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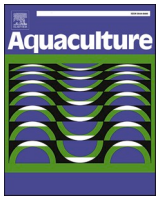

Enzymatic treatment of plant proteins in combination with algae-based nutraceutical inclusion in aquafeeds improves growth performance and physiological traits in the greater amberjack (*Seriola dumerili*)

Luis Molina-Roque [a,1], Paula Simó-Mirabet [a,*], André Barany [a,2], Anyell Caderno [a], Carmen Navarro-Guillén [b], Alba Galafat [c], Miguel Torres [a], Juan Fuentes [b], Juan Miguel Mancera [a], Erick Perera [b], Francisco Javier Alarcón-López [c,d], Juan Antonio Martos-Sitcha [a]

[a] *Department of Biology, Faculty of Marine and Environmental Sciences, Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEI·MAR), University of Cádiz, 11510 Puerto Real, Cádiz, Spain*
[b] *Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Campus Universitario Rio San Pedro s/n, Puerto Real 11519, Spain*
[c] *Department of Biology and Geology, Campus de Excelencia Internacional del Mar (CEI·MAR), University of Almería, 04120 Almería, Spain*
[d] *Lifebioencapsulation SL., Parque Científico PITA, 04131 Almería, Spain*

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ABSTRACT

This study aimed to evaluate, following two different and sequential feeding trials, the effects of partial substitution of fish meal by 44.5 % of plant proteins (~50 % of total protein inclusion) on growth performance, intermediate metabolism, well-being, and muscle chemical composition in the greater amberjack (*Seriola dumerili*). Additionally, the benefits of performing a previous enzymatic treatment on the plant ingredients before elaborating experimental aquafeeds (Trial II) compared to using untreated plant ingredients were also assessed. Three isoproteic (63 % protein, CP) and isolipidic (18 % crude lipid, CL) diets were used in each experiment: i) a control diet (CTRL), with 75.5 % of protein from marine origin; ii) two experimental diets, with 44.5 % of plant proteins (PP and PPe for Trial I and II, respectively); and iii) the PP/PPe diets supplemented with 3 % of an algae-based functional additive produced by Lifebioencapsulation S.L. (PP-LB and PP-LBe for Trial I and II, respectively). The results showed that using vegetable ingredients enzymatically treated before the production of aquafeed allows the partial substitution of dietary animal marine protein without affecting, or even improving the fish growth performance. The algal-based functional additive did not improve the fish growth when incorporated in plant protein-based diets, although it seems to provide a protective effect to overcome the impairment produced by the first contact of pre-treated vegetalized aquafeeds with the gastrointestinal tract in juvenile amberjack. Moreover, the LB additive could provide other benefits in the long term both in fish fed on diets elaborated with untreated and pre-treated plant ingredients, as evidenced by the level of cortisol released, the protection against oxidative stress, and the improvement in the chemical composition of muscle compared to the fish fed the plant-based diet without the functional additive. These findings demonstrate that the combination of an enzymatic pre-treatment of plant proteins together with the use of nutraceuticals from algae-based additives is a potential tool for more sustainable aquaculture of greater amberjack, a carnivorous species of high commercial interest.

* Corresponding author.
E-mail addresses: luis.molina@ulpgc.es (L. Molina-Roque), paula.simo@uca.es (P. Simó-Mirabet), abarany@ucm.es (A. Barany), anyell.caderno@uca.es (A. Caderno), carmen.navarro@csic.es (C. Navarro-Guillén), agd056@ual.es (A. Galafat), miguel.torres@uca.es (M. Torres), xoan.fuentes@csic.es (J. Fuentes), juanmiguel.mancera@uca.es (J.M. Mancera), erick.perera@csic.es (E. Perera), falarcon@ual.es (F.J. Alarcón-López), juanantonio.sitcha@uca.es (J.A. Martos-Sitcha).
[1] Current address: Aquaculture Research Group (GIA), IU-ECOAQUA, University of Las Palmas de Gran Canaria, 35214 Telde, Las Palmas, Canary Islands, Spain.
[2] Current address: Department of Genetics, Physiology and Microbiology, Faculty of Biological Sciences, Complutense University of Madrid, 28040 Madrid, Spain.

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1. Introduction

Aquaculture is the fastest-growing and most diverse animal production system in terms of production yield, employability, number of species, environments, and technology used. Recently, and particularly in the European Union, species diversification strategies have been impulsed to improve the sector's resilience and sustainability to deal with environmental, economic, and technical challenges such as climate change, diseases, or market fluctuations (Cai et al., 2023). However, the full establishment of new species is a slow process because the industry considers cautiously all technical, financial, economic, and time-consuming challenges that involve the farming of a new species, instead preferring to concentrate efforts on the already established and most advantageous species for rapid growth (Harvey et al., 2016). The greater amberjack (*Seriola dumerili*) is a pelagic teleost with increasing interest in the marine fish farming industry because of its outstanding biological and economic potential (Robles and Mylonas, 2017). Among its potential features are fast growth, flesh quality, average price, and acceptance by the global market (Nijssen et al., 2019). This species is strictly carnivorous, being an active predator from its earliest stages (García-Gómez, 2000) to juveniles (Takakuwa et al., 2006), which is the reason for its high energy and protein requirements in adapted aquafeeds (Navarro-Guillén et al., 2019).

Although fishmeal (FM) is the ideal source of dietary protein for predatory fish, its continuous demand, unstable supply, and high prices due to the expansion of aquaculture, has led the scientific community to focus on the search for alternatives to make this activity more profitable and sustainable. Primary efforts are focused on the replacement of FM with vegetable sources, mainly enhanced by the strict European legislation on the use of insects and terrestrial animal by-products, such as poultry blood, meat, feather, and bone meals (Rodehutscord et al., 2002; Tomás et al., 2005; Gasco et al., 2018, 2020). Against this background, and based on their nutritional potential, plant feedstuffs are widely used as an alternative to FM being the main plant-based alternatives soybean, pea, canola, lupin, or linseed (Drew et al., 2007). Nevertheless, including relatively high levels of these materials in diets becomes more challenging when dealing with carnivorous species, such as *S. dumerili*, affecting their growth performance and health (Oliva-Teles et al., 2015). Among other parameters, plant-based diets can cause adverse effects on nutrient digestibility and bioavailability, antioxidant defense (Olsvik et al., 2011; Minářová et al., 2021), and gut functionality and health (Diwan et al., 2022). In this regard, the literature has shown that plant proteins can present drawbacks for fish nutrition, such as the presence of anti-nutritional factors (ANFs) (Chakraborty et al., 2019), a high carbohydrate content, deficiency in certain essential amino acids or low palatability (Gatlin III et al., 2007). All these can affect nutrient availability, digestibility, and absorption, leading to subsequent harmful effects on whole fish health and welfare (Glencross, 2016; Daniel, 2018; Hua et al., 2019).

These handicaps can be overcome with different dietary techniques, such as the addition of functional additives (Sarker et al., 2007; Abdel-Latif et al., 2022), the combination of different plant sources (Torstensen et al., 2008; Martínez-Llorens et al., 2012; López-Elías et al., 2015) or the application of biotechnological treatments, including physical (microwave), chemical (enzymes), and biological (microbial fermentation) (Ghosh and Mukhopadhyay, 2006; Thongprajukaew et al., 2011; Jiang et al., 2014; Encarnação, 2016; Sansuwan et al., 2017; Jannathulla et al., 2019; Hua et al., 2019) or even the combination of some of them (Fonseca et al., 2023; Vizcaíno et al., 2024). Specifically, in the case of chemical treatments, exogenous enzymes improve fish growth performance (Zheng et al., 2020; Molina-Roque et al., 2022; Flores-Moreno et al., 2024) as they increase the nutritional value of plant sources, or even microalgal biomasses, by breaking down cellular walls, and therefore make nutrients available to easily absorbable fractions. Furthermore, applying enzymes like proteases can neutralize the trypsin inhibitors present in soybean meal, enabling greater utilization of dietary protein (Jannathulla et al., 2019). On the other hand, supplementation of diets with a high percentage of FM substitution with functional additives can reverse the consequences of including plant proteins. For example, supplementation with microalgae can improve the nutritional value of aquafeeds due to the presence of high-quality proteins, long-chain polyunsaturated fatty acids (LC-PUFA), vitamins, and micronutrients (Yaakob et al., 2014; Ansari et al., 2021). Including microalgae in diets at low percentages has been shown to enhance growth performance, immune response, feed digestibility, and final product quality (Nagarajan et al., 2021; Molina-Roque et al., 2022; Sáez et al., 2024). Moreover, microalgae are characterized by the production of bioactive compounds (da Silva Vaz et al., 2016) that may act as nutraceutical ingredients that evoke benefits in the growth rate of aquatic species through higher triglyceride and protein deposition in muscle, disease resistance, nitrogen output, omega-3 fatty acid content, physiological activity, and carcass quality (Shah et al., 2018). Thus, there is extensive information on the use of algae in fish feed. For example, many of the secondary metabolites produced by the algae, including functional proteins, antioxidants, minerals, and vitamins, have been found to have antiviral and antimicrobial properties improving the immune status, gut functioning, and stress resistance in fish (Saleh, 2020). Moreover, from an environmental perspective, the use of microalgae has been considered a sustainable approach for mitigating anthropogenic CO_2 (Becker, 2007) or wastewater (Kamyab et al., 2018); thus, microalgae biomass can be a valuable candidate as an eco-friendly functional additive.

Under this framework, the present study aims to evaluate the effects of diets with a high inclusion of plant ingredients (44.5 %) on growth, intermediate metabolism, well-being, and muscle composition in *S. dumerili,* when including a dietary algal-based functional additive. The benefits of using an enzymatic pre-treatment on the vegetable ingredients before their inclusion into aquafeeds compared with those diets elaborated with the untreated feedstuffs were also assessed.

2. Material and methods

2.1. Experimental design and diet formulation

Two feeding trials were conducted (henceforth named Trial I and Trial II) with identical diets, using the same vegetable ingredients and including an algae-based functional additive composed of a blend of hydrolysed algae and yeast. In Trial I the aquafeed contained vegetable ingredients, whereas in Trial II these ingredients were enzymatically pre-treated. Then, three isoproteic (63 % crude protein, CP) and isolipidic (18 % crude lipid, CL) diets were elaborated for each feeding assay: i) Control diet (CTRL), mimicking the ingredient composition of commercial diets, including 75.5 % of animal protein from marine sources; ii) Experimental diets with a high inclusion of plant proteins (44.5 %, i.e. ~50 % of total protein inclusion) named as PP and PPe for Trial I and II, respectively; and iii) PP/PPe diets supplemented with 3 % of an algae-based additive (PP-LB and PP-LBe for Trial I and II, respectively). All diets were formulated and developed by Life-Bioencapsulation S.L. (Almería, Spain; Ingredients and chemical composition of the diets used in both feeding trials are shown in Table 1. Note that for Trial II, previous to pellet extrusion, plant protein ingredients included in the three experimental aquafeeds (wheat, pea protein, and soybean protein concentrate), were previously enzymatically hydrolysed for improving the bioavailability of carbohydrates breaking the vegetable cell walls. Briefly, for the enzymatic hydrolysis, plant ingredients were suspended (100 g dry weight/L) in 50 mM sodium citrate buffer (pH 5.0) and incubated at 40 °C under continuous agitation for 6 h in presence of a blend of phytases (ePhyt®, 1309. Phytase activity units/mL, Global Feed S.L. Tervalis Group, Huelva, Spain) and carbohydrases (xylanase 20,000 U/g; glucanase; 30,000 U/g; cellulase 10,000 U/g, and protease 10,000 U/g) providing a 0.05 enzyme to plant ingredients ([E]/[S]) ratio. Immediately after the

Table 1
Ingredients and proximal composition (% dry matter) of the experimental diets (Control, CTRL; Plant protein diet, PP; PP with 3 % of an algal-based additive, PP-LB). Vegetable ingredients in diets PPe and PP-LBe were pre-treated by enzymatic hydrolysis.

Ingredient composition	CTRL	PP/PPe	PP-LB/PP-LBe
Fishmeal LT94 [1]	50.5	41.3	41.3
Squid meal [2]	6.0		
CPSP90 [3]	6.0	0.5	0.5
Krill meal [4]	5.0	0.5	0.5
Shrimp meal [5]	2.0	–	–
Mussel meal [6]	6.0	–	–
Wheat gluten [7]	5.0	13.0	12.4
Pea protein concentrate [8]	1.8	14.9	14.5
Soybean protein concentrate [9]	1.8	14.0	13.5
GreenBoost Plus [10]	–	–	3.0
Fish oil [11]	6.7	9.4	9.1
Soybean lecithin [12]	1.0	1.0	1.0
Wheat meal [13]	4.2	1.4	0.2
Choline cloride [14]	0.2	0.2	0.2
Betain [15]	0.2	0.2	0.2
Vitamin and Mineral premix [16]	1.5	1.5	1.5
Vitamin C [17]	0.1	0.1	0.1
Guar gum [18]	2.0	2.0	2.0
Crude protein	63.9	62.9	63.5
Crude lipid	18.7	18.4	18.2
Ash	12.9	10.8	11.0
Moisture	5.9	6.1	5.7

[1] 69.4 % crude protein, 12.3 % crude lipid (Norsildemel, Bergen, Norway). [2, 3, 4, 5, 6] purchased from Bacarel (UK). CPSP90 is enzymatically pre-digested fishmeal. [7] 78 % crude protein (Lorca Nutrición Animal SA, Murcia, Spain). [8] Pea protein concentrate, 85 % crude protein, 1.5 % crude lipid (Emilio Peña SA, Spain). [9] Soybean protein concentrate, 62 % crude protein, 2% crude lipid (LorcaNutrition, Spain). [10] *GreenBoost Plus* additive composed by hydrolysed yeasts (40 %), a blend of freshwater and marine microalgae (40 %) and *Alaria esculenta* (20 %) enzymatically hydrolysed. [11] AF117DHA (Afamsa, Spain). [12] P700IP (Lecico, DE). [13] Local provider (Almería, Spain). [14, 15] Lorca Nutrición Animal SA (Murcia, Spain). [16] *Lifebioencapsulation* SL (Almería, Spain). Vitamins (mg kg^{-1}): vitamin A (retinyl acetate), 2000,000 UI; vitamin D3 (DL-cholecalciferol), 200,000 UI; vitamin E (Lutavit E50), 10,000 mg; vitamin K3 (menadione sodium bisulphite), 2500 mg; vitamin B1(thiamine hydrochloride), 3000 mg; vitamin B2 (riboflavin), 3000 mg; calcium pantothenate, 10,000 mg; nicotinic acid, 20,000 mg; vitamin B6 (pyridoxine hydrochloride), 2000 mg; vitamin B9 (folic acid), 1500 mg; vitamin B12 (cyanocobalamin), 10 mg vitamin H (biotin), 300 mg; inositol, 50,000 mg; betaine (Betafin S1), 50,000 mg. Minerals (mg kg^{-1}): Co (cobalt carbonate), 65 mg; Cu (cupric sulphate), 900 mg; Fe (iron sulphate), 600 mg; I (potassium iodide), 50 mg; Mn (manganese oxide), 960 mg; Se (sodium selenite), 1 mg; Zn (zinc sulphate) 750 mg; Ca (calcium carbonate), 18.6 %; (186,000 mg); KCl, 2.41 %; (24,100 mg); NaCl, 4.0 % (40,000 mg). [17] TECNOVIT, Spain. [18] EPSA, Spain.

hydrolysis, the reaction mixture was heated at 80 °C for 15 min to inactivate the enzymes.

2.2. Animal maintenance and ethics

Greater amberjack juveniles were supplied by Futuna Blue España S. L. (El Puerto de Santa María, Cádiz, Spain) and acclimated to the indoor experimental facilities at the *Servicios Centrales de Investigación en Cultivos Marinos* (SCI-CM, CASEM, University of Cádiz, Puerto Real, Cádiz, Spain; Spanish Operational Code REGA ES11028000312) in a flow-through system under controlled conditions of salinity (37 ‰), temperature (20 ± 1 °C), and natural photoperiod at our latitude (36°31′45″ N, 6°11′31″ W, from October to December 2020 for Trial I and from October to December 2021 for Trial II). All experimental procedures were performed following the guidelines of the Animal Welfare and Ethics Committee of the University of Cádiz for the protection of animals used in scientific experiments, according to the principles published in the European Animal Directive (2010/63/EU) and Spanish laws (Royal Decree RD53/2013). In addition, the Ethical Committee from the Autonomous Andalusian Government approved the experiments (Junta de Andalucía reference number 23/10/2019/176).

2.3. Feeding protocol and sampling procedures

Fish with an initial mean body mass of 9.4 ± 0.1 for Trial I and 6.4 ± 0.1 g for Trial II were distributed in 9 tanks of 400 L capacity but using a volume of ~200 L to maintain the initial stocking density near 1 kg/m^3. Experimental groups (CTRL, PP/PPe, and PP-LB/PP-LBe) were tested in triplicate for 62 days (Trial I) and 69 days (Trial II) under the conditions described above.

During the feeding trials, fish were kept in a flow-through circulatory system as described above. Fish were then manually fed with experimental diets five times per day (9.00 h, 10.30 h, 12.00 h, 13.30 h, and 18.00 h) until apparent satiety (ad libitum), ensuring that the amount offered in each experimental unit was fully ingested. The feeding tests were performed blindly, as the aquafeeds were labeled with different colours without reference to their composition, eliminating any source of subjectivity when feeding the animals. No mortality was detected in any experimental group during the feeding trial.

Biometric samplings were carried out every three weeks using a sedative dose (0.3 mL/L seawater) of 2-phenoxyethanol to assess growth performance in both feeding trials, and feed intake was recorded weekly. From this data, we calculated different zootechnical and biometric indices (see Section 2.4). At the end of both feeding trials, 12 overnight fasted fish from each experimental diet (4 fish per tank) were randomly selected, deeply anesthetized with a lethal dose (1 mL/L seawater) of 2-phenoxyethanol, and then individually weighed and measured. Blood was drawn from the caudal vessels with heparinized syringes and centrifuged at 3000 ×*g* for 20 min at 4 °C to obtain plasma. Fish were then cervically sectioned and sampled to obtain biopsies of different tissues. Liver and perivisceral fat were weighed from each individual. The entire intestine was removed from the pyloric caeca down to the rectum for length measurement. Plasma and liver samples were taken in both experiments for the analysis of biochemical parameters, whereas white skeletal muscle was obtained for proximal composition. Additionally, in Trial II, liver and muscle biopsies were sampled to assess oxidative stress biomarkers and biochemical parameters, respectively. Samples were snap-frozen in liquid nitrogen and stored at −80 °C until further analysis. Finally, the remaining fish of each experimental unit were also weighted and measured to obtain the growth performance and biometric parameters described below.

2.4. Growth performance and biometric parameters

The growth parameters evaluated were: i) Specific growth rate (SGR) = (100 × (ln final body weight - ln initial body weight)/days; ii) Weight gain (WG) = (100 × (body weight increase)/initial body weight; iii) Feed efficiency (FE) = weight gain/total feed intake; and iv) Condition factor (K) = (100 × body weight)/fork $length^3$.

Organosomatic indices were calculated as the ratio of tissue to body weight or fork length for liver, perivisceral fat and intestine with the following equations: i) Hepatosomatic index (HSI) = (100 × liver weight)/fish weight; and ii) Intestine length index (ILI) = (100 × intestine length)/fork body length); and iii) Mesenteric index (MSI) = (100 × perivisceral fat weight)/fish weight.

2.5. Biochemical and hormonal parameters of plasma

Metabolic parameters were spectrophotometrically analyzed using commercial kits (SpinReact SA, St. Esteve d' en Bas, Girona, Spain), adapted to 96-well microplates. These parameters included glucose (Ref. 131,001,200), lactate (Ref. 1,001,330), cholesterol (Ref. 41,021), and triglycerides (Ref. 1,001,311). Plasma total protein concentration was determined with the bicinchoninic acid method using the commercial BCA kit (BCA™ Protein assay kit, Pierce, Rockford, USA).

Plasma total bile acids were evaluated with the commercial TBA Kit (SpinReact SA; Ref. 1,001,030) using a serum calibrator for total bile acids assay (TBA CAL; Ref. 1,002,290) according to the manufacturer's indications. Plasma cortisol levels were assessed with the commercial Cortisol Enzyme Immunoassay Kit (Arbor Assays, K003-H1W; Ann Arbor, Michigan, USA), whereas plasma levels of Igf-i were measured through competitive inhibition ELISA also using a commercial kit (CSB-E12122Fh for IGF-I, CUSABIO), both according to the manufacturer's indications. All assays were performed with a PowerWave™ 340 microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA), controlled by KCjunior Software for Microsoft® Windows.

2.6. Biochemical parameters of liver and muscle

Frozen biopsies used for the assay of metabolites were mechanically homogenized (blender) in 7.5 volumes ice-cold 0.6 N $HClO_4$, neutralized using 1 M KCO_3, centrifuged (30 min, 3,220 ×*g* at 4 °C), and the isolated supernatants were used to determine tissue metabolites. Triglycerides (TAG) and lactate levels were determined spectrophotometrically with commercial kits (SpinReact, see above). Glycogen concentration was quantified using the method described by Keppler and Decker (1974), where glucose obtained after glycogen breakdown with amyloglucosidase (Sigma-Aldrich, Ref. A7420) was determined with the same glucose commercial kit (SpinReact, see above).

2.7. Proximal composition

The proximal analysis of the different experimental diets, as well as of the fish muscle was determined following the AOAC (2000) procedures for dry matter and ash. Crude protein (N × 6.25) was determined by elemental analysis (C: H: N) with a Fisons EA 1108 analyzer (Fisons Instruments, USA). Values were expressed as percentage of dry matter (% DM). The amount of total lipids was determined following the procedure described by Folch et al. (1957).

2.8. Oxidative stress biomarkers

Liver antioxidant status was assessed by measuring catalase activity (CAT), total antioxidant capacity (TAC), protein carbonylation (PC), lipid peroxidation (LPO), and mitochondrial reactive oxygen species production (mtROS). Frozen samples were homogenized in 500 μL ultrapure water using an Ultra-Turrax® disperser (IKA®-Werke, Germany). One aliquot containing 4 % butylated hydroxytoluene (BHT) in methanol was used for the determination of LPO. The remaining homogenate was diluted (1:1) in 0.2 M K-phosphate buffer, pH 7.4, and centrifuged for 10 min at 10,000 ×*g* (4 °C). The post-mitochondrial supernatant (PMS) was kept at −80 °C for the analysis of catalase activity, total antioxidant capacity, and protein carbonylation. CAT was determined by measuring the decomposition of the substrate H_2O_2 at 240 nm (Claiborne, 1985). TAC was assessed following the protocol described by Erel (2004), using colored 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{+}$). PC was measured by the reaction of 2,4-dinitrophenylhydrazine (DNPH) with carbonyl groups, according to the DNPH alkaline method (Mesquita et al., 2014). Endogenous LPO was determined by measuring thiobarbituric acid-reactive substances (TBARS) (Bird and Draper, 1984).

For mtROS determination, samples were homogenized in 200 μL ice-cold mitochondria isolation buffer (225 mM mannitol, 75 mM sucrose, 1 mM EGTA, and 4 mM HEPES, pH 7.2). Then, the homogenate was centrifuged for 10 min at 600 ×*g* and 4 °C. The supernatant was picked off and centrifuged again for 10 min, at 11,000 ×*g* and 4 °C. The pellet was resuspended in a buffer containing 250 mM sucrose and 5 mM HEPES (pH 7.2). mtROS production was assessed by the dihydrodichloro-fluorescein diacetate method, H(2)DCF-DA (Van Der Toorn et al., 2009).

The protein content of PMS (CAT, TAC, LPO, and PC determinations) and mtROS samples was determined according to Bradford's method (Bradford, 1976) using bovine serum albumin as standard. All biomarkers were determined in 96 well flat bottom microplates using a temperature-controlled microplate reader (Synergy H1, BioTek Instrument, Inc., USA).

2.9. Statistical analyses

Data on feed intake and growth indices are represented as the mean ± SEM (standard error of the mean) of triplicate tanks, data on somatic indices are the mean ± SEM of 12 fish, and data on body mass are the mean ± SEM of 60–75 fish per group. All data were checked for normality and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests, respectively, with $p < 0.05$. Differences among treatments in all parameters were analyzed by one-way analysis of variance (ANOVA, $p < 0.05$). Tukey's test was applied to those parameters with significant group differences ($p < 0.05$). The software package GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analyses and figures.

3. Results

3.1. Growth performance and biometric parameters

Growth evolution (body mass, g) and weight gain (%) of the experimental groups during both feeding trials are represented in Fig. 1 (A, Trial I; B, Trial II). Growth performance and somatic indices from both experiments are compared in Table 2. The initial body weight was the same among experimental groups in the Trial I (~ 9 g) and the Trial II (~ 6 g). In Trial I, individuals of the CTRL, PP, and PP-LB groups grew to a final mean body weight of 61.5 g, 42.3 g, and 38.9 g, respectively, being statistically lower (~31–36 %) in fish-fed both plant protein diets

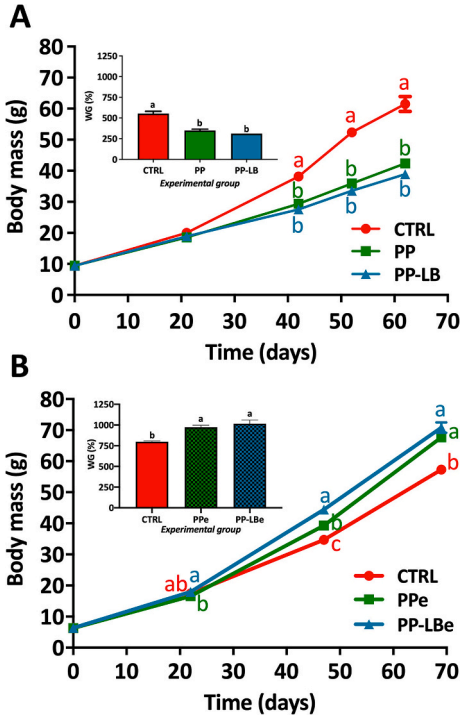

Fig. 1. Evolution of growth and final weight gain (WG, %) of the three experimental groups (CTRL, PP/PPe, and PP-LB/PP-LBe) from Trial I (A) and Trial II (B) after the long-term feeding trials. CTRL control diet; PP/PPe: plant protein diet; PP-LB/PP-LBe: PP/PPe diet with 3 % of an algae-based additive. Data are the mean ± SEM of 36 fish/treatment. Different letters in each panel mean statistical differences after one-way ANOVA and Tukey test ($p < 0.05$).

Table 2
Growth performance and somatic indices of *S. dumerili* juveniles fed different experimental diets[1]. Data on feed intake and growth indices are the mean ± SEM of triplicate tanks. Data on somatic indices are the mean ± SEM of 12 fish, whereas data on initial and final body mass are the mean ± SEM of 36 fish. Different superscript letters in each row indicate significant differences among dietary treatments based on one-way ANOVA and Tukey's test ($p < 0.05$).

	Trial I				Trial II			
	CTRL	PP	PP-LB	p[2]	CTRL	PPe	PP-LBe	p[2]
Initial body mass (g)	9.41 ± 0.01	9.42 ± 0.01	9.46 ± 0.01	0.4464	6.37 ± 0.01	6.35 ± 0.01	6.34 ± 0.01	0.1715
Final body mass (g)	61.5 ± 4.2^{a}	42.3 ± 4.2^{b}	38.9 ± 4.2^{b}	0.0040	57.3 ± 2.1^{b}	67.7 ± 2.1^{a}	70.8 ± 2.1^{a}	0.0004
SGR (%)[3]	3.03 ± 0.06^{a}	2.42 ± 0.05^{b}	2.28 ± 0.03^{b}	0.0001	3.18 ± 0.01^{b}	3.44 ± 0.02^{a}	3.49 ± 0.03^{a}	0.0001
FI[4]	47.7 ± 0.7^{a}	39.3 ± 0.3^{b}	38.3 ± 0.7^{b}	0.0001	44.7 ± 2.8^{b}	59.1 ± 0.7^{a}	63.1 ± 0.7^{a}	0.0007
FE[5]	1.19 ± 0.04^{a}	0.86 ± 0.08^{b}	0.82 ± 0.03^{b}	0.0058	1.14 ± 0.07	1.04 ± 0.03	1.02 ± 0.03	0.2056
K[6]	1.66 ± 0.02	1.65 ± 0.02	1.67 ± 0.04	0.8959	1.63 ± 0.02^{b}	1.70 ± 0.01^{a}	1.67 ± 0.01^{ab}	0.0108
HSI (%)[7]	1.06 ± 0.11	1.35 ± 0.14	1.33 ± 0.18	0.2040	1.33 ± 0.08	1.34 ± 0.07	1.27 ± 0.11	0.8300
ILI (%)[8]	78.2 ± 2.7	75.9 ± 3.9	75.0 ± 2.3	0.8020	63.5 ± 2.1^{b}	75.0 ± 1.5^{a}	70.8 ± 1.6^{a}	0.0002

[1] Control, CTRL; Plant protein diet, PP/PPe; PP/PPe with 3 % of an algal-based additive, PP-LBe.
[2] Values resulting from one-way analysis of variance (ANOVA).
[3] Specific growth rate = 100 × (ln final body weight − ln initial body weight)/days.
[4] Feed Intake = (grams of aquafeed consumed/tank)/week.
[5] Feed Efficiency = weight gain/total feed intake.
[6] Condition factor = (100 × body weight)/fork length^{3}.
[7] Hepatosomatic index = (100 × liver weight)/fish weight.
[8] Intestine length index = (100 × intestine length)/fork length.

compared to those fish-fed the CTRL diet. In contrast, in Trial II, fish-fed plant-based diets (PPe and PP-LBe) grew faster than CTRL fish, with a final body weight increase of ~121 % compared to the CTRL group. This improvement was already observed in the PP-LBe group after the first three weeks from the beginning of the feeding trial. A similar pattern was observed in SGR and feed intake (FI), which were statistically lower in fish-fed plant-based diets (PP and PP-LB) in Trial I, whereas in Trial II, fish from the PPe and PP-LBe groups achieved significantly higher SGR and FI compared to the CTRL group. In line with these results, in Trial I, the plant protein diets (PP and PP-LB) produced a significantly lower FE compared to the CTRL diet, whereas no differences in this parameter were found among the experimental groups of Trial II. Regarding the condition factor (K), no significant differences were observed among the experimental groups in Trial I, although a significant increase was detected in this parameter in fish-fed the PPe diet compared to the CTRL group in Trial II. In terms of somatic indices, neither the HSI nor the ILI showed differences among groups in Trial I. In contrast, in Trial II a significantly higher ILI was observed in fish-fed plant protein diets (PPe and PP-LBe) compared to the fish-fed CTRL diet.

3.2. Plasma, liver and muscle analyses

Results on plasma, liver, and muscle parameters are shown in Table 3. Plasma glucose revealed no significant differences among experimental groups in Trial I. However, a significant increase in this metabolite was observed with the plant protein diets (PPe and PP-LBe) in Trial II. Lactate levels decreased in the plasma of fish-fed PP diet compared to the PP-LB and CTRL groups in Trial I, but in Trial II, no differences were detected among groups for this metabolite. Similarly, the PP diet caused a significant decrease in plasma TAG levels concerning the other two groups in Trial I, while in Trial II, the levels were significantly higher in both PPe and PP-LBe diets compared to the CTRL

Table 3
Plasma, liver, and muscle biochemistry of *S. dumerili* juveniles fed different experimental diets[1]. Data are the mean ± SEM of 12 fish. Different superscript letters in each row indicate significant differences among dietary treatments based on one-way ANOVA and Tukey's test ($p < 0.05$).

	Trial I				Trial II			
	CTRL	PP	PP-LB	p[2]	CTRL	PPe	PP-LBe	p[2]
Plasma								
Glucose (mM)	6.12 ± 0.17	6.43 ± 0.35	6.89 ± 0.29	0.2001	4.87 ± 0.15^{b}	5.62 ± 0.24^{a}	5.54 ± 0.16^{a}	0.0113
Lactate (mM)	2.02 ± 0.08^{a}	1.58 ± 0.10^{b}	2.17 ± 0.21^{a}	0.0055	2.25 ± 0.16	2.43 ± 0.10	2.38 ± 0.13	0.4712
Triglycerides (mM)	0.49 ± 0.04^{a}	0.34 ± 0.04^{b}	0.51 ± 0.04^{a}	0.0152	1.18 ± 0.04^{b}	1.51 ± 0.07^{a}	1.51 ± 0.08^{a}	0.0011
Cholesterol (mM)	8.05 ± 0.39^{a}	7.18 ± 0.23^{ab}	6.78 ± 0.19^{b}	0.0231	5.15 ± 0.22	5.11 ± 0.14	5.21 ± 0.14	0.0997
Total bile acids (μM)	28.1 ± 3.2	23.8 ± 2.4	30.3 ± 4.9	0.4039	15.7 ± 2.4	17.9 ± 2.5	16.1 ± 1.9	0.2761
Proteins (mg/mL)	29.5 ± 0.9^{a}	15.4 ± 0.8^{b}	14.7 ± 0.4^{b}	0.0001	32.8 ± 1.4^{b}	37.8 ± 1.2^{a}	39.4 ± 1.2^{a}	0.0027
Cortisol (ng/mL)	7.02 ± 0.86^{b}	9.66 ± 1.08^{ab}	11.8 ± 1.50^{a}	0.0027	8.08 ± 1.12	13.9 ± 3.10	10.8 ± 2.80	0.2676
Igf-i (ng/mL)					4.16 ± 0.04^{b}	5.34 ± 0.11^{a}	5.37 ± 0.19^{a}	<0.0001
Liver								
Triglycerides (mg/g tissue)	38.2 ± 1.3	44.1 ± 2.8	44.9 ± 2.9	0.1004	28.7 ± 3.3^{b}	61.7 ± 4.4^{a}	59.8 ± 3.3^{a}	0.0001
Glucose (mg/g tissue)	1.39 ± 0.17	1.14 ± 0.13	1.41 ± 0.26	0.2574	2.80 ± 0.32	3.35 ± 0.14	3.02 ± 0.17	0.2181
Glycogen (mg/g tissue)	6.17 ± 0.49^{a}	4.13 ± 0.27^{b}	3.85 ± 0.25^{b}	0.0003	8.22 ± 1.03	6.99 ± 0.63	8.43 ± 0.44	0.3517
Lactate (mg/g tissue)	0.08 ± 0.01^{ab}	0.07 ± 0.01^{b}	0.13 ± 0.03^{a}	0.0362	0.07 ± 0.02^{b}	0.16 ± 0.03^{a}	0.11 ± 0.02^{ab}	0.0236
Muscle								
Triglycerides (mg/g tissue)	–	–	–	–	5.59 ± 0.36	6.13 ± 0.38	6.48 ± 0.68	0.4509
Glucose (mg/g tissue)	–	–	–	–	0.94 ± 0.25	1.56 ± 0.43	1.07 ± 0.40	0.4875
Glycogen (mg/g tissue)	–	–	–	–	4.47 ± 0.68	5.40 ± 1.07	6.08 ± 0.88	0.4466
Lactate (mg/g tissue)	–	–	–	–	2.89 ± 0.19	3.04 ± 0.24	3.03 ± 0.22	0.8690

[1] Control, CTRL; Plant protein diet, PP/PPe; PP/PPe with 3 % of an algal-based additive, PP-LBe.
[2] Values resulting from one-way analysis of variance (ANOVA).

group. Cholesterol levels only changed in fish fed on diets without enzymatic pre-treatment. Specifically, PP-LB diet evoked a significant reduction in cholesterol levels compared to the CTRL diet. No differences were found in plasma total bile acids among groups in neither experiment. Comparing both experiments, protein plasma levels showed opposite patterns, being reduced in fish-fed plant protein diets (PP and PP-LB, Trial I) and increased in fish fed with enzymatically pre-treated plant-based diets (PPe and PP-LBe, Trial II) compared to their respective CTRL groups. Besides, changes in cortisol levels were only observed in Trial I, increasing in fish fed the PP-LB diet compared to the other two experimental groups. Finally, in Trial II, Igf-i levels were raised in fish-fed pretreated vegetable diets compared to the CTRL fish.

In the liver, no differences in free glucose levels were observed among groups in any feeding trials performed. However, in Trial I (but not in Trial II), both plant protein diets (PP and PP-LB) produced a decrease in glycogen levels compared to the CTRL group. Hepatic TAG levels did not differ among groups in Trial I whereas the plant protein diets in Trial II (PPe and PP-LBe) increased TAG deposited in the liver compared to the CTRL group. Lastly, in Trial I, an increase in lactate levels was observed in the liver of the fish-fed PP-LB diet compared to the other two groups, while the pre-treatment of vegetable ingredients (Trial II) produced a significant increase of lactate in fish-fed PPe compared to the CTRL group. Note that muscle analyses were only assessed for Trial II, in which no significant differences were found in any of the parameters analyzed.

3.3. Proximal composition of muscle

Proximal composition of greater amberjack muscle fed with the different experimental diets in both Trial I and II is detailed in Table 4. In Trial I, protein content in fish fed with PP and PP-LB diets was similar to the CTRL group, but the lipid content was lower in those fish fed with diets enriched in plant protein (PP and PP-LB). Muscle protein content did not differ among dietary treatments in Trial II but the lipid content tended to increase slightly in the groups fed the diets elaborated with a high content of the enzymatically hydrolysed vegetable ingredients (PPe and PP-LBe groups). Ash content in CTRL group was higher compared fish fed on PP and PP-LB in Trial I, but lower in CTRL compared to PPe in Trial II. Concerning moisture, fish fed CTRL showed lower values only in Trial I.

3.4. Oxidative stress

Results of liver antioxidant status assessed for Trial II are shown in Table 5. CAT showed a significant decrease in fish fed the PPe diet compared to the CTRL group, while the PP-LBe group had intermediate values. The opposite trend is observed in mtROS. However, in parameters such as TAC, protein carbonylation, and LPO no differences were found among the CTRL diet and the other two diets with a high content of vegetable protein sources.

4. Discussion

Several studies have demonstrated that pre-treatments by enzymatic hydrolysis of vegetable sources, including algae, can improve digestibility and growth performance in different fish species (Ai et al., 2007; Cao et al., 2007; Kalhoro et al., 2018; Maas et al., 2018, 2019, 2020; Martínez et al., 2019; Martínez-Antequera et al., 2021; Molina-Roque et al., 2022) but this issue has never been examined before in the greater amberjack. Likewise, results obtained revealed that the enzymatic treatment of plant proteins allows the inclusion of at least 44.5 % plant proteins substituting animal marine protein, resulting in ~50 % of total protein included in aquafeeds, without affecting, or even improving, the growth performance of a carnivorous fish species of high commercial interest such as the greater amberjack (*S. dumerili*). Moreover, the effects of exogenous enzymes are not only beneficial when feeding carnivorous species with vegetable feedstuffs but also produce the same improvements in herbivorous species, as it has also been confirmed in Nile tilapia (*Oreochromis niloticus*) (Lin et al., 2007; Goda et al., 2012) or in grass carp (*Ctenopharyngodon idella*) (Zhou et al.,

Table 4
Proximate composition (g 100 g^{-1} wet weight) in the muscle of *S. dumerili* juveniles fed different experimental diets[1]. Data are the mean ± SEM of 12 fish. Different superscript letters in each row indicate significant differences among dietary treatments based on one-way ANOVA and Tukey's test ($p < 0.05$).

	Trial I				Trial II			
	CTRL	PP	PP-LB	p[22]	CTRL	PPe	PP-LBe	p[2]
Total protein	18.43 ± 0.05	18.31 ± 0.08	18.28 ± 0.02	0.1570	21.10 ± 0.08	21.02 ± 0.07	20.86 ± 0.04	0.2350
Total lipid	1.16 ± 0.02^{a}	0.95 ± 0.02^{b}	0.96 ± 0.02^{b}	0.0002	1.08 ± 0.02^{b}	1.13 ± 0.01^{b}	1.19 ± 0.01^{a}	0.0023
Ash	1.66 ± 0.01^{a}	1.54 ± 0.02^{b}	1.54 ± 0.02^{b}	0.0116	1.56 ± 0.01^{b}	1.63 ± 0.01^{a}	1.61 ± 0.02^{ab}	0.0153
Moisture	77.03 ± 0.018^{b}	78.54 ± 0.14^{a}	78.06 ± 0.20^{a}	0.0025	75.38 ± 0.67	75.05 ± 0.04	75.28 ± 0.15	0.8854

[1] Control, CTRL; Plant protein diet, PP/PPe; PP/PPe with 3 % of an algal-based additive, PP-LBe.
[2] Values resulting from one-way analysis of variance (ANOVA).

Table 5
Liver oxidative status in *S. dumerili* juveniles fed different experimental diets[1]. Data are the mean ± SEM of 12 fish. Different superscript letters in each row indicate significant differences among dietary treatments based on one-way ANOVA and Tukey's test ($p < 0.05$). Prot: protein.

	Trial II			
	CTRL	PPe	PP-LBe	p[2]
CAT (Act/μg prot)[3]	8.37 ± 0.58^{a}	6.68 ± 0.45^{b}	7.05 ± 0.36^{ab}	0.0436
TAC (μmol trolox/mg prot)[4]	0.31 ± 0.08	0.31 ± 0.07	0.19 ± 0.05	0.3742
PC (μmol carbonyl/μg prot)[5]	3.56 ± 0.75	7.53 ± 1.45	7.12 ± 1.59	0.0684
LPO (nmol TBARS/μg prot)[6]	0.12 ± 0.02	0.21 ± 0.04	0.19 ± 0.02	0.1193
mtROS (Act/μg prot)[7]	17.5 ± 3.9^{b}	50.3 ± 8.3^{a}	37.2 ± 7.5^{ab}	0.0059

[1] Control, CTRL; Plant protein diet, PP/PPe; PP/PPe with 3 % of an algal-based additive, PP-LBe.
[2] Values resulting from one-way analysis of variance (ANOVA).
[3] Catalase activity.
[4] Total antioxidant capacity.
[5] Protein carbonylation.
[6] Lipid peroxidation.
[7] Mitochondrial reactive oxygen species production.

2013). Although previous studies, such as those performed by Jover et al. (1999) with *Seriola dumerili* or by Watanabe et al. (1995) with *Seriola quinqueradiata*, showed positive growth results with 20 % soybean meal inclusion, Tomás et al. (2005) and Dawood et al. (2015) set this limit at 30 % without affecting the productive performance of *S. dumerili*. Even so, recent studies seem to be promising in terms of growth performance by a 100 % replacement of FM and fish oil in this species by other vegetable and animal meals, although a clear reduction in survival rates was obtained in the most extreme diet (Milián-Sorribes et al., 2024). In the present study, the aquafeeds used in both trials contained no more than 15 % soybean protein concentrate. This moderate inclusion might explain the promising results in feed efficiency, which was better in Trial II after submitting the plant ingredients to an enzymatic pre-treatment before aquafeed elaboration. However, other factors such as legume varieties, protein extraction methods (Decroos et al., 2007), or the presence and interaction among different ANFs cannot be discarded. Although the accurate reason for the adverse effects of soybean derivatives is not fully established, some research points out that the interaction among ANFs, antigens, and intestinal microbiota could be the cause of impaired nutrient digestibility and absorption, leading to intestinal inflammation (Hu et al., 2021; Zhang et al., 2021), and protein and lipid metabolism affection (Lazzarotto et al., 2018).

Fish-fed diets with vegetable ingredients pre-treated enzymatically (PPe) reached an SGR of 3.5 % compared to fish-fed diets with untreated ingredients (SGR = 2.5 %). This indicates an improved growth during the experimental period, which is confirmed by the increase of plasma insulin-like growth factor I (Igf—i) in fish fed pre-treated diets since this hormone, together with growth hormone (Gh), participates in growth regulation and they are the main muscle-accretion regulatory factors (Vélez et al., 2016; Perelló-Amorós et al., 2021). Also, fish-fed with the PPe diet showed the highest condition factor, reaching values of 1.70 ± 0.01, similar to other studies in wild and cultured specimens of *S. dumerili* (Fernández-Montero et al., 2018) which may indicate a good health status. Contrary to what occurred with untreated vegetable diets with or without the algae-based nutraceutical, inducing a lower feed intake and feed efficiency when compared to the CTRL diet, the application of enzymatic hydrolysis on vegetable ingredients increased feed intake without changes in feed efficiency. Thus, it could be suggested that the enhanced growth of fish-fed these diets was not only due to better bioavailability, assimilation, and utilization of the nutrients but also to better palatability that allowed the high feeding rates observed. Even more importantly, this observation also suggests that nutritional intervention does not produce any detrimental effects on feed acceptability since voluntary feeding cessation is one of the first problems observed when a nutritional imbalance is denoted (Bendiksen et al., 2011; Sun et al., 2016). Indeed, fish with carnivorous habits, such as *S. dumerili*, are not able to digest and use optimally the carbohydrates present in vegetables due to low activities of enzymes such as amylase (Hidalgo et al., 1999). In some cases, these compounds can be considered as ANFs and cannot be used for producing energy, relying on fats and proteins as energy sources, or even behaving as undigested and excreted molecules. This is why the application of enzymes benefits the use of these alternative ingredients to improve growth performance without affecting their gut microbial diversity, as has been recently described by Flores-Moreno et al. (2024) and Peralta-Sánchez et al. (2024) in European seabass (*Dicentrarchus labrax*), as it enhances the bioavailability of these compounds improving absorption in the proximal intestine (Kamalam et al., 2017), and allowing an optimal and faster growth without affecting animal health. In addition to that, another interesting finding is related to the LB nutraceutical which did not have any effect in Trial I and does not seem to be too relevant in a long-term feeding period since this supplemented diet produces almost the same growth performance as PPe at the end of the Trial II. Even so, during the first weeks of the feeding trial, the algae-based additive seemed to stimulate growth faster, as shown in the evolution of biomass along the feeding trial (Fig. 1B), which could be used as a preventive treatment for putative nutritional impacts due to changes in aquafeed formulation, especially in the first contact (short periods) to new feeds. Then to fully understand the underlying cause or consequence of these observations, further studies will be conducted at the intestinal level, in terms of intestinal functionality, microbiota, or molecular and endogenous intestinal mechanisms.

Regarding organosomatic indices, HSI values in Trial I and II (above 1 %) are in line with those shown in previous nutritional studies with this species when animal marine protein is replaced by vegetable protein (Dawood et al., 2015; Hossain et al., 2018; Monge-Ortiz et al., 2018; Takakuwa et al., 2020). Similar HSI have also been reported for greater amberjack fed with a commercial diet at the same culture temperature (Fernández-Montero et al., 2018). All these indicate that using vegetable ingredients per se would not affect the energy status of the liver or fish health, regardless of whether they are pre-treated. It is mainly related to the fine formulation covering nutritional species-specific traits. On the other hand, the diets used in the present study provoke an increment of fat accumulation (TAG) in the liver, especially in Trial II, which is a typical consequence of aquafeeds formulated with a high content of vegetable ingredients (Gu et al., 2014; Zhang et al., 2019; Yao et al., 2024). Likewise, the slight TAG increase (non-significant) in fish-fed diets with untreated plant ingredients could be the result of the reduced feed intake, in which the energy ingested was used for basal metabolism avoiding an excess accumulation in the liver. On the other hand, it is well-known that fish gut length strongly depends on feeding habits, with carnivorous species having shorter guts. However, omnivorous species such as gilthead seabream (*Sparus aurata*) can elongate their intestine to increase the absorption surface when the diet contains more vegetable feedstuffs (Santigosa et al., 2008; Perera et al., 2020; Molina-Roque et al., 2022). Contrary to that observed in other studies regarding the lack of an intestinal adaptation in *S. dumerili* (Takakuwa et al., 2020), the results of the present work demonstrated the intestinal adaptability in this species when ingesting aquafeeds with a high proportion of vegetable sources, as evidenced the increase of intestine length (ILI) in fish-fed with pre-treated vegetable ingredients compared to the CTRL group. However, this plasticity is only evidenced in fish fed diets with the enzymatic treatment which probably caused an increase in nutrient bioavailability, though more analyses are needed to fully understand the intestinal plasticity and functionality of the greater amberjack. The results obtained strongly suggest that this species can only benefit from nutrients easily digestible by their natural enzymatic capacity. In fact, low carbohydrase activity is still expected in fish species with carnivorous preferences, as previously described in *S. dumerili* where authors did not detect amylase activity in juveniles in previous studies (Navarro-Guillen et al., 2022), being only detected in first-feeding larvae using fluorescence techniques (Gamberoni et al., 2021). This may be the cause or the consequence of why this capacity remained hidden in previous studies in this species and the lack of changes in the intestinal length of fish-fed diets without a pre-treatment (Trial I). This agreed with previous studies in other species, such as rainbow trout (*Oncorhynchus mykiss*), when comparing a commercial diet with a vegetable-based diet (Gatesoupe et al., 2018), in striped catfish with different percentages of FM replacement with grains (Allam et al., 2020) or gilthead seabream when varying levels of substitution using vegetable protein sources (Sitjà-Bobadilla et al., 2005).

As stated before, natural feeding habits affect the ability to use digestible carbohydrates in farmed fish. Herbivorous and omnivorous species tend to have a more efficient carbohydrate metabolism due to greater amylase activity, intestinal glucose uptake capacity, and control of glycemia compared to carnivorous fish (Kamalam et al., 2017). In addition, glycemia in fish is known to be highly dependent on the rate of feed intake (Polakof et al., 2012) which is in line with fish fed diets with untreated (reduced FI) and pre-treated (increased FI) vegetable ingredients. In fact, the glucose increase in fish-fed pre-treated diets has also been described when replacing FM with fermented soybean pulp in African catfish (*Clarias gariepinus*) (Kari et al., 2021). This may be a

consequence of the treatment of plant proteins which produces a higher bioavailability of carbohydrates together with an increased feed intake. This could be explained by a relative increase not only in carbohydrates but also in their bioavailability since, as mentioned in Basto-Silva et al. (2021), an increase in carbohydrate content in diets can lead to less satiety sensation due to an increase in leptin receptor (*lepr*) and a decrease of cholecystokinin (*cck*) intestinal expression (orexigenic and anorexigenic hormones, respectively). Those results were obtained at 5 h after feeding, so, considering that we fed fish every 1.50 h, the increase in feed intake in the groups with plant proteins could be explained by a lower satiety sensation. Moreover, recent studies in the yellowtail kingfish (*Seriola lalandi*) conclude that starch is poorly digested by yellowtail kingfish probably due to low amylase activity, secretion, and biosynthesis or the relatively short gut length of yellowtail kingfish (Horstmann et al., 2023; Zuther et al., 2024). In addition, RNA-Seq studies in larvae of *Seriola quinqueradiata* despite revealing the existence of two α-amylase genes, exhibited low or undetected expression levels along the gastrointestinal tract (stomach, intestine, and rectum tissues), while showing higher proteolytic enzyme activities according to the transcriptional signatures exhibited by carnivorous fish (Yasuike et al., 2018). Considering all described above, plasma lactate levels were not affected in fish fed with pre-treated plant-based diets (Trial II), whereas a decrease in lactate occurred in fish fed the untreated plant protein diet (PP, Trial I), as described in previous studies (Resende et al., 2023). Higher lactate levels can also result from carbohydrate-enriched diets that contribute to increased glycogen stores and preferential glycogen degradation for energy requirements (Maruhenda Egea et al., 2015). In the present study, the algae-based nutraceutical seems to have a positive effect in re-establishing the values, similar to the rest of the groups in Trial I.

Parallelly, the increase in TAG plasma levels in fish-fed pre-treated vegetable diets compared to the CTRL diet mainly reflected the increased feed intake observed (Table 2). However, the levels of this metabolite also depend on other parameters, such as the species tested, diet composition, stage of development, and the type of dietary plant ingredients used (Ye et al., 2019). Accordingly, some studies reported an increase in plasma TAG in fish fed with plant-based aquafeeds (Slawski et al., 2011; Takakuwa et al., 2020; Shen et al., 2020), whereas others showed a decrease in this metabolite (Regost et al., 1999; Moradi et al., 2013; Slawski et al., 2012; Rahmdel et al., 2018). In the present study, and considering the results obtained in terms of growth performance, it seems that the pre-treatment of vegetable ingredients also induced a better use of the energy supplied by dietary lipids. In general, the pre-treatment of ingredients performed in this study seems to allow a better availability of both glucose and TAG metabolites which fish might use as fuel for growth. Another widely described consequence of the use of plant proteins is the reduction of cholesterol levels regardless of the species (Regost et al., 1999; Kaushik et al., 2004; Moradi et al., 2013; Gatesoupe et al., 2018; Ye et al., 2019) or developmental stage (Rahmdel et al., 2018), as it was observed in fish-fed untreated plant-based diets. However, pre-treated vegetable-based diets can maintain stable cholesterol levels in plasma. According to Sitjà-Bobadilla et al. (2005), hypocholesterolaemia in fish when feeding plant proteins may be due to increased bile salt excretion, or the inhibition of cholesterol absorption in the intestine. Moreover, it is expected that lower dietary cholesterol level in diets formulated with a high content of plant protein ingredients for replacing FM can contribute to lowering the concentration of this metabolite in plasma. Considering that experimental diets tested in both feeding trials had the same amount of FM and that the plasma total bile acids levels did not vary among groups in either trial, the results may indicate an improved bioavailability and absorption of dietary cholesterol when the vegetable ingredients are enzymatically hydrolysed before inclusion in aquafeeds. Remarkably, and similar to previous studies (Sitjà-Bobadilla et al., 2005; Moradi et al., 2013; Allam et al., 2020), plasma protein levels were reduced in fish-fed untreated plant-based diets, probably due to the lower digestibility capacity of plant proteins (Regost et al., 1999). However, when vegetable ingredients were pre-treated enzymatically in our study, a higher mobilization of plasma proteins was observed, which may suggest higher bioavailability and bioaccessibility of dietary proteins, as has been shown with the fermentation of soybean pulp (Kari et al., 2021) or rapeseed meal (Dossou et al., 2018) before their inclusion in the feed. Since proteins are related to structural components for tissue development and growth in addition to energy sources (García-Márquez et al., 2023, and references herein), the higher plasma proteins could also be correlated with growth performance (i.e. the highest levels of plasma proteins were found in fast-growing groups, and vice versa).

Stress is one of the factors to consider in aquaculture production, as it can affect hormone secretion rates, intermediary metabolism, immunity, and nutrient utilization (Hossain et al., 2017). The use of vegetable ingredients in fish diets affects cortisol release, likely due to the different PUFA contents (Montero et al., 2003; Montero and Izquierdo, 2010; Ganga et al., 2011; Montero et al., 2015). In carnivorous species, these ingredients can cause enteritis (Jutfelt, 2011; Stone et al., 2018; Liu et al., 2020; Seibel et al., 2022), leading to cortisol release into the bloodstream (Carabotti et al., 2015). However, only fish-fed diets without pre-treatment showed increased cortisol levels with respect to fish fed CTRL diet, indicating a clear positive effect of enzymatic treatment of vegetable proteins on overall metabolism and well-being in this fish species. Cortisol is not only a biomarker of stress but also a master regulator of metabolism playing an important role in growth, osmoregulation, and reproduction (Mommsen et al., 1999) and even more at the physiological levels detected in our experimental approach. This underscores the importance of hydrolysing vegetable ingredients to enhance the digestion and absorption of lipid content.

Accordingly, the liver is probably the most critical organ for evaluating the metabolic effects of the diet, primarily related to lipid metabolism (Ye et al., 2019), since it is one of the main energy reservoirs and the target tissue for metabolic hormonal control (e.g. cortisol). In this regard, an increase in TAG deposition in the liver of fish-fed pre-treated vegetable ingredients diets is in line with previous studies in other fish species (Sitjà-Bobadilla et al., 2005; Zhou et al., 2005; Rahmdel et al., 2018). Replacement of animal marine ingredients with plant proteins can cause liver damage (Takakuwa et al., 2020) and, among other effects, can lead to steatosis (Siddik et al., 2021) or affect the immune response by decreasing complement proteins production (Sitjà-Bobadilla et al., 2005). On the other hand, carbohydrate metabolism was little affected by rich-plant protein diets except for the lower glycogen content in the liver of fish-fed untreated vegetable diets (Trial I). This could be due to different factors, as i) the lower feed intake observed in these two experimental groups; ii) a lower bioavailability of carbohydrates from the diet, and/or iii) the potential stimulation of glycolytic pathways by cortisol action (Mommsen et al., 1999). Therefore, applying exogenous enzymes as carbohydrases may avoid the reduction of hepatic glycogen storage (Castillo and Gatlin III, 2015), which is an important energy source and essential for physiological processes. Lastly, the production of reactive oxygen species (ROS) is a natural process caused by the cellular metabolism itself but can be modulated by its interaction with the environment, such as dietary changes. An increase in ROS leads to oxidative damage, which triggers enzyme inactivation, protein carbonylation, and lipid peroxidation, among others. Balanced concentrations of anti- and pro-oxidant factors are continuously generated during regular cellular metabolism to avoid this situation (Lushchak, 2016). Among these antioxidant factors, catalases reduce H_2O_2 to H_2O in the peroxisomes (Betancor et al., 2012). Dietary changes can cause an imbalance in ROS production and removal, which may lead to a reduction in fish growth performance (Betancor et al., 2012; Roo et al., 2019). In gilthead seabream, dietary vegetable oil mixtures differentially modified the intestinal oxidative status (García-Meilán et al., 2023) although this has not been observed in the greater amberjack (Milián-Sorribes et al., 2023). Thus, these results suggest that dietary fatty acid composition effects on oxidative metabolism are

species-specific. In Atlantic salmon (*Salmo salar*), a change from a marine-based to a plant-based diet affected the antioxidant defense, with lower catalase activity in fish transferred to a plant-based diet (Olsvik et al., 2011). These findings align with those of the present study, where the dietary inclusion of pre-treated vegetable ingredients significantly increased mtROS production in liver. However, in our study, this outcome may be related to a higher metabolic activity associated with faster growth which does not necessarily involve an imbalance in oxidative status in the liver, judging by the absence of relevant changes in lipid peroxidation and other indicators of oxidative stress. Moreover, even in this scenario, it is interesting to note that the addition of the nutraceutical to the plant-based diet provided additional protection against oxidative stress, with a tendency to decrease ROS production and increase catalase activity in the PP-LBe group. Thus, it is still worth studying if the use of this nutraceutical in aquafeeds with a high nutritional value, as those tested in Trial II of the present study, can provide some advantages when this fish species is fed with plant protein diets concurrently with other stressors known to boost oxidative stress under farming conditions. In addition, our results pointed out that the use of the nutraceutical should be further evaluated in feeds with fish oil replacement by vegetable oils.

Finally, in Trial II, vegetable protein inclusion did not significantly affect muscle metabolism. In a previous study in Senegalese sole (*Solea senegalensis*), Rodiles et al. (2015) did not observe any changes in the proximate composition of muscle, fatty acid profile, or muscular metabolites with a 30 % FM replacement using plant protein sources. Additionally, no adverse effects were observed on the whole body and muscle proximate composition in Atlantic cod (*Gadus morhua*) when fed diets rich in plant proteins (Hansen et al., 2007). This suggests that the use of plant protein sources, at least enzymatically pre-treated, does not affect muscle metabolism and function, regardless of species and feeding habits. Besides, the chemical composition of fish muscle constitutes a valuable indicator of its quality and nutritional values (Lanza et al., 2001; Ahmed et al., 2022). In this context, results obtained in Trial I did not show a significant increase in muscle protein content in fish-fed PP diet despite the fact the increased body moisture level which might be associated to nutritionally unbalanced diet and the observed suboptimal growth. These findings were consistent with other studies where has been demonstrated that replacing FM protein with soybean meal in diets did not affect the proximate composition in rainbow trout (Yang et al., 2010) or European seabass (Tibaldi et al., 2006). However, the results obtained disagree with those reported in Bastard halibut (*Paralichthys olivaceus*; Shen et al., 2024), catfish (*Pseudobagrus ussuriensis*; Wang et al., 2015), Nile tilapia (Ajani, 2016), and European seabass (Kaushik et al., 2004). Fish fed the PP/PPe and PP-LB/PP-LBe diet showed a protein content similar to that observed in the CTRL groups, which might suggest that the dietary inclusion of the algae-based functional additive did not affect protein accretion when feeding fish on diets with a high content of plant ingredients. However, several studies have documented an increase in muscle protein in other cultured fish species, attributing this phenomenon to the dietary inclusion of algae (Abdel-Warith et al., 2016; Roohani et al., 2019; Galafat et al., 2020). Regarding muscle lipid content, our results showed a significant decrease in fish-fed PP and PP-LB diets, which is reversed in fish-fed diets including the vegetable ingredients enzymatically treated (PPe and PP-LBe). This fact could be related to the lower availability of nutrients in plant sources, which could increase the use of lipids as an energy source. Similar results have been described for different aquaculture fish species when replacing FM with vegetable meals (Gaber, 2006; Wang et al., 2015; Song et al., 2020; Peng et al., 2022). In this sense, previous works reported that the use of algae in aquafeeds could also cause an impact on lipid turnover, which could lead to a reduction in lipid storage in fish muscle (Vizcaíno et al., 2016).

5. Conclusions

The results obtained in the present study demonstrate that the inclusion of a high % of plant protein in the diet (44.5 %) partially replacing marine animal protein can affect the growth performance in *S. dumerili* juveniles. However, the enzymatic treatment of the plant protein sources provides a potential tool for improving nutritional bioaccessibility and value. Moreover, the supplementation of the plant-based diet with the algae-based additive in Trial II allowed a faster adaptation of fish to vegetable diets compared to those fish-fed on diets that were devoid of it. Although supplementation with this additive does not seem to provide significant benefits to the culture of *S. dumerili* in a short- term (62 and 69 days), it may help to attenuate the negative effects of using high levels of plant proteins on other aspects, such as orchestrate the physiological and metabolic responses or ameliorate the hepatic oxidative stress. It would be interesting to conduct further studies to evaluate the impact of this and other nutraceutical compounds in a longer-term feeding trial in this fast-growing species, and also to assess its value for intestinal plasticity and adaptation to the nutritional impacts caused by these newer aquafeed formulations.

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CRediT authorship contribution statement

Luis Molina-Roque: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Paula Simó-Mirabet:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Visualization, Writing – review & editing. **André Barany:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Anyell Caderno:** Data curation, Formal analysis, Writing – review & editing. **Carmen Navarro-Guillén:** Formal analysis, Methodology, Writing – review & editing. **Alba Galafat:** Formal analysis, Funding acquisition, Writing – review & editing. **Miguel Torres:** Formal analysis, Writing – review & editing. **Juan Fuentes:** Conceptualization, Writing – review & editing. **Juan Miguel Mancera:** Resources, Writing – review & editing. **Erick Perera:** Formal analysis, Methodology, Writing – review & editing. **Francisco Javier Alarcón-López:** Conceptualization, Funding acquisition, Investigation, Resources, Writing – review & editing. **Juan Antonio Martos-Sitcha:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are all presented in the figures and tables, as well as available from the authors upon reasonable request.

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References

Abdel-Latif, H.M., Abdel-Daim, M.M., Shukry, M., Nowosad, J., Kucharczyk, D., 2022. Benefits and applications of *Moringa oleifera* as a plant protein source in aquafeed: a review. Aquaculture 547, 737369. https://doi.org/10.1016/j.aquaculture.2021.737369.

Abdel-Warith, A.W.A., Younis, E.M.I., Al-Asgah, N.A., 2016. Potential use of green macroalgae *Ulva lactuca* as a feed supplement in diets on growth performance, feed utilization and body composition of the African catfish, *Clarias gariepinus*. Saudi J. Biol. Sci. 23 (3), 404–409. https://doi.org/10.1016/j.sjbs.2015.11.010.

Ahmed, I., Jan, K., Fatma, S., Dawood, M.A.O., 2022. Muscle proximate composition of various food fish species and their nutritional significance: a review. J. Anim. Physiol. Anim. Nutr. 106 (3), 690–719. https://doi.org/10.1111/jpn.13711.

Ai, Q., Mai, K., Zhang, W., Xu, W., Tan, B., Zhang, C., Li, H., 2007. Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 147 (2), 502–508. https://doi.org/10.1016/j.cbpa.2007.01.026.

Ajani, E.K., 2016. Total replacement of fishmeal by soybean meal with or without methionine fortification in the diets of Nile tilapia, *Oreochromis niloticus*. J. Fish. Aquat. Sci. 11 (3), 238–343. https://doi.org/10.3923/jfas.2016.238.243.

Allam, B.W., Khalil, H.S., Mansour, A.T., Srour, T.M., Omar, E.A., Nour, A.A.M., 2020. Impact of substitution of fish meal by high protein distillers dried grains on growth performance, plasma protein and economic benefit of striped catfish (*Pangasianodon hypophthalmus*). Aquaculture 517, 734792. https://doi.org/10.1016/j.aquaculture.2019.734792.

Ansari, F.A., Guldhe, A., Gupta, S.K., Rawat, I., Bux, F., 2021. Improving the feasibility of aquaculture feed by using microalgae. Environ. Sci. Pollut. Res. 28 (32), 43234–43257. https://doi.org/10.1007/s11356-021-14989-x.

AOAC, 2000. Official Methods of Analysis. The Association of Official Analytical Chemisths, p. 17.

Basto-Silva, C., Enes, P., Oliva-Teles, A., Balbuena-Pecino, S., Navarro, I., Capilla, E., Guerreiro, I., 2021. Dietary protein source and protein/carbohydrate ratio affects appetite regulation-related genes expression in gilthead seabream (*Sparus aurata*). Aquaculture 533, 736142. https://doi.org/10.1016/j.aquaculture.2020.736142.

Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv. 25 (2), 207–210.

Bendiksen, E.Å., Johnsen, C.A., Olsen, H.J., Jobling, M., 2011. Sustainable aquafeeds: progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 314 (1–4), 132–139. https://doi.org/10.1016/j.aquaculture.2011.01.040.

Betancor, M.B., Caballero, M., Terova, G., Saleh, R., Atalah, E., Benítez-Santana, T., Bell, J.G., Izquierdo, M., 2012. Selenium inclusion decreases oxidative stress indicators and muscle injuries in sea bass larvae fed high-DHA microdiets. Br. J. Nutr. 108, 2115–2128. https://doi.org/10.1017/s0007114512000311.

Bird, R.P., Draper, H.H., 1984. Comparative studies on different methods of malonaldehyde determination. In: Methods in Enzymology, vol. 105. Academic Press, pp. 299–305. https://doi.org/10.1016/S0076-6879(84)05038-2.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Cai, J., Chan, H.L., Yan, X., Leung, P., 2023. A global assessment of species diversification in aquaculture. Aquaculture, 739837. https://doi.org/10.1016/j.aquaculture.2023.739837.

Cao, L., Wang, W., Yang, C., Yang, Y., Diana, J., Yakupitiyage, A., Li, D., 2007. Application of microbial phytase in fish feed. Enzym. Microb. Technol. 40 (4), 497–507. https://doi.org/10.1016/j.enzmictec.2007.01.007.

Carabotti, M., Scirocco, A., Maselli, M.A., Severi, C., 2015. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann. Gastroenterol. 28 (2), 203. http://www.ncbi.nlm.nih.gov/pmc/articles/pmc4367209/.

Castillo, S., Gatlin III, D.M., 2015. Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. Aquaculture 435, 286–292. https://doi.org/10.1016/j.aquaculture.2014.10.011.

Chakraborty, P., Mallik, A., Sarang, N., Lingam, S.S., 2019. A review on alternative plant protein sources available for future sustainable aqua feed production. Int. J. Chem. Stud. 7, 1399–1404.

Claiborne, A., 1985. Catalase activity. In: Greenwald, R.A. (Ed.), CRC Handbook of Methods in Oxygen Radical Research. CRC Press, Boca Raton, pp. 283–284.

da Silva Vaz, B., Moreira, J.B., de Morais, M.G., Costa, J.A.V., 2016. Microalgae as a new source of bioactive compounds in food supplements. Curr. Opin. Food Sci. 7, 73–77. https://doi.org/10.1016/j.cofs.2015.12.006.

Daniel, N., 2018. A review on replacing fish meal in aqua feeds using plant protein sources. Int. J. Fish. Aquat. Stud. 6 (2), 164–179.

Dawood, M.A., Koshio, S., Ishikawa, M., Yokoyama, S., 2015. Effects of partial substitution of fish meal by soybean meal with or without heat-killed *lactobacillus plantarum* (LP20) on growth performance, digestibility, and immune response of amberjack, *Seriola dumerili* juveniles. Biomed. Res. Int. 2015. https://doi.org/10.1155/2015/514196.

Decroos, K., Vincken, J.P., Van Koningsveld, G.A., Gruppen, H., Verstraete, W., 2007. Preparative chromatographic purification and surfactant properties of individual soyasaponins from soy hypocotyls. Food Chem. 101 (1), 324–333. https://doi.org/10.1016/j.foodchem.2006.01.041.

Diwan, A.D., Harke, S.N., Gopalkrishna, & Panche, A. N., 2022. Aquaculture industry prospective from gut microbiome of fish and shellfish: an overview. J. Anim. Physiol. Anim. Nutr. 106 (2), 441–469. https://doi.org/10.1111/jpn.13619.

Dossou, S., Koshio, S., Ishikawa, M., Yokoyama, S., Dawood, M.A., El Basuini, M.F., Olivier, A., 2018. Effect of partial replacement of fish meal by fermented rapeseed meal on growth, immune response and oxidative condition of red sea bream juvenile, *Pagrus major*. Aquaculture 490, 228–235. https://doi.org/10.1016/j.aquaculture.2018.02.010.

Drew, M.D., Borgeson, T.L., Thiessen, D.L., 2007. A review of processing of feed ingredients to enhance diet digestibility in finfish. Anim. Feed Sci. Technol. 138 (2), 118–136. https://doi.org/10.1016/j.anifeedsci.2007.06.019.

Encarnação, P., 2016. Functional feed additives in aquaculture feeds. In: Aquafeed Formulation. Academic Press, pp. 217–237. https://doi.org/10.1016/B978-0-12-800873-7.00005-1.

Erel, O., 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin. Biochem. 37 (4), 277–285. https://doi.org/10.1016/j.clinbiochem.2003.11.015.

Fernández-Montero, A., Caballero, M.J., Torrecillas, S., Tuset, V.M., Lombarte, A., Ginés, R.R., Montero, D., 2018. Effect of temperature on growth performance of greater amberjack (*Seriola dumerili* Risso 1810) juveniles. Aquac. Res. 49 (2), 908–918. https://doi.org/10.1111/are.13537.

Flores-Moreno, S., Vizcaíno, A.J., Sáez, M.I., Macías-Vidal, J., Martínez, T.F., Martos-Sitcha, J.A., Alarcón-López, F.J., 2024. Effects of phytase and microalgae supplementation on the utilization of aquafeeds for European seabass (*Dicentrarchus labrax*) formulated with a high inclusion level of plant protein. Aquac. Res. 2024 (1), 4775004. https://doi.org/10.1155/2024/4775004.

Folch, J., Lees, M., Stanley, G.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226 (1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5.

Fonseca, F., Fuentes, J., Vizcaíno, A.J., Alarcón, F.J., Mancera, J.M., Martínez-Rodríguez, G., Martos-Sitcha, J.A., 2023. From invasion to fish fodder: inclusion of the brown algae *Rugulopteryx okamurae* in aquafeeds for European sea bass *Dicentrarchus labrax* (L., 1758). Aquaculture 568, 739318. https://doi.org/10.1016/j.aquaculture.2023.739318.

Gaber, M.M., 2006. Partial and complete replacement of fish meal by broad bean meal in feeds for Nile tilapia, *Oreochromis niloticus*, L., fry. Aquac. Res. 37, 986–993. https://doi.org/10.1111/j.1365-2109.2006.01517.x.

Galafat, A., Vizcaíno, A.J., Sáez, M.I., Martínez, T.F., Jérez-Cepa, I., Mancera, J.M., Alarcón, F.J., 2020. Evaluation of *Arthrospira* sp. enzyme hydrolysate as dietary additive in gilthead seabream (*Sparus aurata*) juveniles. J. Appl. Phycol. 32 (5), 3089–3100. https://doi.org/10.1007/S10811-020-02141-0.

Gamberoni, P., Yúfera, M., de Las Heras, V., Siguero, I., Gilannejad, N., Martínez-Rodríguez, G., Navarro-Guillén, C., 2021. Ontogeny and diurnal patterns of molecular gene expression and activity of digestive enzymes in developing greater amberjack. Aquaculture 534, 736330. https://doi.org/10.1016/j.aquaculture.2020.736330.

Ganga, R., Montero, D., Bell, J.G., Atalah, E., Ganuza, E., Vega-Orellana, O., Izquierdo, M., 2011. Stress response in sea bream (*Sparus aurata*) held under crowded conditions and fed diets containing linseed and/or soybean oil. Aquaculture 311 (1–4), 215–223. https://doi.org/10.1016/j.aquaculture.2010.11.050.

García-Gómez, A., 2000. Recent advances in nutritional aspects of *Seriola dumerili*. Cah. Options Mediterr. 47, 249–257.

García-Márquez, J., Domínguez-Maqueda, M., Torres, M., Cerezo, I.M., Ramos, E., Alarcón, F.J., Balebona, M.C., 2023. Potential effects of microalgae-supplemented diets on the growth, blood parameters, and the activity of the intestinal microbiota in *Sparus aurata* and *Mugil cephalus*. Fishes 8 (8), 409. https://doi.org/10.3390/fishes8080409.

García-Meilán, I., Fontanillas, R., Gutiérrez, J., Capilla, E., Navarro, I., Gallardo, Á., 2023. Effects of dietary vegetable oil mixtures including soybean oil on intestinal oxidative stress in gilthead sea bream (*Sparus aurata*). Animals 13 (6), 1069. https://doi.org/10.3390/ani13061069.

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., Caruso, G., 2018. Fishmeal alternative protein sources for aquaculture feeds. In: Feeds for the Aquaculture Sector: Current Situation and Alternative Sources, pp. 1–28. https://doi.org/10.1007/978-3-319-77941-6_1.

Gasco, L., Acuti, G., Bani, P., Dalle Zotte, A., Danieli, P.P., De Angelis, A., Roncarati, A., 2020. Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. Ital. J. Anim. Sci. 19 (1), 360–372. https://doi.org/10.1080/1828051X.2020.1743209.

Gatesoupe, F.J., Fauconneau, B., Deborde, C., Madji Hounoum, B., Jacob, D., Moing, A., Médale, F., 2018. Intestinal microbiota in rainbow trout, *Oncorhynchus mykiss*, fed diets with different levels of fish-based and plant ingredients: A correlative approach with some plasma metabolites. Aquac. Nutr. 24 (5), 1563–1576. https://doi.org/10.1111/anu.12793.

Gatlin III, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38 (6), 551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x.

Ghosh, K., Mukhopadhyay, P.K., 2006. Application of enzymes in aqua feeds. Aqua Feeds Formul. Beyond 3 (4), 7–10.

Glencross, B., 2016. Understanding the nutritional and biological constraints of ingredients to optimize their application in aquaculture feeds. In: Aquafeed Formulation. Academic Press, pp. 33–73.

Goda, A.M.A., Mabrouk, H.A.H.H., Wafa, M.A.E.H., El-Afifi, T.M., 2012. Effect of using baker's yeast and exogenous digestive enzymes as growth promoters on growth, feed utilization and hematological indices of Nile tilapia, *Oreochromis niloticus* fingerlings. J. Agric. Sci. Technol. B 2 (1B).

Gu, M., Kortner, T.M., Penn, M., Hansen, A.K., Krogdahl, Å., 2014. Effects of dietary plant meal and soya-saponin supplementation on intestinal and hepatic lipid droplet accumulation and lipoprotein and sterol metabolism in Atlantic salmon (*Salmo salar L.*). Br. J. Nutr. 111 (3), 432–444. https://doi.org/10.1017/s0007114513002717.

Hansen, A.C., Rosenlund, G., Karlsen, Ø., Koppe, W., Hemre, G.I., 2007. Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I—effects on growth and protein retention. Aquaculture 272 (1–4), 599–611. https://doi.org/10.1016/j.aquaculture.2007.05.010.

Harvey, B., Soto, D., Carolsfeld, J., Beveridge, M., Bartley, D.M., 2016. Planning for aquaculture diversification: the importance of climate change and other drivers. In: FAO Technical Workshop, pp. 23–25.

Hidalgo, M.C., Urea, E., Sanz, A., 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. Aquaculture 170 (3–4), 267–283. https://doi.org/10.1016/S0044-8486(98)00413-X.

Horstmann, P., Maas, R.M., de Boer, X.V., Staessen, T.W., Kokou, F., Schrama, J.W., 2023. Faecal waste characteristics of yellowtail kingfish (*Seriola lalandi*) fed with pelleted and natural feed. Anim. Feed Sci. Technol. 299, 115625. https://doi.org/10.1016/j.anifeedsci.2023.115625.

Hossain, M.S., Koshio, S., Ishikawa, M., Yokoyama, S., Sony, N.M., Kader, M.A., Fujieda, T., 2017. Effects of dietary administration of inosine on growth, immune response, oxidative stress and gut morphology of juvenile amberjack, *Seriola dumerili*. Aquaculture 468, 534–544. https://doi.org/10.1016/j.aquaculture.2016.11.020.

Hossain, S., Koshio, S., Ishikawa, M., Yokoyama, S., Sony, N.M., Islam, J., Fujieda, T., 2018. Substitution of dietary fishmeal by soybean meal with inosine administration influences growth, digestibility, immunity, stress resistance and gut morphology of juvenile amberjack *Seriola dumerili*. Aquaculture 488, 174–188. https://doi.org/10.1016/j.aquaculture.2018.01.037.

Hu, Y., Zhang, J., Xue, J., Chu, W., Hu, Y., 2021. Effects of dietary soy isoflavone and soy saponin on growth performance, intestinal structure, intestinal immunity and gut microbiota community on rice field eel (*Monopterus albus*). Aquaculture 537, 736506. https://doi.org/10.1016/j.aquaculture.2021.736506.

Hua, K., Cobcroft, J.M., Cole, A., Condon, K., Jerry, D.R., Mangott, A., Strugnell, J.M., 2019. The future of aquatic protein: implications for protein sources in aquaculture diets. One Earth 1 (3), 316–329. https://doi.org/10.1016/j.oneear.2019.10.018.

Jannathulla, R., Rajaram, V., Kalanjiam, R., Ambasankar, K., Muralidhar, M., Dayal, J.S., 2019. Fishmeal availability in the scenarios of climate change: inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. Aquac. Res. 50 (12), 3493–3506. https://doi.org/10.1111/are.14324.

Jiang, T.T., Feng, L., Liu, Y., Jiang, W.D., Jiang, J., Li, S.H., Zhou, X.Q., 2014. Effects of exogenous xylanase supplementation in plant protein-enriched diets on growth performance, intestinal enzyme activities and microflora of juvenile Jian carp (*Cyprinus carpio var. Jian*). Aquac. Nutr. 20 (6), 632–645. https://doi.org/10.1111/anu.12125.

Jover, M., Garcıa-Gómez, A., Tomas, A., de la Gándara, F., Pérez, L., 1999. Growth of Mediterranean yellowtail (*Seriola dumerilii*) fed extruded diets containing different levels of protein and lipid. Aquaculture 179 (1–4), 25–33. https://doi.org/10.1016/S0044-8486(99)00149-0.

Jutfelt, F., 2011. Barrier function of the gut. In: Encyclopedia of Fish Physiology: From Genome to Environment, 2, pp. 1322–1331.

Kalhoro, H., Zhou, J., Hua, Y., Ng, W.K., Ye, L., Zhang, J., Shao, Q., 2018. Soy protein concentrate as a substitute for fish meal in diets for juvenile *Acanthopagrus schlegelii*: effects on growth, phosphorus discharge and digestive enzyme activity. Aquac. Res. 49 (5), 1896–1906. https://doi.org/10.1111/are.13645.

Kamalam, B.S., Medale, F., Panserat, S., 2017. Utilisation of dietary carbohydrates in farmed fishes: new insights on influencing factors, biological limitations and future strategies. Aquaculture 467, 3–27. https://doi.org/10.1016/j.aquaculture.2016.02.007.

Kamyab, H., Friedler, F., Klemes, J.J., Chelliapan, S., Rezania, S., 2018. Bioenergy production and nutrients removal by green microalgae with cultivation from agro-wastewater palm oil mill effluent (POME)-a review. Chem. Eng. Trans. 70, 2197–2202.

Kari, Z.A., Kabir, M.A., Mat, K., Rusli, N.D., Razab, M.K.A.A., Ariff, N.S.N.A., Wei, L.S., 2021. The possibility of replacing fish meal with fermented soy pulp on the growth performance, blood biochemistry, liver, and intestinal morphology of African catfish (*Clarias gariepinus*). Aquac. Rep. 21, 100815. https://doi.org/10.1016/j.aqrep.2021.100815.

Kaushik, S.J., Coves, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture 230 (1–4), 391–404. https://doi.org/10.1016/S0044-8486(03)00422-8.

Keppler, D., Decker, K., 1974. Glycogen determination with amyloglucosidase. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Academic Press, New York, pp. 1127–1131.

Lanza, M., Priolo, A., Biondi, L., Bella, M., Salem, H.B., 2001. Replacement of cereal grains by orange pulp and carob pulp in faba bean-based diets fed to lambs: effects on growth performance and meat quality. Anim. Res. 50 (1), 21–30. https://doi.org/10.1051/animres:2001101.

Lazzarotto, V., Médale, F., Larroquet, L., Corraze, G., 2018. Long-term dietary replacement of fishmeal and fish oil in diets for rainbow trout (*Oncorhynchus mykiss*): effects on growth, whole body fatty acids and intestinal and hepatic gene expression. PLoS One 13 (1), e0190730. https://doi.org/10.1371/journal.pone.0190730.

Lin, S., Mai, K., Tan, B., 2007. Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus* x *O. aureus*. Aquac. Res. 38 (15), 1645–1653. https://doi.org/10.1111/j.1365-2109.2007.01825.x.

Liu, H., Dong, X., Tan, B., Du, T., Zhang, S., Yang, Y., Liu, H., 2020. Effects of fish meal replacement by low-gossypol cottonseed meal on growth performance, digestive enzyme activity, intestine histology and inflammatory gene expression of silver sillago (*Sillago sihama Forsskál*) (1775). Aquac. Nutr. 26 (5), 1724–1735. https://doi.org/10.1111/anu.13123.

López-Elías, J.A., Moreno-Arias, A., Miranda-Baeza, A., Martínez-Córdova, L.R., Rivas-Vega, M.E., Márquez-Ríos, E., 2015. Proximate composition of bioflocs in culture systems containing hybrid red tilapia fed diets with varying levels of vegetable meal inclusion. N. Am. J. Aquac. 77 (1), 102–109. https://doi.org/10.1080/15222055.2014.963767.

Lushchak, V.I., 2016. Contaminant-induced oxidative stress in fish: a mechanistic approach. Fish Physiol. Biochem. 42, 711–747. https://doi.org/10.1007/s10695-015-0171-5.

Maas, R.M., Verdegem, M.C., Dersjant-Li, Y., Schrama, J.W., 2018. The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. Aquaculture 487, 7–14. https://doi.org/10.1016/j.aquaculture.2017.12.040.

Maas, R.M., Verdegem, M.C., Schrama, J.W., 2019. Effect of non-starch polysaccharide composition and enzyme supplementation on growth performance and nutrient digestibility in Nile tilapia (*Oreochromis niloticus*). Aquac. Nutr. 25 (3), 622–632. https://doi.org/10.1111/anu.12884.

Maas, R.M., Verdegem, M.C., Stevens, T.L., Schrama, J.W., 2020. Effect of exogenous enzymes (phytase and xylanase) supplementation on nutrient digestibility and growth performance of Nile tilapia (*Oreochromis niloticus*) fed different quality diets. Aquaculture 529, 735723. https://doi.org/10.1016/j.aquaculture.2020.735723.

Martínez, F.P., Bermúdez, L., Aznar, M.J., Moyano, F.J., 2019. Evaluation of enzyme additives on the nutritional use of feeds with a high content of plant ingredients for *Mugil cephalus*. Fishes 4 (4), 56. https://doi.org/10.3390/fishes4040056.

Martínez-Antequera, F.P., Barranco-Ávila, I., Martos-Sitcha, J.A., Moyano, F.J., 2021. Solid-state hydrolysis (SSH) improves the nutritional value of plant ingredients in the diet of *Mugil cephalus*. Fishes 7 (1), 4. https://doi.org/10.3390/fishes7010004.

Martínez-Llorens, S., Vidal, A.T., Cerdá, M.J., 2012. A new tool for determining the optimum fish meal and vegetable meals in diets for maximizing the economic profitability of gilthead sea bream (*Sparus aurata, L.*) feeding. Aquac. Res. 43 (11), 1697–1709. https://doi.org/10.1111/j.1365-2109.2011.02977.x.

Maruhenda Egea, F.C., Toledo-Guedes, K., Sanchez-Jerez, P., Ibanco-Cañete, R., Uglem, I., Saether, B.S., 2015. A metabolomic approach to detect effects of salmon farming on wild saithe (*Pollachius virens*) populations. J. Agric. Food Chem. 63 (49), 10717–10726. https://doi.org/10.1021/acs.jafc.5b04765.

Mesquita, C.S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J.V., Marcos, J.C., 2014. Simplified 2, 4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Anal. Biochem. 458, 69–71. https://doi.org/10.1016/j.ab.2014.04.034.

Milián-Sorribes, M.C., Peres, H., Tomás-Vidal, A., Moutinho, S., Peñaranda, D.S., Jover-Cerdá, M., Martínez-Llorens, S., 2023. Hepatic, muscle and intestinal oxidative status and plasmatic parameters of greater amberjack (*Seriola dumerili*, Risso, 1810) fed diets with fish oil replacement and probiotic addition. Int. J. Mol. Sci. 24 (7), 6768. https://doi.org/10.3390/ijms24076768.

Milián-Sorribes, M.C., Martínez-Llorens, S., Peñaranda, D.S., Jauralde, I., Jover-Cerdá, M., Tomás-Vidal, A., 2024. Growth, survival, and intestinal health alterations in Mediterranean yellowtail (*Seriola dumerili*) due to alternatives to fishmeal and fish oil. Curr. Issues Mol. Biol. 46 (1), 753–772. https://doi.org/10.3390/cimb46010049.

Minářová, H., Bláhová, L., Kalina, J., Papežíková, I., Mareš, J., Kopp, R., Palíková, M., 2021. Plant-based and immunostimulant-enhanced diets modulate oxidative stress, immune and haematological indices in rainbow trout (*Oncorhynchus mykiss*). Acta Vet. Brno 90 (2), 233–253. https://doi.org/10.2754/avb202190020233.

Molina-Roque, L., Bárany, A., Sáez, M.I., Alarcón, F.J., Tapia, S.T., Fuentes, J., Martos-Sitcha, J.A., 2022. Biotechnological treatment of microalgae enhances growth performance, hepatic carbohydrate metabolism and intestinal physiology in gilthead seabream (*Sparus aurata*) juveniles close to commercial size. Aquac. Rep. 25, 101248. https://doi.org/10.1016/j.aqrep.2022.101248.

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9, 211–268. https://doi.org/10.1023/A:1008924418720.

Monge-Ortiz, R., Tomás-Vidal, A., Gallardo-Álvarez, F.J., Estruch, G., Godoy-Olmos, S., Jover-Cerdá, M., Martínez-Llorens, S., 2018. Partial and total replacement of fishmeal by a blend of animal and plant proteins in diets for *Seriola dumerili*: effects

on performance and nutrient efficiency. Aquac. Nutr. 24 (4), 1163–1174. https://doi.org/10.1111/anu.12655.
Montero, D., Izquierdo, M., 2010. Welfare and health of fish fed vegetable oils as alternative lipid sources to fish oil. In: Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds, pp. 439–485. https://doi.org/10.1201/9781439808634.
Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M.J., Izquierdo, M.S., 2003. Vegetable lipid sources for gilthead seabream (*Sparus aurata*): effects on fish health. Aquaculture 225 (1–4), 353–370. https://doi.org/10.1016/S0044-8486(03)00301-6.
Montero, D., Terova, G., Rimoldi, S., Tort, L., Negrin, D., Zamorano, M.J., Izquierdo, M., 2015. Modulation of adrenocorticotrophin hormone (ACTH)-induced expression of stress-related genes by PUFA in inter-renal cells from European sea bass (*Dicentrarchus labrax*). J. Nutr. Sci. 4, e16. https://doi.org/10.1017/jns.2015.6.
Moradi, N., Imanpoor, M., Taghizadeh, V., 2013. Hematological and biochemical changes induced by replacing fish meal with plant protein in the *Cyprinus carpio Linnaeus* (1785). Glob. Vet. 11 (2), 233–237. https://doi.org/10.5829/idosi.gv.2013.11.2.7533.
Nagarajan, D., Varjani, S., Lee, D.J., Chang, J.S., 2021. Sustainable aquaculture and animal feed from microalgae–nutritive value and techno-functional components. Renew. Sust. Energ. Rev. 150, 111549. https://doi.org/10.1016/j.rser.2021.111549.
Navarro-Guillén, C., Conceição, L.E.C., Pinto, W., Siguero, I., Urrutia, P., Moyano, F.J., Yúfera, M., 2019. Fast growing greater amberjack post-larvae require a high energy-high protein weaning diet. Aquaculture 499, 195–202. https://doi.org/10.1016/j.aquaculture.2018.09.037.
Navarro-Guillen, C., Yufera, M., Perera, E., 2022. Biochemical features and modulation of digestive enzymes by environmental temperature in the greater amberjack, *Seriola dumerili*. Front. Mar. Sci. 9, 960746.
Nijssen, E.J., Reinders, M.J., Krystallis, A., Tacken, G., 2019. Developing an internationalization strategy using diffusion modeling: the case of greater amberjack. Fishes 4 (1), 12. https://doi.org/10.3390/fishes4010012.
Oliva-Teles, A., Enes, P., Peres, H., 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In: Feed and Feeding Practices in Aquaculture, pp. 203–233. https://doi.org/10.1016/B978-0-08-100506-4.00008-8.
Olsvik, P.A., Torstensen, B.E., Hemre, G.I., Sanden, M., Waagbø, R., 2011. Hepatic oxidative stress in Atlantic salmon (*Salmo salar L.*) transferred from a diet based on marine feed ingredients to a diet based on plant ingredients. Aquac. Nutr. 17 (2), e424–e436. https://doi.org/10.1111/j.1365-2095.2010.00778.x.
Peng, K., Fu, B., Li, J., Zhao, H., Cao, J., Huang, W., Chen, B., Li, Z., Peng, Z., Wei, M., 2022. Effects of replacing soybean meal and rapeseed meal with faba bean meal on growth performance and muscle quality of tilapia (*Oreochromis niloticus)*. Aquac. Rep. 26, 101328. https://doi.org/10.1016/j.aqrep.2022.101328.
Peralta-Sánchez, J.M., Rabelo-Ruiz, M., Martín-Platero, A.M., Vizcaíno, A.J., Flores-Moreno, S., Macías-Vidal, J., Martínez-Bueno, M., 2024. Microalgae and phytase dietary supplementation improved growth and gut microbiota in juvenile European seabass (*Dicentrarchus labrax*). BMC Genomics 25 (1), 1–12. https://doi.org/10.1186/s12864-024-10760-x.
Perelló-Amorós, M., García-Pérez, I., Sánchez-Moya, A., Innamorati, A., Vélez, E.J., Achaerandio, I., Gutiérrez, J., 2021. Diet and exercise modulate GH-IGFs axis, proteolytic markers and myogenic regulatory factors in juveniles of gilthead sea bream (*Sparus aurata*). Animals 11 (8), 2182. https://doi.org/10.3390/ani11082182.
Perera, E., Sánchez-Ruiz, D., Sáez, M.I., Galafat, A., Barany, A., Fernández-Castro, M., Martos-Sitcha, J.A., 2020. Low dietary inclusion of nutraceuticals from microalgae improves feed efficiency and modifies intermediary metabolisms in gilthead sea bream (*Sparus aurata*). Sci. Rep. 10 (1), 18676. https://doi.org/10.1038/S41598-020-75693-3.
Polakof, S., Panserat, S., Soengas, J.L., Moon, T.W., 2012. Glucose metabolism in fish: a review. J. Comp. Physiol. B. 182, 1015–1045. https://doi.org/10.1007/s00360-012-0658-7.
Rahmdel, K.J., Noveirian, H.A., Falahatkar, B., Lashkan, A.B., 2018. Effects of replacing fish meal with sunflower meal on growth performance, body composition, hematological and biochemical indices of common carp (*Cyprinus carpio*) fingerlings. Fish. Aquat. Life 26 (2), 121–129. https://doi.org/10.2478/aopf-2018-0013.
Regost, C., Arzel, J., Kaushik, S.J., 1999. Partial or total replacement of fish meal by corn gluten meal in diet for turbot (*Psetta maxima*). Aquaculture 180 (1–2), 99–117. https://doi.org/10.1016/S0044-8486(99)00026-5.
Resende, D., Pereira, R., Domínguez, D., Pereira, M., Pereira, C., Pintado, M., Velasco, C., 2023. Stress response of European seabass (*Dicentrarchus labrax*) fed plant-based diets supplemented with swine blood hydrolysates. Aquac. Rep. 30, 101600. https://doi.org/10.1016/j.aqrep.2023.101600.
Robles, R., Mylonas, C.C., 2017. Exploring the biological and socio-economic potential of new/emerging candidate fish species for the expansion of the European aquaculture industry. Impact 2017 (1), 14–16. https://doi.org/10.21820/23987073.2017.1.14.
Rodehutscord, M., Abel, H.J., Friedt, W., Wenk, C., Flachowsky, G., Ahlgrimm, H.J., Breves, G., 2002. Consequences of the ban of by-products from terrestrial animals in livestock feeding in Germany and the European Union: alternatives, nutrient and energy cycles, plant production, and economic aspects. Arch. Anim. Nutr. 56 (2), 67–91. https://doi.org/10.1080/00039420214180.
Rodiles, A., Herrera, M., Hachero-Cruzado, I., Ruiz-Jarabo, I., Mancera, J.M., Cordero, M. L., Alarcón, F.J., 2015. Tissue composition, blood biochemistry and histology of digestive organs in Senegalese sole (*Solea senegalensis*) juveniles fed diets containing different plant protein ingredients. Aquac. Nutr. 21 (6), 767–779. https://doi.org/10.1111/anu.12207.
Roo, J., Hernández-Cruz, C.M., Mesa-Rodriguez, A., Fernández-Palacios, H., Izquierdo, M.S., 2019. Effect of increasing n-3 HUFA content in enriched *Artemia* on growth, survival and skeleton anomalies occurrence of greater amberjack *Seriola dumerili* larvae. Aquaculture 500, 651–659. https://doi.org/10.1016/j.aquaculture.2018.09.065.
Roohani, A.M., Kenari, A.A., Kapoorchali, M.F., Borani, M.S., Zoriezahra, S.J., Smiley, A. H., Esmaeili, N., Rombenso, A.N., 2019. Effect of spirulina *Spirulina platensis* as a complementary ingredient to reduce dietary fish meal on the growth performance, whole-body composition, fatty acid and amino acid profiles, and pigmentation of Caspian brown trout (*Salmo trutta caspius*) juveniles. Aquac. Nutr. 25, 633–645. https://doi.org/10.1111/anu.12885.
Sáez, M.I., Galafat, A., Tapia Paniagua, S.T., Martos-Sitcha, J.A., Alarcón-López, F.J., Martínez Moya, T.F., 2024. Feed additives based on *N. gaditana* and *A. platensis* blend improve quality parameters of aquacultured gilthead seabream (*Sparus aurata*) fresh fillets. Fishes 9 (6), 205. https://doi.org/10.3390/fishes9060205.
Saleh, H.H., 2020. Review on using of macro algae (seaweeds) in fish nutrition. J. Zool. Res. 2 (2). https://doi.org/10.30564/jzr.v2i2.2054.
Sansuwan, K., Kovitvadhi, S., Thongprajukaew, K., Ozório, R.O., Somsueb, P., Kovitvadhi, U., 2017. Microwave irradiation and pelleting method affected feed chemical composition and growth performance and feed utilization of sex-reversed Nile tilapia, *Oreochromis niloticus* (L.). Aquac. Res. 48 (4), 1836–1848. https://doi.org/10.1111/are.13021.
Santigosa, E., Sánchez, J., Médale, F., Kaushik, S., Pérez-Sánchez, J., Gallardo, M.A., 2008. Modifications of digestive enzymes in trout (*Oncorhynchus mykiss*) and sea bream (*Sparus aurata*) in response to dietary fish meal replacement by plant protein sources. Aquaculture 282 (1–4), 68–74. https://doi.org/10.1016/j.aquaculture.2008.06.007.
Sarker, M.S.A., Satoh, S., Kiron, V., 2007. Inclusion of citric acid and/or amino acid-chelated trace elements in alternate plant protein source diets affects growth and excretion of nitrogen and phosphorus in red sea bream *Pagrus major*. Aquaculture 262 (2–4), 436–443. https://doi.org/10.1016/j.aquaculture.2006.10.007.
Seibel, H., Chikwati, E., Schulz, C., Rebl, A., 2022. A multidisciplinary approach evaluating soybean meal-induced enteritis in rainbow trout *Oncorhynchus mykiss*. Fishes 7 (1), 22. https://doi.org/10.3390/fishes7010022.
Shah, M.R., Lutzu, G.A., Alam, A., Sarker, P., Kabir Chowdhury, M.A., Parsaeimehr, A., Daroch, M., 2018. Microalgae in aquafeeds for a sustainable aquaculture industry. J. Appl. Phycol. 30, 197–213. https://doi.org/10.1007/s10811-017-1234-z.
Shen, J., Liu, H., Tan, B., Dong, X., Yang, Q., Chi, S., Zhang, S., 2020. Effects of replacement of fishmeal with cottonseed protein concentrate on the growth, intestinal microflora, haematological and antioxidant indices of juvenile golden pompano (*Trachinotus ovatus*). Aquac. Nutr. 26 (4), 1119–1130. https://doi.org/10.1111/anu.13069.
Shen, N., Song, Z., Xia, C., Mu, H., Chen, M., Cheng, H., Xu, J., Sun, Y., Wie, C., Zhang, L., 2024. Comparative evaluation of soybean meal vs. extruded soybean meal as a replacer for fishmeal in diets of olive flounder (*Paralichthys olivaceus*): effects on growth performance and muscle quality. Aquaculture 578, 740136. https://ui.adsabs.harvard.edu/link_gateway/2024Aquac.57840136S/. https://doi.org/10.1016/j.aquaculture.2023.740136.
Siddik, M.A., Pham, H.D., Francis, D.S., Vo, B.V., Shahjahan, M., 2021. Dietary supplementation of fish protein hydrolysate in high plant protein diets modulates growth, liver and kidney health, and immunity of barramundi (*Lates calcarifer*). Aquac. Nutr. 27, 86–98. https://doi.org/10.1111/anu.13404.
Sitjà-Bobadilla, A., Peña-Llopis, S., Gómez-Requeni, P., Médale, F., Kaushik, S., Pérez-Sánchez, J., 2005. Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*). Aquaculture 249 (1–4), 387–400. https://doi.org/10.1016/j.aquaculture.2005.03.031.
Slawski, H., Adem, H., Tressel, R.P., Wysujack, K., Koops, U., Wuertz, S., Schulz, C., 2011. Replacement of fish meal with rapeseed protein concentrate in diets fed to wels catfish (*Silurus glanis L.*). Aquac. Nutr. 17 (6), 605–612. https://doi.org/10.1111/j.1365-2095.2011.00857.x.
Slawski, H., Adem, H., Tressel, R.P., Wysujack, K., Koops, U., Kotzamanis, Y., Schulz, C., 2012. Total fish meal replacement with rapeseed protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss Walbaum*). Aquac. Int. 20 (3), 443–453. https://doi.org/10.1007/s10499-011-9476-2.
Song, D., Tun, Y., Mi, J., Luo, J., Min, J., Nie, G., Zhou, Q., 2020. Effects of faba bean on growth performance and fillet texture of Yellow River carp, *Cyprinus carpio* haematopterus. Aquac. Rep. 17, 100379. https://doi.org/10.1016/j.aqrep.2020.100379.
Stone, D.A., Bellgrove, E.J., Forder, R.E., Howarth, G.S., Bansemer, M.S., 2018. Inducing subacute enteritis in yellowtail kingfish *Seriola lalandi*: the effect of dietary inclusion of soybean meal and grape seed extract on hindgut morphology and inflammation. N. Am. J. Aquac. 80 (1), 59–68. https://doi.org/10.1002/naaq.10002.
Sun, M., Hassan, S.G., Li, D., 2016. Models for estimating feed intake in aquaculture: a review. Comput. Electron. Agric. 127, 425–438. https://doi.org/10.1016/j.compag.2016.06.024.
Takakuwa, F., Fukada, H., Hosokawa, H., Masumoto, T., 2006. Optimum digestible protein and energy levels and ratio for greater amberjack *Seriola dumerili* (Risso) fingerling. Aquac. Res. 37 (15), 1532–1539. https://doi.org/10.1111/j.1365-2109.2006.01590.x.
Takakuwa, F., Suzuri, K., Horikawa, T., Nagahashi, K., Yamada, S., Biswas, A., Tanaka, H., 2020. Availability of potato protein concentrate as an alternative protein source to fish meal in greater amberjack (*Seriola dumerili*) diets. Aquac. Res. 51 (3), 1293–1302. https://doi.org/10.1111/are.14480.
Thongprajukaew, K., Kovitvadhi, U., Kovitvadhi, S., Somsueb, P., Rungruangsak-Torrissen, K., 2011. Effects of different modified diets on growth, digestive enzyme activities and muscle compositions in juvenile Siamese fighting fish (*Betta splendens*

Regan, 1910). Aquaculture 322, 1–9. https://doi.org/10.1016/j.aquaculture.2011.10.006.
Tibaldi, E., Hakim, Y., Uni, Z., Tulli, F., de Francesco, M., Luzzana, U., Harpaz, S., 2006. Effects of the partial substitution of dietary fish meal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass (*Dicentrarchus labrax*). Aquaculture 261 (1), 183–193. https://doi.org/10.1016/j.aquaculture.2006.06.026.
Tomás, A., de la Gándara, F., Garcia-Gomez, A., Perez, L., Jover, M., 2005. Utilization of soybean meal as an alternative protein source in the Mediterranean yellowtail, *Seriola dumerili*. Aquac. Nutr. 11 (5), 333–340. https://doi.org/10.1111/j.1365-2095.2005.00365.x.
Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.I., Berntssen, M.H.G., 2008. Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. Aquaculture 285 (1–4), 193–200. https://doi.org/10.1016/j.aquaculture.2008.08.025.
Van Der Toorn, M., Rezayat, D., Kauffman, H.F., Bakker, S.J., Gans, R.O., Koëter, G.H., Slebos, D.J., 2009. Lipid-soluble components in cigarette smoke induce mitochondrial production of reactive oxygen species in lung epithelial cells. Am. J. Phys. Lung Cell. Mol. Phys. 297 (1), L109–L114. https://doi.org/10.1152/ajplung.90461.2008.
Vélez, E.J., Azizi, S., Millán-Cubillo, A., Fernandez-Borras, J., Blasco, J., Chan, S.J., Gutiérrez, J., 2016. Effects of sustained exercise on GH-IGFs axis in gilthead sea bream (*Sparus aurata*). Am. J. Phys. Regul. Integr. Comp. Phys. 310 (4), R313–R322. https://doi.org/10.1152/ajpregu.00230.2015.
Vizcaíno, A.J., Mendes, S.I., Varela, J.L., Ruiz-Jarabo, I., Rico, R., Figueroa, F.L., Alarcón, F.J., 2016. Growth, tissue metabolites and digestive functionality in *Sparus aurata* juveniles fed different levels of macroalgae, *Gracilaria cornea* and *Ulva rigida*. Aquac. Res. 47 (10), 3224–3238. https://doi.org/10.1111/ARE.12774.
Vizcaíno, A.J., Sáez, M.I., Galafat, A., Galindo-Melero, R., Perera, E., Casal-Porras, I., Alarcón, F.J., 2024. Effects of feeding European seabass (*Dicentrarchus labrax*) juveniles with crude, hydrolysed and fermented biomass of the invasive macroalga *Rugulopteryx okamurae* (Ochrophyta). Aquac. Rep. 34, 101877. https://doi.org/10.1016/j.aqrep.2023.101877.
Wang, Y., Yu, S., Wang, Y., Che, J., Zhao, L., Bu, X., Yang, Y., 2015. Effect of replacing fish meal with soybean meal on growth, feed utilization and nitrogen and phosphorus excretion of juvenile *Pseudobagrus ussuriensis*. Aquac. Res. 47 (10), 3145–3155. https://doi.org/10.1111/are.12765.
Watanabe, T., Aoki, H., Viyakarn, V., Maita, M., Yamagata, Y., Satoh, S., Takeuchi, T., 1995. Combined use of alternative protein sources as a partial replacement for fish meal in a newly developed soft-dry pellet for yellowtail. Aquac. Sci. 43 (4), 511–520.
Yaakob, Z., Ali, E., Zainal, A., Mohamad, M., Takriff, M.S., 2014. An overview: biomolecules from microalgae for animal feed and aquaculture. J. Biol. Res.-Thessalon. 21 (1), 1–10. https://doi.org/10.1186/2241-5793-21-6.
Yang, Y.-H., Wang, Y.-Y., Lu, Y., Li, Q.-Z., 2010. Effect of replacing fish meal with soybean meal on growth, feed utilization and nitrogen and phosphorus excretion on rainbow trout (*Oncorhynchus mykiss*). Aquac. Int. 19, 405–419. https://doi.org/10.1007/s10499-010-9359-y.
Yao, S., Li, W., Cai, C., Wang, C., Kang, J., Hu, H., Ye, Y., 2024. Comparative study on the effects of four plant protein sources on the liver and intestinal health of largemouth bass, *Micropterus salmoides*. Aquac. Nutr. 2024. https://doi.org/10.1155/2024/6337005.
Yasuike, M., Iwasaki, Y., Nishiki, I., Nakamura, Y., Matsuura, A., Yoshida, K., Fujiwara, A., 2018. The yellowtail (*Seriola quinqueradiata*) genome and transcriptome atlas of the digestive tract. DNA Res. 25 (5), 547–560. https://doi.org/10.1093/dnares/dsy024.
Ye, H., Xu, M., Chen, L., Tan, X., Chen, S., Zou, C., Wang, A., 2019. Effects of dietary plant protein sources influencing hepatic lipid metabolism and hepatocyte apoptosis in hybrid grouper (*Epinephelus lanceolatus*♂ × *Epinephelus fuscoguttatus*♀). Aquaculture 506, 437–444. https://doi.org/10.1016/j.aquaculture.2019.03.075.
Zhang, Y., Chen, P., Liang, X.F., Han, J., Wu, X.F., Yang, Y.H., Xue, M., 2019. Metabolic disorder induces fatty liver in Japanese seabass, *Lateolabrax japonicas* fed a full plant protein diet and regulated by cAMP-JNK/NF-kB-caspase signal pathway. Fish Shellfish Immunol. 90, 223–234. https://doi.org/10.1016/j.fsi.2019.04.060.
Zhang, W., Tan, B., Deng, J., Haitao, Z., 2021. Multiomics analysis of soybean meal induced marine fish enteritis in juvenile pearl gentian grouper, *Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂. Sci. Rep. 11 (1), 23319. https://doi.org/10.1038/s41598-021-02278-z.
Zheng, C.C., Wu, J.W., Jin, Z.H., Ye, Z.F., Yang, S., Sun, Y.Q., Fei, H., 2020. Exogenous enzymes as functional additives in finfish aquaculture. Aquac. Nutr. 26 (2), 213–224. https://doi.org/10.1111/anu.12995.
Zhou, Q.C., Mai, K.S., Tan, B.P., Liu, Y.J., 2005. Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum*). Aquac. Nutr. 11 (3), 175–182. https://doi.org/10.1111/j.1365-2095.2005.00335.x.
Zhou, Y., Yuan, X., Liang, X.F., Fang, L., Li, J., Guo, X., He, S., 2013. Enhancement of growth and intestinal flora in grass carp: the effect of exogenous cellulase. Aquaculture 416, 1–7. https://doi.org/10.1016/j.aquaculture.2013.08.023.
Zuther, P.H., Maas, R.M., Blok, T., Kals, J., Nederlof, M.A., Prakash, S., Schrama, J.W., 2024. Effect of dietary starch on digestion kinetics and nutrient utilization in yellowtail kingfish (*Seriola lalandi*). Aquaculture 591, 741149. https://doi.org/10.1016/j.aquaculture.2024.741149.