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Growth, Fatty Acid Profile and Malondialdehyde Concentration of Meagre *Argyrosomus regius* Fed Diets with Different Lipid Content

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Abstract: The aim of the study was to evaluate the growth, fatty acid profile and concentration of malondialdehyde of muscle tissue of meagre *Argyrosomus regius* fed diets with different lipid content. The long-term experiment was conducted in three feeding groups: A (CP = 52.0; CL = 21.0), B (CP = 56.0; CL = 18.0), C (CP = 48.0; CL = 16.0) with two replicates in marine net cages on Bisage Island, Adriatic Sea over 20 months. At the beginning of the experiment, fish were of equal weight (6.83 ± 1.03 g) and length (8.57 ± 0.49 cm) and were fed to satiation during the experiment. At the end of the experiment, the fish from each feeding group (n = 110) were measured and muscle tissue was collected (n = 60) and stored at −80 °C until analysis. The final weight and condition factor were significantly different ($p < 0.05$) between the groups. The highest ratio of crude fats and n-3/n-6-fatty acids was found in the muscle tissue of group A. Fish fed diet A also exhibited higher MDA levels compared to fish in the other feeding groups, indicating elevated levels of lipid peroxidation in muscle tissues. Experimental feeding group A showed better growth performance, a higher content of the beneficial fatty acids EPA and DHA and a more favorable n-3/n-6 ratio than feeding groups B and C. Continuously monitoring and adjusting feeding protocols in accordance with lipid content and fatty acid composition could maximize growth and health outcomes in meagre farming.

Keywords: aquaculture; oxidative stress; fatty acids; malondialdehyde; *Argyrosomus regius*

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1. Introduction

There is increased interest among aquaculturists around the world in fast-growing species, such as cobia (*Rachycentrom canadum*), greater amberjack (*Seriola dumerili*), meagre (*Argyrosomus regius*), common dolphin fish (*Coryphaena hippurus*), various species of grouper (*Epinephelus* sp.) and tuna (*Thunnus* sp.) [1,2]. The meagre (*A. regius*) is a sciaenid distributed in the Mediterranean and Black Seas and along the Atlantic coasts of Europe and Africa [3]. The fish can grow up to 2 m in length and reach 50 kg in the wild [4]. The species also possesses the biological characteristics required for commercial aquaculture using established farming technologies [5]. These characteristics include rapid growth of ~1 kg per year [6–8], low feed conversion ratio of 0.9–1.2 [6,9], and good processing yield and nutritional value [6,10]. In addition, it has a high meat quality and is suitable for industrial processing [11,12] which emphasizes meagre as a highly marketable species. Due to the highlighted characteristics, meagre has also become an increasingly important fish species due to the need for species diversification in Mediterranean mariculture [13,14].

Fish nutrition is one of the most demanding technological processes and plays a direct key role in intensive aquaculture as it affects the production cost, growth and health of the rearing organism [15]. There is a close relationship between the quality of fish feed (or pollution load) and the nutritional value of the species, i.e., protein content, phospholipid composition and the proportion of unsaturated fatty acids, especially DHA and EPA [16]. Dietary lipids are not only an important source of energy, but also a source of fatty acids needed for the synthesis of new cell lipids for growth and reproduction and for the turnover of existing lipids [17]. The type and amount of dietary lipids can influence fish metabolism, immune function, and the fatty acid composition of tissues [18]. Determining the basic nutrient requirements of a species, especially the optimal ratio between proteins and lipids, along with the inclusion of optimal amounts of fish oil, is essential for a balanced diet [7,19].

A current trend in fish feeding is the use of higher proportions of lipids in the diet. While increasing the lipid content in the diet can help reduce high feed costs by partially saving protein in the feed, problems such as excessive fat deposits in the liver can affect fish health, quality and shelf life of the final product [20]. Marine fish oil is the richest available source of long-chain polyunsaturated fatty acids (PUFAs), which are important in human nutrition for a variety of important functions [21,22]. However, the Marine Ingredients Organisation (IFFO) estimates that about 75% of annual fish oil production still goes into aquaculture feeds [23].

The fatty acid composition of individual fish oils varies considerably and is influenced by factors such as age, size, species, reproductive status, geographic location, and the season in which they are harvested. The major fatty acids found in the highest concentrations in fish oil include 14:0, 16:0, 16:1n-7, 18:1n-9, 20:5n-3 and 22:6n-3 [24]. Moreover, the oils of capelin, sand eel, mackerel and herring, among other high latitude North Atlantic species, contain a higher proportion of monounsaturated fatty acids (MUFA) compared to the other fatty acid classes, which is exceptional in the chemical composition of fish oil. In particular, these species have much higher proportions (>5%) of 20:1n-9 and 22:1n-11, derived from a diet rich in zooplankton (calanoid copepods and euphausiids) containing an abundance of waxy esters [25]. Fishmeal and fish oil are still considered the most nutritious and digestible ingredients for farmed fish feeds, but their share in compound feeds for aquaculture has shown a clear downward trend, mainly due to supply and price fluctuations. They are increasingly used selectively, for example for specific phases of production, particularly for hatchery, broodstock and finishing feeds [26].

In recent years, the term “dietary oxidative stress” has been coined to describe an imbalance between pro-oxidants and antioxidants resulting from an inadequate supply of nutrients [27]. Many antioxidants that contribute to the antioxidant capacity of a fish are derived from its diet, particularly fat-soluble antioxidants, as they cannot be synthesized de novo by animals [28]. Consequently, fish with different diets cope with oxidative stress in different ways. One of the most commonly used methods to determine oxidative stress is the TBARS test (thiobarbituric acid substances) which determines the formation of reactive aldehydes, malondialdehyde (MDA) as a biomarker and secondary lipid peroxidation product [29,30]. Understanding the relationship between dietary lipids, fatty acid composition, and oxidative stress markers such as MDA is essential for optimizing fish diets to enhance growth performance, improve health, and ensure product quality in aquaculture practices [31]. By measuring MDA levels, researchers can evaluate the oxidative damage and adapt feeding strategies accordingly to mitigate adverse effects and promote better health and growth outcomes in fish. The aim of the study was to evaluate growth, fatty acid profile and the concentration of malondialdehyde as a bioindicator of oxidative stress and the fatty acid profile of muscle tissue of meagre *Argyrosomus regius* fed diets containing varying amounts of fish oil. The research assumes that diets with different lipid content will have a significant effect on the fatty acid profile of muscle tissue and the concentration of malondialdehyde in muscle tissue. In addition, the experimental feed will likely have a higher proportion of lipids in the muscle tissue of the fish and a more favorable fatty acid composition due to the higher lipid content in its chemical composition.

2. Materials and Methods

2.1. Research Location

The study was conducted near Bisage Island in the Adriatic Sea (44°01′28.6″ N 15°13′11.2″ E) (Figure 1). Sea temperature was measured daily with a fixed thermometer at a depth of 2 m. The oxygen content was measured daily and was not below 80% saturation, and salinity was stable at 38‰ throughout the study. In order to maintain favorable zoo-hygienic breeding conditions, the nets in the cages were changed at least once a month, or more often if necessary. The mesh size was adapted to the size of the fish.

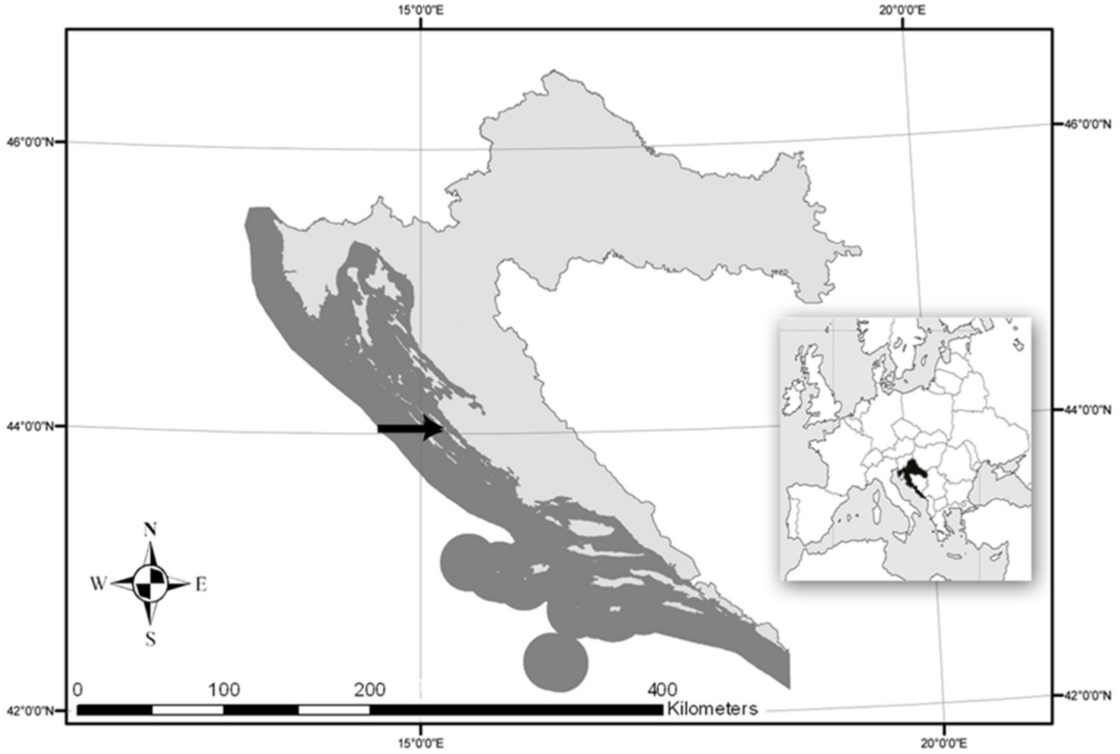

Figure 1. The location of the research—Republic of Croatia (arrow—Bisage Island).

2.2. Experimental Fish and Feeding Trial

Meagre fingerlings originate from a French hatchery. At the beginning of the experiment, the density was 0.28 kg/m^3. When the density exceeded 10 kg/m^3, fish were transferred to a larger cage volume. At the end of the experiment, the density was a maximum of 8 kg/m^3. The overall survival rate of the fish was 90–95%. Mortality was up to 20 g fish and was caused by *Ceratothoa oestroides*.

The feeding experiment was conducted in three feeding groups: A (CP = 52.0; CF = 21.0; fish oil = 15%), B (CP = 56.0; CF = 18.0; fish oil = 10%), C (CP = 48.0; CF = 16.0; fish oil = 7%) (CP = Crude protein; CL = Crude lipid) (Table 1) with two replicates (3 × 2) in marine net cages (9 × 5 × 5 m; V = 225 m^3) during 20 months (May 2016–December 2017). Depending on the study period and fish category, 1.5, 1.9, 3.5, 4.5 and 6.5 mm pellets were used. The ingredients used in the research for the production of the experimental feed (A) are classified and covered by intellectual property with the rights established in the European Directive [32]. The research complied with the guidelines of the national principles of the Animal Welfare Act [33] and European Directive [34] on the protection of animals used for scientific purposes.

Table 1. Proximal composition of experimental feed (A); commercial feed for sole (B); commercial feed for meagre (C).

	Feed Mixtures		
Proximal Composition (% of Diet)	**A**	**B**	**C**
Crude protein	52.0	56.0	48.0
Crude fat	21.0	18.0	16.0
Crude fiber	0.7	0.4	3.0
Ash	11.4	10.4	8.3
P	1.6	1.65	1.12
Ca	2.4	2.11	1.87
Na	0.9	0.68	0.38
Vitamins (UI/kg) *			
Vit. A	15,000	15,000	15,000
Vit D_3	2500	800	800
Vit C	500	150	200
Vit E	300	150	250
Microelements (mg/kg)			
Cu	4.7	1.5	4.1
Mn	-	12	12
Na (μg/kg)	438	-	-
Zn	301	75	75
I	4.6	1.8	1.8
Antioxidants (mg/kg)			
Propyl galate	22	100	100
BHA ¥	-	100	100
Citric acid	20	-	-

* UI—international unit code, ¥ BHA—Butyl—hydroxyanisole.

2.3. Sample Preparation and Analysis

During the experiment, there were around 9000 fish in each cage. For the basic morphometric analysis, 100–110 specimens were sampled from each cage to measure total length (TL, cm) and wet weight (W, g). Before measurement, the fish were anesthetized with 0.2 g/L tricaine methanesulfonate (MS-222, Sandoz, Switzerland). The biometric measurements were performed monthly, and after the measurement the fish were returned to the cage. At the end of the study, samples were taken for the fatty acid profile. The Fulton's condition factor (K) was calculated on the basis of the measurements:

$$K = W/TL^3 \times 100.$$

The biomass in the cages was estimated from the weight measurements and the total number of fish per cage at the beginning and the end of the experiment. The fish were fed twice daily from May to December until obvious satiation, and once daily during the remaining months. Feed intake was recorded daily for each cage. Growth and nutrient utilization indices were calculated based on the collected data:

$$\text{Specific growth rate (SGR)} = \ln\text{(final weight} - \text{initial weight)/duration of the experiment (days)},$$

$$\text{Thermal growth coefficient (TGC)} = \text{final weight } 1/3 - \text{initial weight } 1/3/(t \times \text{duration of the experiment (days))} \times 1000,$$

$$\text{Feed conversion ratio (FCR)} = \text{feed offered (g)/weight gain (g)}.$$

Twenty samples were collected from each experimental group (A, B, C) (10 from each experimental unit, N = 60). Fish were stored at −80 °C before further processing. Samples were dissected and 10 g of muscle was separated from the remaining tissue and homogenized in a blender to determine the composition of malondialdehyde, lipids and fatty acids. The total MDA concentration in 10% of the homogenates in 0.14 M potassium chloride was measured by high-performance liquid chromatography (HPLC) according to the modified method of [35]. RP HPLC analysis was performed on the TSP −130 system (Thermo Separation Products, Inc, Thermo Fisher Scientific, Inc, Waltham, MA, USA) using the Waters symmetry® C18 column (Waters, Milford, MA, USA) with a length of 25 cm, a radius of 4.6 mm, and a particle size of 5 µm. The mobile phase consisted of methanol and 50 mM potassium dihydrogen phosphate in a 50:50 ratio. The flow rate of the mobile phase was 1 mL/min, the analysis time was 10 min, and the wavelength of the UV detector was 532 nm. Under these conditions, the retention time is 3 min. The linearity of this method was 0.99 and the reliability was 99%. Total lipids (TL) of homogenized muscle were extracted according to the method of [36]. The extract layers were separated at least three times with the addition of a salt solution to maximize lipid recovery, and the organic phases were combined and evaporated to dryness. The total lipid content was weighed after evaporation of the solvent. Total extracts were saponified (1.2 M NaOH in methanol), acidified (6 M HCl), methylated (14% BF_3 in methanol), and extracted in dichloromethane [37]. Fatty acid methyl esters (FAMEs) were analyzed using the Agilent gas–liquid chromatography (GLC) 6890 N GC system equipped with a 5973 Network Mass Selective Detector, a capillary column (25 m × 0.3 mm × 0.25 m; cross-linked 5% phenylmethylsiloxane), and ultra-high-purity helium as the carrier gas. The GLC setting was programmed to increase the column temperature from 145 °C by 4 °C min^{-1} to 270 °C at a constant column pressure of 2.17 kPa. Retention times, peak areas and mass spectra were recorded using Chemstation software. FAMEs were identified using the mass spectral data and the family plot of equivalent chain length data for GC standards for the GC column used. The bacterial FAME standard mix, FAME mix C18–C20, polyunsaturated fatty acid standards (PUFA1 and PUFA3), cod liver oil, and several individual pure standards from FAME were used.

2.4. Statistical Analysis

To test the differences between initial and final weight, the Kolmogorov–Smirnov and Lilliefors tests for normality and the Levene test for homogeneity of variance were performed. The initial weight was homogeneous and normally distributed, so that an ANOVA was performed. For the final weight and the condition factor (K), the variances were inhomogeneous, so that a non-parametric test (Kruskal–Wallis ANOVA by ranks) was carried out by group ($p < 0.05$). ANOVA was used for fatty profile data analysis with Fisher’s LSD test ($p < 0.05$). A multivariate principal component analysis (PCA, Primer) was used to identify the predominant effect of diet quality and growth parameters on FAME structure; in particular, PUFA content and oxidative stability of each group were tested. The analysis was based on correlation matrices (constructed using the C16:1, C18:1, C18:2, C18:3, EPA, DHA, TL, MDA, SGR, Kfin, n-3/n-6) involving the normalization of all variables due to their different scales. Only the principal components with eigenvalues > 1 were considered to account for much of the parameter variability. PCA was performed using the software Primer 7.

3. Results

The results of growth, condition factor and feed conversion ratio of meagre fed with three experimental diets are shown in Table 2. The initial masses do not differ, while the final weight and K are significantly different between the groups. The average mass (gain) of meagre by group and month and the average sea temperature for the periods between the two biometric studies are shown in Figure 2.

The mean sea temperature was 19.54 ± 2.60 °C in 2016, while in 2017 it was 18.3 ± 4.38 °C. The average sea temperature at the end of the trial was 14.38 ± 0.38 °C. The fat content (g/100 total weight of fish) in each group of fish was: A = 6.80; B = 7.20; C = 4.30.

The fatty acid profile of the meagre muscle tissue, total lipid content and relevant proportions of FA and ratios between polyunsaturated DHA and EPA fatty acids and sum of n-6 and n-3 fatty acids (n-3/n-6) were analyzed. The results are presented in Table 3.

Table 2. Growth parameters of meagre fed diets with different lipid content.

	Group A	Group B	Group C
Initial weight (g)	6.74 ± 0.99	6.91 ± 1.07	6.85 ± 1.05
Final weight (g)	1899.51 ^{a} ± 289.10	1760.40 ^{b} ± 270.04	1105.13 ^{c} ± 227.16
Final K	1.28 ^{a} ± 0.07	1.25 ^{b} ± 0.07	1.17 ^{c} ± 0.10
SGR	1.184	1.177	1.137
TGC	1.223	1.216	1.118
FCR	2.04	2.25	2.41

Group A, Experimental feed for meagre; Group B, Commercial feed for sole; Group C, Commercial feed for meagre; K—condition factor; SGR—Specific growth rate; TGC—Thermal growth coefficient; FCR—Feed conversion ratio; Values within the same row with different superscripts are significantly different at the level, $p < 0.05$.

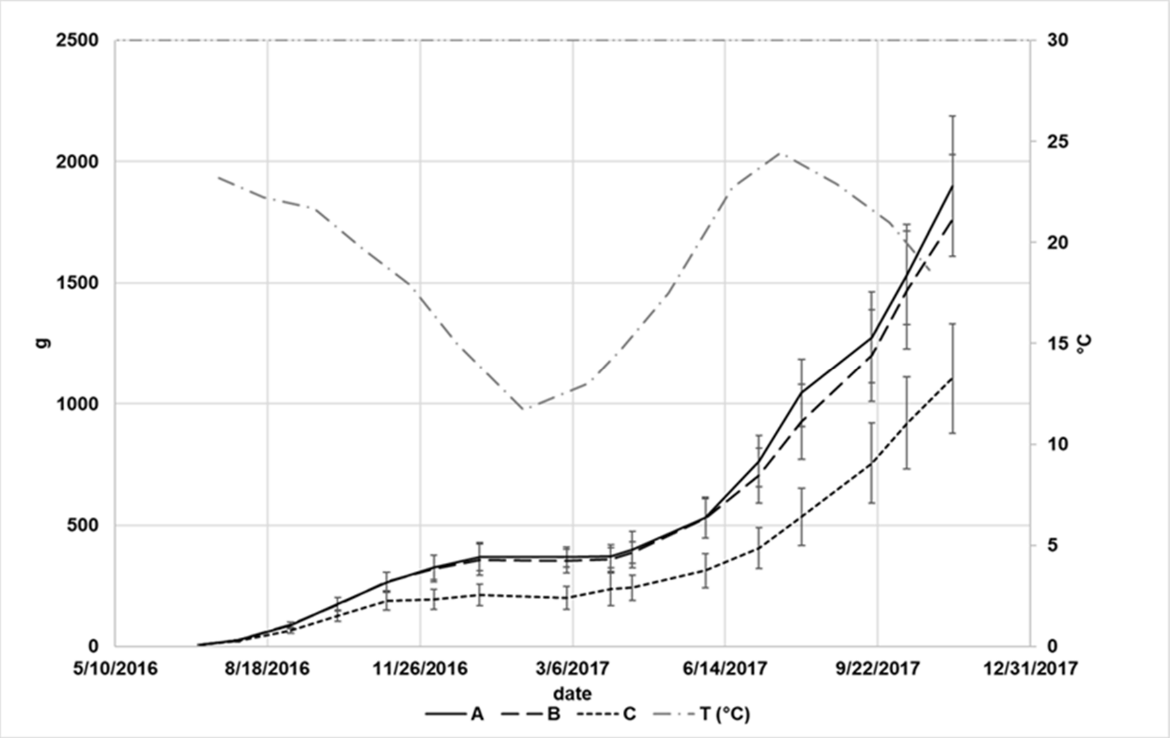

Figure 2. Growth of meagre in groups A, B and C from the beginning to the end of the experiment. A, Experimental feed for meagre; B, Commercial feed for sole; C, Commercial feed for meagre. Error bars show the standard deviation.

The results of fatty acid profile of muscle tissues of meagre fed diets with different fish oil content showed that fish fed diet A had the highest proportion of C20:5 (n-3) (EPA) and C22:6 (n-3) DHA with a significantly higher n-3/n-6 ratio, compared to diets B and C. The proportions of saturated fatty acids ranged in all three groups within 25.43–27.61%. The generally high proportion of PUFA (≥45.62 ± 4.44%) was the highest commercial feed C (46.83 ± 5.13%). Whereas no significant difference was expressed between the PUFA contents in each feeding group, there is noticeable difference in the proportions of sum of C18 UNSFA, C20 UNSFA and C22 UNSFA between the groups. Group C fed by commercial meagre feed contained higher proportions of C18:1 (21.05%) and C18:2 (10.48%) and substantially high proportion of C18:3 (3.05%) contributing to the highest sum of C18 unsaturated fatty acids in the experiment (34.58%). The descending order of the sum of C18 UNSFA from B (28.91%) to A (26.03%) was followed by the ascending summary proportions of DHA + EPA, with maximum content in experimental feeding group A (33.24%). The resulting DHA/EPA ratio, as well as the n-3/n-6 ratio were also the highest in the group A, reaching 1.96 and 3.86, respectively.

Table 3. Fatty acid profile of muscle tissue of meagre *A. regius* fed diets containing different levels of fish oil (% of total fatty acids).

	Feed Mixtures						**p Level**
	A		**B**		**C**		
Fatty Acid	$\bar{x}$	**SD**	$\bar{x}$	**SD**	$\bar{x}$	**SD**	
C14:0	3.03^{a}	0.68	2.80^{b}	0.70	1.97^{c}	0.413	***
C15:0	0.42^{a}	0.11	0.34^{b}	0.16	0.33^{b}	0.07	**
C16:3 (n-3)	1.00^{ab}	0.39	1.21^{a}	0.35	0.79^{b}	0.20	***
C16:1 (n-7)	4.10^{a}	0.89	3.95^{a}	0.93	3.24^{b}	0.51	**
C16:0	17.27^{a}	1.06	17.45^{a}	1.19	16.03^{b}	0.87	***
C17:1 (n-9)	0.74	0.45	0.86	0.64	0.62	0.26	ns
C18:3 (n-3)	2.20	1.18	3.72	2.11	3.05	2.24	ns
C18:2 (n-6)	5.86^{b}	2.18	7.76^{b}	2.23	10.48^{a}	2.62	*
C18:1	17.97^{ab}	3.00	17.43^{b}	2.80	21.05^{a}	4.77	*
C18:0	6.50^{b}	0.67	6.71^{a}	0.59	6.66^{b}	0.77	*
C20:4 (n-6)_ARA	0.55^{a}	0.07	0.53^{a}	0.05	0.44^{b}	0.05	***
C20:5 (n-3)_EPA	11.65^{a}	1.56	11.21^{a}	1.10	9.34^{b}	1.10	***
C20:3 (n-6)	1.40	0.29	1.38	0.32	1.34	0.18	ns
C20:1 (n-7)	1.56^{a}	0.31	1.17^{b}	0.21	1.26^{b}	0.16	***
C20:0	0.27^{ab}	0.11	0.23^{b}	0.08	0.33^{a}	0.06	***
C22:6 (n-3)_DHA	21.59^{a}	3.46	19.69^{a}	3.61	17.29^{b}	3.55	**
C22:4 (n-6)	1.92^{b}	0.17	1.65^{a}	0.23	1.83^{b}	0.25	*
C22:1 (n-9)	1.98^{a}	0.54	1.17^{b}	0.38	1.22^{b}	0.29	***
C22:0	0.23	0.12	0.15	0.018	0.19	0.043	ns
C24:1	0.68^{a}	0.22	0.49^{b}	0.13	0.46^{b}	0.09	***
SFA	27.49^{a}	1.25	27.61^{a}	1.50	25.43^{b}	1.35	***
UNSFA	72.51^{b}	1.25	72.39^{b}	1.50	74.57^{a}	1.35	***
MUFA	26.90	4.19	25.76	3.71	27.74	4.47	ns
PUFA	45.62	4.44	46.63	4.65	46.83	5.13	ns
n-6	9.19^{b}	4.58	12.34^{ab}	4.42	14.50^{a}	7.16	**
n-3	35.48^{a}	6.12	34.56^{a}	6.28	30.65^{b}	6.82	*
n-3/n-6	3.86^{a}	1.54	2.80^{b}	1.13	2.11^{c}	0.86	**
DHA/EPA	1.96	0.40	1.70	0.28	1.83	0.22	ns
Total lipids	16.81	7.84	13.04	5.78	11.18	3.59	ns

A, Experimental feed for meagre; B, Commercial feed for sole; C, Commercial feed for meagre; SFA—Saturated fatty acids; UNSFA—Unsaturated fatty acids; MUFA—Monounsaturated fatty acids; PUFA-Polyunsaturated fatty acid; DHA—Docosahexaenoic acid; EPA-Eicosapentaenoic acid. Values represent mean ± SD of two repetitions; values within the same row with different superscripts are significantly different at the level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns $p > 0.05$.

The results of the oxidative stress in meagre muscle tissue, measured as the concentration of malondialdehyde (MDA) are presented in Figure 3. The mean values of MDA concentration in skeletal muscle tissue ranged from the highest value (0.031 ± 0.01 μmolg^{-1}) in group A to the lowest value of 0.008 ± 0.006 μmolg^{-1} in group B (Figure 3). The mean results of MDA concentration in feeding group C were 0.025 ± 0.01 μmolg^{-1}. A significant difference in MDA concentration was observed between feeding groups A and B ($p < 0.05$).

To clarify the relative importance of varying diet quality on the lipid composition and physiological condition of the meagre during marine cage farming, Principal Component Analysis (PCA) was performed.

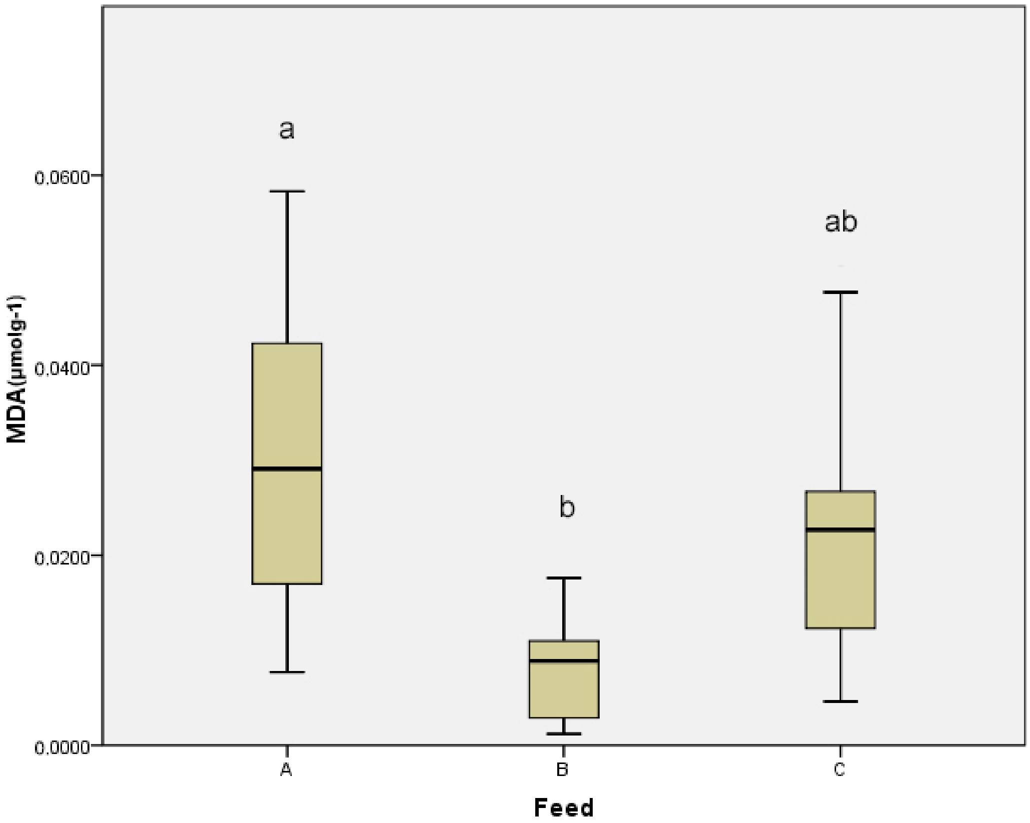

Figure 3. Malondialdehyde (MDA) concentration (µmolg^{-1}) in skeletal muscle tissue of meagre (*Argyrosomus regius*) of all feeding groups ($p < 0.05$); A, Experimental feed for meagre; B, Commercial feed for sole; C, Commercial feed for meagre; Boxes within different superscripts are significantly different at the level, $p < 0.05$.

The relationship among feed quality (Prot/Fat), unsaturated FA content, oxidative stress (MDA) and physiological condition indices (Kfin, SGR) in meagre groups fed different diets, are shown on the principal component plot (Figure 4) where PC1 and PC2 explained 66.6% and 26.0% of total variance, respectively. Meagre groups fed diets A and C were strongly positive for PC2 (DHA/EPA), but separated into positive (C18:2n-6) and negative (C22:6n-3) relationships with PC1. The highest loadings for negative relationship were obtained for DHA (−0.333), Kfin (−0.330), TL (−0.323), n-3/n-6 (−0.320), and SGR (−0.319) on PC1 whereas on Feed Prot/Fat (−0.345), C16:1 (−0.343) and C18:3 (−0.291) on PC2. The growth variables, Kfin and SGR are strongly related mutually and linked to the EPA, DHA, total lipids and n-3/n-6. This relationship indicates that higher lipid content and polyunsaturated DHA and EPA content result in the faster growth and better condition of meagre.

For the positive relationship on PC2 the highest loadings, as well as strong mutual relationship, were shown by DHA/EPA ratio (0.476) and MDA (0.524) as indicator of lipid peroxidation. The positive relation between DHA/EPA and MDA, and negative with C18:3 and Protein/Fat ratio in the feed indicate high DHA/EPA and low C18:3 favoring higher peroxidation level in meagre fed by Commercial Feed C, compared to B. The positive relationship between C16:1 and C18:3, and mutually negative with MDA indicated that shorter C-chain, and lower unsaturation degree in fatty acids correlate with less oxidative stress, occurring in meagre fed by Commercial Feed B.

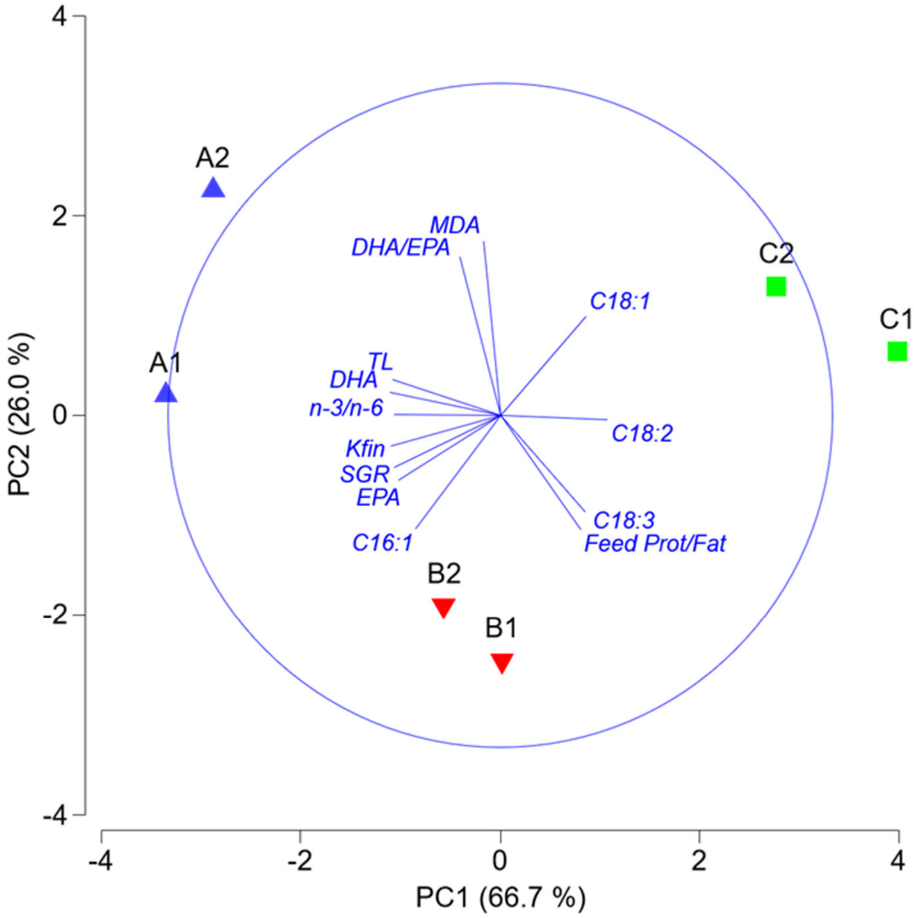

Figure 4. Principal Component Analysis for total lipid content, dominant unsaturated fatty acids (C16:1, C18:3, C18:2, C18:1, EPA and DHA), ratios (n-3/n-6, DHA/EPA) and peroxidation level (MDA) in meagre fed diets A, B and C (results for both replicates are included). The Feed quality in terms of Protein/Fat ratio, condition index (Kfin) and specific growth rate (SGR) are included as well. Projections of variables on the PC1–PC2 plane are given in circles.

4. Discussion

Meagre production is quite novel to the Mediterranean aquaculture and is recently coming to its rise, contributing significantly to the diversity of the domestic seafood offer, and to sustainability of the fisheries. There are very few studies trying to optimize nutritional requirements of the farmed meagre with the end-product quality, sustaining the economic sustainability of the process. One of the modalities to lower the production cost is in elevating the fat content and lowering the protein content in the fish feed.

The results presented herein are in accordance with the study of [38], that demonstrated significant improvement in SGR and FCR due to the increase in dietary crude lipids from 12 up to 17%. Ref. [18] indicate the low presence of mesenteric fat and intramuscular fat in meagre, even in the fish fed diet with the highest fat content. This finding could encourage the use of high-fat diets and, given the high growth potential of meagre, the production of a large commercial size useful for the processing industry. However, high proportion of lipids in the diet (≥17%) can result in higher fat accretion, impaired growth performance and finally increased level of lipid peroxidation in fish [39].

The ability of fish to thrive on diets rich in C18:1 fatty acids, mostly derived from vegetable oils varies among species [40–42] causing in many cases consequently higher cellular levels of C18:2 (n-6). The experimental diet used in the present study resulted in significantly lower levels of undesirable linoleic acid C18:2 (n-6), compared to both commercial feeds, for sole and for meagre. It is well documented that teleosts have different enzyme capacities to desaturate 18C fatty acids into 20–22C LC -PUFAs [41,43], with generally higher conversion efficiency in freshwater fish species, especially carnivores that feed on organisms rich in HUFA [40].

The proportions of fatty acids measured in the present experiment are partially consistent with [11] ranging 25.43–27.61% for SFA and 45.62–46.83% for PUFA. However, all three diets applied herein resulted in higher proportions of PUFA and lower proportions of SFA, with the consequent remarkably high n-3 PUFA proportion. The highest proportion of n-3 PUFA (≥35%), and consequently the highest n-3/n-6 ratio (3.86) are observed in the meagre fed with experimental diet A. The detection of similar levels of n-3 in fish meat by [12,44] suggests that meagre retains n-3 PUFA well. This is also a good indication that EPA converts to other n-3 fatty acids in fish meat. FAO/WHO [45] recommend intake ratio of n-3/n-6 ratios in the range of 1:8 to 1:5 as ideal because 5 times higher n-6 concentrations do not interfere with the conversion of the n-3 PUFA alpha-linolenic acid (18:3n-3, ALA) to n-3 highly unsaturated fatty acids (HUFA) [46]. This is due to the higher affinity of elongase and desaturase enzymes for n-3 than for n-6 fatty acid substrates. Therefore, even with a fivefold lower amount of n-3 fatty acids, PUFAs can be equally converted [47]. This explains the better results of the ratio of n-3/n-6 fatty acids in diet A. Lipids from marine fish oils can be deposited in fish muscle. People who consume these fillets could enjoy the health benefits of eating foods rich in omega-3 fatty acids, such as reduced symptoms of depression and improved cardiovascular health [20,48]. Amongst other fatty acids, EPA and DHA are very important for human consumption because they can significantly affect human health early development and the prevention of some diseases; therefore, consuming foods containing these fatty acids is highly recommended [49].

In human nutrition, fish is the main source of polyunsaturated fatty acids, which unfortunately are very susceptible to degradation processes such as oxidation. Polyunsaturated fatty acids often serve as targets for nascent free radicals that trigger lipid peroxidation [50], threatening the quality of foods, especially those containing highly unsaturated fats [51]. The values for MDA recorded in our study were overall lower than the values determined in the similar study reported by [52] for fish fed with fish oil with a peroxide value of 11.5 meqkg^{-1} (corresponding to 0.037 µmolg^{-1}).

Although the experimental diet group (A) demonstrated the highest value of MDA (0.031 µmolg^{-1}), all three groups had values that can be described as low peroxidation levels in muscle tissue. No health problems or differences in survival were observed. Differences in MDA values between groups may be the result of different amounts of fat. Ref. [53] found that different areas of muscle tissue in fish can have different MDA values and that it is correlated with the amount of fat. Since the samples of fish muscle tissue in the study were collected immediately after harvest and stored properly, external influence in the form of oxidation is excluded, and the dispersion of the data within the feeding groups can be attributed to the suboptimal winter periods of sampling (December; T = 14.38 ± 0.38 °C) since the best temperature for growth of meagre is between 17–21 °C, with an acceptable range of 14–23 °C [4]. This would be in accordance to [54], who found that the lowest stress levels (MDA and catalase as indicators) occurred on *Dicentrarchus labrax* at the optimal temperature (24 °C) and increased outside the upper and lower optimal thermal limits of this species. Otherwise, no relative importance of the temperature seasonality was observed over growth, lipid composition and overall condition of meagre.

In fish, the biosynthesis of lipids involves key mechanisms such as the elongation and desaturation of fatty acids, which are critical for maintaining cellular membrane integrity and function [55]. Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, can be influenced by the lipid content in their diet. Diets high in certain lipids can exacerbate oxidative stress, impacting fish welfare by impairing growth, immune function, and overall health [56]. Studies have shown that a balanced lipid intake is essential for optimizing fish health and welfare, minimizing oxidative damage, and ensuring efficient metabolic function [56,57].

The PCA indicated a clear distinction between meat quality of the fish fed three different diets. The fish were grouped according to their fatty acid content and clearly separated according to the total lipid content and HUFA proportions and ratios. The overall effect of the protein enrichment in the commercial feed for sole (B) was positive on C18:3 (n-3) and EPA, resulting in high content of n-3 fatty acids, and at the same time the lowest indication of the oxidative stress. The meagre fed experimental feed A enriched in both the fat and the protein contents demonstrated expectedly high proportions of HUFA as well as the highest n-3/n-6 ratio, qualifying for the highest meat quality in the presented experiment. Since marine fish have restricted enzymatic capacity for elongation and desaturation, EPA and DHA are considered essential FA in marine fish species, meaning their presence in the muscle tissue depends mostly on the dietary intake [8]. Although the experimental diet A resulted in the highest growth parameters and the highest FA quality from the nutritional and health perspective, it had a strong positive relationship to the peroxidation products development. Lipid peroxidation can be generally described as a process in which oxidants such as free radicals attack lipids containing carbon–carbon double bond (s), especially PUFAs, resulting in lipid peroxyl radicals and hydroperoxides [58]. At intermediate or high rates of lipid peroxidation, the extent of oxidative damage exceeds the repair capacity of the fish cell, eventually leading to molecular cellular damage, which can promote the development of various pathological conditions [50]. Ref. [52] reported lower survival, inflammation and hemorrhages of the dorsal, pectoral and tail fins in fish fed with oxidized fish oil (peroxide value 277 and 555 meqkg^{-1}), which had 0.050–0.053 MDA µmolg^{-1} in the muscle. Although values obtained in the presented experiment are significantly below the abovementioned ranges, further advancement of the experimental diet is required in order to inhibit free radical formation and further peroxidation of the HUFA.

5. Conclusions

Compared to B and C, fish fed diet A had the most promising results in terms of LC-PUFA profile and ratio of n-3/n-6 fatty acids in terms of promoting human health. It is recommended to consider incorporating experimental feed for meagre (diet A), which showed superior growth performance, higher levels of beneficial fatty acids EPA and DHA, and a favorable n-3/n-6 ratio, into the feeding regimen for meagre aquaculture. Additionally, monitoring and adjusting feeding regimes based on lipid content and fatty acid composition could optimize growth and health outcomes in meagre farming. Further research into the long-term effects of different dietary compositions on meagre health and production efficiency would provide valuable insights for sustainable aquaculture practices. Some of the research proposed for the future would include whole body and fillet composition of meagre with additional liver histology and a taste panel.

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References

1. Anastasiades, G. Biodiversity in Eastern Mediterranean Marine Aquaculture: An Approach to New Species. Ph.D. Thesis, University of Insubria, Department of Biotechnology and Molecular Sciences, Varese, Italy, 2010; p. 112.
2. Mosqueira, M.; Pombo, A.; Borges, C.; Brito, A.C.; Zacarias, N.; Esteves, R.; Palma, C. Potential for Coastal and Offshore Aquaculture in Portugal: Insights from Physico-Chemical and Oceanographic Conditions. *Appl. Sci.* **2022**, *12*, 2742. [CrossRef]
3. Haffray, P.; Malha, R.; Sidi, M.O.T.; Prista, N.; Hassan, M.; Castelnaud, G.; Karahan-Nomm, B.; Gamsiz, K.; Sadek, S.; Bruant, J.S.; et al. Very high genetic fragmentation in a large marine fish, the meagre *Argyrosomus regius* (Sciaenidae, Perciformes): Impact of reproductive migration, oceanographic barriers and ecological factors. *Aquat. Living Resour.* **2012**, *25*, 173–183. [CrossRef]
4. FAO (2005–2011). Cultured Aquatic Species Information Programme. *Argyrosomus regius*. Cultured Aquatic Species Information Programme. Text by Stipa, O. Angelini, M In: FAO Fisheries and Aquaculture Department (Online). Rome. Updated 10 February 2005. Available online: http://www.fao.org/fishery/culturedspecies/Argyrosomus_regius/en (accessed on 10 October 2023).
5. Papadakis, I.; Kentouri, M.; Divanach, P.; Mylonas, C.C. Ontogeny of the digestive system of meagre *Argyrosomus regius* reared in a mesocosm, and quantitative changes of lipids in the liver from hatching to juvenile. *Aquaculture* **2013**, *388–391*, 76–88. [CrossRef]
6. Duncan, N.J.; Estévez, A.; Fernández-Palacios, H.; Gairin, I.; Hernández-Cruz, C.M.; Roo, J.; Schuchardt, D.; Vallés, R. Aquaculture production of meagre (*Argyrosomus regius*): Hatchery techniques, ongrowing and market. In *Advances in Aquaculture Hatchery Technology*; Alan, G., Gavin, B., Eds.; Woodhead Publishing: Cambridge, UK, 2013; pp. 519–541.
7. Fountoulaki, E.; Grigorakis, K.; Kounna, C.; Rigos, G.; Papandroulakis, N.; Diakogeorgakis, J.; Kokou, F. Growth performance and product quality of meagre (*Argyrosomus regius*) fed diets of different protein/lipid levels at industrial scale. *Ital. J. Anim. Sci.* **2017**, *16*, 685–694. [CrossRef]
8. Carvalho, M.; Peres, H.; Saleh, R.; Fontanillas, R.; Rosenlund, G.; Oliva-Teles, A.; Izquierdo, M. Dietary requirement for n-3 long-chain polyunsaturated fatty acids for fast growth of meagre (*Argyrosomus regius*, Asso 1801) fingerlings. *Aquaculture* **2018**, *488*, 105–113. [CrossRef]
9. Monfort, M.C. Present market situation and prospects of meagre (*Argyrosomus regius*), as an emerging species in Mediterranean aquaculture. In *Studies and Reviews, General Fisheries Commission for the Mediterranean No. 89*; FAO: Roma, Italy, 2010; p. 28.
10. Martinez Llorens, S.; Espert Real, J.; Moya, V.; Moya Salvador, V.J.; Jover Cerda, M.; Tomas Vidal, A. Growth and nutrient efficiency of meagre (*Argyrosomus regius*, Asso, 1801) fed extruded diets with different protein and lipid levels. *Int. J. Fish. Aquac.* **2011**, *3*, 195–203.
11. Hernandez, M.D.; Lopez, M.B.; Alvarez, A.; Ferrandini, E.; Garcia Garcia, B.; Garrido, M.D. Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. *Food Chem.* **2009**, *114*, 237–245. [CrossRef]
12. Grigorakis, K.; Fountoulaki, E.; Vasilaki, A.; Mittakos, I.; Nathanailides, C. Lipid quality and filleting yield of reared meagre (*Argyrosomus regius*). *Int. J. Food Sci. Technol.* **2011**, *46*, 711–716. [CrossRef]
13. Parisi, G.; Teroa, G.; Gasco, L.; Piccolo, G.; Roncarati, A.; Moretti, V.M.; Centoducati, G.; Gatta, P.P.; Pais, A. Current status and future perspectives of Italian finfish aquaculture. *Rev. Fish Biol. Fish.* **2014**, *24*, 15–73. [CrossRef]
14. Kružić, N.; Mustać, B.; Župan, I.; Čolak, S. Meagre (*Argyrosomus regius* Asso, 1801) aquaculture in Croatia. *Croat. J. Fish.* **2016**, *74*, 14–19. [CrossRef]
15. Hixson, S.M. Fish nutrition and current issues in aquaculture: The balance in providing safe and nutritious seafood, in an environmentally sustainable manner. *J. Aquac. Res. Dev.* **2014**, *5*, 1000234. [CrossRef]
16. Ayala, M.D.; López-Albors, O.; Blanco, A.; Garcia-Alcázar, A.; Abellán, E.; Ramirez-Zarzosa, G.; Gil, F. Structural and ultrastructural changes on muscle tissue of sea bass, *Dicentrarchus labrax* L., after cooking and freezing. *Aquaculture* **2005**, *250*, 215–231. [CrossRef]
17. Sargent, J.R.; Tocher, D.R.; Bell, G.J. The lipids. In *Fish Nutrition*; Halver, J.E., Hardy, R.W., Eds.; Academic Press: Cambridge, MA, USA, 2002; pp. 182–257, An Imprint of Elsevier Science; Available online: http://hdl.handle.net/1893/2926 (accessed on 23 November 2023).
18. Glencross, B.D. A feed is still only as good as its ingredients: An update on the nutritional research strategies for the optimal evaluation of ingredients for aquaculture feeds. *Aquac. Nutr.* **2020**, *26*, 1871–1883. [CrossRef]
19. Piccolo, G.; Bovera, F.; De Riu, N.; Marono, S.; Salati, F.; Cappuccinelli, R.; Moniello, G. Effect of two different protein/fat ratios of the diet on meagre (*Argyrosomus regius*) traits. *Ital. J. Anim. Sci.* **2008**, *7*, 363–371. [CrossRef]
20. Craig, S. *Understanding Fish Nutrition, Feeds, and Feeding*; College of Agriculture and Life Sciences, Virginia Tech: Blacksburg, VA, USA, 2017; Volume 420, pp. 1–6.
21. Kaur, G.; Guo, X.; Sinclair, A.J. Short update on docosapentaenoic acid: A bioactive long-chain n-3 fatty acid. *Curr. Opin. Clin. Nutr. Metab. Care* **2016**, *19*, 88–91. [CrossRef]
22. Senadheera, T.R.L.; Hossain, A.; Shahidi, F. Marine Bioactives and Their Application in the Food Industry: A Review. *Appl. Sci.* **2023**, *13*, 12088. [CrossRef]
23. Auchterlonie, N. The continuing importance of fishmeal and fish oil in aquafeeds. In Proceedings of the Aquafarm Conference, Pordenone, Italy, 15–16 February 2020; Available online: www.iffo.net/iffo-presentations (accessed on 10 November 2023).
24. De Silva, S.; Francis, D.S.; Tacon, A.G.J. Fish oil in aquaculture: In retrospect. In *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*; Turchini, G.M., Ng, W.-K., Tocher, D.R., Eds.; CRC Press: Boca Raton, FL, USA, 2011; pp. 1–20. [CrossRef]

25. Ackman, R.G. Fatty acids in fish and shellfish. In *Fatty Acids in Foods and Their Health Implications*, 3rd ed.; Chow, C.K., Ed.; CRC Press: Boca Raton, FL, USA, 2008; pp. 155–185. [CrossRef]
26. FAO. *The State of World Fisheries and Aquaculture 2018—Meeting the Sustainable Development Goals*; FAO: Rome, Italy, 2018; p. 227.
27. Sies, H.; Stahl, W.; Sevanian, A. Nutritional, dietary and postprandial oxidative stress. *J. Nutr.* **2005**, *135*, 969–972. [CrossRef] [PubMed]
28. Goodwin, T.W. *The Biochemistry of the Carotenoids, Volume II Animals*; Chapman and Hall: London, UK, 1984; p. 224. [CrossRef]
29. Draper, H.H.; Squires, E.J.; Mahmooch, H.; Wu, J.; Agarwal, S.; Handley, M. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. *Free Radic. Biol. Med.* **1993**, *15*, 353–363. [CrossRef] [PubMed]
30. Janero, D.P. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic. Biol. Med.* **1990**, *9*, 515–540. [CrossRef]
31. Cordiano, R.; Di Gioacchino, M.; Mangifesta, R.; Panzera, C.; Gangemi, S.; Minciullo, P.L. Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update. *Molecules* **2023**, *28*, 5979. [CrossRef]
32. *Directive 2004/48/EC (2004)*; Corrigendum to Directive 2004/48/EC of the European Parliament and of the Council of 29 April 2004 on the Enforcement of Intellectual Property Rights. Edward Elgar Publishing: Cheltenham, UK, 2017.
33. NN 102/2017 Animal Protection Act—Narodne Novine. Available online: https://narodne-novine.nn.hr/clanci/sluzbeni/2017_10_102_2342.html (accessed on 4 November 2023). (In Croatian).
34. European Commission. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Off. J. Eur. Union* **2010**, *50*, 33–79.
35. Groto, D.; Santa Maria, L.D.; Boeira, S.; Valentini, J.; Charao, M.F.; Moro, A.M.; Nascimento, P.C.; Pomblum, V.J.; Garcia, S.C. Rapid quantification of malondialdehyde in plasma by high performance liquid chromatography-visible detection. *J. Pharm. Biomed. Anal.* **2007**, *42*, 619–624. [CrossRef] [PubMed]
36. Bligh, E.D.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [CrossRef] [PubMed]
37. Morrison, W.R.; Smith, L.M. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* **1964**, *5*, 600–608. [CrossRef]
38. Chatzifotis, S.; Panagiotidou, M.; Divanach, P. Effect of protein and lipid dietary levels on the growth of juvenile meagre (*Argyrosomus regius*). *Aquac. Int.* **2012**, *20*, 91–98. [CrossRef]
39. Fei, S.; Xia, Y.; Chen, Z.; Liu, C.; Liu, H.; Han, D.; Jin, J.; Yang, Y.; Zhu, X.; Xie, S. A high-fat diet alters lipid accumulation and oxidative stress and reduces the disease resistance of overwintering hybrid yellow catfish (*Pelteobagrus fulvidraco*♀× *P. vachelli*♂). *Aquac. Rep.* **2022**, *23*, 101043. [CrossRef]
40. Tocher, D.R. Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. *Rev. Fish. Sci.* **2003**, *11*, 107–184. [CrossRef]
41. Leaver, M.J.; Bautista, J.M.; Björnsson, B.T.; Jönsson, E.; Krey, G.; Tocher, D.R.; Torstensen, B. Towards Fish Lipid Nutrigenomics: Current State and Prospects for Fin-Fish Aquaculture. *Rev. Fish. Sci.* **2008**, *16*, 73–94. [CrossRef]
42. Castro, C.; Corraze, G.; Panserat, S.; Oliva-Teles, A. Effects of fish oil replacement by a vegetable oil blend on digestibility, postprandial serum metabolite profile, lipid and glucose metabolism of European sea bass (*Dicentrarchus labrax*) juveniles. *Aquac. Nutr.* **2014**, *21*, 592–603. [CrossRef]
43. Cook, H.W.; McMaster, R.C.R. *Biochemistry of Lipids, Lipoproteins, and Membranes*; Vance, D.E., Vance, J.E., Eds.; Elsevier: Amsterdam, The Netherlands, 2004; pp. 181–204. [CrossRef]
44. Poli, B.M.; Parisi, G.; Zampacavallo, G.; Iurzan, F.; Mecatti, M.; Lupi, P.; Bonelli, A. Preliminary results on quality and quality changes in reared meagre (*Argyrosomus regius*): Body and fillet traits and freshness changes in refrigerated commercial-size fish. *Aquac. Int.* **2003**, *11*, 301–311. [CrossRef]
45. FAO/WHO. *Diet, Nutrition, and the Prevention of Chronic Diseases*; WHO Technical Reper Series; WHO: Geneva, Switzerland, 2003; p. 916.
46. Biandolino, F.; Prato, E.; Grattagliano, A.; Parlapiano, I. Effect of Different Cooking Methods on Lipid Content and Fatty Acid Profile of Red Mullet (*Mullus barbatus*). *Pol. J. Food Nutr. Sci.* **2023**, *73*, 59–69. [CrossRef]
47. Simopoulos, A.P. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. *Biomed. Pharmacother.* **2006**, *60*, 502–507. [CrossRef]
48. Simopoulos, A.P. Omega-3 fatty acids in health and disease and in growth and development, a review. *Am. J. Clin. Nutr.* **1991**, *54*, 438–463. [CrossRef] [PubMed]
49. Jobling, J.; Leknes, O. Cod liver oil: Feed oil influences on fatty acid composition. *Aquac. Int.* **2010**, *18*, 223–230. [CrossRef]
50. Ayala, A.; Muñoz, M.F.; Argüelles, S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Med. Cell. Longev.* **2014**, *31*, 360438. [CrossRef] [PubMed]
51. Secci, G.; Parisi, G. From farm to fork: Lipid oxidation in fish products. A review. *Ital. J. Anim. Sci.* **2016**, *15*, 124–136. [CrossRef]
52. Chen, Y.J.; Liu, Y.J.; Yang, H.J.; Yuan, Y.; Liu, F.J.; Tian, L.X.; Liang, G.Y.; Yuan, R.M. Effect of dietary oxidized fish oil on growth performance, body composition, antioxidant defence mechanism and liver histology of juvenile largemouth bass Micropterus salmoides. *Aquac. Nutr.* **2012**, *18*, 321–331. [CrossRef]
53. Halamičkova, A.; Vorlova, L.; Smutna, M.; Svobodova, Z.; Buchtova, H. Comparison of malondialdehyde concentration in different muscle areas of tench *Tinca tinca* L. *Fish Physiol. Biochem.* **2003**, *29*, 305–312. [CrossRef]

54. Vinagre, C.; Madeira, D.; Narciso, L.; Cabral, H.N.; Diniz, M. Effect of temperature on oxidative stress in fish: Lipid peroxidation and catalase activity in the muscle of juvenile seabass, *Dicentrarchus labrax*. *Ecol. Indic.* **2012**, *23*, 274–279. [CrossRef]
55. Tocher, D.R. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquac. Res.* **2010**, *41*, 717–732. [CrossRef]
56. Montero, D.; Mathlouthi, F.; Tort, L.; Afonso, J.M.; Fernández-Vaquero, A.; Izquierdo, M.S. Replacement of dietary fish oil by vegetable oils affects humoral immunity and oxidative stress in gilthead seabream (*Sparus aurata*). *Fish Shellfish Immunol.* **2010**, *29*, 1073–1081. [CrossRef] [PubMed]
57. Bou, M.; Berge, G.M.; Baeverfjord, G.; Sigholt, T.; Østbye, T.-K.; Romarheim, O.H.; Hatlen, B.; Lee, Y.-S.; Ruyter, B. Requirement of n-3 very long-chain PUFA in Atlantic salmon (*Salmo salar* L.) post-smolt fed vegetable oil blends. *Aquaculture* **2017**, *479*, 638–649. [CrossRef]
58. Yin, H.; Xu, L.; Porter, N.A. Free radical lipid peroxidation: Mechanisms and analysis. *Chem. Rev.* **2011**, *111*, 5944–5972. [CrossRef] [PubMed]