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Effect of Mussel Meal Feed Supplement on Growth, Health Status, Proximate Composition and Fatty Acid Profile of Gilthead Seabream (*Sparus aurata*)

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Abstract: To evaluate the effects of mussel meal, as a sustainable ingredient for fish feed, on the growth, health status, proximate composition, and fatty acid profile of gilthead seabream, mussel meal was included in commercial feed formulations. Sunflower oil (2%) was used as a binding agent. Four groups of gilthead seabream were fed either with control feed (commercial feed, commercial feed and sunflower oil) or mussel-meal-supplemented formulations (commercial feed, sunflower oil, and 2.5 or 5% mussel meal) for six weeks. In this experiment, a total of 180 specimens of gilthead seabream juveniles were included. The initial weight and length of the gilthead seabream specimens were, on average, 13.04 g and 9.57 cm, respectively. The average temperature of the seawater ranged between 25 and 26 °C during the experiment. The results of this study indicated a higher relative weight gain and a slightly lower feed conversion ratio in the control group fed with commercial feed, probably because of macronutrient imbalances introduced by the addition of mussel meal and sunflower oil. The groups fed with mussel-supplemented diets had a slightly lower crude protein content compared to the group fed with a commercial diet. The addition of sunflower oil and mussel meal decreased the saturated fatty acid content while increasing the monounsaturated and polyunsaturated fatty acid content compared to the control group. However, the high content of DHA and EPA in the mussel meal resulted in a proportional increase of these fatty acids in the muscle tissue of gilthead seabream, although the overall effect was not statistically significant. The findings of this study suggest that mussel meal is a promising source of protein and lipids for sustainable fish feed production, but under the experimental setup, mussel meal did not act as an attractant for increasing fish feed intake during the summer conditions.

Keywords: fish feed; mussel meal; gilthead seabream; growth performance; proximate composition; fatty acid profile

Key Contribution: The results of this study suggest that mussel meal, which is produced from mussels using a bioremediation model, has significant potential as a sustainable nutrient supplement for gilthead seabream diets, providing beneficial fatty acids that are further deposited in the dorsal muscle of gilthead seabream. The control group fed with a commercial feed formulation showed significantly higher relative growth and weight gain, a higher condition index and a slightly lower feed conversion factor, confirming the high quality and balanced nutritional composition of the feed used.

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1. Introduction

Gilthead seabream (*Sparus aurata* Linnaeus, 1758) is the main fish species reared in the Black and Mediterranean Seas [1]. Gilthead seabream is one of the most economically valuable species in Mediterranean aquaculture, with the growing demand attributed to its quality, nutritional value, and adaptability to farmed conditions [2]. Its adaptability to varied environmental conditions across the Mediterranean makes it ideal for aquaculture. This supports large-scale production in most Mediterranean countries, where the optimal water temperatures are among the major factors influencing fish productivity and profitability [3]. Although gilthead seabream farming plays a critical role in meeting the demand for healthy seafood, expanding aquaculture in the Mediterranean raises sustainability concerns. This challenge is similar to those faced by many other intensive aquaculture systems worldwide.

Sustainability in aquaculture faces major challenges, particularly with the use of fish meal and fish oil in feeds, as these ingredients are sourced from wild-caught fish, primarily small pelagic species. Over-reliance on fish meal and fish oil contributes to the depletion of marine fish stocks, putting pressure on biodiversity and increasing the risk of ecosystem imbalances [4,5]. Additionally, fish meal and fish oil production has significant carbon and environmental footprints, making it increasingly incompatible with sustainable aquaculture goals [6]. To address these challenges, the aquaculture industry is exploring alternative ingredients, such as plant proteins [7–9], insect meals [10,11], and algae [12–14], which are more sustainable and renewable. However, challenges remain, as many alternatives may lack essential nutrients, such as amino acids and omega-3 fatty acids, or may impact feed palatability, digestibility, and growth performance [15]. Efforts to balance nutritional requirements with sustainability have led to innovations in feed formulations, but finding economically viable and nutritionally complete replacements for fish meal and fish oil remains a key focus of research [9].

Among various fish meal substitutes, mussel meal has gained attention for several reasons, including its relatively high protein and lipid content, favorable amino acid and fatty acid profile, and potential role as an attractant that can enhance feed palatability, potentially leading to improved growth rates and feed conversion in different marine species [16–18]. Mussels are high in protein (50–70% dry weight) and lipids (5–16% dry weight) and have essential amino acid and fatty acid profiles similar to those of fish meal [19,20], making them a promising fish meal substitute. However, studies on this subject are limited and have shown varied results across fish species. Berge and Austreng [21] reported reduced growth, protein digestibility, and adverse pigmentation effects with increased blue mussel supplementation in the diets of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). Similar trends, including a lower specific growth rate and feed intake, were observed with higher mussel levels in other species, such as turbot (*Scophthalmus maximus* Linnaeus, 1758) [22] and Ussuri catfish (*Pseudobagrus ussuriensis* Dybowski, 1872) [23]. In contrast, replacing 25–75% of fish meal with mussel meal significantly improved the specific growth rate, feed conversion ratio, protein efficiency ratio, and viscero-somatic index in juvenile common sole (*Solea solea* Linnaeus, 1758) [24]. The benefits of mussel meal addition to diets, without adverse effects, were also confirmed for Arctic char (*Salvelinus alpinus* Linnaeus, 1758) [25].

Wild gilthead seabream largely rely upon shellfish as a natural food source [26,27], suggesting that mussel meal could be well suited as a nutrient source for farmed seabream [28]. Moreover, mussel meal could act as an attractant to increase the feed intake during the winter growth stagnation, caused by the low water temperatures present in the western Mediterranean, which negatively affects farm production and profitability [3]. As filter feeders, mussels offer numerous sustainability advantages compared to other protein sources, including a lower environmental footprint on a per-unit protein basis [29]. Additional sustainability advantages include carbon fixation from the atmosphere, positive effects on ocean eutrophication, and lower environmental impacts in terms of the accumulated surplus food debris, localized benthic organic enrichment, and oxygen depletion [30,31].

Mussels from the *Mytilus edulis* complex are widely used as bioindicators due to their ability to filter and bioaccumulate pollutants [32–34]. Farming undersized mussels in non-

commercial mariculture areas with less favorable environmental conditions can provide a sustainable source of mussel meal. In this context, mussels are not only considered a safe and nutritive future food source [35] but also have a positive impact on the local marine environments through the ecosystem services they provide [36].

In this context, the purpose of this work was to examine the addition of mussel meal obtained from undersized Mediterranean mussels (*Mytilus galloprovincialis* Lamarck, 1819), produced as part of a bioremediation model, to commercial fish feed and its effect on the growth and health parameters of gilthead seabream juveniles, as well as on the fillet quality in terms of the proximate composition and fatty acid profile.

2. Materials and Methods

2.1. Mussel Meal Production and Experimental Feed Preparation

Biomass production and bioremediation of the local marine environment through ecosystem services provided by *M. galloprovincialis* preceded this experiment (ERA-NET BlueBio HRZZ—MuMiFaST project, 2022–2024). The biomass was produced from undersized mussels grown in the Lim Bay mariculture area (Istrida d.o.o.) and in the vicinity of the wastewater treatment plant outlet (UPOV Cuvi). These locations are situated in the north-east Adriatic Sea near the city of Rovinj (Region of Istria, Croatia).

Mussel biomass production was conducted during a nine-month period, from August 2022 to May 2023. Mussels were harvested at the end of the biomass production and bioremediation period. After harvesting and sorting, the mussels were transported to the laboratory for further analysis and production of mussel meal. The potential contamination levels were assessed for 8 heavy metal(loid)s, 16 polycyclic aromatic hydrocarbons, the total polychlorinated biphenyls, and microplastics. Although the mussel meal from both locations complied with the EU regulations on the permissible contaminant levels in seafood, mussels from the mariculture area were selected for this experiment. A second experiment is planned for the low-temperature winter period using mussel meal produced from mussels grown near the WWTP outfall.

Mussel meal was produced from the mussels' soft tissue after briefly boiling them in a microwave oven until the shells were opened (2 min). The collected soft tissue was dried in a heating oven with circulating air (80 °C for 24 h) and ground in a laboratory mill (final fineness < 2 mm). Composite samples of mussel meal were used for the determination of the proximate composition and fatty acid content (n = 2).

Mussel meal was added at 2.5 and 5% to commercial fish feed (Perla < 1 mm, Crobo 1.5 mm and 1.9 mm, Skretting, Norway) using 2% sunflower oil (Zvijezda dd, Zagreb, Croatia) as a mixture binder to obtain two different feed formulations for further trials.

2.2. Fish Feeding Trials

Gilthead seabream juveniles were obtained from Cromaris d.d. (Croatia). Upon their arrival in the experimental laboratory, the fish juveniles were acclimated to the conditions present in our laboratory (experimental conditions) and treated in a potassium permanganate bath.

The gilthead seabream specimens were kept in 6 tanks (6 × 50 L volume, 30 fish/tank) with open water circulation, constant aeration and daily monitoring of salinity (38.31 ± 0.31), temperature and oxygen concentration (70–85%). The fish trials were conducted under a natural light photoperiod (14–15 h light in July; 10–9 h night in August). The feeding experiment was conducted for 6 weeks (July–August 2023) at high water temperatures (1–2 week: 26 ± 0.04 °C; 2–4 week: 25 ± 0.02 °C; 4–6 week: 26 ± 0.12 °C), during which the fish were divided into 4 groups depending on the feed formulation: Control 1—C1 (commercial feed, 1 tank), Control 2—C2 (commercial feed with 2% sunflower oil, 1 tank), Feed 1—F1 (commercial feed with 2.5% mussel meal and 2% sunflower oil, 2 tanks) and Feed 2—F2 (commercial feed with 5% mussel meal and 2% sunflower oil, 2 tanks). The fish were fed ad libitum three times a day during the experiment and the fish feed granulation was adapted to the fish growth requirements. Fecal waste was removed after each feeding.

2.3. Biometric Measurements

In all the samplings, the fish were submitted to anesthesia (MS-222, 15 mg/L SW) prior to conducting biometric measurements (30 fish specimens/experimental tank). The initial weight and length of the gilthead seabream specimens were similar in all the groups (C1—12.94 ± 1.69 g, 9.46 ± 0.29 cm; C2—13.14 ± 2.23 g, 9.61 ± 0.14 cm; F1—13.02 ± 1.39 g, 9.52 ± 0.41 cm; F2—13.05 ± 1.94 g, 9.52 ± 0.25 cm). The length, weight and condition index were determined at the start and after 2, 4, and 6 weeks of the experiment. The fish growth performance was evaluated by calculating the relative growth rate (%), relative weight gain (%), specific growth rate, feed conversion ratio (%), protein efficiency ratio (%) and condition index [37]. The hepatosomatic index, viscero-somatic index, survival rate (%) and dorsal muscle yield (%) were recorded at the end of the experiment [38,39].

In addition to the periodic measurements, the specific observations in terms of health monitoring included surveillance for morphological and behavioral changes, as well as the occurrence of injuries or symptoms of diseases, throughout the acclimation and experiment duration.

2.4. Proximate Composition Analysis

The proximate composition of the mussel meal produced in the laboratory, commercial feed (C1), control feed (C2) and feed F1 and F2 with added mussel meal (2.5 and 5%, respectively) is presented in Table 1.

Table 1. Proximate composition of mussel meal and commercial feed Perla < 1 mm (0–2 weeks), Crobo 1.5 mm (2–4 weeks) and Crobo 1.9 mm (4–6 weeks) used for the diet composition in this experiment.

	Mussel Meal *	**Perla < 1 ****	**Crobo 1.5 ****	**Crobo 1.9 ****
Dry matter (%)	92.91	n.p.	n.p.	n.p.
Crude protein (%)	52.07	55.0	55.0	53.0
Crude fat (%)	6.59	15.0	16.5	17.0
Crude carbohydrates (%)	22.45	n.p.	n.p.	n.p.
Ash (%)	11.8	10.1	9.0	8.0

* values were obtained by laboratory analysis. ** values were obtained from the product specification sheet. n.p. not provided.

At the end of the experiment, 25 fish specimens from each tank were filleted and the dorsal muscle tissue without skin and bones was pooled for each experimental group, minced using a handheld blender, and used for all the further analyses. The crude composition of the mussel meal was determined directly on prepared samples of mussel meal prior to mixing with commercial feed.

The moisture, protein and ash content were determined according to the methods recommended by the AOAC [40]. The lipid content was determined by the two-step extraction with cyclohexane and propan-2-ol mixtures as solvents, according to the method of Smedes [41].

Lipids for the determination of the fatty acid composition of the mussel meal and fillets were extracted according to Smedes [41]. To preserve the unsaturated fatty acids from oxidation, the final step of the Smedes method, i.e., drying at 103 °C, was excluded from the protocol. Fatty acid methyl esters were prepared by transesterification with methanol according to the ISO 5509:2000 method [42]. Briefly, 60 mg of extracted lipids was dissolved in 4 mL of isooctane and 200 µL of potassium hydroxide in methanol (2 mol/L) was added. The mixture was vortexed for 30 s and left for a few minutes at room temperature to react. Afterwards, 1 g of sodium hydrogen sulphate monohydrate was added, mixed, and the clear supernatant containing methyl esters was transferred into the vial.

For the gas chromatographic analysis, 1 µL of prepared methyl esters was injected into an Agilent Technologies 6890 N Network GC system (Santa Clara, CA, USA) equipped with a flame ionization detector. The fatty acid methyl esters (FAME) were separated on a DB-23 capillary column (Agilent Technologies). Helium was used as the carrier gas, with a constant flow of 1.5 mL/min. The temperature of the injector was set at 250 °C

and that of the detector at 280 °C. The oven temperature was programmed to increase by 7 °C/min from the initial 60 °C to the final temperature of 220 °C, where it was maintained for 17 min. The split ratio was 30:1, and the fatty acids were identified by comparing their retention times with the retention times of the 37 Component FAME Mix (Supelco, Sigma-Aldrich, St. Louis, MO, USA). The surface normalization method was used to determine the quantitative composition of the fatty acids, expressed as a percentage of the total fatty acids. All the analyses were carried out in duplicate.

2.5. Nutritional Indices of Lipid Quality

The fish nutritional value was evaluated for all the investigated groups in terms of the fillet fatty acid composition, calculating 12 different indices. Commonly adopted indices like the total saturated fatty acids (∑SFA), total monounsaturated fatty acids (∑MUFA), total polyunsaturated fatty acids (∑PUFA), total omega-3 fatty acids (∑n-3), DHA/EPA, EPA + DHA, total omega-3/omega-6 fatty acids (∑n-3/∑n-3) and total PUFA/SFA were calculated [43].

Additional indices like the polyene index (PI), thrombogenic index (IT), unsaturation index (UI) and fish lipid quality (FLQ) were taken into consideration as well [43].

2.6. Statistical Analysis

The results of all the measurements are presented as the mean ± SD. The normality of the parameter distribution was tested by the Shapiro–Wilk test and the homogeneity of variance was tested by Levene's test. Data were compared using a one-way ANOVA and the significance of the differences between the investigated groups was evaluated using Tukey's HSD post hoc test ($p < 0.05$). When the normality and/or homogeneity were not confirmed, the data were analyzed using the non-parametric Kruskal–Wallis test and the significance of the differences between the investigated groups was estimated using Dunn's post hoc test ($p < 0.05$). Smaller sample size variables ($n < 5$) were analyzed using the Kruskal–Wallis test accompanied by the Monte Carlo permutation test (1000 permutations).

A multiple linear regression model with an interaction was used to describe the relation between the fish length and weight measured across the different treatment groups, with the length and group as predictors. The values of the weight and length were ln-transformed. The model includes the 95% confidence intervals for the parameters, along with the R^2 estimation and slope b values. Differences between groups were tested using Student's *t*-test for comparison of the estimated slopes [44].

The Pearson's correlations were calculated between all the reported variables and significant correlations were determined at $p < 0.05$.

Statistical analyses and plotting were carried out in the R environment (v.4.4.1) with RStudio (v.2024.09.1+394) using the basic, "coin", "rstatix", "pheatmap", and "mdatools" packages [45–49].

2.7. Ethical Statements

All the fish-handling procedures were conducted following the EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the Commission's Implementing Decision 2020/569, Croatian government legal acts (Animal Protection Act NN 102/2017, NN 32/2019; Ordinance on the Protection of Animals Used for Scientific Purposes NN 55/2013, 39/17, 116/19) in the fish experimental facilities of the Ruđer Bošković Institute, Center for Marine Research—Rovinj (HR POK-029, UP/I-322-01/21-01/46, Ministry of Agriculture). The experiments were conducted in accordance with the approvals received from the Bioethics Committee of the Ruđer Bošković Institute, Ethics Committee for animal protection (EP 361/2022), and decision UP/I/322-01/22-01/15 (Project "The effect of feed enriched with mussel meal on the growth and vitality of gilthead seabream (*Sparus aurata*) during winter and summer periods") released from the Croatian Ministry of Agriculture.

3. Results

3.1. Growth Performance, Health Status, and Survival

A total of 150 specimens representing four different feeding groups (C1, C2, F1 and F2) were analyzed for the length–weight relationship (Figure 1). A significant relationship between the length–weight parameters was noted for the feeding groups investigated (R^2 = 0.981, $p < 0.0001$). The growth was a positive allometric, as the weight of the gilthead seabream specimens increased more than their length (b > 3) (Figure 1). Significant differences between the investigated groups were not observed for the obtained b-values ($p > 0.05$).

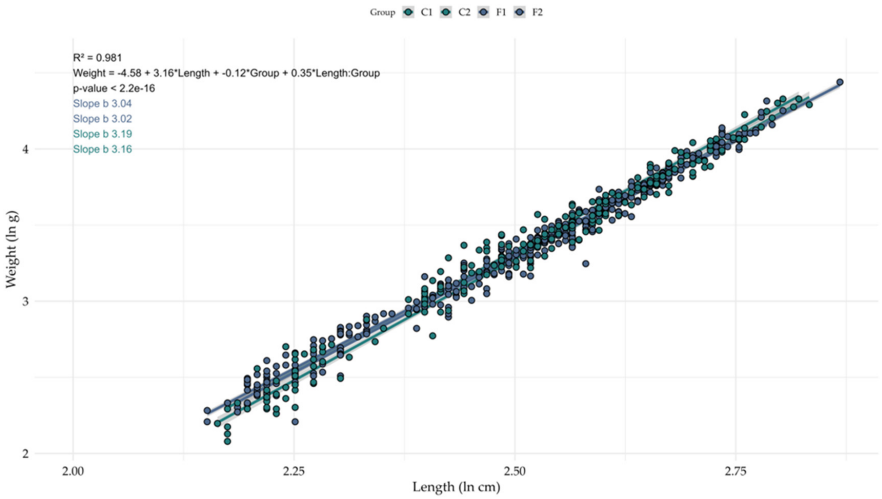

Figure 1. The length–weight relationship for gilthead seabream fed with different feed formulations (C1, C2, F1, F2) measured during the experiment (0–6 weeks).

The growth performance in terms of the relative growth, relative weight gain, specific growth rate, and condition index is presented at intervals (comparison between successive biometric measurements; Supplementary Table S1) and cumulatively (comparison between final and initial measurements; Supplementary Tables S2 and S3, Figures 2 and 3).

Initially, no significant differences were observed between different groups in terms of the average body length and weight. Over the six-week experiment, the control group C1 showed a significantly higher condition index after four (K4) and six (K6) weeks. Although this group had the highest values when comparing the last two measurements (K6), group F1 showed a significantly higher condition index (K6) compared to the control group C2 at that interval (Supplementary Table S1).

The highest relative growth was observed in groups C1 and F1 during the first interval (RG2); however, this trend changed over time, and no significant differences were found between the last two intervals (RG6). The highest weight gain and growth were observed in groups C1 and C2, which had significantly higher relative weight gain and specific growth rate compared to the mussel-fed groups during the first interval (RWG2, SGR2). In contrast, no significant differences were noted between the experimental groups during the later measurements (RWG4, RWG6, SGR4, SGR6) (Supplementary Table S1).

In contrast to the short time-dependent observations, the overall effect of the different feed supplementation on gilthead seabream development can be observed when comparing the initial (prior experiment) and final (end of the experiment) measurements (Figure 2). Longer exposure to different diets significantly affected the relative growth (C1 > C2, F1, F2), relative weight gain (C1, C2 > F1, F2), specific growth rate (C1 > C2, F1, F2), and condition index (C1 > F1, F2 > C2), even if no differences were noted in the feed conversion rate and protein efficiency ratio.

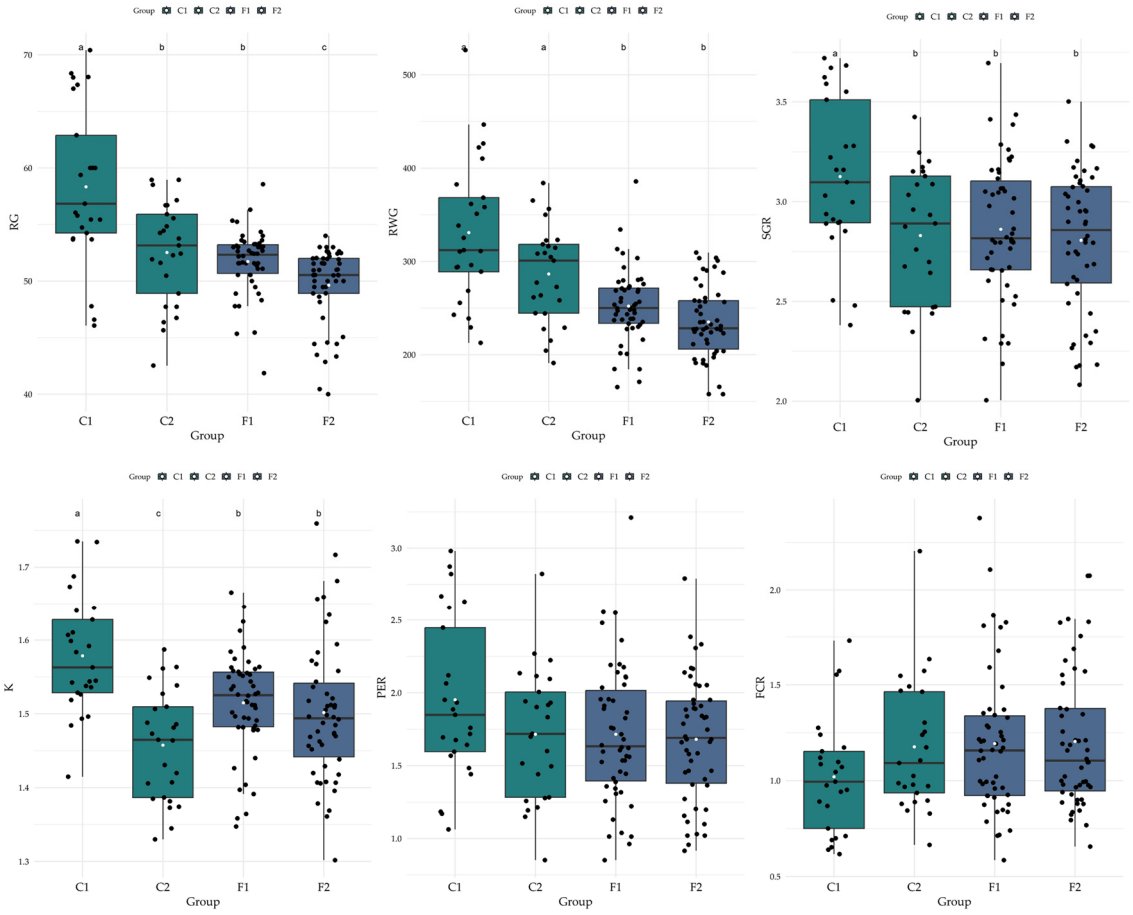

Figure 2. Relative growth (RG), relative weight gain (RWG), condition index (K), specific growth rate (SGR), protein efficiency ratio (PER) and feed conversion rate (FCR) of gilthead seabream fed with different feed formulations (C1, C2, F1, F2) at the end of the experiment (6 weeks). Different letters indicate significant differences among different groups ($p < 0.05$).

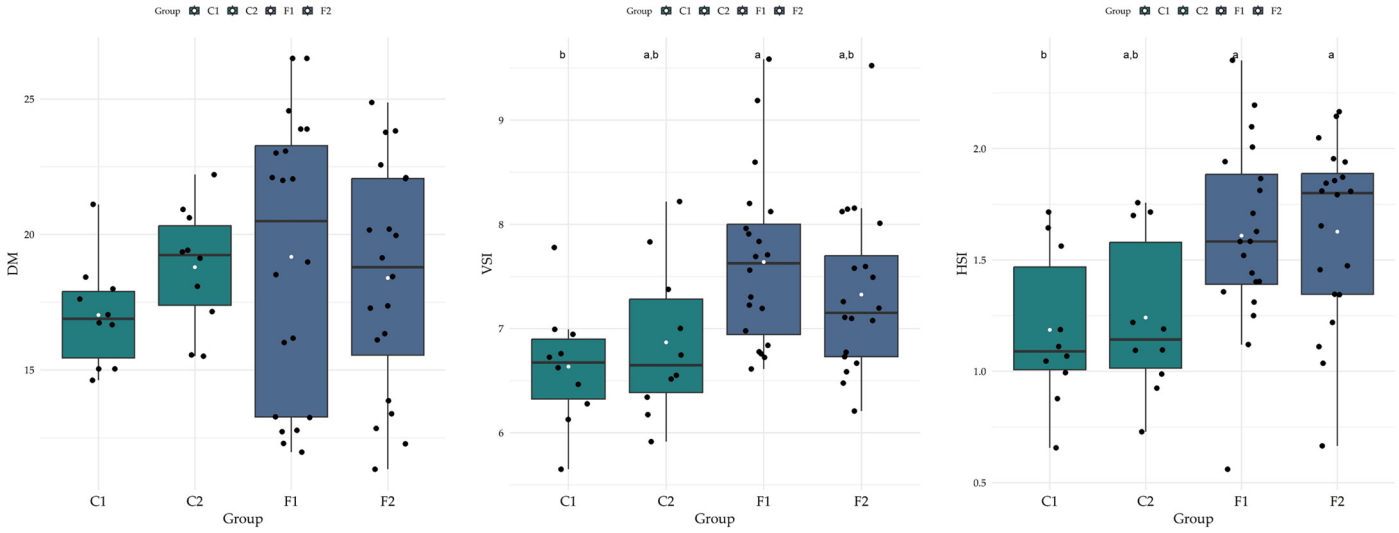

Figure 3. Dorsal muscle proportion (DM), viscero-somatic index (VSI) and hepatosomatic index (HSI) of gilthead seabream fed with different feed formulations (C1, C2, F1, F2) at the end of the experiment (6 weeks). Different letters indicate significant differences among different groups ($p < 0.05$).

It should be noted that no malformations or discolorations on the gilthead seabream specimens were noted at the end of the experiment (Supplementary Figure S1). The survival of the gilthead seabream at the end of the experiment was C1—90.32%, C2—93.33%, F1—96.55 ± 0.12%, and F2—93.49 ± 3.2%.

The proportion of dorsal mussel in the gilthead seabream weight was similar in all the investigated groups (Figure 3), but generally, a lower proportion of dorsal muscle in the total weight was noted for smaller specimens. Significant differences were noted in the viscero-somatic index between the control group C1 and mussel-fed group F1, while both mussel-fed groups (F1, F2) had a higher hepatosomatic index when compared to the control C1 group (Figure 3).

3.2. Proximate Composition of Dorsal Muscle

Significant differences were observed in the protein content between the control groups C1 and C2 with a higher protein content (20.37% and 20.41%, respectively) and groups F1 and F2 with a lower protein content (19.95% and 19.80%, respectively). Control group C2 had a significantly higher ash content (1.50%) than group F2 (1.42%), while all the other parameters showed no significant differences between groups (Supplementary Table S4).

3.3. Fatty Acid Profile and Fatty Acids Indices

The fatty acid composition of the supplemented feed is reported (Supplementary Table S5). The fatty acid composition of the gilthead seabream's dorsal muscle was characterized by three predominant fatty acids—C18:1 cis (32.95–34%), C16:0 (18.11–20.83%), and C18:2 cis (12.47–18.22%) (Supplementary Table S6, Figures 4 and 5).

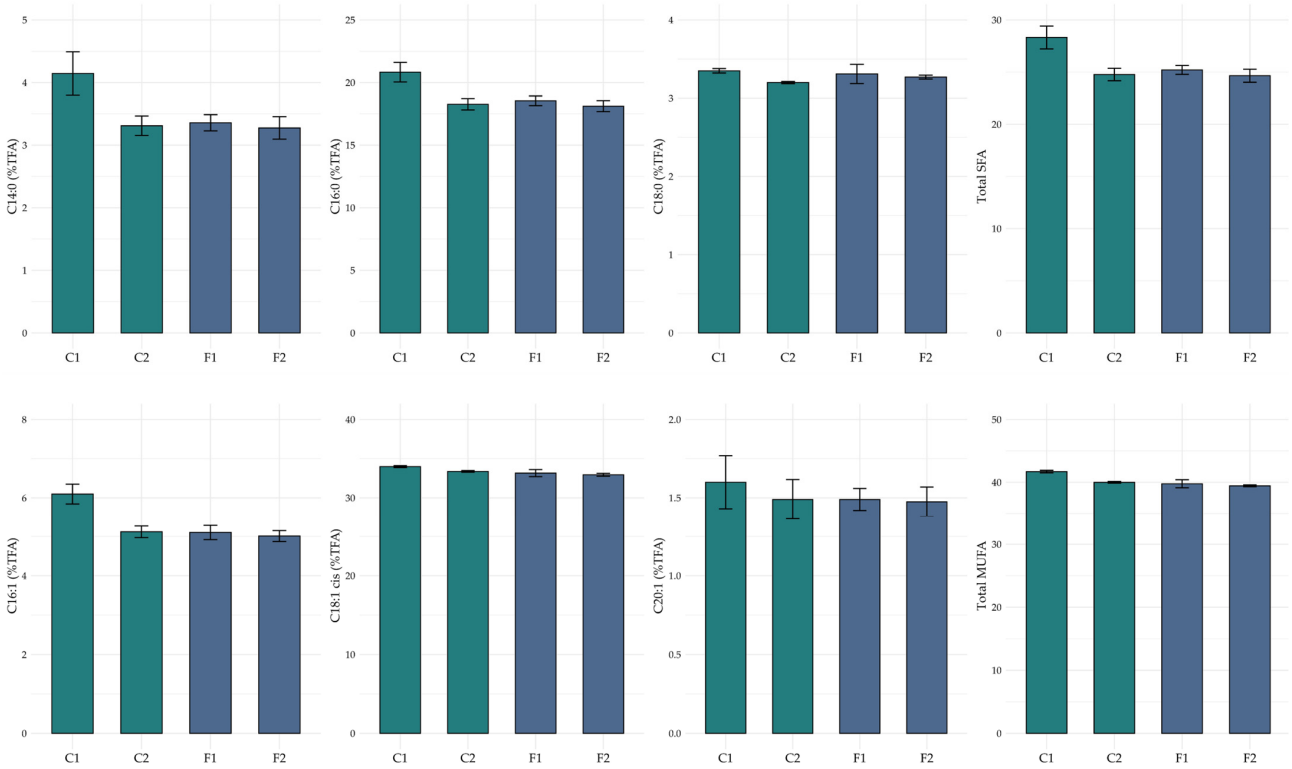

Figure 4. Saturated (SFA) and monounsaturated fatty acid (MUFA) profiles (mean ± SD) expressed as the % of total fatty acids (TFAs) of the dorsal muscle of gilthead seabream fed with different feed formulations (C1, C2, F1, F2) at the end of the experiment (6 weeks).

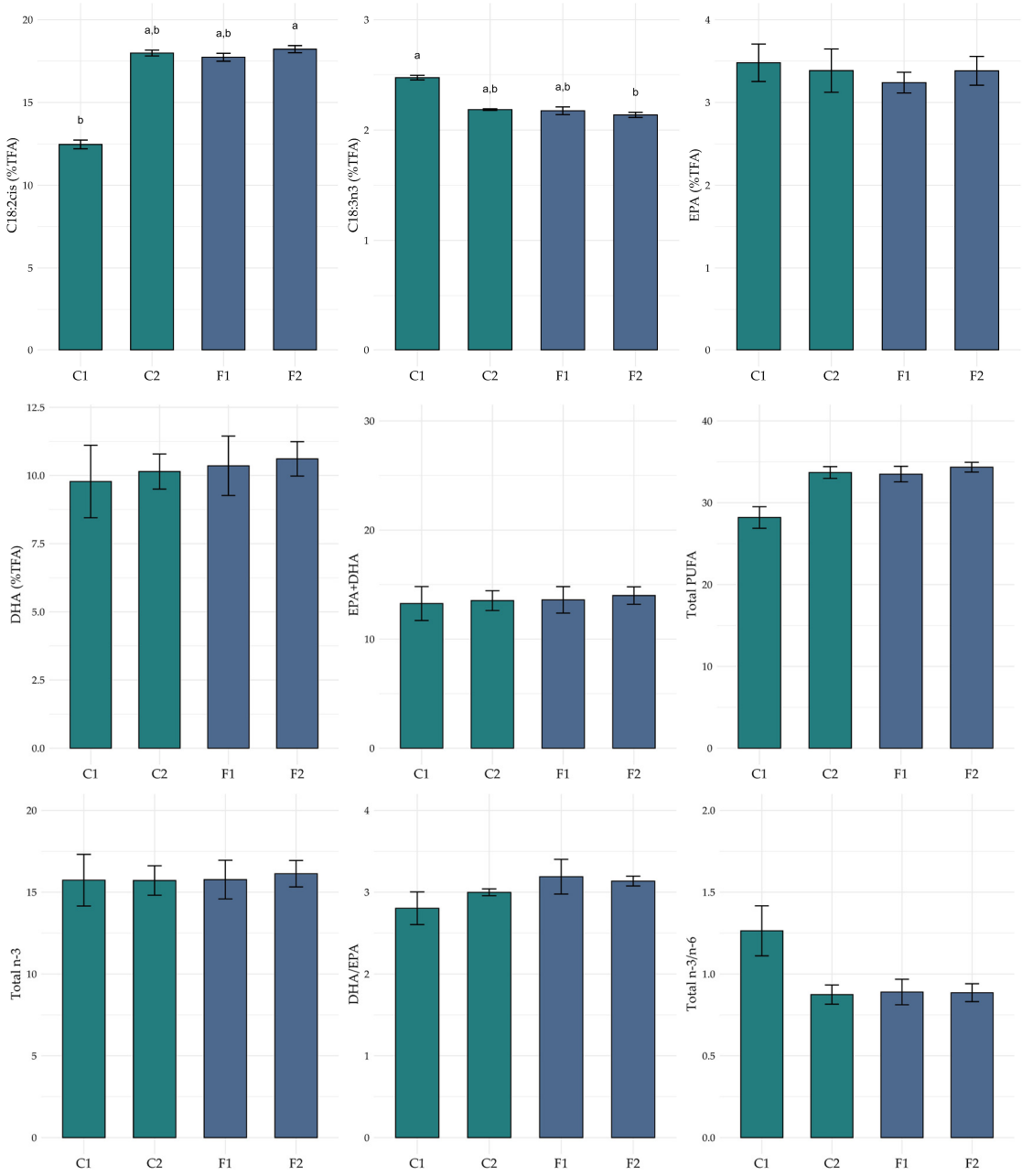

Figure 5. Polyunsaturated fatty acid (PUFA) profiles (mean ± SD) expressed as the % of total fatty acids (TFAs) of the dorsal muscle of gilthead seabream fed with different feed formulations (C1, C2, F1, F2) at the end of the experiment (6 weeks). Different letters indicate significant differences among different groups ($p < 0.05$).

When analyzing the fatty acid profile of gilthead seabream fed with different diets (C2, F1, F2), significant variations were observed compared to the control group (C1). Specifically, diets supplemented with either sunflower oil (C2) or both sunflower oil and mussel meal (F1, F2), contributed to a general decrease in the C18:0, C18:1cis, C18:3n3 ($p < 0.05$), and C20:1 content. An opposite trend was noted for the C18:2 cis ($p < 0.05$) and DHA content, as they were generally increased in these (C2, F1, F2) feeding groups. The content of EPA varied depending on the diet, with lower levels present in the C1 and F1 groups (Supplementary Table S6, Figures 4 and 5).

The profile of fatty acids was dominated by monounsaturated fatty acids (39.44–41.7%), followed by polyunsaturated fatty acids (28.2–34.35%) and saturated fatty acids (24.66–28.33%).

An exception was noted for the fatty acid groups analyzed for gilthead seabream fed with commercial feed (C1), where the PUFA content (28.2%) was lower and the SFA content (28.33%) was higher than the trends described in the other feeding groups (C2–F2) (Supplementary Table S7, Figures 4 and 5).

In the present study, no significant differences were noted in the fatty acid indices between the differently fed groups of gilthead seabream (Supplementary Table S7), indicating that the overall quality of the dorsal muscle meat was not affected by the different diets.

When comparing the fatty acid groups of mussel-fed gilthead seabream to those of mussel meal, or between different groups for the gilthead seabream's dorsal muscle, differences in their distribution can be noted (Figure 6, Supplementary Table S8). The polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs) were more abundant in the mussel meal than in the gilthead seabream, while the monounsaturated fatty acids (MUFAs) were more abundant in the gilthead seabream than in the mussel meal. Furthermore, the n-3 fatty acids were more pronounced in the mussel meal, whereas the n-6 fatty acids had a higher content in the gilthead seabream.

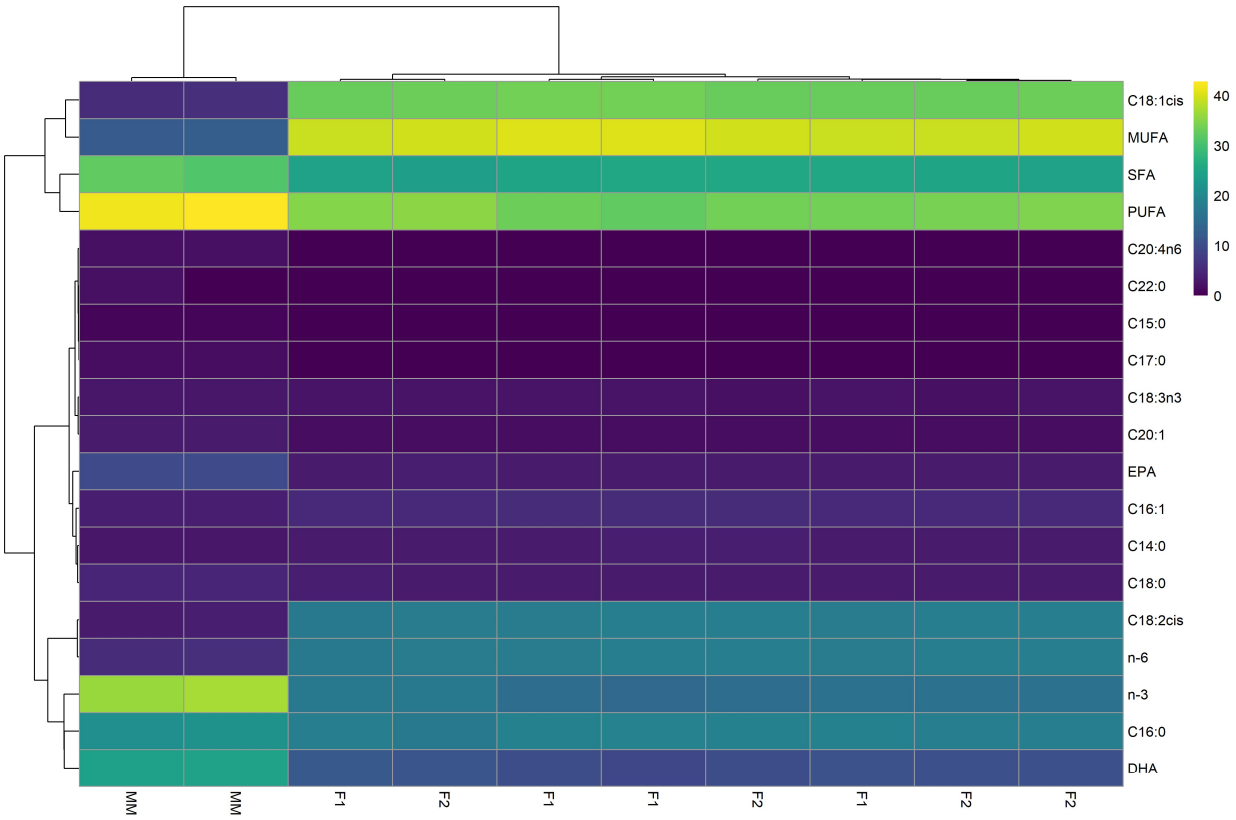

Figure 6. Fatty acid profile expressed as the % of total fatty acids (TFAs) of mussel meal (MM) and the dorsal muscle of gilthead seabream fed with mussel-supplemented feed formulations (F1, F2) at the end of the experiment (6 weeks).

The specific fatty acids EPA and DHA both had a higher content in the mussel meal compared to the gilthead seabream, while the content of C18:2 cis was higher in the gilthead seabream than in the mussel meal (Figure 6).

4. Discussion

Being the dominant fish species reared in aquaculture systems across many areas, considerable research efforts were devoted to finding ideal and sustainable dietary formulations that would benefit both the growth rates and quality of gilthead seabream [13,50–54]. Despite this, the relationship between specific environmental conditions, dietary intake,

growth indices, and fatty acid composition remains highly variable and is often only partially understood for this species.

Different factors can influence the length–weight relationship, some of which are the fish health, condition, and feed intake [44,51]. The b-values reported for gilthead seabream in this study were higher ($b > 3$) when compared to the results obtained for this species in other studies [50,51]. These groups of authors have reported b-values ($b < 3$) for gilthead seabream either collected from purse-seiners or farm-grown specimens fed with different protein contents, respectively. The results of our study indicate that both the commercial feed and the mussel-supplemented commercial feed did not negatively affect this parameter.

Previous studies have reported that gilthead seabream exhibits the highest specific growth rates during the initial growth phase under variable environmental conditions [55]. Similar trends in the specific growth rate, relative growth and relative weight gain were observed in this study (Supplementary Table S1). The reported variations could be attributed to the generally higher initial growth, natural fluctuations in temperatures and photoperiods during the summer, as indicated in this study. Seasonal factors such as the temperature and light are known to affect the physiology and growth rate of this ectothermic species and are related to the up- or down-regulation of growth hormones [56]. Kir [57] reported that the optimal temperature for gilthead seabream production ranges between 25 and 26 °C, aligning with the high sea temperatures present during summer in the Mediterranean area. In contrast, a reduction in the light periods may negatively affect the growth hormone regulation and contribute to slower overall growth, as observed in this study. The reported results further indicate that the feed composition may play a significant role in enhancing the growth and weight gain in gilthead seabream, particularly under increased seawater temperatures and decreasing light duration. This trend was more evident in the time-dependent condition index monitoring, where longer exposure to mussel-supplemented feed resulted in an increased condition index for the F1 group when compared to the control group C2 (Supplementary Table S1).

Even if previous reports indicate that the vegetable and animal oils used in fish feed do not compromise the growth of different fish species [58], these results are not in accordance with the results obtained in this study. Even a low addition of sunflower oil (2%) negatively affected several growth indices (C1 vs. C2 groups) in gilthead seabream (Figure 2). In this context, a higher feed conversion rate may indicate reduced digestible energy from the diet, which could further decrease the feed efficiency [58]. In this experiment, no significant differences were observed between the feeding groups (C1–F2), suggesting that the overall feed efficiency was adequate or slightly reduced. Studies conducted on different fish species reported that an increased intake of the polyunsaturated/saturated fatty acid ratio in fish diets contributed to feed digestibility due to the high specificity of fish digestive lipases for polyunsaturated lipids [58]. Despite the lower PUFA/SFA ratio in the mussel-supplemented feed diets (Supplementary Table S5), the overall growth performance was generally lower in the F1 and F2 groups when compared to the C1 feeding group. This can be explained by the similar ratios of PUFA/SFA in commercial feed (1.38) and mussel meal (1.33), and the notably higher ratio in sunflower oil (4.65), which prevailed and contributed to an overall loss of dietary balance in feeds C1, F1 and F2. This is in agreement with other studies, which have shown that the substitution of fish meal with mussel meal does not affect the growth performance significantly [16,18,24,28].

The elevated values for both somatic indices suggest that the nutrient absorption capacity and fish metabolism were influenced by the experimental diets (Figure 3). Higher values for the somatic indices may indicate elevated energy (lipid) storage, accompanied by the enlargement of both the visceral organs and the liver specifically [37,39]. On the one hand, the increased fat deposition observed in these organs can be an indicator of the high-caloric diets provided in the C1, F1 and F2 groups. On the other hand, when combined with the higher condition index present in the mussel-fed groups (F1, F2), as compared to the control group C1, these findings may suggest an overall negative effect of vegetable

oil addition in the mussel-supplemented diets on fish metabolism. These findings may indicate that gilthead seabream, unlike some other fish species, has a limited capacity to oxidize specific groups of lipids and transform them into metabolic energy [58–60].

The proximate composition revealed a significantly higher protein content in both control groups compared to groups F1 and F2 fed with mussel meal (Supplementary Table S4). The analysis of the ash content showed significant differences between control group C2 and group F2 fed with 5% mussel meal. The observed variations in the proximate composition can be attributed to differences in the feed ingredients, which may affect the protein or lipid content of fish muscle [6,28,54,61]. The cited studies have shown that gilthead seabream fed with diets containing plant-based lipids have a higher lipid content in the fish muscle, which was the case in this study, although the observed differences were not statistically significant.

The differences in some fatty acid values noted between the control group C1 and all the other dietary groups can be attributed to the addition of 2% sunflower oil to their formulations (Figures 4 and 5, Supplementary Tables S6 and S7). Sunflower oil, being composed of pure fats, has a greater effect on the total fatty acid profile of the feed compared to the addition of mussel meal, which contains a lower fat content. The large difference in the linoleic acid (C18:2) content between the control group C1 and the other groups (C2, F1 and F2) confirms the earlier conclusion, as linoleic acid is the predominant fatty acid in sunflower oil, with an average content of well over 50% TFA (Supplementary Table S6). The same is true for the total polyunsaturated fatty acids, as their content in sunflower oil is in the range of almost 60% TFA (Supplementary Table S5). While previous studies have reported that vegetable oils, such as sunflower oil, may represent a valuable replacement for fish oil in some inland and marine fish species [59,60,62], these results cannot be confirmed for gilthead seabream. As previously indicated, gilthead seabream potentially lacks not only the enzymes necessary to efficiently oxidize specific lipids but also those needed to synthesize fatty acids from precursors present in sunflower oil. Therefore, the higher deposition and abundance of linoleic and α-linoleic acid (C18:3n3) in dorsal muscle are related to the inability of gilthead seabream to synthesize long-chain polyunsaturated acids, like DHA and EPA, from these substrates [62]. These results highlight the need for further research that should address the age-dependent enzymatic specificity of gilthead seabream, with the aim being to better understand its ability to take up and deposit fatty acids from different dietary sources at different growth stages.

Although the general metabolic pathways of fatty acids in marine fish species were previously described, current knowledge confirms that fatty acid metabolism varies significantly among lower and higher trophic fish levels, genotypes of the same species, dietary intakes, and conditions in the marine environment [52,53,62,63]. Studies conducted on gilthead seabream have demonstrated that this higher trophic omnivorous species has a deficiency in desaturating and elongating essential fatty acids. Consequently, the diet of gilthead seabream must include specific long-chain polyunsaturated fatty acids to support its optimal growth [64]. The results of this study confirmed increased levels of EPA and DHA in mussel meal (Figure 6, Supplementary Table S5), suggesting it as a sustainable alternative to traditionally used fish oil [24,28,62]. As such, mussels represent a valuable source of fatty acids for marine fish species of higher trophic levels (>3), particularly given their limitation in long-chain polyunsaturated fatty acid bioconversion [62]. This work further sustains previous hypotheses, as gilthead seabream diets supplemented with mussel-derived EPA and DHA enhanced the deposition of these fatty acids in the tissues (Figure 5). Due to the high content of DHA and EPA in mussel meal, the content of these fatty acids in the muscle tissue was proportional to the content of mussel meal supplement in the feed formulation, although the overall effect was not statistically significant. A similar effect of mussel meal on the DHA and EPA content had been observed in previous studies [24,28]. When it comes to the metabolic relationship between EPA and DHA, a positive correlation ($r = 0.64$, $p = 0.025$) in their accumulation was noted in this study (Supplementary Table S8). This could indicate that no competition in their deposition was present in gilthead seabream

fed with different diets during increased summer temperatures. Since the EPA and DHA content is generally declining in farmed gilthead seabream in the Mediterranean area [65], the presented results are even more significant, as mussel meal could be adopted to both stimulate the feed intake and increase the content of these valuable fatty acids.

Despite the higher content of saturated fatty acids in both commercial feed and mussel meal, their deposition decreased in gilthead seabream fed with mussel-supplemented diets (Figures 4 and 6, Supplementary Tables S5 and S6). Nevertheless, the inverted correlation between saturated ($r = -0.98$, $p < 0.001$) and monounsaturated ($r = -0.95$, $p < 0.001$) with polysaturated fatty acids suggests that the deposition of the later ones was favored (Supplementary Table S8). Vallecillos et al. [52] assumed that variations in saturated fatty acid deposition are related to differences in gilthead seabream intramuscular fattening. Generally, lower lipid levels were present in C1 and F2 compared to C2 and F1, with similar trends present for the saturated fatty acid deposition (Supplementary Tables S4 and S7). The fatty acid indices can be grouped into those having a positive impact on human health when present at lower (e.g., index of thrombogenicity) or higher values (e.g., unsaturation index, EPA + DHA, and fish lipid quality). The fatty acid indices measured for gilthead seabream were similar across all the dietary groups (Supplementary Table S7). However, when compared to gilthead seabream analyzed across different seasons [66], the specimens in this experiment had nearly half the value of the index of thrombogenicity. This finding indicates that the fatty acid profile of gilthead seabream farmed under the investigated dietary regimes could contribute to a reduced risk of blood clot formation and coronary heart disease development when included in the human diet. The unsaturation index, often used in macroalgal fatty acid description, can also be applied to other food sources [43]. The values reported for gilthead seabream in this study were comparable to or exceeded those of several seaweed species, reflecting a fatty acid profile favorable for cardiovascular health. Fatty acids such as EPA and DHA are essential for human biological processes, reducing the risk of hypertension and inflammation while increasing cognitive functions [43]. In this study, the reported EPA + DHA content was within the range reported for gilthead seabream farmed in the Mediterranean area [65]. While Senso et al. [66] noted significant seasonal variations in the fish lipid quality, our results suggest that well-planned diets or dietary supplements may, even if only moderately, improve the values of this index in gilthead seabream. Altogether, the fatty acid indices evaluated in this study support previous findings that gilthead seabream offers distinct nutritional benefits, with the potential to improve human health if included in the diet.

5. Conclusions

Fish diets including 2.5 and 5% mussel meal as an additive to commercial feed, along with 2% of sunflower oil as a binding agent, resulted in slightly lower weight gain and slightly higher feed conversion ratios. These effects were attributed to changes in the balance of macronutrients in the feed formulation. Gilthead seabream groups fed with mussel-meal-supplemented diets had a slightly lower protein content in their dorsal muscle. The addition of sunflower oil and mussel meal to the feed formulation increased the rates of monounsaturated and polyunsaturated fatty acid, while decreasing the saturated fatty acid content, compared to the control group fed with commercial feed. Due to the high content of DHA and EPA in mussel meal, the muscle tissue content of these fatty acids was proportional to the amount of mussel meal supplement in the feed formulation, although the overall effect was not statistically significant. These results suggest that mussel meal could be a potential source of proteins and lipids for fish feed production. To gain clearer insight into this topic, future studies should be conducted using a minimal amount of binding agent in the feed formulation or substitution of a proportion of fishmeal with mussel meal to mitigate the undesirable effects of the binding agent observed in this study.

The findings of this study highlight the potential of adopting mussels in bioremediation processes of the local marine environment and their reuse in producing targeted diets. However, the contamination levels in such bioremediation-based feed production models

should be considered in detail. Additionally, the nutritive quality of gilthead seabream fed with sustainably produced mussel supplements resulted in the produced fillets having fatty acid indices desirable in human nutrition.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fishes9120524/s1, Figure S1. Gilthead seabream specimens fed with different feed formulations (C1, C2, F1, F2) after 6 weeks of growth, Supplementary Table S1. Condition index (K 2–6), relative growth (RG 2–4), relative weight gain (RWG 2–4), specific growth rate (SGR 2–4) of gilthead seabream (mean ± SD) fed with different feed formulations (C1, C2, F1, F2) during the experiment (0–6 weeks), Supplementary Table S2. Relative growth (RG), relative weight gain (RWG), condition index (K), specific growth rate (SGR), protein efficiency ratio (PER) and feed conversion rate (FCR) of gilthead seabream (mean ± SD) fed with different feed formulations (C1, C2, F1, F2) at the end of the experiment (6 weeks), Supplementary Table S3. Dorsal muscle proportion (DM), viscerasomatic index (VSI) and hepatosomatic index (HSI) of gilthead seabream (mean ± SD) fed with different feed formulations (C1, C2, F1, F2) at the end of the experiment (6 weeks), Supplementary Table S4. Proximate composition (mean ± SD) of dorsal muscle of gilthead seabream fed with different feed formulations (C1, C2, F1, F2) at the end of the experiment (6 weeks), Supplementary Table S5. Fatty acid profile (mean ± SD) expressed as % of total fatty acids (TFAs) of diet formulations used for gilthead seabream feeding experiment, Supplementary Table S6. Fatty acid profile (mean ± SD) expressed as % of total fatty acids (TFA) of the dorsal muscle of gilthead seabream fed with different feed formulations (C1, C2, F1, F2) at the end of the experiment (6 weeks), Supplementary Table S7. Fatty acid indices (mean ± SD) of the dorsal muscle of gilthead seabream fed with different feed formulations (C1, C2, F1, F2) at the end of the experiment (6 weeks), Supplementary Table S8. Pearson's correlation coefficient for fatty acid content of the dorsal muscle of gilthead seabream fed with different feed formulations at the end of the experiment (6 weeks).

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Data Availability Statement: Dataset available on request from the authors.

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References

1. *The State of Mediterranean and Black Sea Fisheries 2023*; FAO: Rome, Italy, 2023; ISBN 978-92-5-138411-4.
2. Mendes, R. Technological Processing of Fresh Gilthead Seabream (*Sparus aurata*): A Review of Quality Changes. *Food Rev. Int.* **2019**, *35*, 20–53. [CrossRef]
3. Llorente, I.; Luna, L. The Competitive Advantages Arising from Different Environmental Conditions in Seabream, *Sparus aurata*, Production in the Mediterranean Sea. *J. World Aquac. Soc.* **2013**, *44*, 611–627. [CrossRef]

4. Fantatto, R.R.; Mota, J.; Ligeiro, C.; Vieira, I.; Guilgur, L.G.; Santos, M.; Murta, D. Exploring Sustainable Alternatives in Aquaculture Feeding: The Role of Insects. *Aquac. Rep.* **2024**, *37*, 102228. [CrossRef]
5. Boyd, C.E.; D'Abramo, L.R.; Glencross, B.D.; Huyben, D.C.; Juarez, L.M.; Lockwood, G.S.; McNevin, A.A.; Tacon, A.G.J.; Teletchea, F.; Tomasso, J.R., Jr.; et al. Achieving Sustainable Aquaculture: Historical and Current Perspectives and Future Needs and Challenges. *J. World Aquac. Soc.* **2020**, *51*, 578–633. [CrossRef]
6. Matos, E.; Dias, J.; Dinis, M.T.; Silva, T.S. Sustainability vs. Quality in Gilthead Seabream (*Sparus aurata* L.) Farming: Are Trade-Offs Inevitable? *Rev. Aquac.* **2017**, *9*, 388–409. [CrossRef]
7. Martínez-Llorens, S.; Vidal, A.T.; Garcia, I.J.; Torres, M.P.; Cerdá, M.J. Optimum Dietary Soybean Meal Level for Maximizing Growth and Nutrient Utilization of On-Growing Gilthead Sea Bream (*Sparus aurata*). *Aquac. Nutr.* **2009**, *15*, 320–328. [CrossRef]
8. Martínez-Llorens, S.; Baeza-Ariño, R.; Nogales-Mérida, S.; Jover-Cerdá, M.; Tomás-Vidal, A. Carob Seed Germ Meal as a Partial Substitute in Gilthead Sea Bream (*Sparus aurata*) Diets: Amino Acid Retention, Digestibility, Gut and Liver Histology. *Aquaculture* **2012**, *338–341*, 124–133. [CrossRef]
9. Vélez-Calabria, G.; Tomás-Vidal, A.; Peñaranda, D.S.; Jover-Cerdá, M.; Llorens, S.M. Effect of Additives Inclusion in Gilthead Seabream (*Sparus aurata* L.) Diets on Growth, Enzyme Activity, Digestibility and Gut Histology Fed with Vegetable Meals. *Animals* **2023**, *13*, 205. [CrossRef] [PubMed]
10. Mastoraki, M.; Katsika, L.; Enes, P.; Guerreiro, I.; Kotzamanis, Y.P.; Gasco, L.; Chatzifotis, S.; Antonopoulou, E. Insect Meals in Feeds for Juvenile Gilthead Seabream (*Sparus aurata*): Effects on Growth, Blood Chemistry, Hepatic Metabolic Enzymes, Body Composition and Nutrient Utilization. *Aquaculture* **2022**, *561*, 738674. [CrossRef]
11. Fabrikov, D.; Barroso, F.G.; Sánchez-Muros, M.J.; Hidalgo, M.C.; Cardenete, G.; Tomás-Almenar, C.; Melenchón, F.; Guil-Guerrero, J.L. Effect of Feeding with Insect Meal Diet on the Fatty Acid Compositions of Sea Bream (*Sparus aurata*), Tench (*Tinca tinca*) and Rainbow Trout (*Oncorhynchus mykiss*) Fillets. *Aquaculture* **2021**, *545*, 737170. [CrossRef]
12. Vizcaíno, A.J.; López, G.; Sáez, M.I.; Jiménez, J.A.; Barros, A.; Hidalgo, L.; Camacho-Rodríguez, J.; Martínez, T.F.; Cerón-García, M.C.; Alarcón, F.J. Effects of the Microalga *Scenedesmus almeriensis* as Fishmeal Alternative in Diets for Gilthead Sea Bream, *Sparus aurata*, Juveniles. *Aquaculture* **2014**, *431*, 34–43. [CrossRef]
13. Carvalho, M.; Montero, D.; Rosenlund, G.; Fontanillas, R.; Ginés, R.; Izquierdo, M. Effective Complete Replacement of Fish Oil by Combining Poultry and Microalgae Oils in Practical Diets for Gilthead Sea Bream (*Sparus aurata*) Fingerlings. *Aquaculture* **2020**, *529*, 735696. [CrossRef]
14. Randazzo, B.; Di Marco, P.; Zarantoniello, M.; Daniso, E.; Cerri, R.; Finoia, M.G.; Capoccioni, F.; Tibaldi, E.; Olivotto, I.; Cardinaletti, G. Effects of Supplementing a Plant Protein-Rich Diet with Insect, Crayfish or Microalgae Meals on Gilthead Sea Bream (*Sparus aurata*) and European Seabass (*Dicentrarchus labrax*) Growth, Physiological Status and Gut Health. *Aquaculture* **2023**, *575*, 739811. [CrossRef]
15. Al-Souti, A.; Gallardo, W.; Claereboudt, M.; Mahgoub, O. Attractability and Palatability of Formulated Diets Incorporated with Chicken Feather and Algal Meals for Juvenile Gilthead Seabream, *Sparus aurata*. *Aquac. Rep.* **2019**, *14*, 100199. [CrossRef]
16. Nagel, F.; von Danwitz, A.; Schlachter, M.; Kroeckel, S.; Wagner, C.; Schulz, C. Blue Mussel Meal as Feed Attractant in Rapeseed Protein-Based Diets for Turbot (*Psetta maxima* L.). *Aquac. Res.* **2014**, *45*, 1964–1978. [CrossRef]
17. Tandler, A.; Berg, B.A.; wm. Kissil, G.; Mackie, A.M. Effect of Food Attractants on Appetite and Growth Rate of Gilthead Bream, *Sparus aurata* L. *J. Fish Biol.* **1982**, *20*, 673–681. [CrossRef]
18. Vidakovic, A.; Langeland, M.; Sundh, H.; Sundell, K.; Olstorpe, M.; Vielma, J.; Kiessling, A.; Lundh, T. Evaluation of Growth Performance and Intestinal Barrier Function in Arctic Charr (*Salvelinus alpinus*) Fed Yeast (*Saccharomyces cerevisiae*), Fungi (*Rhizopus oryzae*) and Blue Mussel (*Mytilus edulis*). *Aquac. Nutr.* **2016**, *22*, 1348–1360. [CrossRef]
19. Albrektsen, S.; Kortet, R.; Skov, P.V.; Ytteborg, E.; Gitlesen, S.; Kleinegris, D.; Mydland, L.-T.; Hansen, J.Ø.; Lock, E.-J.; Mørkøre, T.; et al. Future Feed Resources in Sustainable Salmonid Production: A Review. *Rev. Aquac.* **2022**, *14*, 1790–1812. [CrossRef]
20. Jusadi, D.; Ekasari, J.; Suprayudi, M.A.; Setiawati, M.; Fauzi, I.A. Potential of Underutilized Marine Organisms for Aquaculture Feeds. *Front. Mar. Sci.* **2021**, *7*, 609471. [CrossRef]
21. Berge, G.M.; Austreng, E. Blue Mussel in Feed for Rainbow Trout. *Aquaculture* **1989**, *81*, 79–90. [CrossRef]
22. Weiß, M.; Buck, B.H. Partial Replacement of Fishmeal in Diets for Turbot (*Scophthalmus maximus*, Linnaeus, 1758) Culture Using Blue Mussel (*Mytilus edulis*, Linneus, 1758) Meat. *J. Appl. Ichthyol.* **2017**, *33*, 354–360. [CrossRef]
23. Luo, C.; Wang, Y.; Tao, S.; Liao, Y.; Yang, C.; Cui, C.; Yang, J.; Yang, Y. Effects of Replacing Fish Meal with Mussel (*Cristaria plicata*) Meat on Growth, Digestive Ability, Antioxidant Capacity and Hepatic IGF-I Gene Expression in Juvenile Ussuri Catfish (*Pseudobagrus ussuriensis*). *Aquac. Res.* **2019**, *50*, 826–835. [CrossRef]
24. Mongile, F.; Mandrioli, L.; Mazzoni, M.; Pirini, M.; Zaccaroni, A.; Sirri, R.; Parma, L.; Gatta, P.P.; Sarli, G.; Bonaldo, A. Dietary Inclusion of Mussel Meal Enhances Performance and Improves Feed and Protein Utilization in Common Sole (*Solea solea*, Linnaeus, 1758) Juveniles. *J. Appl. Ichthyol.* **2015**, *31*, 1077–1085. [CrossRef]
25. Wagner, L.; Gómez-Requeni, P.; Moazzami, A.A.; Lundh, T.; Vidakovic, A.; Langeland, M.; Kiessling, A.; Pickova, J. 1H NMR-Based Metabolomics and Lipid Analyses Revealed the Effect of Dietary Replacement of Microbial Extracts or Mussel Meal with Fish Meal to Arctic Charr (*Salvelinus alpinus*). *Fishes* **2019**, *4*, 46. [CrossRef]
26. Richard, M.; Forget, F.; Mignucci, A.; Mortreux, S.; Le Gall, P.; Callier, M.; Weise, A.; McKindsey, C.; Bourjea, J. Farmed Bivalve Loss Due to Seabream Predation in the French Mediterranean Prevost Lagoon. *Aquacult. Environ. Interact.* **2020**, *12*, 529–540. [CrossRef]

27. Avignon, S.; Tastard, E.; Weston, S.; Duhamel, G.; Denis, F. Morphological Identification and DNA Barcoding Used for Diet Analysis of Gilthead Seabream (*Sparus aurata*) in Its Expanding Northerly Range. *Aquat. Living Resour.* **2017**, *30*, 1. [CrossRef]
28. Jaeger, C.; Corraze, G.; Gayet, V.; Larroquet, L.; Surget, A.; Terrier, F.; Aubin, J. Discarded Blue Mussel (*Mytilus edulis*): A Feed Ingredient That Maintains Growth Performance of Juvenile Gilthead Seabream (*Sparus aurata*) While Fishmeal and Fish Oil Are Removed. *J. Appl. Aquac.* **2024**, 1–17. [CrossRef]
29. Tamburini, E.; Turolla, E.; Fano, E.A.; Castaldelli, G. Sustainability of Mussel (*Mytilus galloprovincialis*) Farming in the Po River Delta, Northern Italy, Based on a Life Cycle Assessment Approach. *Sustainability* **2020**, *12*, 3814. [CrossRef]
30. McKindsey, C.W.; Thetmeyer, H.; Landry, T.; Silvert, W. Review of Recent Carrying Capacity Models for Bivalve Culture and Recommendations for Research and Management. *Aquaculture* **2006**, *261*, 451–462. [CrossRef]
31. Suplicy, F.M. A Review of the Multiple Benefits of Mussel Farming. *Rev. Aquac.* **2020**, *12*, 204–223. [CrossRef]
32. Hamer, B.; Korlević, M.; Durmiši, E.; Nerlović, V.; Bierne, N. Nuclear marker Me 15/16 analyses of *Mytilus galloprovincialis* populations along the eastern Adriatic coast. *Cahiers de Biologie Marine* **2012**, *53*, 35–44. [CrossRef]
33. Goldberg, E.D.; Bowen, V.T.; Farrington, J.W.; Harvey, G.; Martin, J.H.; Parker, P.L.; Risebrough, R.W.; Robertson, W.; Schneider, E.; Gamble, E. The Mussel Watch. *Environ. Conserv.* **1978**, *5*, 101–125. [CrossRef]
34. Gosling, E. *Marine Mussels: Ecology, Physiology, Genetics and Culture*; John and Wiley and Sons: Hoboken, NJ, USA, 2021.
35. Parodi, A.; Leip, A.; De Boer, I.J.M.; Slegers, P.M.; Ziegler, F.; Temme, E.H.M.; Herrero, M.; Tuomisto, H.; Valin, H.; Van Middelaar, C.E.; et al. The Potential of Future Foods for Sustainable and Healthy Diets. *Nat. Sustain.* **2018**, *1*, 782–789. [CrossRef]
36. Filgueira, R.; Byron, C.J.; Comeau, L.A.; Costa-Pierce, B.; Cranford, P.J.; Ferreira, J.G.; Grant, J.; Guyondet, T.; Jansen, H.M.; Landry, T.; et al. An Integrated Ecosystem Approach for Assessing the Potential Role of Cultivated Bivalve Shells as Part of the Carbon Trading System. *Mar. Ecol. Prog. Ser.* **2015**, *518*, 281–287. [CrossRef]
37. Mendes, R.; Rema, P.; Dias, J.; Gonçalves, A.T.; Teodósio, R.; Engrola, S.; Sánchez-Vázquez, F.J.; Conceição, L.E.C. Socially Acceptable Feed Formulations May Impact the Voluntary Feed Intake and Growth, but Not Robustness of Nile Tilapia (*Oreochromis niloticus*). *Fishes* **2024**, *9*, 361. [CrossRef]
38. Campbell, K.B.; McLean, E.; Barrows, F.T. In Pursuit of Fish-Free Feeds: A Multi-Species Evaluation. *Fishes* **2022**, *7*, 336. [CrossRef]
39. Ayisi, C.L.; Zhao, J.; Rupia, E.J. Growth Performance, Feed Utilization, Body and Fatty Acid Composition of Nile Tilapia (*Oreochromis niloticus*) Fed Diets Containing Elevated Levels of Palm Oil. *Aquac. Fish.* **2017**, *2*, 67–77. [CrossRef]
40. AOAC. *Official Methods of Analysis*, 16th ed.; AOAC International: Arlington, WA, USA, 1995.
41. Smedes, F. Determination of Total Lipid Using Non-Chlorinated Solvents. *Analyst* **1999**, *124*, 1711–1718. [CrossRef]
42. *ISO 12966-2:2017*; Animal and Vegetable Fats and Oils—Preparation of Methyl Esters of Fatty Acids. ISO: Geneva, Switzerland, 2017. Available online: https://www.iso.org/standard/72142.html (accessed on 24 April 2024).
43. Chen, J.; Liu, H. Nutritional Indices for Assessing Fatty Acids: A Mini-Review. *Int. J. Mol. Sci.* **2020**, *21*, 5695. [CrossRef]
44. Sinovčić, G.; Franičević, M.; Zorica, B.; Čikeš-Keč, V. Length–Weight and Length–Length Relationships for 10 Pelagic Fish Species from the Adriatic Sea (Croatia). *J. Appl. Ichthyol.* **2004**, *20*, 156–158. [CrossRef]
45. Hothorn, T.; Hornik, K.; van de Wiel, M.A.; Zeileis, A. Implementing a Class of Permutation Tests: The Coin Package. *J. Stat. Softw.* **2008**, *28*, 1–23. [CrossRef]
46. Kolde, R. Pheatmap: Pretty Heatmaps 2019. Available online: https://cran.ms.unimelb.edu.au/web/packages/pheatmap (accessed on 1 December 2024).
47. R Core Team. Available online: https://cran.r-project.org/manuals.html (accessed on 1 December 2024).
48. Kassambara, A. Rstatix: Pipe-Friendly Framework for Basic Statistical Tests 2023. Available online: https://cran.r-project.org/web/packages/rstatix/index.html (accessed on 1 December 2024).
49. Kucheryavskiy, S. Mdatools—R Package for Chemometrics. *Chemom. Intell. Lab. Syst.* **2020**, *198*, 103937. [CrossRef]
50. Akyol, O.; Gamsiz, K. Age and Growth of Adult Gilthead Seabream (*Sparus aurata* L.) in the Aegean Sea. *J. Mar. Biol. Assoc. United Kingd.* **2011**, *91*, 1255–1259. [CrossRef]
51. Korkut, A.K.A.Y.; Gurkan, S. Length-Weight Relationship and Condition Factor as an Indicator of Growth and Feeding Intensity of Sea Bream (*Sparus aurata* L., 1758) given Feed with Different Protein Contents. *Indian J. Anim. Res.* **2018**, *53*, 510–514.
52. Vallecillos, A.; Marín, M.; Bortoletti, M.; López, J.; Afonso, J.M.; Ramis, G.; Arizcun, M.; María-Dolores, E.; Armero, E. Genetic Analysis of the Fatty Acid Profile in Gilthead Seabream (*Sparus aurata* L.). *Animals* **2021**, *11*, 2889. [CrossRef]
53. Montero, D.; Moyano, F.J.; Carvalho, M.; Sarih, S.; Fontanillas, R.; Zamorano, M.J.; Torrecillas, S. Nutritional Innovations in Superior Gilthead Seabream (*Sparus aurata*) Genotypes: Implications in the Utilization of Emerging New Ingredients through the Study of the Patterns of Secretion of Digestive Enzymes. *Aquaculture* **2023**, *577*, 739958. [CrossRef]
54. Benedito-Palos, L.; Navarro, J.C.; Sitjà-Bobadilla, A.; Bell, J.G.; Kaushik, S.; Pérez-Sánchez, J. High Levels of Vegetable Oils in Plant Protein-Rich Diets Fed to Gilthead Sea Bream (*Sparus aurata* L.): Growth Performance, Muscle Fatty Acid Profiles and Histological Alterations of Target Tissues. *Br. J. Nutr.* **2008**, *100*, 992–1003. [CrossRef]
55. Velázquez, M.; Zamora, S.; Martínez, F.J. Influence of Environmental Conditions on Demand-Feeding Behaviour of Gilthead Seabream (*Sparus aurata*). *J. Appl. Ichthyol.* **2004**, *20*, 536–541. [CrossRef]
56. Mhalhel, K.; Levanti, M.; Abbate, F.; Laurà, R.; Guerrera, M.C.; Aragona, M.; Porcino, C.; Briglia, M.; Germanà, A.; Montalbano, G. Review on Gilthead Seabream (*Sparus aurata*) Aquaculture: Life Cycle, Growth, Aquaculture Practices and Challenges. *J. Mar. Sci. Eng.* **2023**, *11*, 2008. [CrossRef]

57. Kır, M. Thermal Tolerance and Standard Metabolic Rate of Juvenile Gilthead Seabream (*Sparus aurata*) Acclimated to Four Temperatures. *J. Therm. Biol.* **2020**, *93*, 102739. [CrossRef] [PubMed]
58. Caballero, M.J.; Obach, A.; Rosenlund, G.; Montero, D.; Gisvold, M.; Izquierdo, M.S. Impact of Different Dietary Lipid Sources on Growth, Lipid Digestibility, Tissue Fatty Acid Composition and Histology of Rainbow Trout, *Oncorhynchus mykiss*. *Aquaculture* **2002**, *214*, 253–271. [CrossRef]
59. López, L.M.; Torres, A.L.; Durazo, E.; Drawbridge, M.; Bureau, D.P. Effects of lipid on growth and feed utilization of white seabass (*Atractoscion nobilis*) fingerlings. *Aquaculture* **2006**, *253*, 557–563. [CrossRef]
60. Bertucci, J.I.; Tovar, M.O.; Unniappan, S.; Navarro, J.C.; Canosa, L.F. Effects of Dietary Sunflower Oil on Growth Parameters, Fatty Acid Profiles and Expression of Genes Regulating Growth and Metabolism in the Pejerrey (*Odontesthes bonariensis*) Fry. *Aquac. Nutr.* **2018**, *24*, 748–757. [CrossRef]
61. Castro, C.; Corraze, G.; Firmino-Diógenes, A.; Larroquet, L.; Panserat, S.; Oliva-Teles, A. Regulation of Glucose and Lipid Metabolism by Dietary Carbohydrate Levels and Lipid Sources in Gilthead Sea Bream Juveniles. *Br. J. Nutr.* **2016**, *116*, 19–34. [CrossRef]
62. Xu, H.; Turchini, G.M.; Francis, D.S.; Liang, M.; Mock, T.S.; Rombenso, A.; Ai, Q. Are Fish What They Eat? A Fatty Acid's Perspective. *Prog. Lipid Res.* **2020**, *80*, 101064. [CrossRef] [PubMed]
63. Torno, C.; Staats, S.; Fickler, A.; de Pascual-Teresa, S.; Izquierdo, M.S.; Rimbach, G.; Schulz, C. Combined Effects of Nutritional, Biochemical and Environmental Stimuli on Growth Performance and Fatty Acid Composition of Gilthead Sea Bream (*Sparus aurata*). *PLoS ONE* **2019**, *14*, e0216611. [CrossRef]
64. Tocher, D.R.; Ghioni, C. Fatty Acid Metabolism in Marine Fish: Low Activity of Fatty Acyl Δ5 Desaturation in Gilthead Sea Bream (*Sparus aurata*) Cells. *Lipids* **1999**, *34*, 433–440. [CrossRef] [PubMed]
65. Vasconi, M.; Caprino, F.; Bellagamba, F.; Moretti, V.M. Fatty Acid Composition of Gilthead Sea Bream (*Sparus aurata*) Fillets as Affected by Current Changes in Aquafeed Formulation. *Turk. J. Fish. Aquat. Sci.* **2017**, *17*, 451–459. [CrossRef]
66. Senso, L.; Suárez, M.D.; Ruiz-Cara, T.; García-Gallego, M. On the Possible Effects of Harvesting Season and Chilled Storage on the Fatty Acid Profile of the Fillet of Farmed Gilthead Sea Bream (*Sparus aurata*). *Food Chem.* **2007**, *101*, 298–307. [CrossRef]