream fed similar microalgal formulations [21]. In contrast, when FO was totally replaced by PO as the single replacer, and without any n-3 LC-PUFA supplementation, the growth, feed utilization, and nutrient retention efficiency (lipid, energy, and protein) of fish were negatively affected. These results denote that the residual amount of n-3 LC-PUFA coming from the 15% FM in the diet- a level that is currently used in modern aquafeeds- was insufficient to cover meagre EFA requirements and to support a balanced performance. Although the present study did not include an economic analysis of the different dietary formulations used, it is expected that the blend of these microalgal oils with lower-cost sources, such as poultry and rapeseed oils, might be a reasonable cost-effective tool for the total FO replacement in meagre current practical diets. Indeed, in the present study, the dietary inclusion levels of the two microalgae oils were defined based on the purpose of achieving similar total dietary n-3 LC-PUFA contents of the control FO diet. Given the high costs of microalgal products, further studies decreasing the inclusion levels of these algal oils in an attempt to increase even more the cost effectiveness of the feeds would be of interest. Therefore, the present replacement mixes can be considered as good strategy for increasing the dietary n-3 LC-PUFA contents in aquafeeds under an "ocean-friendly" approach (low FM and FO-free diets), that were then reflected in the composition of fish whole-body and fillets.

Indeed, the fatty acid composition of aquaculture feeds is usually reflected in fish cells and body tissues. In the present study, liver fatty acid profiles showed to be more influenced by the different treatments, whereas in muscle or whole body, those differences among fish fed the different treatments were smaller, although following the same tendency. This confirms that the liver is a highly-sensitive tissue to differences in the dietary composition and highlights the high activity metabolism of fatty acids in meagre livers, as observed for other fish species [34]. In addition, despite meagre of the present study did not reach the commercial size, the FA contents and lipid quality indices were estimated in fish fillets to have a first insight of the potential impact of the tested dietary lipid blends on fillet nutritional quality,

Table 7: Fillet fatty acid composition (% total identified fatty acids) of meagre juveniles fed the experimental diets for 30 days.

	Diets			
	FO	ED	DD	PO
14 : 0	0.94 ± 0.18^{a}	0.46 ± 0.04^{ab}	0.75 ± 0.09^{ab}	0.42 ± 0.05^{b}
15 : 0	0.19 ± 0.02^{a}	0.15 ± 0.01^{ab}	0.15 ± 0.01^{ab}	0.12 ± 0.01^{b}
16 : 0	12.61 ± 0.52	13.41 ± 0.47	13.61 ± 0.18	12.90 ± 0.31
16:1n-7	1.99 ± 0.31	1.24 ± 0.12	1.44 ± 0.13	1.41 ± 0.10
16:2n-4	0.14 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
17 : 0	0.12 ± 0.02	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
16:3n-4	0.13 ± 0.01^{a}	0.11 ± 0.00^{b}	0.11 ± 0.00^{ab}	0.12 ± 0.00^{ab}
16:3n-3	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.00
16:3n-1	0.42 ± 0.05^{b}	0.45 ± 0.01^{b}	0.40 ± 0.05^{b}	0.70 ± 0.07^{a}
16:4n-3	0.45 ± 0.07	0.52 ± 0.07	0.48 ± 0.06	0.53 ± 0.04
18 : 0	5.34 ± 0.22^{b}	5.71 ± 0.32^{b}	5.28 ± 0.43^{b}	7.37 ± 0.36^{a}
18:1n-9	28.12 ± 1.96	29.67 ± 0.81	27.98 ± 1.34	29.50 ± 0.78
18:1n-7	2.99 ± 0.05^{a}	2.73 ± 0.05^{b}	2.46 ± 0.05^{c}	2.80 ± 0.06^{ab}
18:1n-5	0.08 ± 0.00^{ab}	0.07 ± 0.00^{c}	0.08 ± 0.00^{bc}	0.09 ± 0.00^{a}
18:2n-9	0.05 ± 0.00^{a}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}
18:2n-6	16.56 ± 0.76^{c}	18.90 ± 0.25^{b}	17.88 ± 0.51^{bc}	21.37 ± 0.32^{a}
18:2n-4	0.13 ± 0.01	0.05 ± 0.00	0.06 ± 0.02	0.05 ± 0.00
18:3n-6	0.11 ± 0.01	0.08 ± 0.00	0.12 ± 0.01	0.10 ± 0.01
18:3n-3	2.92 ± 0.32	2.70 ± 0.26	2.45 ± 0.16	2.05 ± 0.15
18:4n-3	0.34 ± 0.05^{a}	0.15 ± 0.02^{ab}	0.20 ± 0.03^{ab}	0.12 ± 0.01^{b}
20 : 0	0.41 ± 0.03^{ab}	0.40 ± 0.01^{a}	0.34 ± 0.01^{b}	0.34 ± 0.01^{b}
20:1n-9	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01
20:1n-7	2.05 ± 0.08	1.85 ± 0.02	1.85 ± 0.02	1.80 ± 0.06
20:1n-5	0.13 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.00
20:2n-9	0.04 ± 0.01^{a}	0.03 ± 0.00^{ab}	0.02 ± 0.00^{b}	0.03 ± 0.00^{ab}
20:2n-6	0.37 ± 0.04	0.38 ± 0.02	0.36 ± 0.02	0.47 ± 0.02
20:3n-9	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}	0.00 ± 0.00^{b}	0.01 ± 0.00^{b}
20:3n-6	0.10 ± 0.01^{ab}	0.09 ± 0.01^{b}	0.14 ± 0.02^{a}	0.12 ± 0.00^{ab}
20:4n-6	1.04 ± 0.16	0.97 ± 0.08	1.05 ± 0.09	1.07 ± 0.07
20:3n-3	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.02	0.14 ± 0.00
20:4n-3	0.27 ± 0.00^{a}	0.15 ± 0.01^{c}	0.19 ± 0.01^{b}	0.12 ± 0.00^{c}
20:5n-3	4.98 ± 0.40^{a}	3.08 ± 0.11^{ab}	2.16 ± 0.09^{b}	2.40 ± 0.10^{b}
22:1n-11	1.05 ± 0.04	0.86 ± 0.05	1.02 ± 0.07	1.02 ± 0.07
22:1n-9	0.56 ± 0.05	0.51 ± 0.03	0.54 ± 0.04	0.63 ± 0.03
22:4n-6	0.13 ± 0.02^{ab}	0.11 ± 0.01^{ab}	0.11 ± 0.00^{b}	0.14 ± 0.00^{a}
22:5n-6	0.68 ± 0.15^{b}	0.63 ± 0.06^{b}	3.55 ± 0.50^{a}	0.46 ± 0.04^{b}
22:5n-3	1.56 ± 0.27^{a}	0.88 ± 0.03^{ab}	0.60 ± 0.03^{b}	0.75 ± 0.02^{b}
22:6n-3	12.20 ± 2.53	12.89 ± 0.74	13.83 ± 1.20	10.13 ± 0.48
EPA + DHA	17.18 ± 2.89	15.97 ± 0.78	16.00 ± 1.29	12.53 ± 0.58
EPA/ARA	4.87 ± 0.35^{a}	3.24 ± 0.34^{ab}	2.08 ± 0.08^{bc}	2.25 ± 0.05^{c}
EPA/DHA	0.43 ± 0.05^{a}	0.24 ± 0.02^{ab}	0.16 ± 0.01^{b}	0.24 ± 0.00^{b}

Table 7: Continued.

	Diets			
	FO	ED	DD	PO
SFA	19.64 ± 0.60	20.19 ± 0.62	20.19 ± 0.17	21.22 ± 0.47
MUFA	37.26 ± 2.11	37.21 ± 0.93	35.68 ± 1.55	37.59 ± 1.00
n-9	28.93 ± 1.91	30.37 ± 0.80	28.73 ± 1.36	30.35 ± 0.82
n-6	18.99 ± 0.42^{c}	21.16 ± 0.10^{b}	23.21 ± 0.18^{a}	23.74 ± 0.30^{a}
n-6 LC-PUFA	2.31 ± 0.37	2.17 ± 0.17	5.21 ± 0.63	2.26 ± 0.12
n-3	22.93 ± 2.87^{ab}	20.56 ± 0.63^{a}	20.10 ± 1.23^{ab}	16.32 ± 0.47^{b}
n-3 LC-PUFA	19.13 ± 3.18	17.12 ± 0.78	16.91 ± 1.33	13.54 ± 0.59
n-6/n-3	0.86 ± 0.11^{b}	1.03 ± 0.03^{b}	1.16 ± 0.06^{ab}	1.46 ± 0.05^{a}
H	13.55 ± 0.69	13.87 ± 0.45	14.36 ± 0.26	13.32 ± 0.33
H	85.71 ± 0.70	85.52 ± 0.45	85.09 ± 0.27	86.15 ± 0.33
h/H	6.36 ± 0.39	6.18 ± 0.22	5.93 ± 0.13	6.48 ± 0.19
AI	0.21 ± 0.02	0.19 ± 0.01	0.21 ± 0.01	0.19 ± 0.01
TI	0.17 ± 0.01^{b}	0.18 ± 0.01^{b}	0.19 ± 0.00^{b}	0.21 ± 0.00^{a}
PI	176.50 ± 24.47	166.30 ± 6.44	183.17 ± 12.95	140.68 ± 4.90

Superscripts with lowercase letters indicate significant differences with $P < 0.05$. ∗Denotes a tendency ($P < 0.09$). The following FA were detected at less than 0.05% and therefore were removed from the table: 14:1n-7, 14:1n-5, 15:1n-5, 16:1n-5, 16:2n-6, 16:4n-1, 18:3n-4, 18:3n-1, and 18:4n-1.

which is an important point for meeting consumers needs and expectations. Therefore, meagre fed microalgae diets showed the highest DHA content in body tissues, including the DHA content in mg/g of edible fillets, in line with the dietary composition of the diet. However, microalgal oils were not able to deposit EPA contents in fish cells as high as FO due to the lower dietary EPA content and, thus decreased EPA in mg/g of edible fillets in meagre. Indeed, the replacement of FO by these microalgae oils was reduced by 38 and 57% EPA in muscle in ED and DD diets, respectively, compared to the meagre fed FO diet. Despite this, fillets from the meagre fed ED diet (Veramaris algal oil) presented slightly higher EPA than those from fish fed the DD diet (DHA Natur oil), even considering that Veramaris algal oil was included at a lower percentage in the diet than DHA Natur oil. Therefore, increasing the inclusion of Veramaris algal oil in the diet would probably significantly rise the EPA levels in fish fillets since this oil has approximately 21% of EPA of the total FA content, compared to 16% of FO, although it could also increase the formulation costs. Consequently, the present results provides a first insight on the potential of these two formulations with microalgae oils in delivering similar EPA + DHA contents to consumers through farmed fish consumption as a modern aquaculture FO diet, with levels that are proximate to the minimum recommended levels by World Health Organizations (2.5 mg/g portion). Furthermore, these microalgae novel diets are likely to maintain similar lipid quality indices, including AI and TI, in fish fillets as a traditional FO diet, which also anticipates the possible high nutritional value of those fillets for the consumers. A feeding trial using real farming conditions and reaching meagre commercial size would certainly corroborate the present results, but would be important to validate the high potential of these microalgae oils in delivering high quality products to consumers.

Contrary to the inclusion of blends of microalgae oils and PO as total replacers of the FO in the diet, when the PO acted as the sole FO replacer, TI of meagre fillet were increased, which is in line with the higher 18 : 0 and slightly higher SFA, prothrombogenic fatty acids, of fish fillets fed this diet. Furthermore, it also agrees with the reduced n-3 LC-PUFA contents like EPA and DHA, known to have antithrombogenic properties [35] in fillets of PO-fed fish. Indeed, fillets from PO-fed fish were not able to maintain the minimum recommended levels to decreasethe risk of human coronary disease (2.5 mg/g of edible portion), delivering only 1.6 mg/g of fillets of EPA + DHA, at this fish size, a tendency that would probably be maintained during the whole grow out production cycle. Therefore, these results, along with the highest n-6/n-3 found in the fillets of these fish, denote that the complete replacement of FO by PO might compromise the nutritional quality of fish products for the consumers, related with a higher risk of lipid deposition on blood vessels (thrombogenicity), in agreement with other studies in fish fed this animal fat [1, 21, 36, 37]. Indeed, livers of fish fed PO showed significant higher lipid content than those fed the other experimental diets, suggesting a deficient lipid mobilization from hepatocytes. This condition is often a symptom of n-3 LC-PUFA deficiency [38], since these FA are known to strongly participate in the formation of lipoproteins and, therefore, in the exportation of lipids to outside the liver [39, 40]. In agreement, in a previous study with meagre, levels of n-3 LC-PUFA below the dietary requirement (2.0%) were associated with excessive hepatic lipid accumulation, jeopardizing liver normal function

Table 8: Liver fatty acid composition (% total identified fatty acids) of meagre juveniles fed the experimental diets for 30 days.

	Diets			
	FO	ED	DD	PO
14 : 0	0.88 ± 0.09^{a}	0.49 ± 0.05^{b}	0.76 ± 0.02^{a}	0.36 ± 0.03^{b}
15 : 0	0.18 ± 0.00^{a}	0.15 ± 0.02^{ab}	0.15 ± 0.00^{b}	0.10 ± 0.01^{b}
16 : 0	13.49 ± 0.22^{a}	13.78 ± 0.98^{a}	13.67 ± 0.23^{a}	10.05 ± 0.54^{b}
16:1n-7	3.35 ± 0.25^{a}	2.39 ± 0.09^{ab}	2.60 ± 0.14^{b}	2.75 ± 0.07^{b}
16:1n-5	0.10 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.01
16:2n-4	0.13 ± 0.03^{a}	0.03 ± 0.00^{ab}	0.04 ± 0.00^{ab}	0.02 ± 0.00^{b}
17 : 0	0.11 ± 0.01^{a}	0.07 ± 0.00^{b}	0.07 ± 0.00^{b}	0.05 ± 0.00^{b}
16:3n-4	0.22 ± 0.01^{a}	0.17 ± 0.00^{b}	0.16 ± 0.01^{b}	0.20 ± 0.01^{a}
16:3n-3	0.06 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
16:3n-1	0.06 ± 0.03	0.16 ± 0.12	0.05 ± 0.01	0.03 ± 0.00
16:4n-3	0.14 ± 0.06	0.18 ± 0.07	0.18 ± 0.03	0.05 ± 0.01
18 : 0	7.36 ± 0.81	6.29 ± 0.40	5.47 ± 0.26	7.61 ± 0.35
18:1n-9	37.54 ± 0.78^{bc}	39.05 ± 0.94^{ab}	34.23 ± 0.88^{c}	42.06 ± 0.28^{a}
18:1n-7	3.05 ± 0.08^{ab}	2.62 ± 0.03^{b}	2.31 ± 0.01^{c}	2.86 ± 0.03^{a}
18:1n-5	0.11 ± 0.01^{ab}	0.08 ± 0.00^{b}	0.09 ± 0.01^{ab}	0.12 ± 0.00^{a}
18:2n-9	0.13 ± 0.02	0.09 ± 0.03	0.08 ± 0.02	0.08 ± 0.01
18:2n-6	16.43 ± 0.81^{c}	19.19 ± 0.36^{ab}	18.28 ± 0.47^{bc}	21.55 ± 0.45^{a}
18:2n-4	0.13 ± 0.02	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
18:3n-6	0.23 ± 0.02	0.18 ± 0.04	0.22 ± 0.02	0.18 ± 0.02
18:3n-4	0.12 ± 0.02^{a}	0.05 ± 0.01^{b}	0.07 ± 0.00^{ab}	0.07 ± 0.01^{b}
18:3n-3	2.68 ± 0.07	2.63 ± 0.22	2.36 ± 0.05	2.35 ± 0.14
18:4n-3	0.25 ± 0.04	0.11 ± 0.01	0.15 ± 0.02	0.08 ± 0.01
20 : 0	0.27 ± 0.02^{ab}	0.24 ± 0.00^{a}	0.20 ± 0.01^{b}	0.21 ± 0.00^{b}
20:1n-9	0.19 ± 0.02^{ab}	0.16 ± 0.01^{b}	0.17 ± 0.01^{ab}	0.23 ± 0.01^{a}
20:1n-7	2.53 ± 0.10	2.36 ± 0.04	2.35 ± 0.10	2.60 ± 0.15
20:1n-5	0.14 ± 0.01	0.09 ± 0.00	0.08 ± 0.00	0.10 ± 0.01
20:2n-9	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.00
20:2n-6	0.49 ± 0.02^{c}	0.54 ± 0.01^{bc}	0.60 ± 0.02^{b}	0.69 ± 0.02^{a}
20:3n-9	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
20:3n-6	0.10 ± 0.00^{b}	0.09 ± 0.01^{b}	0.16 ± 0.01^{a}	0.12 ± 0.00^{b}
20:4n-6	0.66 ± 0.17^{ab}	0.65 ± 0.07^{ab}	1.02 ± 0.14^{a}	0.42 ± 0.02^{b}
20:3n-3	0.11 ± 0.00	0.11 ± 0.01	0.14 ± 0.01	0.12 ± 0.01
20:4n-3	0.18 ± 0.03	0.10 ± 0.02	0.17 ± 0.02	0.10 ± 0.02
20:5n-3	1.72 ± 0.03^{a}	1.08 ± 0.13^{b}	0.80 ± 0.08^{bc}	0.57 ± 0.08^{c}
22:1n-11	0.77 ± 0.06^{ab}	0.65 ± 0.04^{b}	0.69 ± 0.01^{b}	0.91 ± 0.04^{a}
22:1n-9	0.86 ± 0.07	0.77 ± 0.08	0.74 ± 0.03	0.85 ± 0.05
22:4n-6	0.10 ± 0.01	0.09 ± 0.01	0.11 ± 0.00	0.10 ± 0.01
22:5n-6	0.24 ± 0.06^{b}	0.24 ± 0.03^{b}	2.66 ± 0.17^{a}	0.07 ± 0.01^{c}
22:5n-3	0.63 ± 0.04^{a}	0.43 ± 0.03^{b}	0.35 ± 0.02^{b}	0.27 ± 0.05^{b}
22:6n-3	4.01 ± 1.31^{ab}	4.43 ± 0.21^{a}	8.55 ± 0.77^{a}	1.79 ± 0.28^{b}

Table 8: Continued.

	Diets			
	FO	ED	DD	PO
EPA + DHA	5.73 ± 1.31^{ab}	5.51 ± 0.32^{ab}	9.36 ± 0.82^{a}	2.36 ± 0.36^{b}
EPA/ARA	2.93 ± 0.61	1.73 ± 0.32	0.81 ± 0.11	1.36 ± 0.19
EPA/DHA	0.52 ± 0.13^{a}	0.24 ± 0.02^{ab}	0.09 ± 0.01^{b}	0.32 ± 0.02^{ab}
SFA	22.34 ± 0.93^{a}	21.04 ± 1.10^{ab}	20.33 ± 0.10^{a}	18.41 ± 0.55^{b}
MUFA	48.70 ± 1.32^{a}	48.24 ± 0.92^{a}	43.33 ± 1.13^{b}	52.58 ± 0.45^{a}
n-9	38.78 ± 0.83^{bc}	40.11 ± 0.91^{ab}	35.27 ± 0.93^{c}	43.27 ± 0.30^{a}
n-6	18.24 ± 1.02^{b}	20.98 ± 0.32^{ab}	23.05 ± 0.55^{a}	23.13 ± 0.46^{a}
n-6 LC-PUFA	1.58 ± 0.24	1.61 ± 0.11	4.54 ± 0.29	1.40 ± 0.05
n-3	9.78 ± 1.23^{a}	9.10 ± 0.49^{ab}	12.74 ± 0.86^{a}	5.37 ± 0.57^{b}
n-3 LC-PUFA	6.66 ± 1.29^{ab}	6.16 ± 0.36^{ab}	10.02 ± 0.84^{a}	2.86 ± 0.43^{b}
n-6/n-3	1.90 ± 0.13^{b}	2.32 ± 0.15^{b}	1.83 ± 0.13^{b}	4.39 ± 0.41^{a}

Superscripts with lowercase letters indicate significant differences with $P < 0.05$. ∗Denotes a tendency ($P < 0.09$). The following FA were detected at less than 0.05% and then were removed from the table: 14:1n-7, 14:1n-5, 15:1n-5, 16:1n-5, 16:2n-6, 16:4n-1, 18:3n-1, and 18:4n-1.

Table 9: Fillet fatty acid composition (mg/g edible fillet) of meagre juveniles fed the experimental diets for 30 days.

	Diets			
	FO	ED	DD	PO
18:1n-9	4.06 ± 0.49	4.12 ± 0.48	3.99 ± 0.41	3.78 ± 0.36
18.2n-6	2.39 ± 0.23	2.62 ± 0.26	2.54 ± 0.22	2.73 ± 0.20
18:3n-3	0.42 ± 0.07	0.38 ± 0.07	0.35 ± 0.04	0.26 ± 0.04
20:4n-6	0.15 ± 0.01	0.13 ± 0.00	0.15 ± 0.01	0.14 ± 0.00
20:5n-3	0.71 ± 0.03^{a}	0.43 ± 0.04^{b}	0.31 ± 0.01^{b}	0.30 ± 0.01^{b}
22:5n-6	0.09 ± 0.01^{bc}	0.09 ± 0.00^{b}	0.50 ± 0.05^{a}	0.06 ± 0.00^{bc}
22:5n-3	0.22 ± 0.02	0.12 ± 0.01	0.08 ± 0.00	0.10 ± 0.01
22:6n-3	1.71 ± 0.24^{ab}	1.76 ± 0.07^{a}	1.94 ± 0.04^{a}	1.28 ± 0.05^{b}
EPA + DHA	2.42 ± 0.25^{a}	2.19 ± 0.11^{a}	2.25 ± 0.04^{a}	1.59 ± 0.06^{b}
SFA	2.82 ± 0.24	2.77 ± 0.16	2.86 ± 0.15	2.70 ± 0.15
MUFA	5.38 ± 0.59	5.16 ± 0.58	5.09 ± 0.51	4.81 ± 0.46
n-3 LC-PUFA	2.69 ± 0.27^{a}	2.35 ± 0.12^{a}	2.38 ± 0.04^{a}	1.72 ± 0.07^{b}
n-6 LC-PUFA	0.33 ± 0.03^{bc}	0.30 ± 0.01^{bc}	0.73 ± 0.06^{a}	0.29 ± 0.01^{c}

and increasing hepatic inflammation [41]. Further morphological analysis are being conducted to evaluate the health condition of the hepatic tissue in meagre fed the present diets. Indeed, PO and other low n-3 LC-PUFA sources, for instance, vegetable oils, led to severe hepatic steatosis by increasing lipogenic activity in the livers of sea bream juveniles [21, 42].

Additionally, the meagre fed DD diet also presented the highest n-6 DPA in body tissues, including in muscle, due to the highest dietary content of this FA in *Schizochytrium* sp., which is found at 20% of the level of DHA [43]. This agrees with other studies in fish that used this microalgae species [21, 44, 45]. Although this FA has been associated with neural and behavior alterations in mammals, particularly at early stages of life, due to its competition with DHA for the incorporation in cell membrane phospholipids [45], some studies concluded that a higher intake of this FA through fish consumption does not impact DHA accumulation in the adult human brain and, thus, higher contents of n-6 DPA in farmed fish fed microalgae diets would probably not constitute a problem for human health [43, 46].

Although the tissue contents of most of FA reflected the diet, some desaturation products through 18:1n-9 or 18:2n-6 were increased in fish fed PO, contrary to their low levels in the diet. These results, together with the upregulation of *fads2* expression in the livers of these fish, suggest an

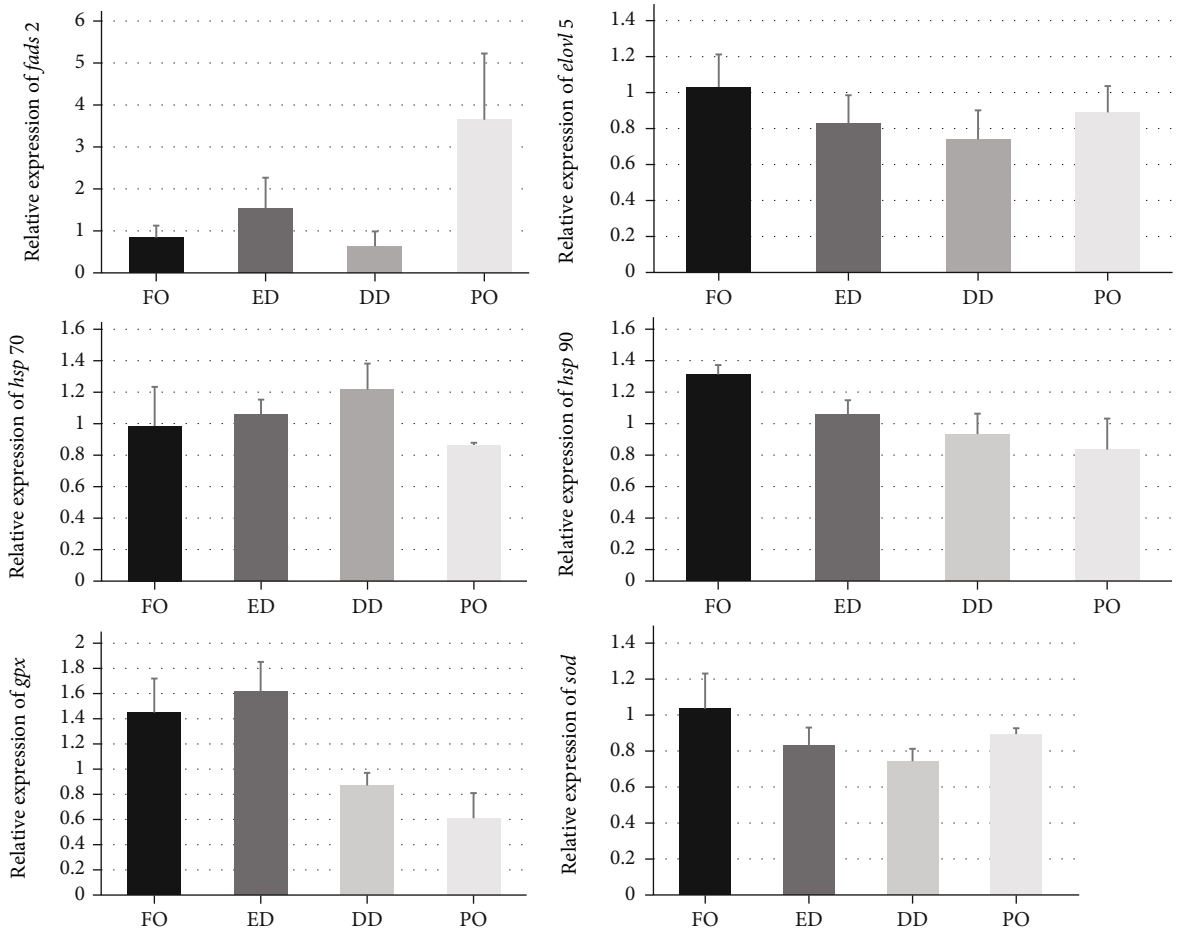

Figure 2: Relative-expression ($2^{-\Delta\Delta ct}$) of health-related genes in livers of meagre juveniles fed the experimental diets for 30 days; ∗ indicates a difference with $0.05 < P < 0.10$. Lower case subindices indicate $P < 0.05$ (*fads2: fatty acyl desaturase 2; elovl5: fatty acyl elongase 5; hsp70/hsp90: heat shock proteins 70 and 90; gpx: glutathione peroxidase; sod: superoxide dismutase).*

activation of desaturation processes in response to a deficient n-3 LC-PUFA diet. This agrees well with the high availability of n-9 (18:1n-9) and n-6 (18:2n-6), that are substrates for FADS2, and this compensatory metabolic response to the decrease of the dietary n-3 LC-PUFA was previously reported in meagre and other marine fish species, for whose n-3 LC-PUFA are considered essential fatty acids [21, 22, 41, 47]. Indeed, FADS2 of meagre was characterized by having a typical $\Delta 6$ as well as$\Delta 8$ functional activity [48]. While $\Delta 6$ pathway typically involves the initial desaturation of 18:3n-3 or 18:2n-6 to 18:4n−3 and 18:3n−6, respectively, the $\Delta 8$ pathway comprises firstly the elongation of 18:3n-3 or 18:2n-6 to 20:3n-3 or 20:2n-6, respectively, which are then desaturated to 20:4n-3 and 20:3n-6. Furthermore, whereas an apparent desaturation towards 18:1n-9 and 18:2n-6 was observed, the same tendency was not observed through n-3 substrate (18:3n-3), which was presented at much lower dietary quantity than 18:1n-9 or 18:2n-6. These results suggest that, despite the higher affinity of the $\Delta 6$ desaturase for n-3 FA, the availability of the substrate seems to have a stronger influence on the desaturation products that are produced [49]. Indeed, in the present study, *fads2* expression was positively correlated with 18:1n-9 and 18:2n-6. Contrary to *fads2*, which is almost always upregulated when the dietary n-3 LC-PUFA contents are low, different studies reported that, under n-3 LC-PUFA deficiency, *elovl5* can be either [22] or either not [21, 42] upregulated. In the present study, despite that some elongation could be speculated by comparing some of the contrary tendencies between FA profiles of diets and fish tissues, *elovl5* relative expression did not differ among fish fed the different dietary treatments.

In addition, the highest dietary PUFA and, consequently, the increase in the risk of peroxidation and oxidative stress could be the reason behind the higher basal expression of *gpx* in the meagre fed ED diet compared with those fed PO, that was posetively correlated with the hepatic EPA contents of fish. Both GPX and SOD are enzymes responsible for protecting biosystems from oxidative damage due to reactive oxygen species [50], although *sod* expression was not altered in the present study by the dietary treatment. In agreement, previous studies in fish have also reported a higher basal activity of different antioxidant enzymes in response to high PUFA diets [51, 52]. Furthermore, a previous study with meagre also reported a higher basal *cat* expression, another gene encoding an antioxidant enzyme, which led to a more controlled stress response to handling [53]. Therefore, this higher basal expression of *gpx* in meagre

fed ED diet is likely to be a compensatory mechanism for the higher pro-oxidant environment. Furthermore, despite the lack of significant effects of dietary treatments on the expression of HSP in the present study, the expressions of *hsp90* and *hsp70* were positively correlated with dietary EPA and EPA/ARA as well as with hepatic ARA, respectively. These results suggest a potential effect of EPA and ARA on HSP metabolism, as described previously both in mammals and fish [54, 55]. HSPs are conserved proteins expressed in all living organisms under normal conditions, and they function as chaperons for maintaining homeostasis as well as are involved in the response to several stressors to avoid and to repair cellular damage [56]. In agreement with the present results, an increased expression tendency of h*sp70* was also observed with the increase of dietary ARA in European sea bass (*Dicentrarchus labrax*) larvae, suggesting a "better prepared" the tissue for dealing with stressful situations [55]. Furthermore, Senegalese sole (*Solea senegalensis*) fed vegetable oils low in ARA similarly presented a reduced expression of *hsp90* and *hsp70* [57], like the meagre fed PO of the present study. Therefore, the slightly higher basal expression *of hsp* in meagre fed with higher dietary ARA and/or EPA diets (as in FO or microalgae diets) might be translated into an optimized and adapted health condition of fish compared to lower dietary ARA and EPA levels (as for instance in PO diet), possibly improving fish welfare. These basal higher expressions of antioxidant defense system and heat shock protein in fish fed diets with higher LC-PUFA levels might thus be particularly important and advantageous for the animal when dealing with stressful events, by avoiding great alterations in tissue biochemistry and, consequently, cell damage. Therefore, studying the response of these genes under stress conditionswhen fish are fed microalgal or PO diets would be of interest in the future.

5. Conclusions

The combination of two commercial microalgae oils (Veramaris algal oil and DHA Natur oil) with poultry oil effectively replaced FO in meagre modern diets with low FM content. Those lipid mixes supported growth, feed utilization, and maintained DHA tissue levels, in a similar way of a current FO diet. Furthermore, microalgae diets showed no alteration of the molecular markers analyzed related with fish health when compared to FO. In contrast, PO did not support fish growth performance and reduced n-3 LC-PUFA tissue contents, whereas upregulated hepatic expression of *fads2* in a response to the n-3 LC-PUFA deficiency in the diet.

Data Availability

The data used to generate the results in this manuscript can be made available if requested to the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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