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RESEARCH

Exploring *Palmaria palmata* as a dietary ingredient for European sea bass (*Dicentrarchus labrax*) juveniles

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Abstract

The red macroalgae *Palmaria palmata* is rich in protein and bioactive compounds and has been identified as a promising dietary ingredient for aquafeeds, with the potential to partially replace selected plant-based feed components. A digestibility trial and a growth trial were conducted to evaluate the potential of untreated and pretreated *P. palmata* as feed ingredients for juvenile European seabass (*Dicentrarchus labrax*), with initial body weights of 63.2 g and 38.0 g, respectively. Five diets were formulated: a control diet without macroalgae, and four diets including either 7.5% or 15% of untreated and pretreated *P. palmata*, replacing plant ingredients. The apparent digestibility coefficients of dry matter, crude protein, lipids, and gross energy did not differ among diets. The dietary inclusion of up to 15% *P. palmata* significantly increased the growth performance and feed efficiency compared to the control diet, without affecting nutrient digestibility, digestive enzyme activities, plasma metabolites, or whole-body composition of the fish. The pretreatment applied to *P. palmata* significantly increased soluble protein content and decreased neutral detergent fiber, but did not further improve growth or feed efficiency. Overall, these results indicate that up to 15% *P. palmata* can be successfully incorporated into the diets of juvenile European seabass, improving growth performance without adverse effects on nutrient digestibility. Further work is required to evaluate the functional properties and implications for fish health of *P. palmata* as well as to assess its economic viability within commercial aquafeed formulations.

Keywords Aquaculture · Macroalgae · High-solid loading hydrolysis · Renewable resources · Sustainably sourced feed components

Introduction

Global aquaculture production must continue to expand to meet the increasing demand for animal protein, driven by a growing population and the urgent need to alleviate poverty and malnutrition while promoting sustainable livelihoods (Madsen et al. 2022). In intensive aquaculture systems, feed costs remain a primary operational concern. Simultaneously,

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sustainability has become an equally critical concern, as the reduced dependency on fishmeal and fish oil has primarily been achieved through the inclusion of terrestrial plant ingredients that compete with human consumption (Glencross et al. 2024b; Tacon et al. 2022). This replacement also introduces economic and environmental limitations, as the shift from fish-based to plant-based feed ingredients has been associated with increased land and water use, higher nutrient discharge, and higher plant ingredient prices due to competition with human food consumption (Cottrell et al. 2025; Glencross et al. 2024a). Consequently, there is a pressing need to identify alternative, renewable, and locally available feed ingredients that can maintain fish health and performance while reducing the sector's environmental footprint (Glencross et al. 2024b; Hoseinifar et al. 2018; Rodiles et al. 2012).

Macroalgae have emerged as promising candidates for inclusion in aquafeed due to their high productivity, minimal cultivation requirements, and reduced environmental footprint. Their cultivation does not require freshwater or arable land and contributes to nutrient bioremediation through the sequestration of nitrogen and phosphorus (Hasselström et al. 2020; Mendez and Kwon 2023). Although the proximate composition of macroalgae varies among species and cultivation conditions, they are generally characterized by moderate protein content, low lipid levels, and high levels of nonstarch polysaccharides, minerals, and vitamins (Zhu et al. 2017; Xie et al. 2019; Fernandes et al. 2022; Vazirzadeh et al. 2022; Manikandan et al. 2022).

The dietary inclusion of macroalgae at low levels (typically below 5%) has been associated with similar or slightly improved growth performance and feed efficiency in several fish species (Wan et al. 2019; Weththasinghe et al. 2026). However, responses are species- and context-dependent (Weththasinghe et al. 2026). However, dietary inclusion levels above 10–20% often reduce growth performance and feed efficiency, likely due to the presence of the complex nonstarch polysaccharides that limit nutrient digestibility (Shpigel et al. 2017; Sotoudeh and Jafari 2017; Vizcaino et al. 2019; Weththasinghe et al. 2026).

Within this context, the red macroalgae *Palmaria palmata*, commonly known as dulse and widely distributed along the North Atlantic shores, has garnered attention as a protein-rich red macroalga (Rhodophyta) (Stévant et al. 2023). Red macroalgae generally have higher protein content than brown and green macroalgae (Fleurence 2016). *P. palmata* generally contains 7–19% of protein (dry weight, DW), with an amino acid profile often higher than many terrestrial sources (Mæhre et al. 2014; Rawiwan et al. 2022; Stévant et al. 2023). Protein levels up to 35% (DW) have also been reported (Morgan et al. 1981), depending on season, habitat, and cultivation method. Carbohydrates in *P. palmata* may account for up to 74% of DW, primarily as xylans (Lahaye et al. 1993; Deniaud et al. 2003), and its lipids have neutral, polar, and free fatty acids proportions of 36%, 37.5%, and 26.5%, respectively (Foseid et al. 2020). With the rising demand for *P. palmata* and the recognition that future sustainable commercial production cannot depend only on wild collection, researchers and industry have focused on cultivating this species within integrated multi-trophic aquaculture (IMTA) systems (Stévant et al. 2023).

Despite its promising composition, only one study to date has evaluated its potential as a feed ingredient for aquaculture species. In that study, dietary inclusion of 5, 10, and 15% *P. palmata* in the diet of Atlantic salmon (*Salmo salar*) did not affect growth performance and feed efficiency, while enhancing the hepatic function, suggesting that *P. palmata* can be safely incorporated as a dietary ingredient for Atlantic salmon (Wan et al. 2016).

The structural complexity of P. palmata cell walls, which are largely indigestible for fish, may limit nutrient bioavailability, thereby constraining its potential as a feed ingredient (Stévant et al. 2023). To improve nutrient bioavailability, polysaccharides in

macroalgae must be hydrolysed into smaller oligomers or individual sugar monomers (Martins et al. 2022). Accordingly, several pretreatment processes have been investigated to enhance nutrient bioavailability, including thermal processing, fermentation, enzymatic or alkaline hydrolysis, and ultrasonication (Brindo and Felix 2014; Güroy et al. 2013; Zhang et al. 2018; Zhu et al. 2016).

Short heat treatments have been reported as a practical and straightforward approach to increasing the protein digestibility of *P. palmata* as evaluated using an in vitro gastrointestinal model (Maehre et al. 2015). Enzymatic and alkaline extractions have also been applied to *P. palmata*, yielding antioxidant-rich extracts with high radical scavenging and metal-chelating activities (Ghelichi et al. 2024a). Optimized alkaline treatments for *P. palmata*, namely hydrolysis with NaOH at low solid-to-liquid ratios (1:20 and 1:15), effectively disrupt the cell wall, thereby increasing protein yield (Harnedy and FitzGerald 2013; Idowu et al. 2024). Alkaline hydrolysis processes performed at low solid loadings require large amounts of water and produce two phases: a liquid and a solid fraction, necessitating an additional separation step. Hydrolysis at high-solid loading yields a single slurry phase that concentrates both soluble and insoluble nutrients in one fraction, with minimal free water, thereby improving processing efficiency and reducing downstream costs (Shiva et al. 2022; Zhang et al. 2023; Shafiei-Alavijeh et al. 2024).

High-solid loading alkaline hydrolysis (1 N NaOH, 30 min) of *P. palmata* (Ramos-Oliveira et al. 2026) reduced fiber fractions and increased soluble protein and phenolic compounds, indicating enhanced extractability of intracellular components; thus, the present study aimed to evaluate the potential of untreated ot treated *P. palmata* as a dietary ingredient for juvenile European seabass (*Dicentrarchus labrax*), specifically as a partial replacement for wheat meal and, to a lesser extent, soybean meal within practical feed formulations.

Material and method

Ethics statement

All experiments were carried out by certified personnel in accordance with FELASA category C guidelines. The trials complied with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes and received approval from the CIIMAR Ethics Committee (reference ORBEA_CIIMAR_27_2019). European seabass juveniles were supplied by Atlantik Fish, Algarve, Portugal, and were quarantined for one month at CIIMAR bioterium. The fish were then transferred to the experimental systems and allowed to acclimate to the experimental conditions for at least 15 days before the beginning of the digestibility and feeding trials.

High-solid loading alkaline autoclave treatment

The high-solid loading alkaline hydrolysis of *P. palmata* was performed by mixing the biomass with an alkaline solution (NaOH 1 N, pH 11) to achieve a final humidity content of 75%. This mixture was then autoclaved at 121 °C for 30 min, dried for 48 h at 60 °C, ground to a particle size of 1 mm, and stored in sealed plastic bags at − 4 °C until use. The proximate composition of untreated and treated *P. palmata* is presented in Table 1.

Table 1 Proximate analysis (% dry matter) and pH of the untreated and high-solid loading alkaline hydrolysis (121 °C, 30 min) of *Palmaria palmata* used in the diets for European seabass

	Palmaria palmata	
	PP	PPT
Proximate analysis and pH		
Dry matter	93.4	97.2
Crude Protein	15.9	13.6
Soluble protein (mg/g)	3.66	8.55
Crude Lipid	1.1	1.1
Acid detergent fiber	4.88	3.45
Neutral detergent fiber	25.9	9.56
pH	6.55	9.95

PP untreated *P. palmata, PPT* alkaline-pretreated *P. palmata*

Experimental diets

A practical diet, with 44% protein and 18% lipids was formulated with fishmeal and a mixture of plant feedstuffs (corn gluten, wheat gluten, soybean meal, pea protein concentrate meal, and wheat meal) as protein sources, and fish oil as lipid source, and was used as a control. Four other diets were formulated similarly to the control diet but partially replacing plant feedstuffs with 7.5% or 15% of untreated *P. palmata* (diets 7.5PP and 15PP) or treated *P. palmata* (diets 7.5PPT and 15PPT). Chromic oxide (Cr_2O_3) was incorporated in the diets as an inert digestibility marker. All ingredients were finely ground, thoroughly mixed, and pelleted using a laboratory pellet mill (California Pellet Mill, Crawfordsville, IN, USA) through a 3-mm die. Afterward, these pellets underwent a 24-h drying process at 65 °C, were placed in plastic bags, and kept at − 4 °C until needed. Dietary composition and proximal composition (% dry matter) are presented in Table 2.

Digestibility trial

The digestibility trial was conducted in a recirculating aquaculture system (RAS) composed of 15 cylindrical fiberglass tanks (60 L capacity). Each tank was equipped with a fecal sedimentation column connected to the outlet, following the design described by Cho et al (1982). The system was supplied with continuously filtered seawater and aerated using air stones. Throughout the experimental period, water temperature was maintained at 22 ± 1 °C, salinity at 20 ± 2.0 ‰, pH 7 ± 0.1, and dissolved oxygen at 7.8 ± 0.2 mg/L. The photoperiod followed a 12-h light:12-h dark cycle, and the water flow was set at 5 L/min.

Six European seabass (*D. labrax*), with an initial average body weight of 63.2 g ± 1.2 g (mean ± SD), were stocked per tank. The diets were randomly assigned to triplicate groups and manually fed to apparent satiety, twice daily, at 9:00 a.m. and 4:00 p.m., 7 days a week. After 7 days of acclimatization to the experimental diets, feces were collected daily for 30 days from the sedimentation columns before morning feeding, centrifuged (4000 × *g*, 10 min), pooled per tank, and stored at –20 °C until analysis. Approximately 30 min after the afternoon meal, tanks, pipes, and sedimentation columns were thoroughly cleaned to remove any residual feces or uneaten feed.

Table 2 Ingredients and proximate composition (% dry matter) of the experimental diets used in the digestibility and feeding trials

	Control	7.5PP	7.5PPT	15PP	15PPT
Ingredients					
Untreated *P. palmata, PP*[1]	-	7.5	-	15	-
Pretreated *P. palmata, PPT*[2]	-	-	7.5	-	15
Fish meal[3]	20	20	20	20	20
Corn gluten[4]	12.5	12.5	12.5	12.5	12.5
Wheat gluten[5]	5.5	5.8	5.8	5.8	5.8
Soybean meal[6]	18.2	17.3	17.3	16.9	16.9
Pea protein concentrate meal[7]	10	10	10	10	10
Wheat meal[8]	15.0	7.7	7.7	0	0
Shrimp hydrolyzate[9]	0.7	0.7	0.7	0.7	0.7
Fish oil	13.7	13.9	13.9	14.2	14.2
Vitamin premix[10]	1	1	1	1	1
Choline chloride 50%[11]	0.5	0.5	0.5	0.5	0.5
Mineral premix[12]	1	1	1	1	1
Binder[13]	1	1	1	1	1
Dicalcium phosphate	0.0	0.2	0.2	0.5	0.5
Methionine	0.1	0.1	0.1	0.1	0.1
Taurine	0.3	0.3	0.3	0.3	0.3
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Proximate analysis and pH					
Dry matter	99.2	98.1	94.7	97.9	99.0
Crude Protein	43.5	44.5	44.3	44.3	44.4
Soluble protein (mg/g)					
Crude Lipid	18.3	18.2	16.6	16.6	16.7
Gross Energy (kJ/g)	26.4	25.9	25.8	25.9	25.6
Acid detergent fiber	3.41	3.45	2.87	4.90	4.06
Neutral detergent fiber	17.1	16.7	15.2	17.2	15.8
Ash	5.80	8.25	6.55	10.6	11.5
pH	5.8	5.6	6.5	5.6	7.0
Essential amino acids					
Arginine, Arg	1.39	1.54	1.57	1.52	1.54
Histidine, His	0.78	0.68	0.71	0.66	0.70
Isoleucine, Ile	1.10	1.20	1.31	1.22	1.29
Leucine, Leu	5.08	5.24	5.08	5.19	5.25
Lysine, Lys	2.51	1.90	1.96	2.00	1.86
Methionine, Met	1.07	1.07	0.79	0.94	0.95
Phenylalanine, Phe	1.70	1.84	1.89	1.99	2.11
Threonine, Thr	1.46	1.40	1.03	1.15	1.09
Valine, Val	1.08	1.32	1.37	1.28	1.32
Non-essential amino acids					
Tyrosine, Tyr	0.77	0.73	0.83	0.82	0.79
Alanine, Ala	4.99	4.86	4.56	4.85	4.68
Aspartic acid, Asp	2.61	2.57	2.65	2.53	2.60
Glutamic acid, Glu	4.73	4.20	4.33	4.17	4.30

Table 2 (continued)

	Control	7.5PP	7.5PPT	15PP	15PPT
Glycine, Gly	5.28	5.15	5.26	5.21	5.12
Serine, Ser	3.60	4.70	4.94	4.98	4.81
Proline, Pro	5.02	5.48	5.37	5.20	5.35

PP, untreated *P. palmata, PPT* alkaline-pretreated *P. palmata,* 7.5 and 15: dietary inclusion levels (%) of *P. palmata*

[1]ALGAplus Lda. Aveiro, Portugal (CP: 15.9%; CL: 1.4%)

[2]High-solid loading alkaline pretreatment of *P. palmata* was performed using 1 N NaOH at a solid-to-liquid ratio of 25:75 (w/w), followed by autoclaving for either 30 min (CP: 15.9%; CL: 1.4%)

[3]Pesquera Centinela, Steam Dried LT, Chile (CP: 77.5%; CL: 11.2%). Sorgal, S.A., Ovar, Portugal

[4]Sorgal, S.A., Ovar, Portugal (CP: 62.6%; CL: 4.1%)

[5]Sorgal, S.A Ovar, Portugal (CP: 76.7%; CL: 1.7%)

[6]Non-GMO; Cargill France SAS, St. Germain-en-Laye, France (CP: 51.6%; CL: 3.7%)

[7]Sorgal, S.A., Ovar, Portugal (CP: 49.4%; CL: 3.3)

[8]Sorgal, S.A., Ovar, Portugal (CP: 12.3%; CL: 3.2%)

[9]Sorgal, S.A. Ovar, Portugal

[10]Vitamins (mg/kg diet): retinol, 18,000 (IU/kg diet); calciferol, 2000 (IU/kg diet); alpha-tocopherol, 35; menadion sodium bis., 10; thiamin, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400

[11]Premix, Viana do Castelo, Portugal

[12]Minerals (mg/kg diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dicalcium phosphate, 8.02 (g/kg diet); potassium chloride, 1.15 (g/kg diet); sodium chloride, 0.4 (g/kg diet)

[13]Aquacube. Agil, UK

Apparent digestibility coefficients (ADCs) of dry matter, crude protein, lipids, gross energy, and amino acids were calculated using the following formula, where nutrient and energy levels correspond to the respective parameter analyzed:

$$ADC_{dry\ matter} = \left(1 - \left(\frac{dietary\ Cr_2O_3\ level}{feces\ Cr_2O_3\ level}\right)\right) \times\ 100$$

$$ADC_{nutrient\ or\ energy} = \left(1 - \left(\frac{dietary\ Cr_2O_3\ level\ \times\ feces\ nutrient\ or\ energy\ level}{feces\ Cr_2O_3\ level\ \times\ dietary\ nutrient\ or\ energy\ level}\right)\right)\ \times\ 100$$

Feeding trial and sampling

The experiment was conducted in a thermoregulated RAS composed of 15 cylindrical fiberglass tanks (each 500 L). During the trial, the water temperature was maintained at 22 °C ± 1 °C, the salinity averaged 20 ± 2.0‰, the pH was kept at 7.1 ± 0.1, and the oxygen levels were maintained at 7.80 ± 0.2 mg/L. Ammonia and nitrites were maintained below 0.1 and 0.3 mg/L, respectively. The photoperiod was controlled at 12 h of light to 12 h of darkness.

At the beginning of the trial, homogenous groups of 16 European sea bass (initial body weight of 38 g ± 0.5 g, mean ± SEM) were constituted and randomly distributed among the experimental tanks. Five individuals from the initial stock were sampled and combined to determine the initial whole-body composition. The experimental diets were randomly allocated to three replicate groups, and the fish were fed manually twice a day, six days per week, to apparent satiety, over a period of 11 weeks. At the end of the feeding trial, the fish were fasted for one day, and bulk weighed for each tank under mild anesthesia (2-phenoxyethanol, 0.3 ml/L). Three fish per tank were randomly sampled, killed with an overdose of anesthetic (2-phenoxyethanol, 10 ml/L). Total length, determined by digital imaging (following Azevedo et al. 2023), as well as whole-body, liver, and visceral weights, were recorded to calculate condition factors and hepatosomatic and visceral indexes. Carcasses, including liver and viscera of these fish, were pooled by tank for final whole-body composition analysis.

The remaining fish were fed for a three-day recovery period, and then three fish per tank were randomly sampled four hours after the first meal. Blood was collected from the caudal vein using heparinized syringes, centrifuged at 10,000 × *g* for 10 min, and the plasma obtained was stored at –80 °C for subsequent analyses. Then, fish were killed with an overdose of an anesthetic (2-phenoxyethanol, 10 ml/L) followed by decapitation, and the whole intestine, including the pyloric caeca, was excised, cleaned of fat and adherent tissues, and stored at –80 °C for subsequent analysis.

Digestive enzymes

For the assessment of digestive enzyme activities, intestinal samples ($n = 9$ per diet) were diluted 1:9 and homogenized in a buffer containing 0.1 M K_2HPO_4 and 0.1 M KH_2PO_4 at pH 7.4. The homogenates were centrifuged at 10,000 × g for 20 min at 4 °C, and the resulting supernatants were aliquoted and stored at –80 °C for subsequent enzyme analyses. Amylase (EC 3.2.1.1) and lipase (EC 3.1.1.3) activities were determined using commercial kits (α-Amylase-LQ, Ref. 1,004,102; and Lipase-LQ, Ref. 1,001,275; Spinreact S.A., Girona, Spain), adapted for kinetic analysis in 96-well microplates. Amylase activity was determined by monitoring the production of 2-chloro-4-nitrophenol at 405 nm, while lipase activity was quantified by measuring the formation of methyl resorufin at 580 nm.

For total alkaline protease activity, samples were incubated for 1 h at 37 °C in a reaction mixture containing 1% (w/v) casein in 0.1 M Tris–HCl buffer, pH 8.0. The reaction was stopped by adding 8% (w/v) trichloroacetic acid, then incubated at 4 °C for 1 h before centrifugation at 1800 × *g* for 10 min. The absorbance of the supernatant was then measured at 280 nm. Protease activity was calculated from a tyrosine standard curve and expressed as U g^{-1} of sample. One unit activity was defined as the amount of enzyme required to release 1.0 µmol of tyrosine per minute.

Trypsin (EC 3.4.21.4) activity was determined using Nα-benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPNA, 0.5 mM) as the substrate, whereas chymotrypsin (EC 3.4.21.1) activity was measured with N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (SAPNA, 0.2 mM). Reaction mixtures were prepared in Tris–HCl buffer supplemented with calcium chloride (50 mM Tris–HCl and 20 mM $CaCl_2$, pH 8.5 for trypsin; 25 mM $CaCl_2$, pH 7.8 for chymotrypsin). The formation of p-nitroaniline was quantified spectrophotometrically at 410 nm using a molar extinction coefficient of 8.8 $mM^{-1} \cdot cm^{-1}$. All enzyme activities were normalized to the soluble protein content of the tissue samples,

measured by the Bradford method (Bradford 1976), using bovine serum albumin as the standard. Digestive enzymes were analyzed using a Multiskan GO microplate reader (model 5111 9200, Thermo Scientific, Nanjing, China).

Plasma metabolites

Plasma concentrations of total protein, albumin, glucose, cholesterol, and triglycerides were determined using commercial assay kits supplied by SpinReact S.A. (Gerona, Spain; refs. 1,001,191, 1,001,091, 41,031, 1,001,291, and 1,001,023, respectively), adapted for use in microplates. Plasma metabolite measurements were performed with a Multiskan GO microplate spectrophotometer (Thermo Scientific, model 5111 9200; Nanjing, China).

Proximate analysis

Chemical analysis of diets, feces, and whole-body composition was performed according to standard methods (Association of Official Analytical Chemists 1980). The initial and final whole-body fish were dried at 100 °C until a constant weight was achieved, ground, and stored in a desiccator until use. The dry matter determined by drying the samples at 105 °C until constant weight; ash by incineration in a muffle furnace at 500 °C for 24 h; protein content (N × 6.25) according to the Kjeldahl method following acid digestion using Kjeltec digestion (Tecator systems, Höganäs, Sweden, models 1015 and 1026, respectively); lipid content by extraction with petroleum ether using a SoxTec (Tecator systems, Höganäs, Sweden, extraction unit model 1043 and service unit model 1046); gross energy by direct combustion in an adiabatic bomb calorimeter (Parr model 6200; Parr Instruments, USA); acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined as described by Van Soest et al. (1991) using a FIBRETHERM FT12 apparatus (13–0026, 12-place, automatic system).

Amino acid composition was determined according to the method described by SSADS-CACTI (University of Vigo), involving acid hydrolysis followed by HPLC–MS/MS analysis (Muñoz-Seijas et al. 2024).

Statistical analysis

Data were presented as the mean of untransformed data and the pooled standard error of the mean. Data were checked for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test). When these assumptions were not met, data were log- or square-root transformed prior to analysis.

Comparisons between the control diet and each test diet were performed using non-orthogonal contrasts. Differences among test diets containing 7.5% or 15% of untreated (PP) or pretreated (PPT) *P. palmata* were analyzed by two-way analysis of variance (ANOVA), with inclusion level (7.5% vs 15%) and treatment (PP vs PPT) as fixed factors. When a significant interaction between inclusion level and treatment was detected, pairwise comparisons were performed using Student's *t*-tests.

A probability level of 0.05 was used to reject the null hypothesis. All analyses were performed using IBM SPSS Statistics version 26 (IBM, Armonk, NY, USA).

Results

The apparent digestibility coefficients of dry matter, crude protein, lipids, gross energy, and neutral detergent fiber did not differ among diets (Table 3). The ADC of several amino acids was higher in fish fed with 7.5PP, 15PP, and 7.5PPT diets than in the control diet. Namely, the ADC of isoleucine, threonine, valine, and serine were significantly higher in fish fed the 7.5PP diet than the control diet (14.2%, 8.4%, 24.5%, 6.2%, respectively). The ADC of arginine, isoleucine, valine, tyrosine, aspartic acid, and serine was significantly higher in fish fed the 7.5PPT diet than the control diet (12.6%, 17.7%, 28.4%, 15.7%, 8.3%, 7.5%, respectively). The ADC of isoleucine, leucine, phenylalanine, valine, tyrosine, alanine, glutamic acid, serine, and proline was significantly higher in fish fed the 15PP diet than in those fed the control diet (13.8%, 7.8%, 16.1%, 32.7%, 17.0%, 3.8%, 12.2%, 8.8%, 7.1%, respectively). In contrast, the ADC of lysine and threonine was significantly lower in fish fed the 15PPT diet than in those fed the control diet (6.1%, 18.8%, respectively).

Irrespective of the dietary inclusion levels, the pretreatment significantly decreased the ADC of isoleucine, leucine, methionine, phenylalanine, alanine, and aspartic acid (Table 3). Regardless of treatment, increasing the dietary inclusion level significantly reduced the ADC of isoleucine, leucine, alanine, and aspartic acid. A significant interaction between pretreatment and inclusion level was observed for the digestibility of arginine, threonine, valine, glutamic acid, and proline. Increasing the inclusion level decreased the digestibility of arginine, valine, and proline in diets including pretreated *P. palmata*. In diets including untreated *P. palmata*, threonine digestibility was significantly higher at a 7.5% inclusion level. In contrast, at a 15% inclusion level, diets containing untreated *P. palmata* showed higher glutamic acid digestibility than the pretreated diet.

European seabass juveniles accepted all diets well, and no mortality occurred (Table 4). In diets containing *P. palmata*, no effects of treatment or dietary inclusion level were observed. Final body weight was significantly higher in fish fed 7.5PP (15.1%), 7.5PPT (13.3%), 15PP (18.1%), and 15PPT (11.8%) diets compared to the control diet. Weight gain and daily growth index were similarly increased in these groups. The final body length was also significantly higher in fish fed the 7.5PP diet (3.2%) and 15PP diet (3.3%), while the final condition index was significantly higher in fish fed the 7.5PPT diet (9.5%) compared to the control diet. Feed intake was higher in fish fed all diets, including *P. palmata*, than in the control; however, the difference was only statistically significant with the 15PPT diet (8.6%). This resulted in feed efficiency being significantly higher in fish fed the 7.5PP, 7.5PPT, and 15PP diets than in those fed the control diet (14.9%, 14.9%, 16.4%, respectively).

The protein efficiency ratio was also higher in fish fed diets containing *P. palmata* than in the control; however, the difference was only statistically significant with the 15PP diet (15.0%) (Table 4). Nitrogen retention (g/kg ABW/day) was significantly higher in fish fed the 7.5PP (42.1%) and 15PP (42.1%) diets compared to the control diet. Energy retention (kJ/kg ABW/day) was similar between the control diet and each test diet. Regardless of inclusion levels, pretreatment of *P. palmata* significantly decreased nitrogen retention (g/kg ABW/day).

No differences were observed among diets for whole-body dry matter, lipid, ash, gross energy, or visceral index (Table 5). Regardless of dietary inclusion level, pretreatment of *P. palmata* resulted in a decrease in whole-body protein content. Compared with the control diet, the hepatosomatic index was significantly lower in fish fed 7.5PP (25%), 7.5PPT (20.9%), 15PP (30.4%), and 15PPT (29.1%) diets.

Table 3 Apparent digestibility coefficients (%) of the diets

	Control	7.5PP	7.5PPT	15PP	15PPT	SEM
Dry matter	66.8	67.9	66.7	68.6	68.8	0.150
Protein	78.3	82.3	79.6	80.4	75.5	0.375
Lipids	80.2	83.1	77.0	87.1	71.8	0.868
Energy	74.6	76.5	76.6	77.1	71.4	0.352
Neutral detergent fiber	49.1	45.8	37.7	43.4	58.4	1.145
Amino acids						
Arginine, Arg	70.5	78.4	80.7$^{■△}$	74.8	66.3$^{▲}$	0.876
Histidine, His	86.2	89.3	90.6	90.2	83.2	0.468
Isoleucine, Ile	66.8	76.3$^{■}$	78.6$^{■}$	76.0$^{■}$	64.9	0.925
Leucine, Leu	84.9	88.6	88.8	91.5$^{■}$	82.7	0.523
Lysine, Lys	93.9	93.3	94.3	93.5	88.2$^{■}$	0.374
Methionine, Met	91.7	94.7	93.5	96.0	88.6	0.428
Phenylalanine, Phe	77.1	83.7	84.7	89.5$^{■}$	77.4	0.784
Threonine, Thr	70.2	76.1$^{■○}$	68.2$^{•}$	71.8	57.0$^{■}$	1.064
Valine, Val	56.0	69.7$^{■}$	71.9$^{■△}$	74.3$^{■}$	55.9$^{▲}$	1.328
Tyrosine, Tyr	70.5	77.5	81.6$^{■}$	82.5$^{■}$	68.2	0.966
Alanine, Ala	91.2	92.4	92.4	94.7$^{■}$	88.3	0.348
Aspartic acid, Asp	75.5	80.6	81.8$^{■}$	80.9	72.1	0.622
Glutamic acid, Glu	77.1	80.8	82.0	86.5$^{■○}$	70.1$^{•}$	0.913
Glycine, Gly	93.4	93.9	94.6	82.1	92.2	0.771
Serine, Ser	84.2	89.4$^{■}$	90.5$^{■}$	91.6$^{■}$	86.0	0.465
Proline, Pro	86.9	90.0	90.5$^{△}$	93.1$^{■}$	85.5$^{▲}$	0.450

Two-way-ANOVA	Variable Source		
	Treatment	Inclusion level	Interaction
Dry matter	ns	ns	ns
Protein	ns	ns	ns
Lipids	ns	ns	ns
Energy	ns	ns	ns
Neutral detergent fiber	ns	ns	ns
Amino acids			
Arginine, Arg	ns	**	**
Histidine, His	ns	ns	ns
Isoleucine, Ile	**	***	ns
Leucine, Leu	**	*	ns
Lysine, Lys	ns	ns	ns
Methionine, Met	*	ns	ns
Phenylalanine, Phe	**	ns	ns
Threonine, Thr	***	***	**
Valine, Val	ns	***	**
Tyrosine, Tyr	ns	ns	ns
Alanine, Ala	***	**	ns
Aspartic acid, Asp	*	**	ns
Glutamic acid, Glu	*	*	**

Table 3 (continued)

Glycine, Gly	ns	ns	ns
Serine, Ser	ns	ns	ns
Proline, Pro	**	**	**

PP untreated *P. palmata, PPT* alkaline-pretreated *P. palmata,* 7.5 and 15: dietary inclusion levels (%) of *P. palmata*

Values are presented as mean ($n = 3$) and pooled standard error of the mean (SEM)

■ Denotes significant differences (non-orthogonal contrast analysis) between the control diet and each test diet

ns-: non-significant ($p \geq 0.05$); $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

○, ● indicate differences between treatments; △, ▲ indicate differences between inclusion levels

The activities of trypsin, chymotrypsin, amylase, and lipase in European seabass were not affected by the experimental diets, except for total protease activity, which was significantly lower in fish fed the 15PPT diet than in those fed the control diet (48.7%) (Table 6).

Plasma cholesterol, total protein, triglyceride, glucose, and albumin levels were not affected by the experimental diets (Table 7).

Discussion

P. palmata has been identified as a promising dietary ingredient for aquaculture that may contribute to the diversification of more sustainable aquafeed formulations. However, its potential as a feed ingredient remains underexplored, particularly in marine fish species. In the present study, the dietary inclusion of up to 15% *P. palmata*, replacing wheat meal, did not compromise the apparent digestibility of the diets. Moreover, its nutrient bioavailability seems to be higher than that of other macroalgae previously tested in European seabass. For example, the dietary incorporation of 8% *Gracilaria gracilis* reduced dry matter, protein, and energy digestibility (Valente et al. 2021), whereas the dietary incorporation of 10% *Gracilaria cornea* resulted in lower dry matter and lipid digestibility (Valente et al. 2006).

In the present study, we employed a novel approach by applying high-solid loading alkaline autoclaving (1 N NaOH for 30 min) to the macroalgae. Although the alkaline autoclave treatment at high solid loading significantly reduced neutral detergent fiber and increased soluble protein of *P. palmata*, the dietary inclusion of pretreated *P. palmata* did not improve overall digestibility. Indeed, it even reduced the digestibility of some amino acids. The reduction in digestibility may be linked to the effects of heat and alkali treatments on the chemical and physical properties of the treated *P. palmata*, which may have limited the access of digestibility enzymes to the digesta. Indeed, in the present study, after treatment, soluble protein increased from 3.66 mg/g in untreated *P. palmata* to 8.55 mg/g in treated *P. palmata*, suggesting that the treatment induced changes in protein conformation and possibly chemical modifications (Ghelichi et al. 2024). Thermal treatment (50 and 90 °C for 15–60 min) has been reported to cause thickening and incipient gel formation of soluble *P. palmata* polysaccharide with a marked increase in viscosity (Lafeuille et al. 2022). Moreover, thermal (temperatures above 60 °C) and alkaline treatments have also been reported to cause extensive restructuring of the

Table 4 Growth performance and feed utilization of European sea bass juveniles fed the experimental diets

	Control	7.5PP	7.5PPT	15PP	15PPT	SEM
Initial body weight (IBW, g)	38.0	38.0	38.0	38.0	38.0	0.001
Final body weight (FBW, g)	68.4	78.7■	77.5■	80.8■	76.5■	1.423
Weight gain (g/kg/day)	7.71	9.39■	9.24■	9.71■	9.07■	0.226
Initial body length (IBL, mm)	160.1	161.1	160.1	159.5	160.5	0.088
Final body length (FBL, mm)	187.0	192.9■	189.1	193.1■	190.8	0.390
Initial condition index[1]	0.93	0.91	0.94	0.93	0.92	0.002
Final condition index[1]	1.05	1.09	1.15■	1.12	1.11	0.016
Daily growth index[2]	0.98	1.24■	1.22■	1.30■	1.19■	0.036
Feed intake (% BW/day)	1.16	1.22	1.20	1.24	1.26■	0.160
Feed efficiency[3]	0.67	0.77■	0.77■	0.78■	0.72	0.015
Protein efficiency ratio[4]	1.53	1.74	1.74	1.76■	1.62	0.032
Nitrogen retention (g/kg ABW/day)[5]	0.19	0.27■	0.24	0.27■	0.21	0.005
Energy retention (kJ/kg ABW/day)[5]	100.9	116.2	114.2	104.0	99.9	3.930
Mortality (%)	0.0	0.0	0.0	0.0	0.0	0.000
Two-way-ANOVA	Variable Source					
	Treatment		Inclusion		Interaction	
Initial body weight (IBW, g)	ns		ns		ns	
Final body weight (FBW, g)	ns		ns		ns	
Weight gain (g/kg/day)	ns		ns		ns	
Initial body length (IBL, mm)	ns		ns		ns	
Final body length (FBL, mm)	ns		ns		ns	
Initial condition index	ns		ns		ns	
Final condition index	ns		ns		ns	
Daily growth index	ns		ns		ns	
Feed intake (% BW/day)	ns		ns		ns	
Feed efficiency	ns		ns		ns	
Protein efficiency ratio[4]	ns		ns		ns	
Nitrogen retention (g/kg ABW/day)[5]	*		ns		ns	
Energy retention (kJ/kg ABW/day)[5]	ns		ns		ns	
Survival (%)	ns		ns		ns	

PP untreated *P. palmata, PPT* alkaline-pretreated *P. palmata,* 7.5 and 15: dietary inclusion levels (%) of *P. palmata*

Values are presented as mean ($n = 3$) and pooled standard error of the mean (SEM)

■Denotes significant differences (non-orthogonal contrast analysis) between the control diet and each test diet (7.5PP; 7.5PPT; 15PP; 15PPT)

Two-way ANOVA: ns: non-significant ($p \geq 0.05$); $^{*}p < 0.05$

[1]Initial or final condition index = (IBW or FWB/IBL^3) × 100

[2]Daily growth index = (FBW$^{1/3}$ − IBW$^{1/3}$)/time in days) × 100

[3]Feed efficiency = wet weight gain/dry feed intake

[4]Protein efficiency ratio = wet weight gain/crude protein intake

[5]Nitrogen or energy retention, NR or ER (g or kJ g/kg/day) = ((FBW × carcass N or energy content)—(IBW × carcass N or energy content))/(ABW × number of days)

Table 5 Whole-body composition (% fresh weight), hepatosomatic, and visceral indices of European sea bass juveniles fed experimental diets

	Initial	Control	7.5PP	7.5PPT	15PP	15PPT	SEM
Dry matter	26.9	35.1	33.6	33.7	33.8	33.5	0.353
Protein	15.0	15.2	16.4	15.6	16.3	14.7	0.120
Lipid	6.87	14.2	12.4	13.2	13.4	12.8	0.325
Ash	6.25	4.32	4.30	4.83	4.84	5.14	0.085
Gross energy (kJ/g)	6.91	9.66	9.72	9.69	8.91	8.97	0.161
Hepatosomatic index[1]	—	2.20	1.65■	1.74■	1.53■	1.56■	0.041
Visceral index[2]	—	11.3	9.74	9.48	10.0	9.20	0.122

Two-way-ANOVA	Variable Source		
	Treatment	Inclusion	Interaction
Dry matter	ns	ns	ns
Protein	*	ns	ns
Lipid	ns	ns	ns
Ash	ns	ns	ns
Gross energy (kJ/g)	ns	ns	ns
Hepatosomatic index[1]	ns	ns	ns
Visceral index[2]	ns	ns	ns

PP untreated *P. palmata, PPT* alkaline-pretreated *P. palmata,* 7.5 and 15: dietary inclusion levels (%) of *P. palmata*

Values are presented as mean ($n = 3$) and pooled standard error of the mean (SEM)

■Denotes significant differences (non-orthogonal contrast analysis) between the control group and each test group (7.5PP; 7.5PPT; 15PP; 15PPT)

Two-way ANOVA: ns: non-significant ($p \geq 0.05$); $^{*}p < 0.05$

[1] Hepatosomatic index = (liver weight/whole body weight) × 100

[2] Visceral index = (visceral weight/whole body weight) × 100

polysaccharide–protein matrix of *P. palmata* and alterations in the tertiary structure of the proteins, promoting protein unfolding and aggregation (Coelho et al. 2025; Harnedy and FitzGerald 2013). Such structural modifications of both polysaccharide and protein fractions likely promote the formation of a more compact, less enzyme-accessible digesta, thereby contributing to the treatment's adverse effect on digestibility, which was more evident at 15% than at 7.5% dietary inclusion.

In line with the apparent digestibility of the diets, dietary inclusion of *P. palmata* did not affect digestive enzyme activities, except for total protease activity in fish fed the 15PPT, which was lower than in the control. Previous studies have reported variable effects of dietary macroalgae inclusion, which may be related to the type of algae tested or differences between fish species. Also, in European sea bass, the dietary inclusion of 5% *Rugulopteryx okamurae* resulted in a marked reduction in digestive enzyme activity, which was partially or almost completely reversed when the macroalgal biomass was hydrolyzed or fermented (Vizcaíno et al. 2024). Similarly, in Nile tilapia (*Oreochromis niloticus*), fermentation of *Enteromorpha prolifera* increased gastrointestinal protease,

Table 6 Intestine enzyme activity (mU/mg protein) of European seabass juveniles fed the experimental diets

	Control	7.5PP	7.5PPT	15PP	15PPT	SEM
Total protease	131.3	105.7	101.8	110.4	67.3■	7.159
Trypsin	59.5	49.0	52.3	52.5	46.8	3.800
Chymotrypsin	3.81	4.12	3.74	3.37	5.22	0.207
Amylase	13.9	20.4	13.6	13.2	14.8	0.911
Lipase	29.3	40.8	27.3	31.7	36.3	1.495
Two-way-ANOVA	Variable Source					
	Treatment	Inclusion	Interaction			
Total protease	ns	ns	ns			
Trypsin	ns	ns	ns			
Chymotrypsin	ns	ns	ns			
Amylase	ns	ns	ns			
Lipase	ns	ns	ns			

PP untreated *P. palmata, PPT*, alkaline-pretreated *P. palmata,* 7.5 and 15: dietary inclusion levels (%) of *P. palmata*

Values are presented as the mean ($n = 9$) and pooled standard error of the mean (SEM)

■Denotes significant differences (non-orthogonal contrast analysis) between the control diet and each test diet (7.5PP; 7.5PPT; 15PP; 15PPT)

Two-way ANOVA: ns: non-significant

Table 7 Plasma metabolite quantification (mg/dL) of European sea bass juveniles fed with the experimental diets

	Control	7.5PP	7.5PPT	15PP	15PPT	SEM
Total protein	159.5	143.2	159.4	156.8	140.3	1.386
Albumin	0.77	0.62	0.77	0.59	0.75	0.013
Cholesterol	122.7	134.4	145.6	128.6	148.5	1.636
Triglycerides	697.2	679.3	793.4	740.7	721.6	6.575
Glucose	64.4	64.1	67.0	71.4	76.2	0.780
Two-way-ANOVA	Variable Source					
	Treatment	Inclusion	Interaction			
Total protein	ns	ns	ns			
Albumin	ns	ns	ns			
Cholesterol	ns	ns	ns			
Triglycerides	ns	ns	ns			
Glucose	ns	ns	ns			

PP untreated *P. palmata, PPT* alkaline-pretreated *P. palmata,* 7.5 and 15: dietary inclusion levels (%) of *P. palmata*

Values are presented as mean ($n = 9$) and pooled standard error of the mean (SEM)

No significant differences were found (non-orthogonal contrast analysis) between the control group versus each test group (7.5PP; 7.5PPT; 15PP; 15PPT)

Two-way ANOVA: ns: non-significant ($p \geq 0.05$)

amylase, and lipase activities (Yang et al. 2016). In red sea bream fed diets containing up to 15% *G. lemaneiformis,* no significant differences were also observed in protease and amylase activities. However, lipase activity decreased at the highest dietary inclusion levels (12–15%) (Xuan et al. 2019). In contrast, rainbow trout (*Oncorhynchus mykiss*) protease activity was decreased in diets containing 12% *G. pygmaea*. Similarly, lipase activity was increased in fish fed 6–12% *G. pygmaea,* while amylase activity was not affected by dietary macroalgae inclusion level (Sotoudeh and Mardani 2018).

Studies evaluating the effect of pretreated macroalgae on digestibility in fish are still limited, and the available evidence suggests that outcomes depend on the fish and macroalgae species, as well as the processing method used. Previously, two technological processes for macroalgae treatment were tested: a physical–mechanical rupture using a vibratory grinding mill to disrupt algal cell walls, and an enzymatic lysis applied after physical disruption using a commercial enzyme cocktail containing lipase, pectinase, cellulase, and amylase. The physical–mechanical rupture treatment did not improve the digestibility of dry matter, protein, lipid, or energy in either *G. gracilis* or *U. rigida* (Batista et al. 2020a, 2020b). In contrast, the enzymatic treatment of *U. rigida* even reduced the digestibility of lipids, protein, and methionine (Batista et al. 2020a, 2020b). On the other hand, a physical–mechanical rupture process based on a vibratory grinding method, followed by controlled drying of the disrupted biomass, was implemented in *G. gracilis*, which increased both protein and energy digestibility (Valente et al. 2021).

Dietary inclusion of *P. palmata* as a replacement for wheat meal significantly improved the growth performance of European seabass compared with the control, with no differences observed between inclusion levels or treatment types. Voluntary feed intake was slightly higher with the diets including *P. palmata* than in the control, although the difference was not statistically significant (except for the 15PPT group). On the other hand, feed efficiency increased (except for the 15PPT group), indicating that the enhanced growth was due to improved nutrient utilization rather than increased feed consumption. Given its limited contribution to total dietary protein, these effects are unlikely to result from protein replacement but rather from the macroalga's functional properties, such as the presence of bioactive compounds or improved palatability.

Accordingly, N retention (g/kg ABW/day) was also higher in fish fed the *P. palmata* diets, and the difference from the control was statistically significant in the groups fed the untreated macroalgae. These results suggest that this red macroalga can be incorporated into diets for European seabass at levels up to 15% as a dietary ingredient with potential to contribute to more sustainable aquafeed formulations. Previously, studies in Atlantic salmon (*Salmo salar*) also reported no adverse effects on growth when up to 15% *P. palmata* was incorporated into the diet (Wan et al. 2016). The effects of dietary inclusion of red macroalgae seem to be species- and level-dependent. For example, in European sea bass, the dietary inclusion of 8% *G. gracilis* did not affect growth and feed utilization, whereas 10% *G. cornea* reduced growth (Valente et al. 2006, 2021). On the other hand, in gilthead sea bream (*Sparus aurata*), dietary inclusion of 2.5–5% *G. gracilis* improved growth performance (Passos et al. 2021). At the inclusion levels tested, these macroalgae should be regarded primarily as a functional dietary ingredient rather than as a conventional protein source, although they may have potential beneficial effects on fish performance, possibly through bioactive compounds that enhance nutrient utilization and metabolic efficiency. Contrary to expectations, dietary inclusion of treated *P. palmata* did not

further improve growth performance and reduced the N retention (g/kg ABW/day). It is known that both the biomass-to-solvent ratio and the alkaline solvent used in the treatment of *P. palmata* affect protein and essential amino acids yield. For example, at low biomass-to-liquid ratios (1:20 and 1:15), KOH and NaOH were shown to extract higher amounts of protein and essential amino acids than Na_2CO_3 (Idowu et al. 2024). However, the heat and alkali treatment applied in this study may have induced the oxidation of some amino acids (e.g., methionine, tyrosine, and threonine) and the formation of protein–protein and protein–carbohydrate crosslinks, thereby decreasing digestibility (Cabral et al. 2022; El Hosry et al. 2025). Furthermore, the feed intake of the 15PPT diet was higher than that of the control diet, which may be related to the lower digestibility of some essential amino acids in the treated diets, leading the animals to increase their voluntary feed intake to meet their nutritional needs, as also previously observed in European seabass (Peres and Oliva-Teles 1999; Boujard et al. 2004).

In the present study, dietary inclusion of *P. palmata* up to 15% did not affect the whole-body proximate composition of European seabass, except for a lower whole-body protein content in fish fed diets containing pretreated *P. palmata* (7.5PPT and 15PPT). This reduction is consistent with the lower nitrogen retention (g/kg ABW/day) observed in fish fed the pretreated diets, regardless of inclusion level, suggesting reduced protein utilization efficiency. This effect may be associated with structural or chemical modifications of proteins induced by the pretreatment, potentially decreasing their bioavailability, as discussed previously. Also, in Atlantic salmon fed diets containing up to 15% *P. palmata*, the whole-body protein, ash, and energy content remained unaffected. However, lipid content was increased in fish fed diets with 5% algae (Wan et al. 2016). Similarly, European seabass fed diets containing up to 10% *Gracilaria bursa-pastoris* or *G. cornea* (Valente et al. 2006) or 8% *G. gracilis* (Batista et al. 2020a, 2020b) showed no significant changes in whole-body proximate composition. Likewise, diets containing 8% *G. gracilis* treated by vibratory grinding did not result in significant differences in whole-body or muscle composition in European seabass (Valente et al. 2021).

The visceral index (VI) also remained unchanged with diet composition. In contrast, the hepatosomatic index (HSI) was lower in fish fed diets containing *P. palmata* than in those fed the control diet. This reduction of HSI may be related to functional properties of *P. palmata*, which may have modulated hepatic lipid deposition. Indeed, in Atlantic salmon, the dietary inclusion of *P. palmata* improved liver health status, despite no changes in HSI or VI being observed (Wan et al. 2016). The functional properties of *P. palmata* require further evaluation.

The plasma metabolite profile of European seabass was also unaffected by either the inclusion level or pretreatment with *P. palmata*. Similarly, European seabass fed 2.5–5% *G. gracilis* showed no changes in plasma glucose, total protein, or triglyceride levels (Passos et al. 2021), while an increase in plasma glucose and a reduction of plasma triglycerides were observed when fed 8% *G. gracilis* (Batista et al. 2020a. 2020b; Valente et al. 2021). On the contrary, when fed a diet including 8% *G. gracilis* subjected to a physical–mechanical rupture treatment, plasma glucose and total protein levels were unaffected. However, plasma triglycerides were decreased compared to the control diet (Valente et al. 2021).

Conclusion

The inclusion of up to 15% *P. palmata* in diets for European sea bass juveniles, replacing wheat meal, was well accepted and improved growth performance and feed efficiency without compromising nutrient digestibility, digestive enzyme activity, or plasma metabolites. The pretreatment applied to the macroalgae in this study, although it significantly increased soluble protein content and decreased neutral detergent fiber, did not enhance fish performance. Overall, these results support *P. palmata* as a promising dietary ingredient for juvenile European seabass and suggest that it may contribute to the development of more sustainable aquafeed formulations when used as a partial replacement for selected plant feed ingredients. Further research should evaluate its functional properties, define optimal inclusion levels in long-term trials, and assess its economic feasibility in practical feeds.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

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References

Association of Official Analytical Chemists (1980) Official methods of analysis of the AOAC, 13th edn.

Azevedo A, Navarro LC, Cavalheri T et al (2023) Morphological traits for allometric scaling of the European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) from Southern Portugal population. J Fish Biol 103:425–438. https://doi.org/10.1111/JFB.15460

Batista S, Pereira R, Oliveira B et al (2020a) Exploring the potential of seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica*, single or blended, as natural dietary ingredients for European seabass *Dicentrarchus labrax*. J Appl Phycol. https://doi.org/10.1007/s10811-020-02118-z

Batista S, Pintado M, Marques A et al (2020b) Use of technological processing of seaweed and microalgae as strategy to improve their apparent digestibility coefficients in European seabass (Dicentrarchus labrax) juveniles. J Appl Phycol 32:5 32:3429–3446. https://doi.org/10.1007/S10811-020-02185-2

Boujard T, Gélineau A, Covès D et al (2004) Regulation of feed intake, growth, nutrient and energy utilisation in European sea bass (*Dicentrarchus labrax*) fed high fat diets. Aquaculture 231:529–545. https://doi.org/10.1016/J.AQUACULTURE.2003.11.010

Bradford MM (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

Brindo RA, Felix N (2014) Evaluation of raw and fermented seaweed, *Ulva lactuca* as feed ingredient in giant freshwater prawn *Macrobrachium rosenbergii*. Int J Fish Aquat Stud 1(3):199–204

Cabral EM, Poojary MM, Lund MN et al (2022) Effect of solvent composition on the extraction of proteins from hemp oil processing stream. J Sci Food Agric 102:6293–6298. https://doi.org/10.1002/JSFA.11979

Cho CY, Slinger SJ, Bayley HS (1982) Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 73:25–41. https://doi.org/10.1016/0305-0491(82)90198-5

Coelho D, Costa DF, Barroca M et al (2025) Simplified, high yielding extraction of Xylan/Xylo-Oligosaccharides from *Palmaria palmata*: the importance of the algae preservation treatment. Marine Drugs 23:302. https://doi.org/10.3390/MD23080302

Cottrell RS, Halpern BS, Cormery M et al (2025) The origins of aquaculture feed ingredients matter more than composition for aquafeed environmental footprint assessments. Nature Food. https://doi.org/10.1038/S43016-025-01236-6

Deniaud E, Quemener B, Fleurence J, Lahaye M (2003) Structural studies of the mix-linked β-(1→3)/β-(1→4)-d-xylans from the cell wall of *Palmaria palmata* (Rhodophyta). Int J Biol Macromol 33:9–18. https://doi.org/10.1016/S0141-8130(03)00058-8

El Hosry L, Elias V, Chamoun V et al (2025) Maillard reaction: mechanism, influencing parameters, advantages, disadvantages, and food industrial applications: a review. Foods 14:1881. https://doi.org/10.3390/FOODS14111881

Fernandes H, Martins N, Vieira L et al (2022) Pre-treatment of *Ulva rigida* improves its nutritional value for European seabass (*Dicentrarchus labrax*) juveniles. Algal Res. https://doi.org/10.1016/j.algal.2022.102803

Fleurence J (2016) Seaweeds as food. Seaweed in Health and Disease Prevention 149–167. https://doi.org/10.1016/B978-0-12-802772-1.00005-1

Foseid L, Natvik I, Devle H, Ekeberg D (2020) Identification of fatty acids in fractionated lipid extracts from *Palmaria palmata*, *Alaria esculenta* and *Saccharina latissima* by off-line SPE GC-MS. J Appl Phycol 32:4251–4262. https://doi.org/10.1007/S10811-020-02193-2/FIGURES/4

Ghelichi S, Sørensen ADM, Hajfathalian M, Jacobsen C (2024) Effect of post-extraction ultrasonication on compositional features and antioxidant activities of enzymatic/alkaline extracts of *Palmaria palmata*. Mar Drugs 22(4):179. https://doi.org/10.3390/MD22040179

Glencross B, Ling X, Gatlin D et al (2024a) A SWOT analysis of the use of marine, grain, terrestrial-animal and novel protein ingredients in aquaculture feeds. Rev Fish Sci Aquac 32:396–434. https://doi.org/10.1080/23308249.2024.2315049

Glencross BD, Bachis E, Robb D, Newton R (2024b) The evolution of sustainability metrics for the marine ingredient sector: moving towards holistic assessments of aquaculture feed. Rev Fish Sci Aquac 32:545–561. https://doi.org/10.1080/23308249.2024b.2337426

Güroy B, Ergün S, Merrifield DL, Güroy D (2013) Effect of autoclaved Ulva meal on growth performance, nutrient utilization and fatty acid profile of rainbow trout, *Oncorhynchus mykiss*. Aquac Int 21:605–615. https://doi.org/10.1007/S10499-012-9592-7/TABLES/7

Harnedy PA, FitzGerald RJ (2013) Extraction of protein from the macroalga *Palmaria palmata*. LWT Food Sci Technol 51:375–382. https://doi.org/10.1016/J.LWT.2012.09.023

Hasselström L, Thomas JB, Nordström J et al (2020) Socioeconomic prospects of a seaweed bioeconomy in Sweden. Sci Rep. https://doi.org/10.1038/s41598-020-58389-6

Hoseinifar SH, Yousefi S, Capillo G et al (2018) Mucosal immune parameters, immune and antioxidant defence related genes expression and growth performance of zebrafish (*Danio rerio*) fed on *Gracilaria gracilis* powder. Fish Shellfish Immunol 83:232–237. https://doi.org/10.1016/j.fsi.2018.09.046

Idowu AT, Amigo-Benavent M, FitzGerald RJ (2024) Impact of alkaline solution, carbohydrase and biomass:solvent on extraction efficiency, and protein and amino acid profiles of *Palmaria palmata* protein extracts. Food Bioprod Process 148:285–297. https://doi.org/10.1016/J.FBP.2024.09.017

Lafeuille B, Francezon N, Goulet C et al (2022) Impact of temperature and cooking time on the physicochemical properties and sensory potential of seaweed water extracts of *Palmaria palmata* and *Saccharina longicruris*. Journal of Applied Phycology 34(3):1731–1747. https://doi.org/10.1007/S10811-022-02731-0

Lahaye M, Michel C, Barry JL (1993) Chemical, physicochemical and in-vitro fermentation characteristics of dietary fibres from *Palmaria palmata* (L.) Kuntze. Food Chem 47:29–36. https://doi.org/10.1016/0308-8146(93)90298-T

Madsen H, Nguyen HM, Lanza GR, Stauffer JR (2022) A one health approach relative to trematode-caused diseases of people and animals associated with aquaculture. Reviews in Fisheries Science & Aquaculture 30(4):542–566. https://doi.org/10.1080/23308249.2022.2090830

Mæhre HK, Malde MK, Eilertsen KE, Elvevoll EO (2014) Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. J Sci Food Agric 94:3281–3290. https://doi.org/10.1002/JSFA.6681

Maehre HK, Edvinsen GK, Eilertsen KE, Elvevoll EO (2015) Heat treatment increases the protein bioaccessibility in the red seaweed dulse (*Palmaria palmata*), but not in the brown seaweed winged kelp (*Alaria esculenta*). J Appl Phycol 28(1):581–590. https://doi.org/10.1007/S10811-015-0587-4

Manikandan DB, Veeran S, Seenivasan S et al (2022) Exploration of marine red seaweed as a dietary fish meal replacement and its potentiality on growth, hematological, biochemical, and enzyme activity in freshwater fish *Labeo rohita*. Trop Anim Health Prod. https://doi.org/10.1007/s11250-022-03392-4

Martins B, Vieira M, Delerue-Matos C et al (2022) Biological potential, gastrointestinal digestion, absorption, and bioavailability of algae-derived compounds with neuro-protective activity: a comprehensive review. Mar. Drugs 20(6):362. https://doi.org/10.3390/md20060362

Mendez RL, Kwon JY (2023) Precipitation and characterization of Pacific dulse (*Devaleraea mollis*) proteins. J Appl Phycol 35:301–315. https://doi.org/10.1007/s10811-022-02853-5

Morgan KC, Simpson FJ, Morgan KC, Simpson FJ (1981) The cultivation of *Palmaria palmata*. Effect of light intensity and nitrate supply on growth and chemical composition. BoMar 24:273. https://doi.org/10.1515/BOTM.1981.24.5.273

Muñoz-Seijas N, Fernandes H, López-Periago JE et al (2024) Characterization of all life stages of *Tenebrio molitor*: envisioning innovative applications for this edible insect. Future Foods 10:100404. https://doi.org/10.1016/J.FUFO.2024.100404

Passos R, Correia AP, Pires D et al (2021) Potential use of macroalgae *Gracilaria gracilis* in diets for European seabass (*Dicentrarchus labrax*): health benefits from a sustainable source. Fish Shellfish Immunol 119:105–113. https://doi.org/10.1016/j.fsi.2021.09.033

Peres H, Oliva-Teles A (1999) Influence of temperature on protein utilization in juvenile European seabass (*Dicentrarchus labrax*). Aquaculture 170:337–348. https://doi.org/10.1016/S0044-8486(98)00422-0

Ramos-Oliveira C, Ferreira M, Belo I, Oliva-Teles A, Peres H (2026) High-solids processing of *Palmaria palmata* for feed applications: effects of alkaline autoclaving and sequential enzymatic treatment. Phycology 6:12. https://doi.org/10.3390/phycology6010012

Rawiwan P, Peng Y, Paramayuda IGPB, Quek SY (2022) Red seaweed: a promising alternative protein source for global food sustainability. Trends Food Sci Technol 123:37–56. https://doi.org/10.1016/J.TIFS.2022.03.003

Rodiles A, Santigosa E, Herrera M et al (2012) Effect of dietary protein level and source on digestive proteolytic enzyme activity in juvenile Senegalese sole, *Solea senegalensis* Kaup 1850. Aquac Int 20:1053–1070. https://doi.org/10.1007/s10499-012-9508-6

Shafiei-Alavijeh R, Aghbashlo M, Tabatabaei M et al (2024) A critical review of sustainable biorefineries utilizing high-solid processing for industrial crop lignocellulosic wastes valorization. Ind Crops Prod 211:118236. https://doi.org/10.1016/J.INDCROP.2024.118236

Shiva, Climent Barba F, Rodríguez-Jasso RM, et al (2022) High-solids loading processing for an integrated lignocellulosic biorefinery: effects of transport phenomena and rheology – a review. Bioresour. Technol. 351, 127044. https://doi.org/10.1016/j.biortech.2022.127044.

Shpigel M, Guttman L, Shauli L et al (2017) *Ulva lactuca* from an integrated multi-trophic aquaculture (IMTA) biofilter system as a protein supplement in gilthead seabream (*Sparus aurata*) diet. Aquaculture 481:112–118. https://doi.org/10.1016/j.aquaculture.2017.08.006

Sotoudeh E, Jafari M (2017) Effects of dietary supplementation with red seaweed, *Gracilaria pygmaea*, on growth, carcass composition and hematology of juvenile rainbow trout, *Oncorhynchus mykiss*. Aquac Int 25(5):1857–1867. https://doi.org/10.1007/S10499-017-0158-6

Sotoudeh E, Mardani F (2018) Antioxidant-related parameters, digestive enzyme activity and intestinal morphology in rainbow trout (*Oncorhynchus mykiss*) fry fed graded levels of red seaweed, *Gracilaria pygmaea*. Aquac Nutr 24:777–785. https://doi.org/10.1111/ANU.12606

Stévant P, Schmedes PS, Le Gall L et al (2023) Concise review of the red macroalga dulse, *Palmaria palmata* (L.) Weber & Mohr. J Appl Phycol 35(2):523–550. https://doi.org/10.1007/S10811-022-02899-5

Tacon AGJ, Metian M, McNevin AA (2022) Future feeds: suggested guidelines for sustainable development. Rev Fish Sci Aquac 30:135–142. https://doi.org/10.1080/23308249.2020.1860474

Valente LMP, Gouveia A, Rema P et al (2006) Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 252:85–91. https://doi.org/10.1016/j.aquaculture.2005.11.052

Valente LMP, Batista S, Ribeiro C et al (2021) Physical processing or supplementation of feeds with phytogenic compounds, alginate oligosaccharide or nucleotides as methods to improve the utilization of *Gracilaria gracilis* by juvenile European seabass (*Dicentrarchus labrax*). Aquaculture. https://doi.org/10.1016/j.aquaculture.2020.735914

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vazirzadeh A, Marhamati A, Chisti Y (2022) Seaweed-based diets lead to normal growth, improved fillet color but a down-regulated expression of somatotropic axis genes in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. https://doi.org/10.1016/j.aquaculture.2022.738183

Vizcaíno AJ, Sáez MI, Galafat A et al (2024) Effects of feeding European seabass (*Dicentrarchus labrax*) juveniles with crude, hydrolysed and fermented biomass of the invasive macroalga *Rugulopteryx okamurae* (Ochrophyta). Aquac Rep 34:10.1016/j.aqrep.2023.101877. https://doi.org/10.1016/j.aqrep.2023.101877

Vizcaino AJ, Fumanal M, Saez MI et al (2019) Evaluation of *Ulva ohnoi* as functional dietary ingredient in juvenile Senegalese sole (*Solea senegalensis*): effects on the structure and functionality of the intestinal mucosa. Algal Res. https://doi.org/10.1016/j.algal.2019.101608

Wan AHL, Soler-Vila A, O'Keeffe D et al (2016) The inclusion of *Palmaria palmata* macroalgae in Atlantic salmon (*Salmo salar*) diets: effects on growth, haematology, immunity and liver function. J Appl Phycol 28:3091–3100. https://doi.org/10.1007/S10811-016-0821-8/FIGURES/1

Wan AHL, Davies SJ, Soler-Vila A, Fitzgerald R, Johnson MP (2019) Macroalgae as a sustainable aquafeed ingredient. Rev Aquac 11:458–492. https://doi.org/10.1111/raq.12241

Weththasinghe P, Thilakarathne HMTP, Herath HMUL, Jayawardana BC (2026) Meta-analysis of the effects of seaweed inclusion in fish diets on growth performance. Aquaculture 611:743085. https://doi.org/10.1016/J.AQUACULTURE.2025.743085

Xie D, Li X, You C et al (2019) Supplementation of macroalgae together with non-starch polysaccharide-degrading enzymes in diets enhanced growth performance, innate immune indexes, and disease resistance against *Vibrio parahaemolyticus* in rabbitfish *Siganus canaliculatus*. J Appl Phycol 31:2073–2083. https://doi.org/10.1007/s10811-018-1662-4

Xuan X, Li W, Zhu W, Wang S (2019) Effects of different levels of macroalga *Gracilaria lemaneiformis* on growth performance and feed utilization on the red sea bream, *Pagrosomus major*. J Appl Phycol 31:3213–3222. https://doi.org/10.1007/s10811-019-01787-9

Yang H, Li Z-B, Chen Q et al (2016) Effect of fermented *Enteromopha prolifera* on the growth performance, digestive enzyme activities and serum non-specific immunity of red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*). Aquac Res 47:4024–4031. https://doi.org/10.1111/are.12856

Zhang X, Wu H, Li Z et al (2018) Effects of dietary supplementation of *Ulva pertusa* and non-starch polysaccharide enzymes on gut microbiota of *Siganus canaliculatus*. J Oceanol Limnol 36:438–449. https://doi.org/10.1007/s00343-017-6235-x

Zhang B, Liu X, Bao J (2023) High solids loading pretreatment: the core of lignocellulose biorefinery as an industrial technology – an overview. Bioresour Technol 369:128334. https://doi.org/10.1016/J.BIORTECH.2022.128334

Zhu D, Wen X, Li S et al (2016) Effects of exogenous non-starch polysaccharide-degrading enzymes in diets containing *Gracilaria lemaneiformis* on white-spotted snapper *Lutjanus stellatus* Akazaki. Aquac Int 24:491–502. https://doi.org/10.1007/S10499-015-9940-5/TABLES/5

Zhu D, Wen X, Li S et al (2017) Evaluation of the red alga *Gracilaria lemaneiformis* and brown alga *Sargassum horneri* as ingredients in diets for white spotted snapper *Lutjanus stellatus* Akazaki juveniles. J Appl Phycol 29:3211–3219. https://doi.org/10.1007/s10811-017-1187-2

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