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Evaluation of Alternative Dietary Ingredients as a Sustainable and Ecological Solution for Meagre (*Argyrosomus regius*) Production in Earthen Ponds

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Abstract: The aquaculture sector is developing sustainability measures to address resource limitations and environmental concerns. A key strategy is replacing fishmeal and fish oil with alternatives that can equally sustain fish health, growth, and water quality. This study compared a standard diet (STD) to an alternative diet (ALT) containing sustainable ingredients, such as plant-based proteins and animal by-products, for meagre raised in earthen ponds within a polyculture system. Over 150 days, 5400 meagre juveniles (174.9 ± 32.8 g) were fed these diets. Fish on the ALT diet showed superior growth, likely due to higher dietary protein content and reduced protein degradation in liver and muscle, leading to increased protein content and reduced levels of dry matter, lipid, ash, energy, and phosphorous. While muscle cohesiveness was affected, fiber area and density were unchanged. ALT-fed fish exhibited higher saturated (SFA) and polyunsaturated (PUFA) fatty acids, reflecting the diet. Water quality indicators, including ammonia, nitrites, nitrates, and phosphates, were similar across diets, though chlorophyll *a* was higher in ponds with STD-fed fish. Overall, the ALT diet emerges as a sustainable alternative to the STD diet, maintaining or enhancing protein levels while reducing fishmeal usage. This approach effectively supports meagre growth and fillet quality without significant additional environmental impact.

Keywords: fish nutrition; sustainability; environment; plant-based ingredients; animal by-products; land-based aquaculture

Key Contribution: The ALT diet, enriched with sustainable ingredients such as plant-based proteins and animal by-products, enhanced growth in meagre raised in earthen ponds within a polyculture system, while ensuring optimal dietary protein levels. This species exhibited higher levels of EPA and DHA, reflecting the composition of the diet, while maintaining nutrient discharge levels comparable to the standard (STD) treatment. This makes the ALT diet a sustainable and effective alternative to the STD diet for meagre production in earthen ponds.

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1. Introduction

Food production is essential to sustain a rapidly expanding global population, projected to reach 10.3 billion by the mid-2080s, up from 8.2 billion in 2024 [1]. Crucial in meeting the demands of this growth are the fisheries and aquaculture sectors, which provide vital access to diverse and nutritious food sources. While capture fisheries remain relatively static, aquaculture stands out as the primary driver of the expansion in the supply of fish for human consumption, a trend that is expected to persist into the foreseeable future [2]. The expansion of aquaculture production has raised both concerns and societal debates, particularly concerning its environmental footprint. Key issues include the inefficient and potentially unsustainable production of fishmeal and fish oil, which are critical sources of protein and lipids for the intensive farming of carnivorous fish species [2–4]. These concerns highlight the need for more sustainable practices within the industry. Effective fish feed management presents a significant opportunity for reducing the environmental impact of aquaculture. According to Wilfart and collaborators [5], feed accounts for 65–95% of the environmental impacts associated with animal production from aquaculture farms and it constitutes 40–70% of the production costs [6]. To decrease reliance on traditional fish meal and oil, various alternative aquafeed ingredients have been developed. These alternatives include plant-based feedstuffs (e.g., soybean, micro, and macroalgae), microbial (e.g., yeast), marine and terrestrial animal ingredients (e.g., squid, krill, insect, poultry), or animal by-products (e.g., heads, bones, viscera) [7–10]. However, the nutritional value of these alternatives varies from that of whole fish, which could significantly affect fish growth, health, and quality. Additionally, environmental impacts may arise if excess nutrients, not utilized by fish, accumulate in the form of uneaten food, feces, and soluble substances such as ammonia, nitrates, nitrites, and phosphates [11]. Therefore, assessing and comparing the nutritional impact of these alternative ingredients on fish physiology, as well as their resulting nutrient discharges, with those of commercial diets is crucial.

Fish meal has traditionally been the primary protein source in feeds for carnivorous species such as meagre (*Argyrosomus regius*). Valued for its suitability in large-scale Mediterranean aquaculture, meagre also exhibits a good tolerance for diets that incorporate alternative protein and oil sources [12–17]. Given these favorable features, we believe meagre could effectively serve as a model to validate diets containing novel ingredients for earthen pond aquaculture production. Several studies have shown that substituting traditional fishmeal and oil in aquafeeds with alternative ingredients can significantly affect the metabolism of some fish species and tissues [18–23]. Therefore, a deeper understanding of the impact of novel ingredients on growth and the nutritional quality of the final product is crucial for evaluating their viability in aquafeeds. Studies on protein degradation systems have shed light on how different dietary protein sources modulate fish somatic growth [24]. Autophagy, for instance, utilizes a variety of hydrolytic enzymes, such as cathepsins, to break down cytosolic materials, including proteins. The ubiquitin–proteasome system (UPS) selectively targets proteins for degradation using a large protease known as the proteasome [25]. Several studies demonstrated changes in the activity of cathepsins and proteasome across various fish tissues under different nutritional conditions [26–29], suggesting that these proteases are valuable biomarkers for assessing protein degradation mechanisms in fish.

Proteins are continually exposed to damage from various factors under both stress and normal physiological conditions, with oxidation being a prevalent type of modification. Accumulation of these oxidized proteins within an organism can lead to various diseases and disorders. Consequently, organisms have developed strategies to eliminate these toxic damaged proteins through proteolytic and repair systems. Sánchez-Nuño and collaborators reported that advanced oxidation protein products (AOPPs) serve as markers of oxidative stress in marine fish [30]. These products result from the direct oxidation of protein amino acid side chains due to damage caused by reactive oxygen species (ROS). Numerous studies have shown that the liver oxidation status of various fish species changes when fed diets containing alternative ingredients to fishmeal and fish oil [22,31–36]. The

findings suggest that such alternative ingredients might influence cellular protein oxidation, potentially affecting the activity of protein degradation systems. Furthermore, fish growth is fundamentally driven by muscle development, which includes both the formation of new muscle fibers and the expansion of existing ones [37]. This muscle cellularity affects fillet texture, a crucial quality trait that significantly influences consumer acceptance of the fish product [38]. Given that the nutritional status of fish can impact its texture and cellular composition, also known as muscle cellularity [16,17,39,40], we considered these parameters as valuable tools for understanding the effect of dietary regimens on meagre growth performance and overall quality.

Proteins and lipids, along with their constituent fatty acids, are the major sources of metabolic energy in fish, essential for growth [41]. Fish are particularly rich in LC-PUFAs, which play crucial roles in both animal and human health. Consequently, it is important to assess the impact of diets on the fatty acid composition of fish tissues, including liver, muscle, and perivisceral fat. Analyzing these changes will offer valuable insights into the quality and nutritional value of the final product for consumers.

In this study, we evaluated a diet containing alternative ingredients to traditional fishmeal and fish oil, comparing it against a commercially available diet commonly used in meagre feeding regimes. The primary objective was to evaluate the alternative diet's impact on fish performance and environmental sustainability, aiming to develop a practical dietary solution for meagre production in earthen ponds that maintains protein levels and growth efficiency without relying on fishmeal. Specifically, we investigated the effects of the two diets on meagre juvenile production, monitoring growth parameters, protein turnover, tissue structure, fatty acid composition, and environmental quality indicators. These findings are critical for enhancing the sustainability of aquaculture practices.

2. Materials and Methods

2.1. Experimental Diets

Two diets were evaluated: a standard commercial diet (STD), commonly used in meagre feeding regimes, and an alternative diet (ALT), formulated and produced by Sparos, Lda. (Olhão, Portugal). The ingredients for each diet are listed in Tables 1 and S1. The fatty acid profiles and biochemical compositions of these two experimental diets are presented in Table 2.

Table 1. Formulation of the ALT diet.

Ingredients, %	ALT Diet
Fishmeal 60 [1]	8.00
Squid meal [2]	2.00
Microalgae meal (*Chlorella vulgaris*) [3]	1.80
Microalgae meal (*Tetraselmis chuii*) [4]	0.20
Soy protein concentrate [5]	12.00
Pea protein concentrate [6]	3.00
Wheat gluten [7]	11.50
Corn gluten [8]	10.00
Guar meal [9]	8.00
Soybean meal 48 [10]	2.00
Rapeseed meal [11]	5.00
Wheat meal [12]	8.00
Wheat bran [13]	2.35
Fish oil (sardine by-products) [14]	5.00
Soybean oil [15]	3.00
Rapeseed oil [16]	5.00
Linseed oil [17]	2.50
Vitamin and mineral premix [18]	1.00
Vitamin C (35%) [19]	0.03
Vitamin E (50%) [20]	0.05

Table 1. *Cont.*

Ingredients, %	ALT Diet
Brewer's yeast [21]	3.00
Macroalgae meal (*Ascophyllum nodosum*) [22]	4.60
Antioxidant [23]	0.30
Monoammonium phosphate [24]	0.40
L-Lysine [25]	0.50
L-Tryptophan [26]	0.15
DL-Methionine [27]	0.40
L-Taurine [28]	0.22

[1] CONRESA: 61.2% crude protein (CP), 8.4% crude fat (CF), Conserveros Reunidos S.A., Spain. [2] 83% CP, 4% CF, Sopropêche, France. [3] 62.5% CP, 9.2% CF, *Chlorella vulgaris*, Allmicroalgae, Portugal. [4] 23.4% CP, 6.2% CF, *Tetraselmis chuii*, Allmicroalgae, Portugal. [5] Soycomil P: 62.2% CP, 0.7% CF, ADM, The Netherlands. [6] Lysamine GPS: 78.1% CP, 8.3% CF, Roquette, France. [7] VITAL: 80.4% CP, 5.8% CF, Roquette, France. [8] 61.2% CP, 5.2% CF, COPAM, Portugal. [9] 55.3% CP, 7.8% CF, Koma, Meelunie B.V., The Netherlands. [10] 47.4% CP, 2.6% CF, Dehulled solvent extracted, CARGILL, Spain. [11] 34.3% CP, 2.1% CF, Solvent extracted, Ribeiro e Sousa Lda, Portugal. [12] 11.7% CP, 1.6% CF, Molisur, Spain. [13] 14.8% CP, 4.7% CF, Ribeiro e Sousa Lda, Portugal. [14] 98.1% CF; 16% EPA, 12% DHA, Univela, Morroco. [15] 98.6% CF, JC Coimbra, Portugal. [16] 98.2% CF, JC Coimbra, Portugal. [17] 98.4% CF, Henry Lamotte Oils GmbH, Germany. [18] Vitamins (IU or mg Kg^{-1} diet): DL-alpha-tocopherol acetate, 100 mg; sodium menadione bisulfate, 25 mg; retinyl acetate, 20,000 IU; DL-cholecalciferol, 2000 IU; thiamine, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium pantothenate, 100 mg; choline chloride, 1000 mg; betaine, 500 mg. Minerals (g or mg kg^{-1} diet): cobalt carbonate, 0.65 mg; copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate, 7.5 mg; sodium chloride, 400 mg; calcium carbonate, 1.86 g; excipient wheat middling's, Premix Lda, Portugal. [19] ROVIMIX Stay C35, DSM Nutritional Product, Switzerland. [20] ROVIMIX E50, DSM Nutritional Product, Switzerland. [21] 41.2% CP, 8.2% CF, Premix Lda, Portugal. [22] 6.8% CP, 4.1% CF, Sopropêche, France. [23] VERDILOX, Kemin Europe NV, Belgium. [24] 26% P, Phosphea, Serbia. [25] 99% Lys, Ajinomoto EUROLYSINE S.A.S., France. [26] 98% Trp, Ajinomoto EUROLYSINE S.A.S., France. [27] Rhodimet NP99: 99% Met, ADISSEO, France. [28] 98% Tau, ORFFA, The Netherlands.

Table 2. Fatty acid and biochemical composition of the experimental diets (STD and ALT).

	Diets	
	STD	**ALT**
Fatty acid (% of total fatty acids)		
14:0	1.8	4.2
16:0	18.6	19.5
18:0	5.0	4.9
Other SFA	1.4	3.0
Total SFA	26.8	31.6
16:1n-7	4.4	4.3
18:1n-9	29.6	13.5
20:1n-9	1.6	3.0
22:1n-9	0.2	0.5
Other MUFAs	4.5	6.6
Total MUFAs	40.3	27.8
18:2n-6	19.1	6.7
18:3n-3	2.4	0.9
20:4n-6	0.9	1.3
20:5n-3 (EPA)	2.1	6.9
22:6n-3 (DHA)	3.7	16.2
Other PUFAs	3.0	5.7
Total PUFAs	31.3	37.7
Total n-3 PUFAs	10.2	27.8
Total n-6 PUFAs	20.8	9.4
EPA + DHA	5.9	23.1
Ratio n-3/n-6	0.5	3.0
n-3 HUFA (EPA + DHA) (% DM)	1.1	3.6
n-3 LC-PUFA (% DM)	1.2	3.8

Table 2. *Cont.*

	Diets	
	STD	**ALT**
Biochemical composition (% DM)		
Dry matter (DM), %	91.3	92.4
Crude protein	44.1	50.3
Crude fat	18.2	15.6
Ash	9.3	9.3
Gross Energy (kJ g^{-1} DM)	21.4	19.5
Total Phosphorous	1.5	0.9

MUFAs—Monounsaturated fatty acids; PUFAs—Polyunsaturated fatty acids; SFAs—Saturated fatty acids; EPA—Eicosapentaenoic acid; DHA—Docosahexaenoic acid; n-3 HUFA (EPA + DHA) (% DM): [(Crude fat, % DM) × (EPA + DHA, %)]/100; n-3 LC-PUFA (% DM): [(Crude fat, % DM) × (20:3n-3 + 20:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3, %)]/100.

2.2. Fish Trial

The animal trial was carried out at the Aquaculture Research Station of the Portuguese Institute of the Sea and Atmosphere (Olhão, Portugal), which is certified by the Direção Geral da Alimentação e Veterinária (DGAV) for animal experimentation under the authorization number 0421/2018. The experiment was conducted by trained scientists adhering to FELASA category C recommendations and followed the European Directive 2010/63/UE on the protection of animals used for scientific purposes. Furthermore, the study followed the World Organization for Animal Health (WOAH) guidelines on animal welfare, specifically concerning farmed fish intended for human consumption.

Four groups of 2700 meagre with 174.9 ± 32.8 g mean body weight were distributed among four sized rectangular earthen ponds, each measuring 500 m^2 with an average depth of 1.5 m. Each pond also housed 500 white seabream (*Diplodus sargus*) with a mean body weight of 236.5 ± 19.1 g, 320 flathead mullet (*Mugil cephalus*) averaging 255.5 ± 94.6 g, and 12,000 oysters (*Crassostrea gigas*) with a mean body weight of 3.85 ± 0.60 g. Additionally, an unknown number of organic species, including phytoplankton and predominantly *Ulva* spp. macroalgae, developed naturally in the ponds. The ponds were operated with continuous water renewal and managed according to the protocols outlined in [41]. Fish were fed three times daily using automatic feeders (NR-A602 model, SINO-AQUA, Taiwan) for 150 days. Feed intake and mortality were recorded daily. To assess fish biomass and growth rates, 50 meagre fish were sampled from each pond at the start (mid-May) and at the end (mid-October) of the trial. For sampling, fish were collected using a seine net and anesthetized with 2-phenoxyethanol (100 mg L^{-1}). All sampled individuals were measured, weighed, and returned to their respective ponds. The average water temperature, dissolved oxygen, and salinity were recorded at 25.8 ± 2.2 °C, 92.1 ± 28.4% saturation and 36.1 ± 2.0 psu, respectively. These parameters were monitored twice daily near the water outlet using an automatic probe system (SINERGIA, Catania, Italy). Levels of ammonia ($NH_3{}^+NH_4{}^+$), nitrites ($NO_2{}^-$), nitrates ($NO_3{}^-$), total phosphates (P-Total/$PO_4{}^{3-}$), and chlorophyll *a* were checked biweekly. Nutrient concentrations in the pond water were analyzed by MARINNOVA (Porto, Portugal) and chlorophyll *a* levels were determined following the method described in [42]. Fish health was regularly monitored to assess the general condition of the group. This action involved weekly rod fishing of individuals to examine ectoparasite levels in the branchial arches using a stereo microscope (UB200i SERIES, Proiser, Spain).

2.3. Sample Collection

Eight meagre fish from the initial stock and from each replicate pond were harvested in an ice water bath at the end of the trial and then stored at −20 °C for whole-body biochemical composition analysis and texture assessments. This method was selected to align with the assessment of fish quality for human consumption, as it remains the most widely practiced method in European slaughterhouses for farmed meagre (*Argyrosomus*

regius) [43] and in fisheries. Fish texture was determined in duplicates in eight fish before analyzing the whole-body biochemical composition. Additionally, five fish per pond were also harvested and samples of perivisceral fat, liver, and white muscle were collected, flash frozen in liquid nitrogen, and stored at −80 °C until further analysis. All dissection procedures were performed on ice to preserve tissue integrity. For histological examinations, a portion of the liver from each fish was fixed with 10% buffered formaldehyde at pH 7.2 for 48 h and subsequently transferred to 70% (m/w) ethanol. Moreover, for muscle cellularity analysis, a cross-section approximately 1 cm thick at the first ray of the dorsal fin from 4 fish per replicate was similarly fixed in 10% buffered formaldehyde at pH 7.2 for 96 h.

2.4. Biochemical Composition Analysis

The analysis of biochemical composition of diets and whole fish was conducted using analytical duplicates and following the methods described in [44]. Dry matter was determined by drying at 105 °C for 24 h; total ash by combustion at 550 °C for 12 h in a muffle furnace (Nabertherm L9/11/B170, Bremen, Germany); crude protein, calculated as nitrogen content (N × 6.25) by a flash combustion technique followed by gas chromatographic separation and thermal conductivity detection using a Leco N Analyzer (Model FP-528, Leco Corporation, St. Joseph, MI, USA); crude lipids, extracted with petroleum ether (40–60 °C) using a SoxtecTM 2055 Fat Extraction System (Foss, Copenhagen, Denmark), with prior acid hydrolysis using 8.3 M HCl; gross energy in an adiabatic bomb calorimeter (Werke C2000, IKA, Königswinter, Germany); and total phosphorous according to the ISO/DIS 6491 method.

2.5. Instrumental Texture

Scaled fillets with skin were selected for analysis because the skin helps to preserve the muscle structure of the fish fillets. The thickest dorsal region was chosen for this purpose. A section of 6 cm × 5 cm (height mean values of 1.0 ± 0.1 and 1.3 ± 0.2 cm for STD and ALT treatments, respectively) was sliced in the front region of the fillet, just behind the first dorsal fin ray. This section was used to measure hardness in its raw state. The analysis was performed using a TA.XTPlus Texture Analyzer (Stable Micro Systems, Surrey, UK), using the double compression test method, also known as Texture Profile Analysis (TPA) [45,46]. The equipment was fitted with a 30 kg load cell and a spherical compression probe (P/0.5S) with a diameter of 12.5 mm. The testing speed was adjusted to 1 mm s^{-1} and the fillets were compressed to 40% of their initial height. Upon completion of the test, parameters such as hardness, cohesiveness, adhesiveness, and chewiness were quantified.

2.6. Muscle Cellularity

Muscle cellularity, which quantifies the area and density of muscle fibers, was analyzed by histology. In this procedure, muscle sections were subjected to a series of dehydration steps and then embedded in paraffin wax. Four 10 µm slices were cut from the paraffin blocks, stained with hematoxylin and eosin [47] and subsequently photographed. Images were captured and analyzed with image analysis software (AxioVision Release 4.8.2. SP2), which was connected to a video camera (AxioCamER5s) and a light microscope (Zeiss-Axioplan). Muscle cellularity was then estimated in four random sections of the epaxial region using the image processing and analysis program Image J v1.49.

2.7. Liver Histology

Fixed liver samples were processed using a standard histologic technique as described by [48]. Sections of 7 mm were prepared using a Leica RM-2155 microtome (Leica, Vienna, Austria) and stained with hematoxylin–eosin before being examined under a light microscope. To capture images, entire slides were scanned using a NanoZoomer-SQ Digital slide scanner (Hamamatsu photonics, Hamamatsu city, Japan) and observations were made at a magnification of ×40. The integrity of the entire tissue in liver sections was assessed using

the NPDview software (Hamamatsu, Japan). The hepatocyte area was analyzed to estimate energy reserves, following the methodology outlined in [49].

2.8. Fatty Acid Profile

The fatty acid methyl esters (FAMEs) in the liver, muscle, and perivisceral fat of fish were determined using the method described in [50]. The results are expressed as a percentage of their relative content.

2.9. Hepatic Protein Oxidation

Advanced oxidation protein products (AOPPs) were determined using the protocol described by [30]. Briefly, liver samples were homogenized in 10 mL g^{-1} cold phosphate saline buffer (PBS) solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) using an Ultra-Turrax homogenizer (IKA, Germany). The homogenates were subsequently centrifuged at 5000× *g* for 10 min at 4 °C. The supernatant was diluted to achieve a concentration of 50 µg of total protein per well in PBS. A volume of 100 µL was dispensed into each well of a 96-well plate, accompanied by chloramine-T (23270; Sigma-Aldrich, St. Louis, MO, USA) standard solutions ranging from 0 to 100 µM. After adding 10 µL of 1.16 M potassium iodide (PO04100250; Scharlau, Spain) and waiting for 5 min, 20 µL of glacial acetic acid was added to all wells. The formation of AOPPs was immediately measured spectrophotometrically at 340 nm. AOPPs concentration was expressed as µM of chloramine–T per µg of total protein. Total protein concentration was measured using the Bradford protein assay (5000006; Bio-Rad, Hercules, CA, USA) [51].

2.10. Proteolytic Markers

The activities of proteasome and cathepsins B and L were measured following the protocol outlined in [27]. Initially, soluble total proteins were extracted from liver and muscle samples (n = 5 per tank) using 4 and 6-mL g^{-1} of cold lysis buffer, respectively. Subsequently, 50 µg of total protein per well was used to determine the activity of cathepsins B and L, which were expressed in relative fluorescence units (RFUs) per microgram of total protein in the sample. Proteasome activity was measured using 50 µg (for liver) and 75 µg (for muscle) of total protein per well, following the protocol described in [49]. The activity of the proteasome was expressed in milliunits per milligram of total protein in the sample.

2.11. Statistical Analysis

All parameters were expressed as the mean of replicates ± standard deviation (SD), with each fish treated as an individual unit for each treatment. Exceptions to this were protease activity and water nutrients, which were expressed as the mean of replicates ± standard error of the mean (SEM). Data were analyzed using Student's *t*-test. The Kolmogorov–Smirnov test was employed to check normality, while Levene's test was used to evaluate variance homogeneity. When the data did not meet the normality assumption, the Mann–Whitney U test was applied. Statistical significance was tested at a 0.05 probability level. All statistical analyses were performed using IBM SPSS Statistics v21 software.

3. Results

3.1. Survival, Growth Performance, Hepatosomatic Index, and Hepatic Oxidized Proteins of Meagre in a Polyculture System Within Earthen Ponds

Both diets were well accepted by the fish. At the end of the trial, the total fish biomass (BS) showed no significant differences between treatments, with the highest values observed in the ALT treatment. Fish densities (D) ranged from 2.08 ± 0.31 to 2.41 ± 0.15 kg m^{-3} (Table 3). Feed conversion ratio (FCR) values were also not significantly different between treatments but tended to be lower in ponds where fish were fed the ALT diet.

Table 3. Growth performance, survival, hepatosomatic index, and hepatic oxidized protein levels in meagre (*Argyrosomus regius*) juveniles fed STD and ALT diets in the earthen pond polyculture system.

	Diets	
	STD	**ALT**
Earthen pond polyculture system		
Biomass (BS) [1]	1562.1 ± 229.6	1805.2 ± 115.6
Density (D) [2]	2.08 ± 0.31	2.41 ± 0.15
Feed Conversion Rate (FCR) [3]	2.37 ± 0.56	2.21 ± 0.02
Argyrosomus regius		
Survival Rate (S) [4]	99.1 ± 0.9	99.8 ± 0.2
Biomass (BS) [1]	1265.2 ± 225.6	1496.0 ± 114.7
Final Body Weight (FBW) [5]	415.8 ± 152.5 [a]	586.3 ± 136.3 [b]
Condition Factor (CF) [6]	1.10 ± 0.10 [a]	1.14 ± 0.05 [b]
Specific Growth Rate (SGR) [7]	0.65 ± 0.12	0.77 ± 0.05
Daily Growth Index (DGI) [8]	2.01 ± 0.43	2.43 ± 0.20
Hepatosomatic Index (HSI) [9]	2.66 ± 0.67 [a]	1.47 ± 0.32 [b]
Hepatic oxidized proteins (AOPPs) [10]	0.64 ± 0.21 [a]	0.45 ± 0.13 [b]

Values are presented as means ± SD of n = 100 for FBW and CF; n = 2 for S, SGR, DGI, BS, D, and FCR; n = 10 for HSI and n = 10 for AOPPs. Means in the same row with different letters indicate significant differences (t-test, $p < 0.05$ for FBW, CF, HSI, and AOPPs; Mann–Whitney, $p < 0.05$ for the other parameters). [1] BS (kg): average fish weight (n = 50) × number of fish in the pond; BSi: Initial weight; BSf: Final weight. [2] D (kg m^{-3}): (BSfA + BfD + BSfM) (kg)/Pond volume (m^3). [3] FCR: total provided dry feed (kg)/[(BSfA + BSfD + BSfM) − (BSiA + BSiD + BSiM)] for each fish species (kg). [4] S (%): (number of fish alive at the end (g)/number of fish stocked initially) × 100. [5] FBW (g): BSfA (g)/final fish number. [6] CF: (wet weight/total lenght^3)/100. [7] SGR (% day^{-1}): [ln (BSf/BSi)/T] × 100. [8] DGI (% day^{-1}): 100 × [$(BSf)^{1/3}$ − $(BSi)^{1/3}$]/T. [9] HSI (%): (wet weight of liver/FBW) × 100. [10] AOPPs (μM chloramine-T per μg of total protein). *A: Argyrosomus regius*; *D: Diplodus sargus*; *M: Mugil cephalus*; T: time duration in days.

Throughout the experimental period, meagre fed the ALT diet exhibited increased agility in capturing feed pellets and displayed prolonged periods of surface lingering in anticipation of feeding. The levels of ectoparasites in the branchial arches were similar across all treatments and within the expected normal range. The overall growth performance, hepatosomatic index, and levels of hepatic oxidized proteins for meagre fed the two diets are detailed in Table 3. Meagre growth, expressed as final body weight (FBW) and condition factor (CF), showed higher values in fish fed the ALT diet (p = 0.000). The specific growth rate (SGR) and the daily growth index (DGI) followed the same trend; however, without significant differences (p = 0.667). Conversely, the hepatosomatic index (HSI) and level of hepatic oxidized proteins (AOPPs) were lower in fish fed this diet ($p < 0.0001$ and p = 0.0281, respectively).

3.2. Experimental Diets and Fish Whole-Body Biochemical Composition

At the end of the trial, significant differences in whole-body biochemical composition were observed between the diets (Table 4). Fish fed the ALT diet had higher protein content (p = 0.000), while their dry matter, lipid, ash, energy, and phosphorus contents were lower (p = 0.001; p = 0.000; p = 0.001; p = 0.026; p = 0.033, respectively).

Table 4. Biochemical composition of meagre (*Argyrosomus regius*) fed with the experimental diets (STD and ALT).

		Diets	
Body Composition (%)	**Initial**	**STD**	**ALT**
Dry matter	28.7 ± 0.9	30.4 ± 1.5 [a]	28.7 ± 1.0 [b]
Protein	53.8 ± 3.4	58.0 ± 3.6 [a]	63.9 ± 1.9 [b]
Lipid	27.8 ± 2.2	28.1 ± 3.7 [a]	23.6 ± 2.2 [b]
Ash	15.4 ± 2.9	14.1 ± 2.0 [a]	11.9 ± 0.9 [b]
Energy (kJ g^{-1})	22.5 ± 0.6	22.8 ± 0.8 [a]	22.1 ± 0.8 [b]
Phosphorous	1.96 ± 0.21	2.04 ± 0.22 [a]	1.89 ± 0.16 [b]

Values are means ± SD of 8 individuals from the initial group and 16 from each experimental diet. Means in the same row with different letters indicate significant differences (t-test, $p < 0.05$).

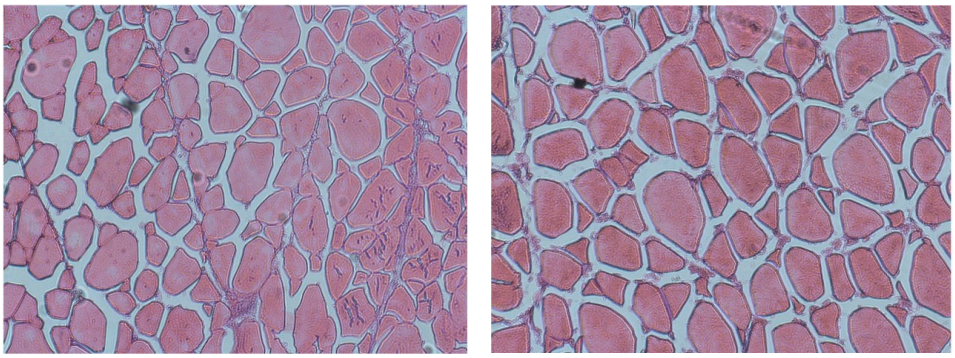
3.3. Flesh Texture and Muscle Cellularity

The analysis of meagre flesh texture revealed that flesh hardness, adhesiveness, and chewiness were similar between the two treatments. However, a significant difference in cohesiveness was observed between the STD and ALT treatments ($p = 0.007$), with the former exhibiting lower values (Table 5). No significant differences were found between the treatments in terms of mean muscle fiber area or density (Figure 1 and Table 5).

Table 5. Flesh texture (muscle fillet) and muscle cellularity (dorsal muscle section) of meagre (*Argyrosomus regius*) juveniles fed the experimental diets (STD and ALT).

	Diets	
	STD	**ALT**
Texture		
Hardness (N)	4.60 ± 1.05	4.90 ± 1.01
Adhesiveness	−0.04 ± 0.01	−0.04 ± 0.01
Cohesiveness	0.55 ± 0.04 [a]	0.51 ± 0.04 [b]
Chewiness (N cm^{-1})	1.73 ± 0.35	1.68 ± 0.25
Muscle cellularity		
Mean Area (mm^2)	0.002556 ± 0.000548	0.003299 ± 0.001219
Fiber density (nr. fibers per mm^2)	267.7 ± 49.1	214.5 ± 52.2

Values presented as mean ± SD ($n = 8$). Means in the same row with different letters indicate significant differences (t-test, $p < 0.05$).



(**a**) (**b**)

Figure 1. Transverse sections of dorsal muscle fibers in meagre (*Argyrosomus regius*) juveniles fed STD (**a**) and ALT (**b**) diets (×100).

3.4. Fatty Acid Composition

3.4.1. Experimental Diets STD and ALT

Palmitic (16:0) and oleic (18:1n-9) acids were the most predominant fatty acids in the experimental diets (Table 2). The STD diet was particularly rich in linoleic acid (18:2n-6), while the ALT diet had higher levels of DHA (22:6n-3). All other fatty acids constituted less than 10% of the total fatty acids in both diets. In the ALT diet, saturated fatty acids (SFAs) accounted for 31.6%, making them the largest group, followed by polyunsaturated fatty acids (PUFAs) at 37.7%. In contrast, the STD diet was highest in monounsaturated fatty acids (MUFAs), comprising 40.3% of its fatty acid profile. In the ALT diet, total n-3 PUFAs made up 27.8% of the total fatty acids, while n-6 PUFAs represented only 9.4%. Conversely, in the STD diet, total n-3 PUFAs accounted for 10.2% and n-6 PUFAs for 20.8%. Additionally, the combined amount of EPA and DHA was substantially higher in the ALT diet (23.1%) compared to the STD diet (5.9%).

3.4.2. Liver, White Muscle, and Perivisceral Fat of Fish

While the proportion of total SFAs remained unchanged in the liver of meagre juveniles fed the experimental diets, significant differences were observed in the levels of MUFAs

and PUFAs (Table 6). The livers of fish fed the STD diet exhibited higher levels of total MUFAs compared to those fed the ALT diet ($p < 0.0001$), while the level of total PUFAs was greater in the livers of fish fed the ALT diet compared to those fed the STD diet ($p < 0.0001$). Specifically, total n-3 PUFAs accounted for 23.9 ± 4.4% of the total fatty acids in the liver of fish fed the ALT diet, significantly higher than that in the liver of fish fed the STD diet ($p < 0.0001$). Conversely, total n-6 PUFAs made up 17.0 ± 1.9% of the total fatty acids in the liver of fish fed the STD diet, significantly exceeding the levels found in the ALT diet group ($p < 0.0001$). Additionally, the combined levels of EPA and DHA were higher in the liver of fish fed the ALT diet compared to those fed the STD diet ($p < 0.0001$).

Table 6. Liver fatty acid profile (% of total fatty acids) of meagre (*Argyrosomus regius*) fed the STD and ALT diets.

	Diets	
	STD	**ALT**
14:0	1.17 ± 0.14 [a]	2.52 ± 0.14 [b]
16:0	20.0 ± 1.5	19.4 ± 0.7
18:0	5.58 ± 0.81 [a]	4.50 ± 0.53 [b]
Other SFA	1.86 ± 0.23 [a]	2.72 ± 0.08 [b]
Total SFA	28.6 ± 2.2	30.1 ± 3.9
16:1n-7	5.65 ± 0.74	5.27 ± 0.29
18:1n-9	32.2 ± 2.0 [a]	17.1 ± 1.5 [b]
20:1n-9	1.91 ± 0.16 [a]	2.62 ± 0.37 [b]
22:1n-9	0.35 ± 0.12 [a]	0.44 ± 0.09 [b]
Other MUFAs	4.11 ± 0.69 [a]	5.45 ± 0.33 [b]
Total MUFAs	44.2 ± 2.1 [a]	30.9 ± 2.0 [b]
18:2n-6	15.1 ± 1.8 [a]	7.33 ± 0.61 [b]
18:3n-3	1.16 ± 0.11 [a]	1.03 ± 0.28 [b]
20:4n-6	0.83 ± 0.19 [a]	1.39 ± 0.21 [b]
20:5n-3 (EPA)	1.39 ± 0.35 [a]	5.11 ± 0.21 [b]
22:6n-3 (DHA)	4.27 ± 0.88 [a]	14.1 ± 4.1 [b]
Other PUFAs	3.02 ± 011 [a]	3.83 ± 0.36 [b]
Total PUFAs	26.5 ± 2.8 [a]	34.3 ± 4.7 [b]
Total n-3 PUFAs	8.34 ± 1.16 [a]	23.9 ± 4.4 [b]
Total n-6 PUFAs	17.0 ± 1.9 [a]	10.3 ± 0.8 [b]
EPA + DHA	5.66 ± 1.09 [a]	19.3 ± 4.1 [b]
Ratio n-3/n-6	0.48 ± 0.08 [a]	2.34 ± 0.46 [b]

MUFAs—Monounsaturated fatty acids; PUFAs—Polyunsaturated fatty acids; SFAs—Saturated fatty acids; EPA—Eicosapentaenoic acid; DHA—Docosahexaenoic acid. Values are means ± SD of 16 individuals from each experimental treatment. Means in the same row with different letters indicate significant differences (*t*-test, $p < 0.05$).

The proportion of SFAs, MUFAs, and PUFAs in the white muscle of meagre juveniles fed the alternative diet (ALT) showed significant differences compared to those fed the standard diets (Table 7). Total SFA and PUFA levels were higher in the muscle of fish fed the ALT diet ($p < 0.0001$), while the level of total MUFAs was higher in the muscle of fish fed the STD diet ($p = 0.0003$). Total n-3 PUFAs were significantly greater in the muscle of fish fed the ALT diet compared to those fed the STD diet ($p < 0.0001$). Conversely, total n-6 PUFAs were higher in the muscle of fish fed the STD diet than in those on the ALT diet ($p < 0.0001$). Furthermore, the overall levels of EPA and DHA in the muscle of fish on the ALT diet were higher than those found in the liver of fish fed the STD diet ($p < 0.0001$).

Similarly, the proportions of SFAs, MUFAs, and PUFAs significantly differed in the perivisceral fat of meagre juveniles fed the experimental diets (Table 8). Total SFAs and PUFAs were higher in the fat of fish fed the ALT diet compared to those fed the STD diet ($p = 0.0025$ for SFAs; $p < 0.0001$ for PUFAs), while total MUFAs were higher in the group of meagre juveniles fed the STD diet ($p < 0.0001$). The total amounts of n-3 PUFAs, n-6 PUFAs, and EPA and DHA in the perivisceral fat mirrored the patterns observed in the white muscle of fish fed the experimental diets ($p < 0.0001$).

Table 7. White muscle fatty acid profile (% of total fatty acids) of meagre (*Argyrosomus regius*) fed the STD and ALT diets.

	Diets	
	STD	**ALT**
14:0	1.31 ± 0.45	1.39 ± 0.28
16:0	18.0 ± 0.6 [a]	18.5 ± 0.4 [b]
18:0	6.54 ± 1.13 [a]	7.39 ± 0.37 [b]
Other SFAs	1.30 ± 0.30 [a]	2.11 ± 0.20 [b]
Total SFAs	27.2 ± 1.0 [a]	29.4 ± 0.4 [b]
16:1n-7	2.39 ± 0.47	2.35 ± 0.35
18:1n-9	21.8 ± 2.0 [a]	13.1 ± 0.9 [b]
20:1n-9	1.21 ± 0.20 [a]	1.83 ± 0.17 [b]
22:1n-9	0.10 ± 0.16	0.08 ± 0.13
Other MUFAs	3.79 ± 0.48	4.08 ± 0.41
Total MUFAs	27.9 ± 6.1 [a]	21.4 ± 1.7 [b]
18:2n-6	17.2 ± 3.1 [a]	7.11 ± 0.70 [b]
18:3n-3	1.08 ± 0.17 [a]	0.73 ± 0.07 [b]
20:4n-6	2.51 ± 0.50	2.59 ± 0.22
20:5n-3 (EPA)	2.79 ± 0.88 [a]	5.44 ± 0.48 [b]
22:6n-3 (DHA)	14.4 ± 4.1 [a]	26.4 ± 1.9 [b]
Other PUFAs	5.11 ± 068 [a]	6.39 ± 0.15 [b]
Total PUFAs	43.1 ± 3.5 [a]	48.6 ± 1.9 [b]
Total n-3 PUFAs	21.4 ± 5.0 [a]	35.9 ± 1.8 [b]
Total n-6 PUFAs	21.4 ± 2.9 [a]	11.9 ± 0.9 [b]
EPA + DHA	17.2 ± 4.8 [a]	31.8 ± 1.8 [b]
Ratio n-3/n-6	1.07 ± 0.60 [a]	3.03 ± 0.26 [b]

MUFAs—Monounsaturated fatty acids; PUFAs—Polyunsaturated fatty acids; SFAs—Saturated fatty acids; EPA—Eicosapentaenoic acid; DHA—Docosahexaenoic acid. Values are means ± SD of 16 individuals from each experimental treatment. Means in the same row with different letters indicate significant differences (*t*-test, $p < 0.05$).

Table 8. Perivisceral fat fatty acid profile (% of total fatty acids) of meagre (*Argyrosomus regius*) fed the STD and ALT diets.

	Diets	
	STD	**ALT**
14:0	1.71 ± 0.09 [a]	3.64 ± 0.36 [b]
16:0	18.4 ± 0.7	18.0 ± 0.9
18:0	5.58 ± 1.22 [a]	4.53 ± 0.16 [b]
Other SFAs	1.49 ± 0.18 [a]	2.54 ± 0.29 [b]
Total SFAs	27.2 ± 1.0 [a]	28.7 ± 1.6 [b]
16:1n-7	4.57 ± 0.37 [a]	5.16 ± 0.41 [b]
18:1n-9	31.7 ± 1.3 [a]	18.4 ± 1.2 [b]
20:1n-9	1.84 ± 0.13 [a]	3.23 ± 0.40 [b]
22:1n-9	0.35 ± 0.03 [a]	0.54 ± 0.06 [b]
Other MUFAs	4.37 ± 0.96 [a]	6.33 ± 0.42 [b]
Total MUFAs	42.8 ± 1.0 [a]	33.6 ± 0.9 [b]
18:2n-6	18.9 ± 2.0 [a]	10.1 ± 3.8 [b]
18:3n-3	1.81 ± 0.13 [a]	1.52 ± 0.26 [b]
20:4n-6	0.61 ± 0.03 [a]	1.05 ± 0.10 [b]
20:5n-3 (EPA)	1.70 ± 0.16 [a]	5.63 ± 0.62 [b]
22:6n-3 (DHA)	3.21 ± 0.22 [a]	10.9 ± 1.1 [b]
Other PUFAs	3.01 ± 0.04 [a]	4.04 ± 0.17 [b]
Total PUFAs	29.2 ± 1.8 [a]	33.3 ± 2.4 [b]
Total n-3 PUFAs	8.41 ± 0.41 [a]	21.9 ± 1.8 [b]
Total n-6 PUFAs	20.3 ± 2.0 [a]	12.5 ± 3.7 [b]
EPA + DHA	4.89 ± 0.36 [a]	16.6 ± 1.7 [b]
Ratio n-3/n-6	0.43 ± 0.06 [a]	1.89 ± 0.49 [b]

MUFAs—Monounsaturated fatty acids; PUFAs—Polyunsaturated fatty acids; SFAs—Saturated fatty acids; EPA—Eicosapentaenoic acid; DHA—Docosahexaenoic acid. Values are means ± SD of 16 individuals from each experimental treatment. Means in the same row with different letters indicate significant differences (*t*-test, $p < 0.05$).

3.5. Histological Examination of the Liver

Meagre fed different dietary treatments exhibited alterations in the normal histological structure of their liver (Figure 2). Histological analysis revealed hepatocytes with a polygonal shape, arranged along sinusoids, displaying varying cytoplasmic volumes and nuclei displaced toward the periphery. Enlarged cells with pale, unstained cytoplasmic regions were indicative of cytoplasmic vacuolization. Despite these observations, no significant differences in hepatocyte energy reserves were observed between groups, as indicated by comparable cytoplasmic vacuolization areas in fish fed the two diets (STD—23.4 ± 6.6%; ALT—29.2 ± 5.8%, $p = 0.050$).

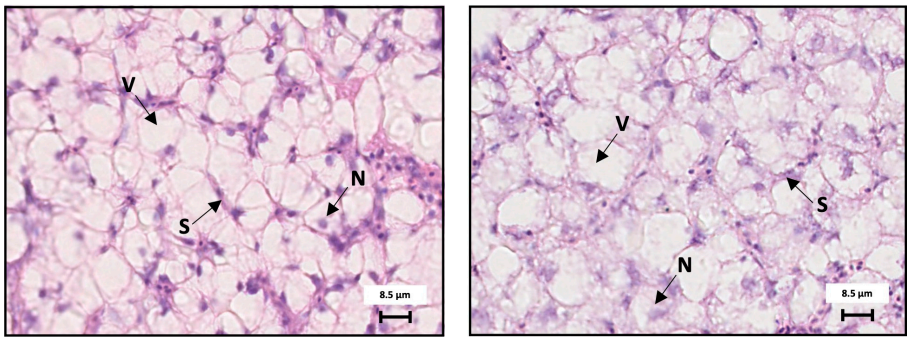


Figure 2. Representative light microphotographs of the liver from meagre (*Argyrosomus regius*) juveniles fed experimental diets: STD (**a**) and ALT (**b**). Each section highlights hepatocyte features, including sinusoids (S), vacuolization (V), and laterally displaced nuclei (N). Scale bar: 8.5 µm; magnification ×40; hematoxylin–eosin staining.

3.6. Proteolytic Activity Markers

The protease activity for the two main protein degradation systems (autophagy–lysosomal and ubiquitin–proteasome) exhibited significant differences between the two dietary treatments in the liver and muscle of meagre (Table 9). Specifically, the activity of cathepsins B and L was significantly lower in both the liver ($p = 0.039$ for cathepsins B; $p = 0.003$ for cathepsin L) and muscle ($p = 0.002$ for cathepsin B; $p = 0.000$ for cathepsin L) of fish fed the ALT diet. A similar pattern was observed for proteasome activity, which was also lower in both the liver ($p = 0.004$) and muscle ($p = 0.004$) of fish fed the ALT diet.

Table 9. Proteolytic markers in the liver and white muscle of meagre (*Argyrosomus regius*) juveniles fed experimental diets (STD and ALT): lysosomal activity (RFU μg^{-1} total protein) was evaluated through the measuring of cathepsins B and L activities, and proteasome activity (mU mg^{-1} total protein) was evaluated through the measuring of the chymotryptic activity of the β5 of the 20S proteasome.

	Diets	
	STD	**ALT**
Cathepsin B		
Liver	488.9 ± 74.1 [a]	306.3 ± 24.5 [b]
White muscle	33.4 ± 3.8 [a]	17.4 ± 0.6 [b]
Cathepsin L		
Liver	770.5 ± 74.2 [a]	451.6 ± 57.4 [b]
White muscle	81.6 ± 7.6 [a]	41.0 ± 4.2 [b]
Proteasome		
Liver	15.1 ± 2.3 [a]	6.41 ± 0.70 [b]
White muscle	12.2 ± 2.1 [a]	4.25 ± 0.54 [b]

Values presented as mean ± SEM ($n = 10$). Means in the same row with different letters indicate significant differences (t-test, $p < 0.05$).

3.7. Pond Water Quality

The levels of ammonia, nitrites, nitrates, and phosphates in the rearing water of the earthen ponds showed no significant variations throughout the entire experimental period (Figure 3). However, an increased concentration of nitrites was observed in the water of fish fed the ALT diet during the month of August ($p = 0.009$). Additionally, the concentration of chlorophyll *a* in the water of fish fed the STD diet was higher during the last three months of the trial compared to the water of fish fed the ALT diet (July, $p = 0.001$; August, $p = 0.001$; September, $p = 0.023$).

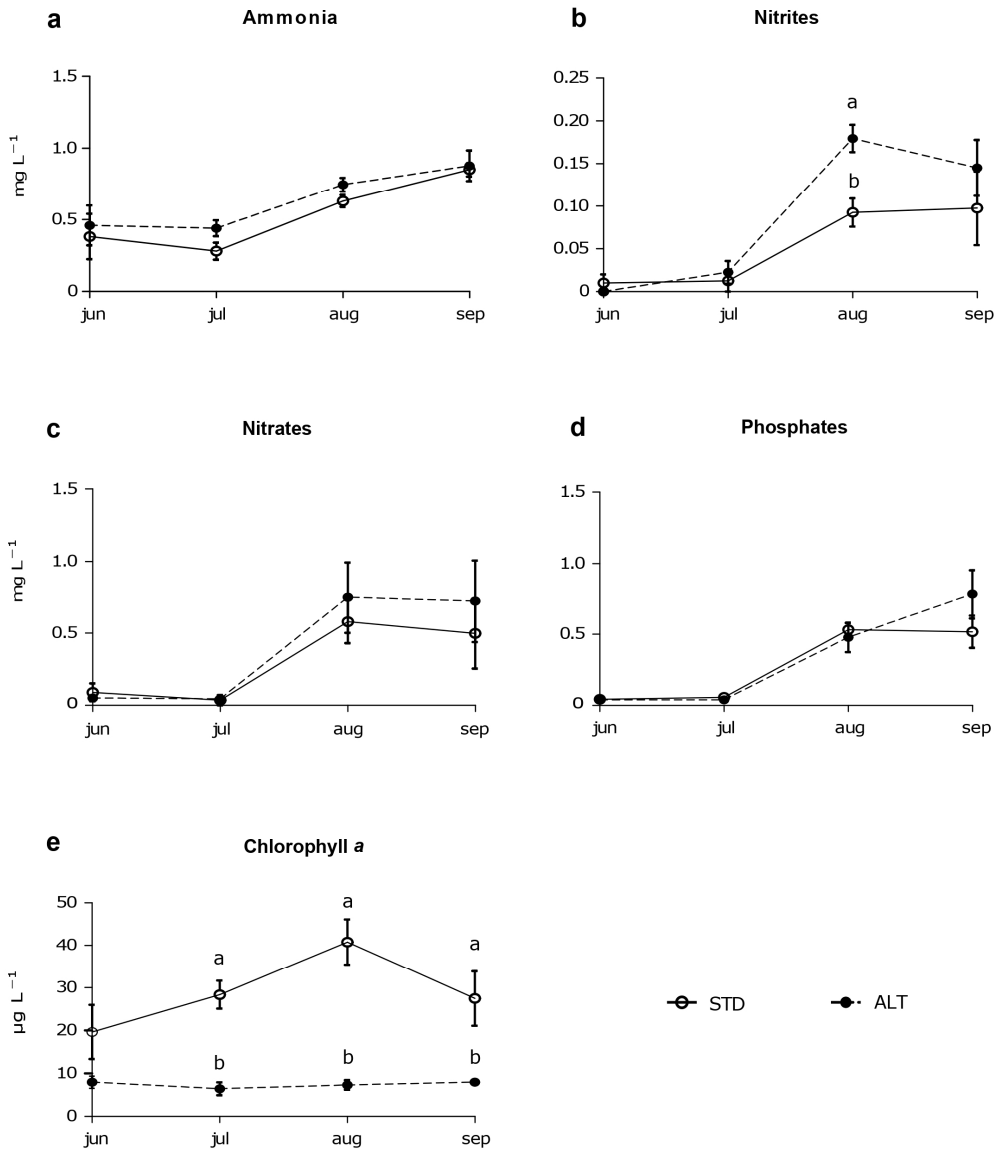


Figure 3. Dissolved nutrients and chlorophyll *a* pattern in the earthen pond water of the two dietary treatments (STD and ALT) during the experimental period (June to September): (**a**) ammonium; (**b**) nitrites; (**c**) nitrates; (**d**) phosphates; and (**e**) chlorophyll *a*. Values are means ± SEM ($n = 4$). Different letters indicate significant differences among treatments (*t*-test, $p < 0.05$).

4. Discussion

The sustainable growth of aquaculture requires the development of highly nutritive and functional novel ingredients to efficiently replace fishmeal and fish oil in aquafeeds. These novel materials, such as plant feedstuffs, microbial, marine, and terrestrial animal ingredients or by-products have significant nutritional limitations compared to fishmeal or

fish oil [8]. Therefore, it is essential to investigate not only the impact of these novel ingredients on overall fish performance and product quality, but also their effect on environmental nutrient discharges, to identify a viable dietary solution for sustainable fish production. Given that earthen ponds are complex, semi-natural systems with environmental factors that are challenging to control or replicate, achieving full triplication is often impractical. Therefore, two replicates were used to balance experimental reliability with the realistic conditions that characterize earthen pond aquaculture. To ensure robust data from these two replicates, we increased sample intensity within each replicate wherever possible, thereby enhancing the reliability of our findings. This approach aligns with established methodologies for providing valid insights within the practical constraints of pond-based research [42,52].

4.1. Evaluating the Overall Production Efficiency in the Earthen Ponds Systems

Polyculture systems in aquaculture provide several advantages, including optimized resource utilization, improved sustainability, and greater resilience of production systems. The fish density achieved at the end of the trial fell within the production levels typically observed in semi-intensive monoculture systems in earthen ponds, ranging from 0.5 to 6 kg m^{-3} [53]. The feed conversion ratio (FCR), a key indicator of production efficiency in aquaculture, showed similar values across the two dietary treatments evaluated in this study. Although the FCR exceeded 2, it remained within the range typically reported for polyculture systems in earthen ponds, which spans from 1.59 to 2.46 [42]. The overall production efficiency is therefore considered adequate for a polyculture system in earthen ponds, as the fish density and FCR values align with typical benchmarks reported for similar systems.

4.2. Comparison of Fish Growth Performance Between STD and ALT Diets

Several studies on meagre (*Argyrosomus regius*) have shown that the inclusion of alternative ingredients in aquafeeds has significant potential to promote fish growth performance, although at controlled proportions [12–15]. For instance, macroalgae, which comprise 4.6% of the ALT diet, are known to enhance the flavor of farmed fish and act as a feeding stimulant. This can increase daily feed intake (DFI) and indirectly contribute to fish growth [54]. However, since other fish species were also reared in each experimental pond, it was not feasible to determine the DFI for meagre juveniles in this study. Indeed, the growth performance of meagre juveniles was not compromised by the alternative diet (ALT) containing sustainable ingredients; instead, it was enhanced. This improvement was evidenced by an increase in final body weight (FBW) and condition factor (CF) in meagre fed the ALT diet compared to those fed the standard diet (STD). These results can be directly attributed to the higher protein content in the ALT diet (50.3%), which is known to enhance meagre growth, compared to the 44.1% protein content in the STD diet [55]. The specific growth rate (SGR) and the daily growth index (DGI), calculated with two replicates per dietary treatment, also supported this positive effect of the ALT diet on fish growth. Protein content was higher in fish fed the ALT diet, while dry matter, lipid, ash, energy, and phosphorus contents were higher in fish fed the STD diet, mirroring the composition of the experimental diets, except for dry matter, which showed minimal variance. These results suggest an effective utilization of dietary components by the fish, with the increased protein content likely being the main factor in the weight gain observed in fish fed the ALT diet. The presence of phytate, an anti-nutritional factor in plant-derived ingredients, may have affected the bioavailability of phosphorous in fish fed the ALT diet, resulting in lower levels compared to those in fish fed the STD diet [56]. Although phosphorous is an essential nutrient for fish growth [57], this potential lower bioavailability did not impact the growth of fish fed the ALT diet. Interestingly, proteasome and lysosomal activity were higher in the liver and muscle of fish fed the STD diet, which corresponded to the highest levels of hepatic oxidized proteins. These data support the hypothesis that the ALT diet may be preventing protein damage in fish tissues, thereby reducing the activity of

protein degradation systems. There are few studies on the dietary modulation of protein degradation systems in fish. However, the autophagy–lysosomal system (ALS) in meagre juveniles has been shown to be modulated by increased levels of taurine in plant-based diets [27], demonstrating the responsiveness of meagre's proteolytic systems to changes in dietary ingredients. A negative correlation between proteasome activity and growth was described in the liver of rainbow trout (*Oncorhynchus mykiss*) [26], which aligns with our findings. This suggests that reduced proteasome activity in fish tissues may account for the increased growth observed in fish fed the ALT diet. All these favorable growth indicators demonstrate that the ALT diet is more effective at supporting the growth of meagre juveniles in earthen ponds than the STD diet. For detailed information about the other species involved in the polyculture system, refer to Table S2. While these species were integrated into the overall systems, their specific performance was not the primary focus of this study.

4.3. Effect of STD and ALT Diets on Fish Health

In our study, fish fed the STD diet exhibited higher hepatosomatic index (HSI) values compared to those fed the ALT diet. Previous research has shown that HSI decreases with increasing dietary EPA and DHA levels in meagre fingerlings [58]. Although these data pertain to an earlier developmental stage, they suggest a potential relationship between dietary EPA and DHA and HSI in this fish species. In fact, a study in juvenile meagre fed diets containing alternative ingredients with varying EPA and DHA levels showed no significant impact on HSI values, despite observing a similar relationship to that reported for meagre fingerlings [13]. The variations in HSI values observed in our study may partly be attributed to histomorphological alterations in liver structures, including differences in the degree of vacuolization. These changes, along with nuclei displaced toward the periphery, may indicate steatosis. However, these alterations did not significantly affect the hepatocyte vacuolization area, suggesting equivalent energy reserves. These findings may be linked to the levels of n-3 HUFA (EPA + DHA) in our experimental diets, which were 1.1% DM for the STD diet and 3.6% DM for the ALT diet. These levels slightly deviate from the range considered optimal for maintaining normal liver histomorphology in meagre fingerlings, reported to be between 2.6 and 3.0% DM n-3 HUFA [58]. Furthermore, it was reported that the dietary n-3 LC-PUFAs requirement for juvenile meagre to ensure healthy liver status lies between 0.7 and 0.8% DM [59]. In our experimental diets, the n-3 LC-PUFA levels exceeded this range with 1.2% DM for the STD diet and 3.8% DM for the ALT diet. These elevated levels may explain the observed liver steatosis in both dietary treatments, without however compromising fish overall health or viability.

The replacement of fish meal and oil by vegetable sources reduces dietary n-3 LC-PUFAs [15,60], which play important roles in fish stress response [61,62]. However, marine microalgae can mitigate this limitation due to their richness in LC-PUFAs and various pigments with antioxidant properties [63]. Indeed, fish fed the ALT diet showed lower levels of hepatic oxidized proteins (AOPPs—advanced oxidized protein products) compared to those fed the STD diet. This result suggests a higher capacity of fish fed the ALT diet to cope with oxidative stress. There is limited data on AOPPs and their role as markers of oxidative damage in marine species. Studies on temperature variations in gilthead seabream have reported that reducing dietary lipid content did not affect the level of oxidized proteins in the liver of fish exposed to warm temperatures (22 °C) [30,64]. In meagre juveniles, low n-3 LC-PUFA diets induced changes in basal levels of oxidative stress-related genes, potentially increasing oxidative stress damage [61]. Conversely, high n-3 LC-PUFA diets may enhance the basal antioxidant response in this fish species [61]. Therefore, the higher amount of n-3 LC-PUFAs, combined with the antioxidant factors derived from the dietary microalgae in the ALT diet, may enhance fish's antioxidant defenses, resulting in lower protein oxidation in the liver compared to fish fed the STD diet.

4.4. Fish Quality in Response to STD and ALT Diets

The differences observed in the whole proximate composition of fish had no effect on the overall texture of the muscle fillet, except for cohesiveness, which was lower in the muscle of fish fed the ALT diet. Indeed, the total substitution of fish oil with rapeseed oil significantly increased the flesh cohesiveness of grass carp; however, this effect has not been observed in other fish species fed similar diets [65]. This property indicates a lower strength of the internal bonds that form the fish fillet, but without any impact on other texture parameters and consequently fish quality. In fact, the two dietary treatments applied in this study did not affect fish muscle cellularity, a key factor influencing flesh texture. However, a previous study reported that plant-based diets affected muscle fiber recruitment in juvenile meagre [16]. Conversely, diets based on insect-derived ingredients appeared to have no effect on muscle cellularity in meagre juveniles [17]. These findings highlight the numerous factors that may influence muscle cellularity, such as fish size and species [66], as well as the amount and type of dietary ingredients.

Fish are the primary food source of n-3 polyunsaturated fatty acids for humans, providing multiple health benefits [67]. Sustainability strategies in the aquaculture industry may reduce the levels of these important fatty acids in fish, thus compromising their nutritional value to consumers [68]. Therefore, it is important to evaluate how the STD and ALT diets may affect the fatty acid composition of meagre. Generally, the fatty acid profiles of farmed fish reflect their diet [68–70]. Indeed, fish fed the ALT diet showed higher levels of total saturated (SFAs) and polyunsaturated (PUFAs) fatty acids in liver, muscle, and perivisceral fat, mirroring the profile of dietary fatty acids. However, the extent of the changes in fatty acid composition depends on the type of tissue, suggesting tissue-specific differences in the catabolic pathway of fatty acids. Short-chain PUFAs, such as α-linoleic acid (LNA, C18:3n-3) and linoleic acid (LA, C18:2n-6), can be used as substrates for synthesizing long-chain PUFAs like EPA and DHA. Interestingly, the higher levels of these short-chain PUFAs in the STD diet did not increase EPA and DHA levels in fish tissues, likely because meagre have a limited capacity to biosynthesize these important long-chain PUFAs [58,59,71]. The higher levels of these fatty acids in the ALT diet were reflected in fish tissues, highlighting the importance of including LC-PUFAs in the diets of marine carnivorous fish like meagre. Most vegetable oils are relatively poor sources of LC-PUFAs compared to marine fish oil [68]. However, they can be partially used [15,72], with their nutritional limitations complemented by other lipid sources derived from aquatic by-products or microalgae, which are rich in LC-PUFAs [13,68]. Therefore, the alternative ingredients to fish meal and oil used in the ALT diet offer a higher intake of LC-PUFAs and a balanced n-3/n-6 ratio, making it more supportive of a healthy human diet, compared to the STD diet.

4.5. Evaluating the Environmental Impacts of Dietary Treatments

One of the major challenges in aquaculture production is achieving a balance between optimal fish growth performance and minimal environmental impact [2]. Feed plays a crucial role in managing these two aspects. Therefore, effective diets should be efficiently utilized by fish to enhance nutrient retention and minimize waste outputs to the environment [73]. Ammonia, end-product of protein catabolism, is primarily excreted through fish gills [74]. Ammonia is then converted into nitrite by naturally occurring bacteria in the water, before being further converted into the less toxic nitrate form by other bacterial species. Feed is also a major contributor to phosphorous discharge in pond water and the surrounding environment [11]. In our study, the concentrations of dissolved ammonia, nitrates, nitrites, and phosphates in the water of experimental ponds were generally unaffected by the dietary treatment. This indicates similar metabolic nutrient management by the fish, efficient pond water renewal, or the presence of other biological factors that consume these metabolic products in the water, such as seaweeds and phytoplankton [52,75]. Studies on gilthead seabream (*Sparus aurata*) have reported an increase in ammonia production in response to high levels of vegetable meal inclusion in diets, possibly due to a lower

utilization of some essential amino acids [76]. Plant-based diets are also associated with increased phosphorous discharge, mainly due to the presence of phytate, which restrict the bioavailability of phosphorous, leading to an increased phosphorous load in the aquatic environment [56,77]. Additionally, nutrient availability limits microalgae growth in aquaculture ponds [78]. Interestingly, higher levels of chlorophyll *a* were found in the water of fish fed the STD diet, suggesting an increased presence of photosynthetic organisms in these ponds, such as microalgae. However, this increase appears to be unrelated to the concentrations of dissolved phosphates, ammonia, nitrites, and nitrates, which were unaffected by the different dietary treatments. Alternatively, the higher presence of photosynthetic organisms in the pond of fish fed the STD diet may result from increased nutrient availability, which might not be immediately evident due to the rapid consumption of these nutrients by the organisms themselves. Other factors, such as higher amounts of carbon sources or micronutrients from the naturally complex and rich environment of an earthen pond, may contribute to the increased presence of photosynthetic organisms in the ponds housing the fish fed the STD diet [52,67,79]. Additionally, higher water transparency in these ponds may allow for increased light penetration, favoring the growth of photosynthetic organisms [52]. Nevertheless, the higher amount of these organisms in the earthen ponds does not seem to benefit the growth performance of meagre.

5. Conclusions

The inclusion of plant-based ingredients, such as micro and macroalgae, along with animal by-products like sardine and fishmeal derivatives in aquafeeds, showed improved effects on fish growth and nutritional value, without causing significant nutrient losses to the environment, compared to the standard diet typically used in meagre production. The ALT diet is therefore a promising strategy to decrease reliance on fishmeal in aquafeeds, thereby enhancing the sustainability of aquaculture, without compromising protein levels or fish growth performance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9120517/s1, Table S1: Ingredient composition of the experimental diets (STD and ALT). Table S2: Survival and growth performance of white seabream (*Diplodus sargus*), mullet (*Mugil cephalus*), and oyster (*Crassostrea gigas*) in the two earthen pond polyculture systems.

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Informed Consent Statement: Not applicable.

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Conflicts of Interest: Author Jorge Dias is employed by the company Sparos Lda.; however, this research was conducted independently of any commercial or financial relationships that could be viewed as a potential conflict of interest. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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