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Effect of feed supplementation with seaweed wracks on performance, muscle lipid composition, antioxidant status, digestive enzyme activities, and plasma biochemistry of gilthead seabream (*Sparus aurata*) juveniles

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ABSTRACT

Macroalgal wracks are frequently considered as waste products. However, macroalgae are a rich source of protein and bioactive compounds with antioxidant and antimicrobial activities whose inclusion in aquafeeds is receiving growing attention. The aim of this study was to assess the use of beach-cast macroalgae as dietary supplement for gilthead seabream (*Sparus aurata)* juveniles. Fish were fed for 93 days with an extruded commercial diet (CD); a CD supplemented with 7% of a dried-powder from a multispecific macroalgal wrack (30.9% *Lobophora* sp., 21.9% *Dictyota* sp., 19.6% *Asparagopsis taxiformis*, 17.5% *Cymopolia* sp., 1.8% *Hypnea* sp., 0.2% *Laurencia* sp., 0.1% *Stypocaulon* sp., and 8% not determined) (CD+MU7); a CD supplemented with a monospecific macroalgal wrack of 85% *Lobophora* sp. (CD+MOL7); and a CD supplemented with a monospecific macroalgal wrack of 85% *Dictyota* sp. (CD+MOD7). Macroalgae were extracted with *n*-hexanes, ethyl acetate and ethanol, and the antioxidant capacity of extracts was assessed, with ethyl acetate leading to the most active extracts ($p < 0.05$). The dietary inclusions of a 7% macroalgal wrack did not negatively affect *S. aurata* survival, growth, body indexes, proximate composition, oxidative status and plasma parameters. Contrarily, fish fed on CD+MU7 and CD+MOD7 showed the highest monoacylglycerols content, while both monospecific diets led to the highest total saturated fatty acids content in muscle ($p < 0.05$). The digestive enzymes profile was slightly modified ($p < 0.05$), but without compromising fish performance. Finally, muscle from fish fed on CD+MOL7 seemed to display a better capacity to modulate the glutathione metabolism ($p < 0.05$). In conclusion, a 7% of macroalgal wracks can be used as a dietary supplement for *S. aurata* juveniles, depicting an attractive alternative for the effective harnessing of this usually discarded biomass. Macroalgal inclusion may also reduce the use of fish-based ingredients for aquafeeds, diminishing the pressure on pelagic fisheries, and contributing to the blue bioeconomy strategy.

Abbreviations: ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); BAL, bile salt-activated lipase; CAT, catalase; CD, control diet; CD+MOD7, control diet with a 7% of monospecific macroalgal wrack inclusion (*Dictyota* sp.); CD+MOL7, control diet with a 7% of monospecific macroalgal wrack inclusion (*Lobophora* sp.); CD+MU7, control diet with a 7% of multispecific macroalgal wrack inclusion; DHA, docosahexaenoic acid; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; DW, dry weight; EPA, eicosapentaenoic acid; FA, fatty acids; FAME, fatty acid methyl esters; GR, glutathione reductase; GST, glutathione-S-transferase; HSI, hepatosomatic index; LC, lipid classes; LC-PUFA, long chain polyunsaturated fatty acids; MDA, malondialdehyde; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PxI, peroxides index; ROS, reactive oxygen species; SD, standard deviation; SFA, saturated fatty acids; SGR, specific growth rate; SOD, superoxide dismutase; TAG, triacylglycerols; TBARS, thiobarbituric acid reactive substances; TL, total lipid; VFI, visceral-fat index; VSI, viscerosomatic index.

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1. Introduction

The feeding of carnivorous fish species is one of the most controversial issues in aquaculture, as it has traditionally relied heavily on the finite global fish stocks. Simultaneously, the increasing costs of marine ingredients compromise the economic profitability of production (Porcino and Genovese, 2022).

The use of terrestrial plant-based components as an alternative to marine ingredients in aquafeeds formulation can reduce the nutritional benefits of fish consumption by humans (Pérez et al., 2014) as they are deprived of the physiologically relevant long chain polyunsaturated fatty acids (LC-PUFA) (Porcino and Genovese, 2022). In addition, some vegetable feeds contain high carbohydrate levels, are deficient in various essential amino acids, and can present antinutritional factors, low digestibility and reduced palatability (Norambuena et al., 2015; Porcino and Genovese, 2022). Nowadays, the extensive demand for natural bioactive products has boosted the search for new sustainable dietary ingredients able to preserve or even improve the nutritional value of farmed fish to consumers (Norambuena et al., 2015). Within this context, micro and macroalgae have been suggested as possible novel alternative sources to lipids and proteins from marine-capture fisheries in aquafeeds (Norambuena et al., 2015). Moreover, algae contain valuable compounds with bioactive properties, such as polysaccharides, vitamins, minerals, proteins, peptides and amino acids, pigments (chlorophylls, carotenoids, xanthophylls and phycobiliproteins), phenolic compounds, sterols and other lipids like n-3 LC-PUFA (Generalić Mekinić et al., 2021; Harb and Chow, 2022; Nunes et al., 2020).

Feeding behavior, dissolved oxygen, temperature, dietary levels of lipid, PUFA, vitamins and minerals, diseases or xenobiotics may influence the risk of oxidative damage in fish (Martínez-Álvarez et al., 2005). In particular, LC-PUFA molecules are highly susceptible to reactive oxygen species (ROS)-induced lipid peroxidation (Carocho and Ferreira, 2013). Fish cells harbor various defence systems to protect themselves against the potential damage caused by ROS, among which are preventing mechanisms by antioxidants; either enzymatic or non-enzymatic (Martínez-Álvarez et al., 2005). The main enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), or glutathione reductase (GR) (Carocho and Ferreira, 2013).

Seaweeds are rich in bioactive metabolites with antioxidant, antimicrobial, anticancer, and antiaging properties (Akbary and Aminikhoei, 2018; Lähteenmäki-Uutela et al., 2021), and its dietary supplementation improved fish stress response (Galindo et al., 2022b), innate immunity (Kim et al., 2013) and flesh quality (Navarro et al., 1999). However, these positive effects are species-specific, dependent on rearing conditions, seaweed species and dose used (Guerreiro et al., 2019; Norambuena et al., 2015), as benefits seem to be closely related to the algal contents of antinutrients such as saponins, tannins, or protease and amylase inhibitors (Norambuena et al., 2015; Vizcaíno et al., 2016; Wassef et al., 2005).

Macroalgae and seagrasses detached from the seabed that frequently strand on the shoreline, play a key role in beach ecosystems (Mossbauer et al., 2012; Rodil et al., 2019). Nonetheless, its accumulation is unpleasant for beach users, affecting the tourism industry (Zárate et al., 2020). The mechanical removal and disposal of this organic matter is costly and complicated, entailing an environmental and economic impact (Mossbauer et al., 2012). As a consequence, new potential industrial applications for this underexploited biomass are being increasingly explored (Harb and Chow, 2022; Zárate et al., 2020), including their inclusion as dietary supplement in aquafeeds (Galindo et al., 2022b).

Gilthead seabream, *Sparus aurata*, is a marine carnivorous fish species with high commercial value. Nowadays, it is the most important finfish aquaculture species in the Mediterranean with a total production of 136,000 tons in 2020 (Savoca et al., 2021). Although dietary inclusion of specific macroalgae has been previously tested in *S. aurata* with variable outcomes (Abdala-Díaz et al., 2021; Emre et al., 2013; Martínez-Antequera et al., 2021; Wassef et al., 2005), supplementation of beach-casts in this species has not yet been studied. The main objective of the present work was to evaluate the use of macroalgal wracks as a feed supplement for *S. aurata* juveniles, giving value to sustainable alternatives for both, the management of this underused waste biomass, and to carnivorous fish feeding.

2. Material and methods

All experimental procedures were performed according to the EU Directive 2010/63/EU for animal experiments, the Spanish Executive Order 53/2013 for the protection of animals for experimentation or other scientific purposes, and were approved by the ULL Ethical Committee (CEIBA2015–0165).

2.1. Macroalgal wracks collection, pre-treatment and extraction

Seaweed wracks were removed from Las Canteras beach (28°08′24″N 15°26′15″W) in Gran Canaria island (Spain) using a bulldozer-like tractor as the routine beach handling and management performed by local public municipality. Taxonomic identification of macroalgae was developed using aliquots of the wet biomass (Zárate et al., 2020). Samples with a minimum weight of 20 kg representing at least 1% of the total biomass collected were cleaned of sand, washed, and naturally dried out in the shadow for 24 h. Dried samples were then ground to a fine powder (1 mm) with a rotor beater mill (SR 30; Retsch GmbH, Haan, Germany), kept in food-grade aluminum foil bags at room temperature during processing, and storaged at 4°C until their analysis and utilization.

In order to evaluate their antioxidant potential, samples of seaweed wracks (25.0 ± 0.5 g) were successively extracted three times with *n*-hexanes, ethyl acetate, and ethanol (250 mL x 24 h) by maceration at room temperature with continuous stirring (Galindo et al., 2022b). Extracts (Table S1, Supplementary Material) were obtained by concentrating the filtered solution under vacuum.

2.2. Total antioxidant activity of macroalgal wracks

Extracts were dissolved in sterile dimethyl sulfoxide (DMSO) (final concentration 50 mg mL^{-1}) using a sonication bath for 3–4 min when needed. The same methodology was developed with a standard solution of Trolox (Zárate et al., 2020).

Antioxidant activity was assayed by the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical (Re et al., 1999; Zárate et al., 2020) and the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical (Blois, 1958) scavenging assays. ABTS reaction was developed by mixing equal volumes of 7 mM ABTS and 2.4 mM potassium persulfate solution for 12–16 h at room temperature in the dark. The resultant solution was then diluted in methanol until an absorbance of 0.7 at 734 nm was reached. Stock solutions of samples in DMSO were dissolved in methanol to a final concentration of 500 μg mL^{-1}. Eleven product concentrations (0.244–250 μg mL^{-1} in methanol) and a standard solution of Trolox (0.098–100 μg mL^{-1}) were tested in 96-well microplates in quadruplicates, using only solvent instead of the sample as control. Samples were allowed to react with ABTS for 8 min at room temperature, and the absorbance was measured at 750 nm with a BioRad Microplate Reader Model 680 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

For the DPPH assay, eleven serial dilutions of samples (0.244–250 μg mL^{-1} in methanol) and Trolox were tested, and a control was also prepared. Absorbance was recorded at 515 nm after adding DPPH dissolved in methanol (90 μg mL^{-1}) and incubation for 30 min in darkness.

The percentage of antioxidant activity for both assays was calculated following the equation:

$$\text{Antioxidant activity (\%)} = [(Abs_{control} - Abs_{sample})/(Abs_{control})] \times 100$$

where $Abs_{control}$, is the absorbance of ABTS or DPPH radical + methanol and Abs_{sample}, is the absorbance of ABTS or DPPH radical + sample/standard.

Concentration yielding 50% scavenging of ABTS or DPPH (IC_{50}) of each sample was calculated by interpolation from the percentage of antioxidant activity *vs*. concentration curve.

2.3. Experimental conditions

Gilthead seabream (*S. aurata*) juveniles (n = 228) of 18.63 ± 1.00 g initial average weight were obtained from Geremar (Santa Cruz de Tenerife, Tenerife, Spain). The experiment was carried out at the facilities of Centro Oceanográfico de Canarias from Instituto Español de Oceanografía (IEO-CSIC). Before the beginning of the feeding trial, fish were acclimatized to the experimental conditions for 3 weeks and fed with the extruded control commercial diet (Inicio Plus 805, Biomar, Palencia, Spain). After this period, fish were randomly distributed in 12 polyethylene 1 m^3-tanks (19 individuals per tank) and reared under a continuous seawater supply of 25 L min^{-1}, natural photoperiod, and ambient daylight of 1600 lux. Water conditions were temperature of 20.2 ± 0.4ºC, dissolved oxygen of 7.1 ± 0.2 mg L^{-1}, and pH 8.3 ± 0.2.

Fish received one of four different dietary treatments in triplicate: the commercial diet (CD, control group), or this diet supplemented with a 7% of a shadow dried powder of either a multispecific (MU) macroalgal wrack (30.9% *Lobophora* sp., 21.9% *Dictyota* sp., 19.6% *Asparagopsis taxiformis*, 17.5% *Cymopolia* sp., 1.8% *Hypnea* sp., 0.2% *Laurencia* sp., 0.1% *Stypocaulon* sp., and 8% not determined) (experimental treatment 1, CD+MU7); a monospecific (MOL) wrack of *Lobophora* sp. (85% *Lobophora* sp.) (experimental treatment 2, CD+MOL7), or a monospecific (MOD) wrack of *Dictyota* sp. (85% *Dictyota* sp.) (experimental treatment 3, CD+MOD7). Animals were fed with 3–5% of their total biomass, three times a day for 93 days. In the manufacturing of diets, the CD was triturated to a fine powder, mixed or not with a 7% of each macroalgal wrack, and repelletized to prevent texture and palatability differences among diets.

2.4. Growth parameters, tissue collection and body indexes

Survival, weight increment and specific growth rate [SGR; ((ln final weight-ln initial weight)/days) × 100] were monitored at the beginning, during (monthly) and at the end of the experimental period. Five individuals from each treatment were randomly chosen, starved for 24 h and sacrificed at the end of the experiment. Clove oil was used to anesthetize the fish, and blood was collected from the caudal vein using heparinized syringes. Samples of muscle, liver and digestive tract were extracted and kept at −80 °C until biochemical analysis. Additionally, hepatosomatic (HSI; (liver weight/body weight) × 100), viscerosomatic (VSI; (viscera weight/body weight) × 100), and visceral-fat (VFI) body indexes were also calculated. VFI was determined from visible fat of organs according to the following scale: 1 (low), 2 (medium) or 3 (high) (Óskarsson, 2008).

2.5. Proximate and lipid composition

The moisture content of diets and fish muscle was determined by drying the samples in an oven at 110ºC until constant weight (AOAC, 2006). Crude protein was determined by conversion of the nitrogen content following Kjeldahl's method, while ash content was estimated by dry-ashing in a furnace at 450ºC for 24 h (AOAC, 2006).

Total lipid (TL) of macroalgal wracks, diets and fish muscle samples was extracted with chloroform/methanol (2:1, v/v) following the Folch method (Folch et al., 1957) with minor modifications (Christie and Han, 2010).

Lipid classes (LC) were analyzed as described by Olsen and Henderson (1989) with minor modifications by Reis et al. (2019). Briefly, a 30 μg aliquot of TL was used to develop a high-performance thin-layer chromatography (HPTLC) in a single-dimensional double-development. LC were quantified by calibrated densitometry using a dual-wavelength flying spot scanner CAMAG TLC Visualizer (Camag, Muttenz, Switzerland).

A 1 mg aliquot of TL was used to obtain fatty acid methyl esters (FAME) by acid-catalyzed transmethylation, using nonadecanoic acid (19:0) as an internal standard. FAME were purified by thin layer chromatography (TLC) (Christie and Han, 2010), and subsequently separated and quantified using a TRACE-GC Ultra Gas Chromatograph (Thermo Scientific, Milan, Italy) as detailed by Galindo et al. (2022a). A mixture of authentic standards (Mix C4-C24 and PUFA No. 3 from menhaden oil (Supelco Inc., Bellefonte, Pennsylvania, USA)) together with a well characterized cod roe oil were used to identify individual FAME. When needed, the identity of FAME was confirmed by GC-MS (DSQ II, Thermo Scientific).

2.6. Antioxidant response

Peroxide index (PxI) was determined in muscle samples from *S. aurata* using a ferric chloride ($FeCl_3$) standard curve following Shantha and Decker (1994). Absorbance was read at 500 nm, and the concentration of lipid peroxides was expressed as $meqO_2$ Kg^{-1} as previously described by Galindo et al. (2022b).

For the analysis of thiobarbituric acid reactive substances (TBARS), and antioxidant enzymes, muscle and liver samples (300 mg) were homogenized in an ice-cold 20 mM Tris-Cl (w/v) buffer (pH 7.4) with protease inhibitors (Complete®, Sigma, Madrid, Spain) (Galindo et al., 2022b).

The content of malondialdehyde (MDA) was evaluated by the TBARS assay following Ohkawa et al. (1979). Samples were measured fluorometrically with excitation at 530 nm and emission at 550 nm employing a multi-well plate reader (Thermo Scientific Appliskan, Thermo Fisher Scientific, Vantaa, Finland), and MDA content (nmol MDA mg $protein^{-1}$) was calculated using a standard curve of 1,1,3,3-tetramethoxipropane, as defined by Galindo et al. (2022b).

For the analysis of antioxidant enzymes, SOD activity was determined using 30 mM pyrogallol as substrate following Mesa-Herrera et al. (2019). One unit of SOD activity was defined as the equivalency to the amount of enzyme that provides a 50% inhibition of the auto-oxidation of pyrogallol. The reaction was measured spectrophotometrically at 420 nm.

Enzymatic activity of CAT was measured at 240 nm adding 485 mM H_2O_2 as substrate according to Clairborne (1985). The molar extinction coefficient of H_2O_2 (Ɛ) used was 42.6 M^{-1} cm^{-1}.

The GR reaction was analyzed using 1 mM GSSG, and 60 μM NADPH as substrates. Oxidation of NADPH was determined at 340 nm, and GR activity was calculated using the molar extinction coefficient of NADPH (Ɛ = −6.22 mM^{-1} cm^{-1}) (Chung et al., 1991).

The GST reaction was determined with 5 mM GSH, and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) as substrates (Habdous et al., 2002). Absorbance was read at 340 nm, and the activity was quantified using the molar extinction coefficient of Mesenheimer complex (Ɛ = 9.6 mM^{-1} cm^{-1}).

One unit of activity (U) was defined as μmol min^{-1} for all antioxidant activities unless otherwise indicated.

2.7. Digestive enzymes

Pancreatic (alpha-amylase, bile salt-activated lipase, total alkaline proteases) and gastric (pepsin) enzyme activities were determined in the gut or stomach of fish, according to Solovyev et al. (2016). Prior to enzymatic activity determination, samples were homogenized in 10 volumes (v/w) of ice-cold Milli-Q water, centrifuged at 3300 x g for 8 min at 4ºC, and the supernatants stored at −80ºC until enzymatic quantification.

Alpha-amylase (E.C. 3.2.1.1) was analyzed using 0.3% soluble starch

dissolved in Na_2HPO_4 buffer (pH 7.4), and the absorbance measured at 580 nm after the addition of 1 N HCl and 2 mL of N/3000 iodine solution (Merck, Darmstadt, Germany) (Métais and Bieth, 1968). Alpha-amylase activity corresponded to the mg of starch hydrolyzed at 37ºC per 30 min per mL.

Bile salt-activated lipase (BAL, E.C. 3.1.1) was assayed as described by Iijima et al. (1998). Samples were incubated with p-nitrophenyl myristate dissolved in 0.25 mM Tris-HCl, pH 9.0, 0.25 mM 2-methoxyethanol, and 5 mM sodium cholate buffer for 30 min at 30ºC. After stopping the reaction with acetone/n-heptane (5:2, v/v), samples were centrifuged at 6000 x g for 2 min at 4 °C, and the absorbance was determined at 405 nm. BAL activity was defined as the μmol of myristate hydrolyzed per min per mL.

Alkaline protease activity was determined following incubation with azocasein (0.5%) in Tris-HCl 50 nmol L^{-1} (pH 9) as substrate for 60 min at 24ºC, and stopping the reaction with 20% trichloroacetic acid (TCA). After centrifugation at 10,000 x g for 5 min, the absorbance of the supernatant was read at 366 nm (García-Carreño and Haard, 1993). One unit of activity was defined as 1 μmol of azo dye released per min per mL.

Pepsin activity was quantified by incubating the extracts with 2% hemoglobin solution in 1 N HCl (pH 2.0) for 60 min at 37ºC. The reaction was stopped with 5% TCA, the extract centrifuged at 4000 x g for 6 min at 4ºC, and the absorbance of the supernatant read at 280 nm (Worthington Biochemical Coorporation, 1972). One unit of activity was defined as the μmol of tyrosine released per min per mL.

The soluble protein of homogenized samples was quantified using bovine serum albumin as standard (Bradford, 1976), and specific activity expressed as mU or U mg $protein^{-1}$.

Absorbances were measured using a spectrophotometer (Beckman Colter DU800 Fullerton, California, USA).

2.8. Plasma parameters

The hematocrit was determined by capillary diffusion and centrifugation. The remaining blood was then centrifuged for 20 min at 700 x g and 4ºC, in a microcentrifuge, and the plasma was collected and stored at −80ºC until further analysis. Using enzymatic colorimetric assays and a BioTek Synergy HT Microplate reader (Winooski, VA, USA), the plasma levels of glucose (BioSystems, Barcelona, Spain), lactate, sodium, and chloride (Spinreact, Girona, Spain) were each determined in duplicate. Finally, cortisol concentration was quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Arbor Assays®Ann Arbor, Michigan, USA).

2.9. Statistical analysis

Before analysis, normality and homoscedasticity within groups were confirmed and, where necessary, variance stabilizing transformations (arcsine and logarithm) were performed. Significant differences between treatments were determined by one-way ANOVA followed by a Tukey HSD post-hoc test. Welch test followed by the Dunnett T3 test were performed for non-homoscedastic data, and Kruskall-Wallis non-parametric test was applied in case of non-normal distribution followed by the pair-wise comparisons Mann-Whitney test with Bonferroni correction.

Results are presented as means ± standard deviation (SD), and the statistical significance was set at $p < 0.05$. All statistical analyses were carried out using the IBM® SPSS Statistics 25.0 software package (IBM Corp., New York, USA).

3. Results

3.1. Total antioxidant activity of macroalgal wracks

All MU wrack extracts showed < 45% of activity for DPPH (Table 1). On the contrary, MOL-ethanol and MOD-ethyl acetate extracts scavenged the DPPH radical by more than 50% at 250 μg mL^{-1}. The IC_{50} values were calculated for these two samples. The most active extract (MOD-ethyl acetate) was nearly 20-fold less active than Trolox (IC_{50} values of 147.54 μg mL^{-1} *vs.* 7.43 μg mL^{-1}, respectively).

With the exception of MU-ethanol, all extracts exceeded 50% activity at the ABTS assay. Overall, MOL wrack comprised the most active extracts, followed by MOD and MU extracts. Thus, MOL-ethyl acetate was the most active of all extracts, with an IC_{50} of 17.42 ± 1.13 μg mL^{-1}, which like being an extract is a very interesting result 20-fold greater than Trolox (0.87 μg mL^{-1}).

3.2. Proximate composition and fatty acid profile of macroalgal wracks and diets

All macroalgal wracks had similar TL content (~3% dry weight, DW), composed mainly of saturated fatty acids (SFA) ranging from 41 to 44% of total fatty acids (FA) (Table 2). Total monounsaturated fatty acids (MUFA) was remarkably low in the MOL wrack (20.75%), while total n-6 and n-3 PUFA were the highest (21.32 and 12.89%, respectively). Eicosapentaenoic acid (EPA, 20:5n-3) represented ~8% of total FA in the MOL wrack and ~4% in the MU and MOD wracks, while docosahexaenoic acid (DHA, 22:6n-3) accounted for ~2% in the MU and < 0.5% in both MOL and MOD wracks.

Proximal and FA composition of diets did not significantly vary with macroalgal wrack supplementation (Table 2). Thus, MUFA was the major group of FA (37–38%) in all diets, followed by PUFA (33–34%) and SFA (27–28%). Total n-6 and total n-3 PUFA comprised 15 and 17–18% of total FA, respectively, with EPA and DHA representing 4.5–5% and 8–9%, respectively (Table 2).

3.3. Survival, growth parameters and body indexes

Regardless of dietary treatment, survival was 100% at the end of the feeding period. In addition, a 7% dietary inclusion of either MU, MOL, or MOD did not affect fish growth parameters or body indexes with respect to the control group (Table 3).

3.4. Proximate and lipid composition of fish muscle

Muscle moisture, protein, ash and TL remained unchanged in muscle samples from all fish groups (75–77%, 81–83% DW, 6–7% DW and 8–9% DW, respectively) (Table 3).

In all cases, neutral lipids represented 61–67% of muscle TL, mainly triacylglycerols (TAG) (45–51% of TL), and cholesterol (9–11% of TL). The only significant difference among groups was that monoacylglycerols (MAG) were more abundant in CD+MU7 (1.43 ± 0.30%) and CD+MOD7-fish (1.15 ± 0.24%), than in CD+MOL7-fish (0.84 ± 0.34%). Phosphatidylcholine (16–20%) and phosphatidylethanolamine (9–11%) were the major phospholipid fractions (Table 4).

Dietary seaweed inclusion did not affect fish muscle FA profile, except total SFA, which increased in CD+MOL7 and CD+MOD7-fish (~26% of total FA) compared to the control group (24.50 ± 0.77%), despite its main component (16:0) did not change (Table 5). MUFA (36–38%), chiefly 18:1, and PUFA (35–37%) were the prevailing FA. Among n-3 PUFA, DHA was the main component (12–13%), doubling EPA proportion (~5%). Finally, ARA represented ~1% of total FA in all treatments.

3.5. Fish antioxidant activities and lipid peroxidation

Antioxidant activity was higher in liver than in muscle samples (Fig. 1). For both tissues, CAT, SOD and GST activities were not altered by the wrack supplementation. By contrast, GR activity was higher in the muscle of CD+MOD7-fish (0.71 ± 0.09) than in the other experimental groups (~0.40 mU mg $protein^{-1}$) but remained unchanged in the liver at around 2 mU mg $protein^{-1}$.

Table 1
DPPH and ABTS activity, and IC_{50} of multispecific (MU), monospecific-*Lobophora* sp. (MOL) and monospecific-*Dictyota sp*. (MOD) macroalgal wracks used in the experiment.

		DPPH				ABTS					
		Activity (%)			IC_{50} (μg mL^{-1})	Activity (%)			IC_{50} (μg mL^{-1})		
MU wrack	*n*-hexanes	19.42 ± 1.27	b	x	> 250	74.80 ± 3.40	b	x	77.85 ± 2.63	b	x
	Ethyl acetate	42.04 ± 2.26	c	x	> 250	73.47 ± 1.24	b	x	70.40 ± 2.59	a	z
	Ethanol	6.53 ± 4.88	a	x	> 250	31.21 ± 1.80	a	x	> 250		
MOL wrack	*n*-hexanes	15.80 ± 10.56	a	x	> 250	68.48 ± 4.57	a	x	132.96 ± 2.85	c	y
	Ethyl acetate	38.99 ± 3.99	b	x	> 250	79.97 ± 1.94	b	y	17.42 ± 1.13	a	x
	Ethanol	54.09 ± 0.86	c	z	207.47 ± 7.31	82.40 ± 1.53	b	y	40.99 ± 2.56	b	x
MOD wrack	*n*-hexanes	30.94 ± 2.62	a	y	> 250	74.11 ± 3.68	a	x	74.17 ± 3.20	b	x
	Ethyl acetate	69.65 ± 3.70	b	y	147.54 ± 8.75	74.26 ± 4.13	a	x	31.67 ± 0.86	a	y
	Ethanol	32.06 ± 1.61	a	y	> 250	81.31 ± 1.17	b	y	80.90 ± 3.71	c	y
Trolox		92.21 ± 0.32			7.43 ± 0.74	83.25 ± 1.28			0.87 ± 0.18		

Results are presented as means ± SD. All determinations were carried out in quadruplicate. DPPH, 1,1-diphenyl-2-picryl-hydrazyl; ABTS, 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid); IC_{50}, Concentration yielding 50% scavenging of each radical. a,b,c within a particular column represent significant differences between solvents for the same wrack ($p < 0.05$). x,y,z within a particular column represent significant differences between wracks for the same solvent ($p < 0.05$). Activity (%) was measured at 250 μg mL^{-1} for macroalgal extracts and at 100 μg mL^{-1} for Trolox standard.

Table 2
Proximate composition (moisture, protein, ash and total lipid content), total fatty acids and main fatty acid composition of MU, MOL and MOD macroalgal wracks, and diets used in the experiment.

	MU wrack	MOL wrack	MOD wrack	Diets			
				CD	CD+MU7	CD+MOL7	CD+MOD7
Moisture (%)	-	-	-	6.15	6.11	6.71	6.63
Protein (% of DW)	-	-	-	47.95	48.80	47.58	46.20
Ash (% of DW)	-	-	-	9.66	12.05	11.79	11.64
Total lipid (% of DW)	2.72	3.35	2.86	17.73	16.07	15.06	16.20
Total FA (μg mg^{-1} DW)	4.64	6.29	3.79	110.44	107.73	92.05	108.17
Fatty acids (% of total FA)							
Total SFA	41.28	41.57	44.30	27.81	27.45	27.73	27.47
14:0	6.28	8.92	8.56	3.50	3.46	3.50	3.46
16:0	30.61	29.73	31.70	18.39	18.17	18.27	18.09
18:0	2.00	1.03	1.52	4.20	4.23	4.25	4.20
Total MUFA	31.33	20.75	27.27	37.58	37.33	37.26	37.34
16:1[1]	8.59	4.79	6.58	5.02	5.01	5.02	4.93
18:1[2]	21.18	14.97	19.18	27.79	27.67	27.55	27.66
20:1[2]	0.62	0.33	0.74	2.50	2.44	2.46	2.49
Total n-6 PUFA	14.01	21.32	15.69	15.35	15.29	15.27	15.25
18:2	7.11	9.74	6.29	13.80	13.78	13.72	13.72
20:4	4.34	7.33	6.14	0.81	0.85	0.86	0.84
Total n-3 PUFA	9.85	12.89	8.65	17.16	17.68	17.50	17.75
18:3	1.31	1.14	1.26	2.18	2.20	2.17	2.18
20:5	4.09	7.84	4.41	4.50	4.63	4.61	4.68
22:5	0.26	0.11	nd	0.91	0.94	0.92	0.94
22:6	1.87	0.39	0.39	8.37	8.68	8.53	8.72
n-3/n-6	0.70	0.60	0.55	1.12	1.16	1.15	1.16
Total n-3 LC-PUFA	6.64	8.75	5.32	14.17	14.65	14.46	14.74

MU: multispecific macroalgal wrack; MOL: monospecific macroalgal wrack (*Lobophora* sp.); MOD: monospecific macroalgal wrack (*Dictyota* sp.); CD, Control diet; CD+MU7, Control diet supplemented with 7% multispecific macroalgal wrack; CD+MOL7, Control diet supplemented with 7% monospecific macroalgal wrack of *Lobophora* sp.; CD+MOD7, Control diet supplemented with 7% monospecific macroalgal wrack of *Dictyota* sp.; DW, dry weight; FA, fatty acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain polyunsaturated fatty acids (≥C20 and ≥2 double bonds). Totals include other minor components not shown. [1] Mainly n-7 isomers for diets, and n-9 for macroalgal wracks; [2] Mainly n-9 isomers. nd, not detected.

The diet did not affect the oxidative status of *S. aurata* juveniles. Thus, PxI comprised 3–4 meq O_2 kg $lipid^{-1}$ in the muscle, while TBARS was ~0.2 and 0.3–0.4 nmol MDA mg $protein^{-1}$ in the muscle and liver, respectively (Fig. 1).

3.6. Digestive enzymes

The activity of digestive enzymes in the gastrointestinal tract of gilthead seabream juveniles varied with dietary macroalgal wrack supplementation (Table 6). Thus, pepsin activity was higher in CD+MOL7-fish (21.25 ± 11.49 mU mg $protein^{-1}$) than in CD+MU7 (6.19 ± 3.49), and CD-fish (4.14 ± 1.34). Furthermore, alkaline proteases showed lower activity when fish were fed CD+MU7 and CD+MOL7 diets (13–14 mU mg $protein^{-1}$) than under control conditions (47.52 ± 11.83), whereas the activity of BAL was maximal in CD+MU7 and CD+MOD7-fish (~7 mU mg $protein^{-1}$). Finally, the highest alpha-amylase values were registered in CD and CD+MU7-fish (0.30–0.31 ± 0.10 U mg $protein^{-1}$), with the lowest in CD+MOD7-fish (0.13 ± 0.06).

3.7. Plasma parameter

Hematocrit (40–52%), cortisol (22.667–41.577 pg dL^{-1}), glucose (20–29 mg dL^{-1}), lactate (32–34 mg dL^{-1}), sodium (460–492 mg dL^{-1}), and chloride (1446–1780 mg dL^{-1}) were not affected by the inclusion of a 7% of macroalgal wrack in the diet (Fig. 2).

Table 3
Growth parameters, body indexes and muscle proximate composition of *S. aurata* juveniles fed the experimental diets.

	CD	CD+MU7	CD+MOL7	CD+MOD7
Growth parameters				
Weight increment (g)	90.58 ± 1.41	87.07 ± 2.91	83.35 ± 5.98	87.09 ± 8.32
SGR (% day^{-1})	1.43 ± 0.04	1.43 ± 0.02	1.38 ± 0.04	1.45 ± 0.04
Body indexes				
HSI (%)	1.25 ± 0.09	1.46 ± 0.22	1.56 ± 0.15	1.45 ± 0.23
VSI (%)	7.32 ± 0.90	7.69 ± 0.72	8.99 ± 2.55	8.22 ± 0.98
VFI	2.60 ± 0.55	2.40 ± 0.55	2.25 ± 0.96	2.00 ± 0.71
Proximate composition				
Moisture (%)	74.56 ± 1.64	75.70 ± 0.82	77.10 ± 2.51	75.64 ± 0.98
Protein (% DW)	81.21 ± 4.32	81.04 ± 0.93	83.08 ± 4.79	81.53 ± 5.19
Ash (% DW)	6.24 ± 0.80	6.54 ± 0.80	5.86 ± 1.60	6.64 ± 0.81
Total lipid (% DW)	9.25 ± 3.06	9.45 ± 3.48	7.81 ± 2.08	8.21 ± 1.69

Results are presented as means ± SD (n = 3 for growth parameters; n = 5 for body indexes and proximate composition). CD, Control diet; CD+MU7, Control diet supplemented with 7% multispecific macroalgal wrack; CD+MOL7, Control diet supplemented with 7% monospecific macroalgal wrack of *Lobophora* sp.; CD+MOD7, Control diet supplemented with 7% monospecific macroalgal wrack of *Dictyota* sp.; SGR, specific growth rate; HSI, hepatosomatic index; VSI, viscerosomatic index; VFI, visceral-fat index; DW, dry weight.

4. Discussion

Algal supplementation in aquafeeds is receiving increasing research interest mainly for their bioactive chemical content, their potential as protein and essential FA nutritional sources, and their sustainability (Chen et al., 2021; Galindo et al., 2022b; García-Ortega et al., 2016; Sáez et al., 2020). However, most studies have been focused on the use of a few green seaweed species, chiefly from the order Ulvales, and in a reduced number of fish species (Güroy et al., 2013; Sáez et al., 2020; Vizcaíno et al., 2019). Despite their potential and importance in food and phycocolloid production, reports testing brown algae species are rather limited (Wan et al., 2019).

To date, the reported effects of macroalgae as feed additives on fish growth performance are highly variable. Thus, previous studies on *S. aurata* demonstrated that a 5% of *Ulva* sp. in the diet enhanced fish weight (Wassef et al., 2005), while a 5% of *Chondrus crispus,* a blend of 5% *Ulva lactuca* and *C. crispus,* a 15% of *U. rigida*, and a 25% of *Gracilaria cornea* resulted in lower growth rates compared to the control diet (Guerreiro et al., 2019; Vizcaíno et al., 2016). By contrast, and in agreement to our study, neither *U. lactuca* (2.6% and 7.8%) nor *U. rigida* (4%) dietary inclusion significantly affected *S. aurata* growth performance (Emre et al., 2013; Shpigel et al., 2017). These results evidence that the effects of dietary macraolgae supplementation do not only depend on fish species, but also on seaweed species and dose used (Guerreiro et al., 2019; Norambuena et al., 2015). Furthermore, the format in which algae is provided is also a relevant issue. For instance, fingerlings of Nile tilapia (*Oreochromis niloticus*) fed a commercial liquid extract based on macroalgae improved growth performance, feed utilization and non-specific immunity (Ashour et al., 2020). Similarly, dietary supplementation with polysaccharides derived from brown macroalgae promoted growth, serum biochemistry and digestive enzyme activities of hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*) fingerlings (Abdelrhman et al., 2022).

The potential effects of macroalgae on fish lipid metabolism have not yet been fully elucidated (Norambuena et al., 2015). Here, SFA increased in muscle samples from *S. aurata* fed diets supplemented with any of the monospecific macroalgal wracks (Table 5), in accordance with previously stated data for the same species fed a diet supplemented with *C. crispus*, or a mix of *U. lactuca* and *C. crispus* (Guerreiro et al., 2019). Increased tissue SFA proportion has been related to a higher dietary SFA content (García-Ortega et al., 2016) which was not the case in our experimental diets. Peixoto et al. (2016a) reported that the inclusion of *Ulva* sp. reduced lipase activity responsible for TAG hydrolysis in European sea bass *Dicentrarchus labrax*. As TAG are usually rich in SFA, a reduction in lipase activity induced by a commercial blend of seaweeds has been proposed to reduce SFA deposition in muscle (Bruni et al., 2020). Therefore, the higher SFA proportions observed in the present work could be caused by an increase in the pancreatic lipase activity (not determined in our study), which has TAG as its preferred substrate (Soumanou et al., 2013). In fact, brown algae has a relevant lipolytic

Table 4
Lipid class composition of muscle from *S. aurata* juveniles fed the different diets.

Lipid class (% of total lipid)	CD	CD+MU7	CD+MOL7	CD+MOD7
SM	0.83 ± 0.31	0.89 ± 0.43	0.47 ± 0.10	0.48 ± 0.18
PC	17.80 ± 3.50	16.49 ± 4.35	19.89 ± 5.07	16.86 ± 2.54
PS	1.83 ± 0.65	1.72 ± 0.49	2.43 ± 1.62	1.98 ± 0.88
PI	3.75 ± 0.70	3.22 ± 1.14	3.91 ± 1.10	3.89 ± 0.71
PG	1.61 ± 0.47	1.55 ± 0.61	1.68 ± 0.77	1.34 ± 0.27
PE	9.71 ± 2.14	9.19 ± 2.08	10.93 ± 3.11	10.53 ± 2.01
TPL	35.54 ± 6.75	33.07 ± 8.26	39.31 ± 10.40	35.09 ± 5.42
MAG	1.35 ± 0.28 ^{ab}	1.43 ± 0.30 ^{b}	0.84 ± 0.34 ^{a}	1.15 ± 0.24 ^{b}
DAG	0.96 ± 0.24	1.07 ± 0.23	1.19 ± 0.39	1.25 ± 0.16
CHO	9.15 ± 1.38	10.82 ± 1.67	10.65 ± 2.05	10.19 ± 1.50
TAG	49.77 ± 7.20	50.74 ± 9.46	44.67 ± 12.18	48.97 ± 7.18
SE	3.23 ± 0.73	2.87 ± 0.88	3.33 ± 1.30	3.36 ± 0.88
TNL	64.46 ± 6.75	66.93 ± 8.26	60.69 ± 10.40	64.91 ± 5.42

Data are presented as means ± SD (n = 5). CD, Control diet; CD+MU7, Control diet supplemented with 7% multispecific macroalgal wrack; CD+MOL7, Control diet supplemented with 7% monospecific macroalgal wrack of *Lobophora* sp.; CD+MOD7, Control diet supplemented with 7% monospecific macroalgal wrack of *Dictyota* sp.; SM, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; TPL, total polar lipids; MAG, monoacylglycerols; DAG, diacylglycerols; CHO, cholesterol; TAG, triacylglycerols; SE, sterol esters; TNL, total neutral lipids. Different letters in superscript within each row denote significant differences ($p < 0.05$).

Table 5
Total fatty acids and main fatty acid composition of muscle from *S. aurata* juveniles fed the different diets.

	CD		CD+MU7		CD+MOL7		CD+MOD7	
Total FA (mg FA 100 g^{-1} wet weight)	1733.30 ± 662.87		1768.03 ± 1004.19		1291.73 ± 386.10		1494.22 ± 269.22	
Fatty acids (% of total FA)								
Total SFA	24.50 ± 0.77	a	25.50 ± 0.46	ab	26.13 ± 0.92	b	26.36 ± 0.55	b
14:0	2.16 ± 0.19		2.25 ± 0.31		2.09 ± 0.45		2.03 ± 0.19	
16:0	17.74 ± 0.49		18.09 ± 0.33		18.50 ± 0.82		18.67 ± 0.43	
18:0	4.00 ± 0.47		4.41 ± 0.44		4.73 ± 0.46		4.74 ± 0.33	
Total MUFA	36.81 ± 2.62		37.80 ± 3.23		36.34 ± 4.33		37.16 ± 3.37	
16:1[1]	5.11 ± 0.52		5.18 ± 0.54		4.88 ± 0.65		4.97 ± 0.43	
18:1[2]	28.18 ± 1.85		28.90 ± 2.59		27.88 ± 3.17		28.50 ± 2.72	
20:1[2]	1.82 ± 0.20		1.89 ± 0.12		1.79 ± 0.78		1.86 ± 0.16	
Total n-6 PUFA	14.19 ± 0.46		13.98 ± 0.59		13.45 ± 0.48		13.25 ± 0.35	
18:2	11.02 ± 0.60		11.00 ± 0.31		10.46 ± 0.64		10.40 ± 0.42	
20:4	1.18 ± 0.22		1.08 ± 0.18		1.20 ± 0.33		1.16 ± 0.26	
Total n-3 PUFA	22.61 ± 2.62		21.07 ± 2.80		22.26 ± 3.97		21.62 ± 3.08	
18:3	1.58 ± 0.16		1.58 ± 0.09		1.47 ± 0.17		1.47 ± 0.10	
20:5	5.00 ± 0.62		4.57 ± 0.52		4.85 ± 1.05		4.74 ± 0.72	
22:5	1.81 ± 0.12		1.70 ± 0.23		1.74 ± 0.12		1.66 ± 0.11	
22:6	13.00 ± 2.26		12.05 ± 2.11		13.08 ± 3.10		12.73 ± 2.37	
n-3/n-6	1.60 ± 0.21		1.50 ± 0.17		1.66 ± 0.33		1.63 ± 0.20	
Total n-3 LC-PUFA	20.45 ± 2.84		18.92 ± 2.91		20.27 ± 4.22		19.62 ± 3.17	

Results are presented as means ± SD (n = 5). CD, Control diet; CD+MU7, Control diet supplemented with 7% multispecific macroalgal wrack; CD+MOL7, Control diet supplemented with 7% monospecific macroalgal wrack of *Lobophora* sp.; CD+MOD7, Control diet supplemented with 7% monospecific macroalgal wrack of *Dictyota* sp.; FA, fatty acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain polyunsaturated fatty acids (≥C20 and ≥2 double bonds). Totalys include other minor components not shown. 1 Mainly n-7 isomer; 2 Mainly n-9 isomers. Different letters in superscript within each row denote significant differences ($p < 0.05$).

activity (Bedoux et al., 2014), contributing to the absorption and tissue deposition of SFA.

Ito et al. (2018) suggested that the content of polyphenols and tocopherols might be the cause of the higher DPPH activity in *Ochrophyta* compared to other phyla. More specifically, and in accordance with our findings, Zárate et al. (2020) described an ethanol extract from a macroalgal wrack formed by 95% *Lobophora* sp. as an attractive antioxidant product; although their data showed a better performance than the results reported in our study (IC_{50}-ABTS 6.21 ± 1.03 *vs.* 40.99 ± 2.56 μg mL^{-1}; Table 1). Brown algae generally possess higher antioxidant activity than green and red algae (Generalić Mekinić et al., 2021), which has been related to their content in phlorotannins, a special group of phenolics involved in protection against oxidative damage. Besides, fucoxanthin has also been described in some *Lobophora* species (Hegazi, 2002; Nunes et al., 2019). Although these bioactive compounds with great antioxidant activity present in algae may mitigate stress responses in fish (Peixoto et al., 2019), seaweed, and seaweed powder or extract supplementation has been described to both increase or decrease lipid peroxidation (Chen et al., 2021; Guerreiro et al., 2019; Passos et al., 2021). GR catalyzes the reduction of GSSG to GSH, representing the antioxidant restoration potential. Here, the antioxidant capacity of macroalgal wrack supplementation with MOD increased GR activity in *S. aurata* juveniles muscle samples (Fig. 1) suggesting a higher capacity to modulate the glutathione metabolism (Peixoto et al., 2016b). Nevertheless, and in spite of the change in GR activity, the global oxidative status of muscle and liver did not significantly differ between fish groups.

Dietary algae inclusion may also influence the activity of enzymes involved in digestive and absorptive processes in several fish species. Thus, while a 20% of *Gracilaria lemaneiformis* inclusion in black sea bream *Acanthopagrus schlegelii*, inhibited pepsin activity, and consequently affected fish growth performance, < 15% supplementation did not (Xuan et al., 2013). In the present study, the activity of pepsin was slightly enhanced in CD+MOL7 and CD+MOD7-fish, suggesting some positive effects of these two macroalgal species.

There were no significant differences in *S. aurata* total fat or TAG deposition (Tables 3–4) despite the fact that BAL activity was higher in CD+MU7-fish. Higher lipolytic activity was previously described for the multispecific wrack in the herbivorous grass carp (*Ctenopharyngodon idella*) (Galindo et al., 2022b), which might be also enhancing BAL activity in fish fed the multispecific wrack in the present study, suggesting that longer experimental periods would be needed in carnivorous fish species like *S. aurata* to detect a clear reduction in lipid deposition.

Regarding alpha-amylase activity, *S. aurata* juveniles showed the lowest values with dietary MOD inclusion (Table 6). Carnivorous fish are believed to have a reduced ability to digest starch (Liang et al., 2022). Macroalgae have a relatively high content of non-starch polysaccharides such as cellulose, xylans, agar, carrageenan, or alginate, which can act as antinutritional factors for some monogastric animals like fish. For instance, the addition of non-starch polysaccharide-degrading enzymes in seaweed feed supplementation to remove or breakdown complex carbohydrates promoted an increase in rabbitfish (*Siganus canaliculatus*) growth (Xie et al., 2019). Furthermore, several studies reporting some beneficial effects of seaweed inclusion used extruded diets (Chen et al., 2021; Martínez-Antequera et al., 2021; Peixoto et al., 2016a, 2016b). Seaweeds processing and dietary extrusion remove or break some of the complex carbohydrates present in seaweeds allowing fish to access them (Guerreiro et al., 2019). Our results point out that at least dietary inclusion of CD+MU7 and CD+MOL7 did not negatively affect starch digestibility in *S. aurata* probably due to the low percentage of inclusion.

The hematological profile of a cultured fish is considered a good indicator of its physiological status and health (Fazio, 2019). Cortisol and hematocrit values, usually used as diagnostic tools in plasma to assess stress (Sadoul and Geffroy, 2019) and anemic features (Ashry et al., 2021), respectively, were in agreement to those previously reported for *S. aurata* (Ashry et al., 2021). Blood glucose, lactate, potassium, sodium and chloride levels change with chronic stress or repeated acute stress (Cnaani et al., 2004; McDonald and Milligan, 1997). In this sense, there is no an apparent detrimental effect on the physiological status, health or well-being of fish fed diets supplemented with the macroalgae assayed in the present work.

5. Conclusion

The dietary supplementation with a 7% of macroalgal wrack rich in Phaeophyceae showed no adverse effects on *S. aurata* survival, growth,

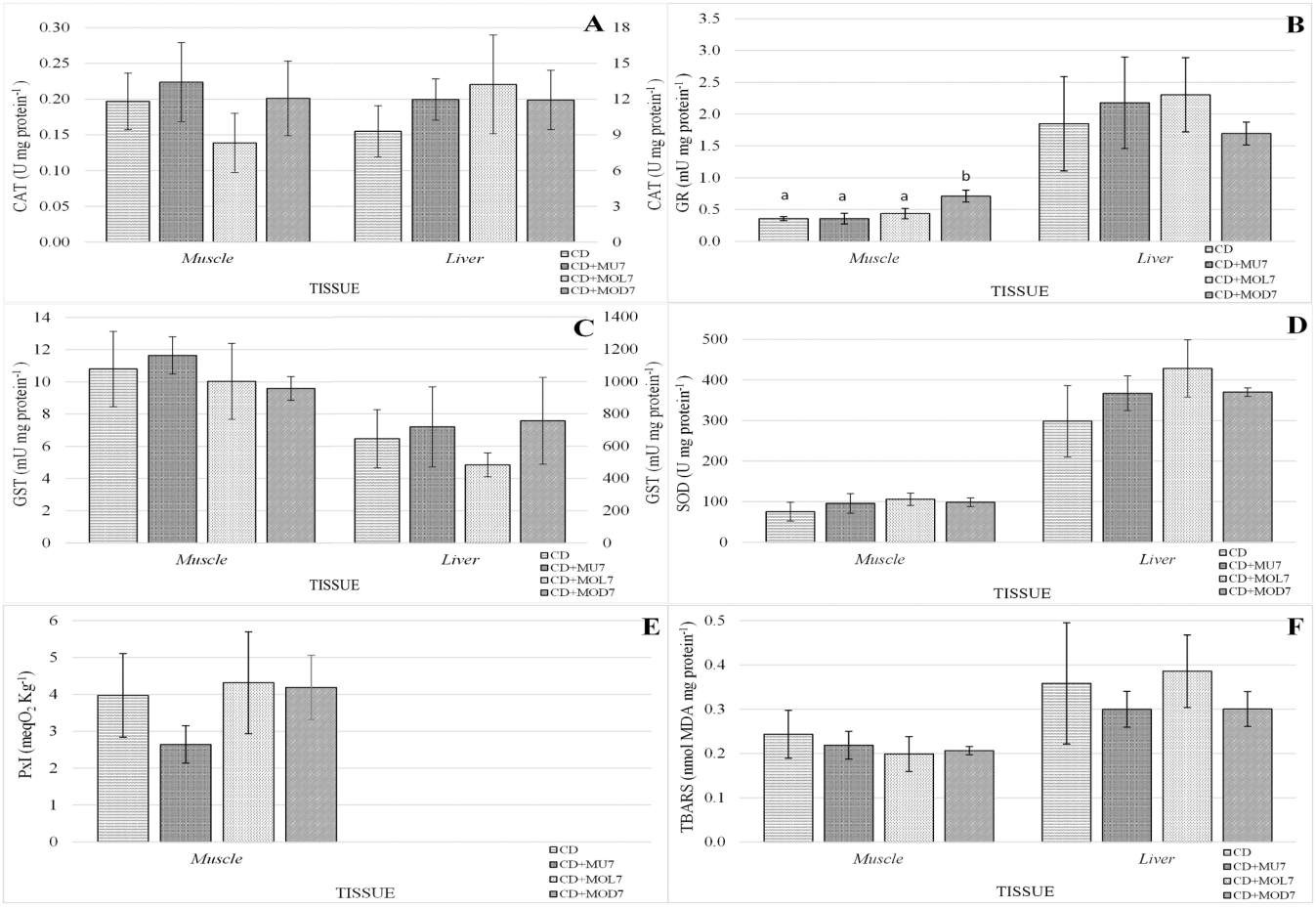

Fig. 1. Antioxidant activities: (A) catalase (CAT); (B) superoxide dismutase (SOD); (C) glutathione-S-transferase (GST); (D) glutathione reductase (GR); (E) peroxides index (PxI) and (F) TBARS. All assays excluding PxI (muscle), were determined in muscle and liver from *S. aurata* juveniles fed the different diets. Results are presented as mean ± SD (n = 5). CD, Control diet; CD+MU7, Control diet supplemented with 7% multispecific macroalgal wrack; CD+MOL7, Control diet supplemented with 7% monospecific macroalgal wrack of *Lobophora* sp.; CD+MOD7, Control diet supplemented with 7% monospecific macroalgal wrack of *Dictyota* sp. Different letters denote significant differences ($P < 0.05$).

Table 6
Digestive enzymes activities determined in *S. aurata* juveniles fed the different diets.

	CD		CD+MU7		CD+MOL7		CD+MOD7	
Pepsin (mU mg protein^{-1})	4.14 ± 1.34	a	6.19 ± 3.49	ab	21.25 ± 11.49	c	15.81 ± 6.12	bc
Alkaline proteases (mU mg protein^{-1})	47.52 ± 11.83	b	12.85 ± 5.81	a	14.07 ± 6.71	a	68.50 ± 41.35	ab
Bile salt-activated lipase (mU mg protein^{-1})	6.33 ± 1.33	ab	7.23 ± 1.34	b	4.61 ± 0.75	a	7.11 ± 2.08	ab
Alpha-amylase (U mg protein^{-1})	0.30 ± 0.08	b	0.31 ± 0.10	b	0.27 ± 0.11	ab	0.13 ± 0.06	a

Results are presented as means ± SD (n = 5). Pepsin was determined in the stomach while alkaline proteases, bile salt-activated lipase and alpha-amylase were determined in the intestine. CD, Control diet; CD+MU7, Control diet supplemented with 7% multispecific macroalgal wrack; CD+MOL7, Control diet supplemented with 7% monospecific macroalgal wrack of *Lobophora* sp.; CD+MOD7, Control diet supplemented with 7% monospecific macroalgal wrack of *Dictyota* sp. Different letters in superscript within each row denote significant differences ($p < 0.05$).

proximate composition, FA and LC profiles, and fish antioxidant status. Despite some minor changes in the digestive capacity, the use of macroalgal wracks as feed additive did not cause any apparent detriment in the physiology of the fish. Additionally, there was also evidence for an improved capacity to restore the antioxidant status of fish fed the MOD diet. Our findings significantly contribute to the sustainable management of beach-cast seaweed biomass promoting blue growth and circular economy strategies in coastal regions. Furthermore, the reduction of aquaculture dependence for finite fisheries resources is also encouraged. The observed effects, however, might depend on the relative abundance of algae species in the collected wracks and their conservation status. Hence, more research with different beach-cast seaweeds is needed in order to determine their true potential to reduce small pelagic-fish-based ingredients in aquafeeds.

CRediT authorship contribution statement

A. Galindo: Writing-original draft preparation, Investigation, Resources, Formal analysis, **J.A. P é rez**: Supervision, Writing – review & editing, **V. Martín:** Methodology, Resources, Writing – review &

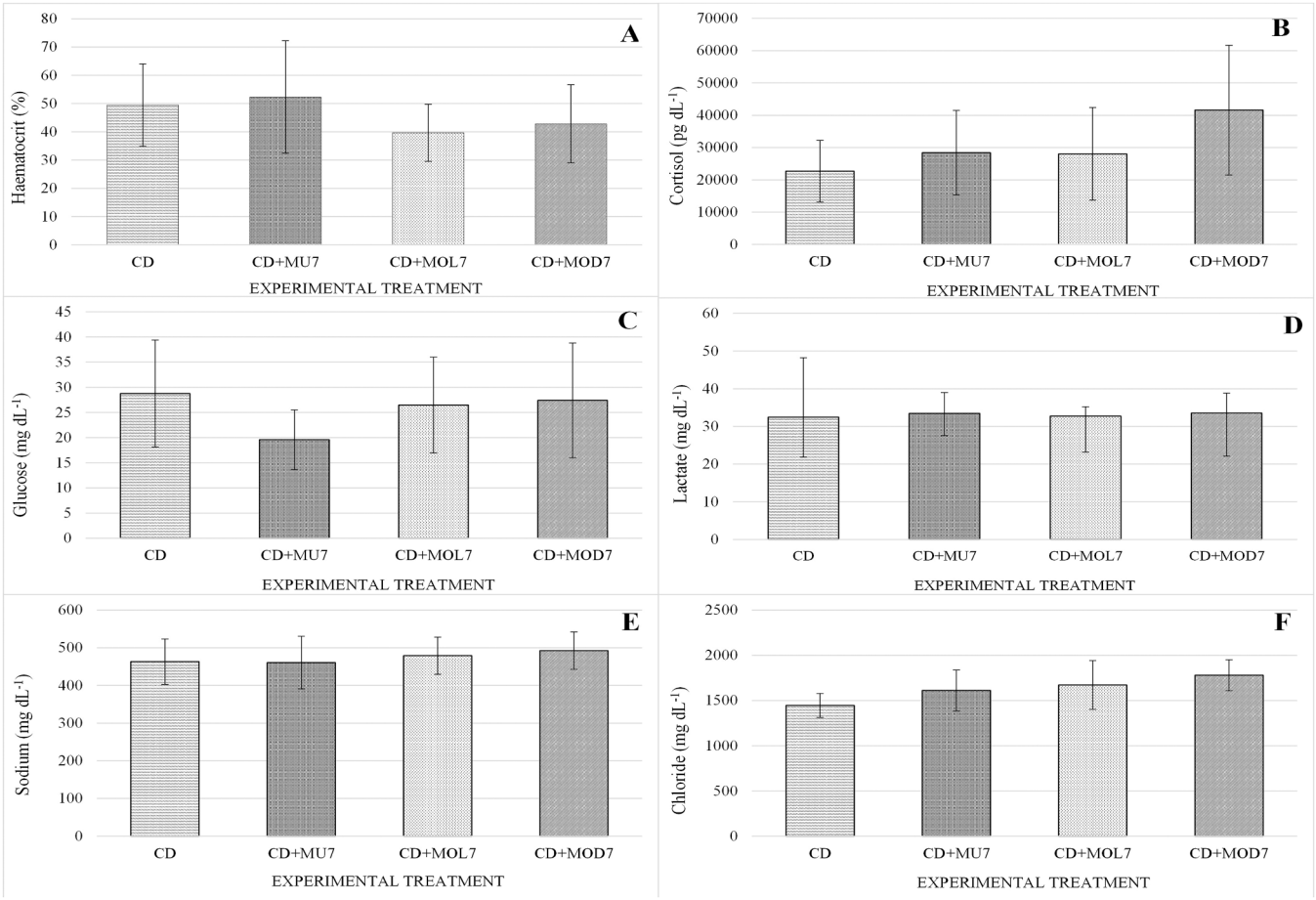

Fig. 2. Plasma parameters: (A) hematocrit; (B) cortisol; (C) glucose; (D) lactate; (E) sodium and (F) chloride from *S. aurata* juveniles fed the different diets. Results are presented as mean ± SD (n = 5). CD, Control diet; CD+MU7, Control diet supplemented with 7% multispecific macroalgal wrack; CD+MOL7, Control diet supplemented with 7% monospecific macroalgal wrack of *Lobophora* sp.; CD+MOD7, Control diet supplemented with 7% monospecific macroalgal wrack of *Dictyota* sp.

editing, **N.G. Acosta:** Formal analysis, Methodology, Supervision, **D.B. Reis:** Methodology, **I.A. Jiménez:** Formal analysis, Methodology, Supervision, **G. Rosa:** Formal analysis, Methodology, Supervision, **M. Venuleo:** Resources, Funding acquisition, Writing – review & editing, **M. Marrero:** Methodology, **C. Rodríguez:** Conceptualization, Project administration, Funding acquisition, 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 authors declare that the data used to support the findings of this study are included within the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2023.101673.

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