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Stress response of European seabass (*Dicentrarchus labrax*) fed plant-based diets supplemented with swine blood hydrolysates

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ABSTRACT

To improve fish welfare, it is essential that aquafeeds are designed to help fish cope with the stressful conditions of fish farms. One effective strategy to achieve this goal is to supplement the diet with bioactive hydrolysates. Here, diet supplementation to modulate oxidative stress after air exposure was investigated in European seabass, using swine blood hydrolysates (BH), obtained either by autohydrolysis (AH) or enzymatically. The enzymatically produced BH were further submitted to a micro- (RMF) and nanofiltration (RNF). Four isolipidic, isoproteic and isoenergetic diets were developed: a plant-based diet with low (12.5%) fishmeal levels (control, CTRL) and three diets where 3% of each BH (RMF, RNF and AH) was added to the CTRL. Diets were assigned to triplicate groups of 71 European seabass juveniles (initial weight 12.3 ± 1.4 g). After 12 weeks, 9 fish per treatment were either immediately sampled or air-exposed for 1 min and let to recover in a new system for 6 h prior to sampling. Stress response increased cortisol levels, followed by an increment in plasma lactate. The challenge increased liver lipid peroxidation (LPO) due to reactive oxygen species (ROS) accumulation. Carbonyls decreased post-stress, maybe due to a possible interaction with the LPO radicals, reducing protein oxidation. None of the BH improved plasma stress response. By reducing catalase levels without increasing LPO, the RNF treatment appears to adjust European seabass' antioxidant defences, indicating its potential to supply exogenous antioxidants to combat oxidative stress induced by ROS. However, this impact was not sufficient to lower LPO levels compared to a control plant-based diet. The tested diets seemed to affect the fish oxidative stress response in the liver, possibly due to the presence of bioactive peptides, which aided in the non-enzymatic modulation of stress response, as observed by the total antioxidant capacity values in the liver.

1. Introduction

The practice of farming marine carnivorous fish used to depend on the inclusion of high levels of marine-based ingredients, such as fishmeal (FM) and fish oil, in their diets. Nevertheless, the scarceness, increased prices and issues concerning the environmental unsustainability of these dietary components have motivated a search for alternative ingredient sources (Naylor et al., 2021). Regarding FM replacement, plant proteins

Abbreviations: ABTS•+, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; ACH50, alternative complement activity; AH-H, hydrolysate obtained by autohydrolysis; AH, diet with AH-H; BH, blood hydrolysates; BHT, butylated hydroxytoluene; BW, body weight; CAT, catalase; CC, carbonyl compounds; CDNB, 1-chloro-2,4-dinitrobenzene; CTRL, control diet; DM, dry matter; DPPH•, 1,1-diphenyl-2-picrylhydrazyl; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid, Ellman's reagent; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; EU, enzyme unit; FM, fishmeal; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; LPO, lipid peroxidation; MDA, malondialdehyde; NADPH, nicotinamide adenine dinucleotide phosphate; NEFA, non-esterified fatty acids; OD, optical density; ORAC, oxygen radical absorbance capacity; PUFAs, polyunsaturated fatty acids; RAS, recirculating aquaculture system; RMF-H, retentate from microfiltration; RMF, diet with RMF-H; RNF-H, retentate from nanofiltration; RNF, diet with RNF-H; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; TG, total glutathione; TNB, 5-thio-2-nitrobenzoic acid.

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are the most used alternatives in aquafeeds (Abdel-Latif et al., 2022; Samuel-Fitwi et al., 2013). However, their use poses some challenges mainly related to food-feed competition, higher water and land requirements and deforestation (Aubin et al., 2019; Hua et al., 2019). Moreover, plant-based diets may also negatively impact fish growth, health and welfare, namely by compromising fish immunity and resistance to stress. Conde-Sieira et al. (2018) found some indications that vegetable diets may hinder fish welfare through alterations in the neurotransmission activity in the hypothalamus and telencephalon. Costa et al. (2020) found that a vegetable-based diet decreased plasmatic lysozyme in European seabass, which may compromise immunity. Torrecillas et al. (2017) found that reducing FM levels to 5% hindered gut associated lymphoid tissue capacity of response after a bacterial infection in European seabass. Finally, in the work of Yin et al. (2020), plant protein diets for hybrid groupers hindered antioxidant capacity and glycolipid metabolism, hampered intestinal development and decreased intestinal flora diversity, which the authors attribute to the presence of anti-nutritional factors. This is a major challenge to aquaculture intensification practices, including increased fish density, regular handling, transport and fishing, all of these being stress sources with negative repercussions on fish health (Sadoul et al., 2021). While stress itself may not be detrimental to fish health in the short term, if it becomes too intense or chronic, it may affect fish physiology at molecular and biochemical levels, impairing animal growth, immunity and survival (Barton, 2002; Sneddon et al., 2016).

Stress responses can be divided into primary, secondary or tertiary (Barton, 2002; Bonga, 1997; Sadoul et al., 2021). Primary responses are due to the activation of endocrine pathways, after recognition of a threat by the central nervous system. Secondary responses include respiratory and cardiovascular alterations due to hormonal action (Barton, 2002; Sadoul et al., 2021). Additionally, metabolic changes, such as increased levels of glucose and lactate and a decrease in tissue glycogen, are observed and the immune system can also be triggered. Finally, tertiary responses reflect the whole animal performance, with stress having ultimately negative impacts on fish growth, disease resistance, behaviour, and even survival (Ashley, 2007; Barton, 2002; Sadoul et al., 2021). Stress may overall lead to the accumulation of reactive oxygen species, ROS (Mohapatra et al., 2013), affecting the balance between ROS production and antioxidant scavenging capacity of tissues, which eventually damages biological molecules such as DNA, lipids and proteins, for example, through an increase in lipid peroxidation (LPO) or carbonyl compounds (CC) (Ko et al., 2014). To counteract the ROS, the organism activates antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) (Kurutas, 2016). However, exogenous antioxidants provided by aquafeeds may also help to maintain the ROS balance and mitigate oxidative damage.

Therefore, and considering the raising concerns regarding fish welfare, it has become clear that well-balanced diets must not only provide the essential nutrients, but also assist fish in coping with the stressful situations they face on fish farms (Ashley, 2007; Machado et al., 2019a). Thus, an emergent strategy for improving fish welfare is the supplementation of aquafeeds with bioactive compounds that provide physiological benefits beyond their pure nutritional value, which have particular relevance when used with plant-based diets (Encarnação, 2016; Olmos-Soto et al., 2015; Siddik et al., 2021). Among these bioactive compounds, protein hydrolysates have raised considerable interest from both the scientific community and the feed industry, as they can be obtained from by-products generated in food industries, which would make them attractive under a circular economy context (Faustino et al., 2019). The biological properties of hydrolysates have been increasingly demonstrated (Manzoor et al., 2022; Okoye et al., 2022; Siddik et al., 2021). These properties are highly dependent on the protein source and on the applied hydrolysis method. For instance, the inclusion of some anchovy hydrolysates in low FM diets for European seabass (*Dicentrarchus labrax*) led to improved lysozyme activity, when compared to a non-supplemented diet, but the usage of the same hydrolysate source with a different enzymatic processing did not lead to any changes in the same parameters (Costa et al., 2020). In juvenile red seabream (*Pagrus major*), Bui et al. (2014) demonstrated that 4.8% of a shrimp hydrolysate powder added to a fishmeal diet (47%) significantly increased plasma immunoglobulin, antiprotease and SOD activities. Additionally, in Japanese seabass (*Lateolabrax japonicus*) and yellow croaker (*Pseudosciaena crocea* R.), lysozyme activity and serum complement were enhanced by inclusion of fish protein hydrolysates (Liang et al., 2006; Tang et al., 2008). Inclusion of a tuna hydrolysate in a diet for barramundi (*Lates calcarifer*), at 10%, in diet with fermented poultry by-product meal led to increased activity of serum GPx (Siddik et al., 2020). Moreover, some hydrolysates have also been shown to improve stress response in mice (Chataigner et al., 2021; Dinel et al., 2021).

Previous work from our group demonstrated that a 3% swine blood hydrolysates (BH; obtained from a by-product of a pig slaughterhouse) inclusion in a plant-based diet led to an eight-fold reduction in European seabass mortality caused by *Tenacibaculum maritimum* infection (Resende et al., 2022), but the physiological mechanisms affected by such bioactive hydrolysates were not explored. Interest in blood hydrolysates arises from the fact that swine blood is an abundant by-product of meat production. Despite the absence of official reports of its global production, an estimation of 859 million L of swine blood can be produced yearly in the European Union (European Commission, 2021; Resende et al., 2022; Toldrà et al., 2019). Usually, it is processed into low-cost blood meal, but it could be valorised through hydrolysis yielding a beneficial mixture rich in bioactive peptides (Bah et al., 2013). In the present study, we propose to further evaluate the ability of BH included in low FM diets to modulate European seabass oxidative stress after a multifactorial acute stress challenge (air exposure followed by transfer to new tanks). This acute stress was chosen as being representative of frequent handling procedures that take place in fish farms, and to which fish response needs improving, through further dietary interventions (Machado et al., 2019a). In particular, some plasmatic metabolites and immune markers, hepatic oxidative stress markers and regulating enzymes, and muscle antioxidant potential were assessed.

2. Materials and methods

2.1. Ethical issues

The animal study protocol was approved by the Ethics Committee of CIIMAR for Managing Animal Welfare (ORBEA-CIIMAR_18_2017), in compliance with the Directive 2010/63/EU (European Union, 2010) and the Portuguese Decree_Law n° 113/2013 on "The protection of animals used for scientific purposes". The present study was performed by accredited scientists in laboratory animal science by the Portuguese Veterinary Authority (1005/92, DGV-Portugal, following FELASA category C recommendations).

2.2. Blood hydrolysates and experimental diets

Hydrolysis of swine blood was performed as described by Resende et al. (2022) and Araújo-Rodrigues et al. (2022). Two different processes were considered: enzymatic hydrolysis and autohydrolysis (the latter yielding the AH-H fraction). The hydrolysate mixture obtained enzymatically was further fractionated with a microfiltration (500 kDa cut-off), with the retentate from the microfiltration being termed RMF-H, while the filtrate was subjected to a nanofiltration (3 kDa cut-off). The retentate from this nanofiltration was called RNF-H. Peptide molecular weight (MW) profile of the hydrolysates is available on Table 1.

Four isonitrogenous (54% protein in dry matter, DM), isolipidic (16% DM) and isoenergetic (22 kJ g^{-1}) diets were produced: a plant-based diet with 12.5% FM was used as a control (control, CTRL) to which 3% of each BH was added, resulting in three further experimental

Table 1
Peptide molecular weight (MW) profile of the blood hydrolysates.

	Ingredients		
	RMF-H	RNF-H	AH-H
Peptide Profile (mAU)			
> 43 kDa	n.d.	n.d.	238137
29–43 kDa	765	32143	138862
13.7–29 kDa	1513	14786	80132
1.2–13.7	48017	373302	349651
< 1.2 kDa	7656	79596	131995

Source:Adapted from Resende et al. (2022).

diets (RMF, RNF, AH). Diets were formulated in compliance with European seabass nutritional requirements (National Research Council, 2011) and were extruded (2 mm) by SPAROS, Lda (Portugal). Table 2 describes the ingredients and proximate composition of diets.

2.3. Experimental design

European seabass juveniles were acquired from a commercial fish farm (Acuinuga, S.L., Spain) and transported to the Fish Culture Experimental Unit of CIIMAR, where the experiment was performed. After an acclimation period (15 days), 12 groups of 71 fish (weight of 12.3 ± 1.4 g, density of 3.5 kg m^{-3}), were randomly distributed by fiberglass tanks (250 L) within a recirculating aquaculture system (RAS). Nitrogenous compounds (NH_4^+ ≤0.05 mg L^{-1}; NO_2^- ≤0.5 mg L^{-1}; NO_3^- ≤5 mg L^{-1}), salinity (35 ± 1‰), temperature (20 ± 1 °C), dissolved oxygen (>90% saturation) and pH (7.5 ≤ pH ≤ 8.5) were monitored and kept at ideal levels for European seabass (Kır et al., 2019). Photoperiod was a cycle of 12 h light/12 h dark. Each tank received filtered saltwater at a flow rate of 16 L min^{-1}. Diets were randomly assigned to triplicate tanks and fish were fed 3 times daily until apparent visual satiety, for 74 days.

Table 2
Ingredients, proximate composition and mineral composition of the diets used in the trial.

	Diets			
	CTRL	RMF	RNF	AH
Ingredients (%)				
Fishmeal (FM)[1]	12.50	12.50	12.50	12.50
Soy protein concentrate[2]	25.00	25.00	25.00	25.00
Wheat gluten[3]	13.50	10.00	10.10	10.20
Corn gluten[4]	15.00	15.00	15.00	15.00
Soybean meal 48[5]	10.00	10.00	10.00	10.00
Wheat meal[6]	7.24	7.44	7.34	7.34
Fish oil[7]	13.40	13.70	13.70	13.60
Vit & Min Premix[8]	0.50	0.50	0.50	0.50
DCP[9]	2.80	2.80	2.80	2.80
L-Tryptophan	0.06	0.06	0.06	0.06
RMF-H[10]	0	3.00	0	0
RNF-H[11]	0	0	3.00	0
AH-H[12]	0	0	0	3.00
Proximate composition (%DM)				
DM	96.24	94.63	97.00	93.96
Ash	7.93	7.73	7.87	8.04
Crude protein	54.24	54.72	54.57	54.63
Crude fat	15.93	15.79	16.21	16.03
Energy (kJ g^{-1})	22.37	22.44	22.55	22.69

CTRL, negative control; RMF, RNF, AH – diets supplemented with the respective hydrolysates. 1FM: 71% crude protein, 11% crude fat, EXALMAR, Peru; 2Soy protein concentrate: 65% protein, 0,7% lipids, ADM, Animal NutritionTM, The Netherlands; 3Wheat gluten: 90.1% DM, 83.8% protein, 1.6% lipids (as DM basis); 4Corn gluten feed: 61% crude protein, 6% crude fat, COPAM, Portugal; 5Dehulled solvent extracted soybean meal: 47.7% crude protein, 2.2% crude fat, CAR-GILL, Spain; 6Wheat meal: 10.2% protein, 1.2% lipids, Casa Lanchinha Lda., Portugal; 7Sardine oil, Sopropêche, France; 8Vitamins (IU or mg/kg diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20,000 IU; DL-cholecalciferol, 2000 IU; thiamine, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium pantothenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg/kg diet): cobalt carbonate, 0.65 mg; copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate,7.5 mg; sodium chloride, 400 mg; calcium carbonate, 1.86 g; excipient wheat middlings. INVIVO 1%, Premix for marine fish, PREMIX Lda, Portugal; 9Di-calcium phosphate; 10Blood hydrolysate: retentate from microfiltration after enzymatic hydrolysis; 11Blood hydrolysate: retentate from nanofiltration after enzymatic hydrolysis; 12Blood hydrolysate: produced by auto-hydrolysis.
Source:Adapted from Resende et al. (2022).

At the end of the trial, fish were fasted for 24 h. Afterwards, 3 fish per tank (9 fish per treatment) were either immediately sampled (non-stressed) or air-exposed for 1 min, placed in 50 L aerated tanks with clean saltwater and sampled (stressed) after a 6-hour recovery period, based on previous literature reports (Machado et al., 2019a; Zheng et al., 2017).

Immediately before sampling, all fish were sacrificed with anaesthetic overdose (0.5 mL L^{-1} of 2-Phenoxyethanol) and individually weighed and measured. Blood was taken from the caudal vein using heparinized syringes and centrifuged at 5000 g for 10 min at 4 °C, for plasma collection. Samples of liver and muscle were also collected and immediately frozen in liquid nitrogen. Plasma and tissue samples were kept at − 80 °C until analysis. The remaining fish were also lightly anesthetized with 2-Phenoxyethanol (60 μL L^{-1}), and individual weight and length were recorded.

2.4. Plasma analysis

All plasma analysis were determined in triplicate on a microplate spectrophotometer (BioTek Synergy HT, Vermont, USA).

2.4.1. Humoral immune parameters

Lysozyme activity (μg mL^{-1} of plasma) was quantified with a turbidimetric assay adapted to microtiter, as described by Hutchinson and Manning (1996) and Costa et al. (2020). A calibration curve with serially diluted, lyophilized hen egg white lysozyme (Sigma) was used.

Total peroxidase activity was measured following the procedure described by Quade and Roth (1997), by defining one unit of peroxidase as the amount which causes an absorbance change of one OD (EU mL^{-1} of plasma).

Alternative complement pathway activity (ACH50) analysis was based on the lysis of rabbit blood cells (Probiológica, Portugal), as described by Sunyer and Tort (1995). ACH50 units were set as the concentration of plasma that caused a cell lysis of 50%.

2.4.2. Metabolites in plasma

Plasma glucose, cholesterol, lactate and non-esterified fatty acids (NEFA) were determined enzymatically using commercial kits (Spinreact, Barcelona, Spain, for glucose, lactate and cholesterol; Wako Chemicals, Neuss, Germany, for fatty acids), adapted to a microplate format (Velasco et al., 2021).

2.5. Liver oxidative stress analysis

Liver samples were homogenized with phosphate buffer (0.1 M, pH 7.4), in a ratio of 1:10 (w/v). To 300 μL of liver homogenate, 5 μL of butylated hydroxytoluene (BHT, 4%, in methanol) were added, after which aliquots for LPO and CC were made and stored at − 80 °C. The remaining homogenate was centrifuged at 10 000 g at 4 °C for 20 min, after which the supernatant was extracted and stored at − 80 °C for antioxidant enzymes' analysis. Before freezing, the protein content of both homogenate and supernatant was measured as described by Bradford (1976) and applied to normalize antioxidant enzymes' activities.

2.5.1. Oxidative stress and antioxidant biomarkers

LPO was assessed in the liver homogenate through the quantification of thiobarbituric acid reactive substances (TBARS), in accordance with Bird and Draper (1984). LPO values were expressed as nmol TBARS/g fresh tissue. Regarding protein oxidation, this was assessed in the liver homogenate through the quantification of carbonyl compounds, using the Protein Carbonyl Content Assay Kit (Sigma-Aldrich MAK094–1KT), following the manufacturer's instructions. Values were expressed as nmol carbonyls per mg protein. The concentration of total antioxidants in liver samples was determined with the Total Antioxidant Capacity (TAC) Assay Kit (Sigma-Aldrich MAK187) and expressed as nmol of Trolox equivalents per g tissue.

Total glutathione (TG) was assessed via the formation of 5-thio-2-nitrobenzoic acid (TNB), at 412 nm, as detailed in Baker et al. (1990) and the results were expressed as nmol conjugated TNB formed per min per mg of protein.

2.5.2. Antioxidant enzyme activities

The activities of CAT, GPx, GR, GST and SOD were assessed in the supernatant, in triplicate using protocols adapted for microplate. CAT activity was evaluated as described by Claiborne (1985), with H_2O_2 30% as substrate. CAT activity was expressed in μmol consumed H_2O_2 per min per mg of protein. GPx activity was estimated considering NADPH oxidation at 340 nm, as stated by Mohandas et al. (1984). GPx activity was expressed as nmol NADPH oxidized per min per mg of protein. To assess GR activity, the method of Cribb et al. (1989) was employed and results were expressed as nmol oxidized NADPH min^{-1} mg $protein^{-1}$ ($\varepsilon = 6.22 \times 10^3\ M^{-1}cm^{-1}$).

The total GST activity was determined as described by Habig et al. (1974). Results were expressed in nmol CDNB conjugate formed per min per mg protein. Finally, SOD activity was measured using a SOD Determination Kit (Sigma-Aldrich 19160–1KT-F). Results were expressed as % of inhibition of the formation of WST-1 formazan per mg protein.

2.6. Muscle antioxidant potential

Muscle antioxidant potential was evaluated as described in Valente et al. (2015). Briefly, the muscle was hydrolyzed by pepsin under acidic conditions, and this reaction was inactivated by boiling at 100 °C. After centrifugation, the supernatant was recovered and analyzed for its antioxidant activity through the 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) and oxygen radical absorbance capacity (ORAC) tests (as it did not present significant 1,1-diphenyl-2-picrylhydrazyl, $DPPH^{\bullet}$, inhibition; data not shown), as described in Ribeiro et al. (2020). A calibration curve was developed with Trolox standards, and the results are expressed as nmol Trolox equivalents per mg muscle.

2.7. Statistical analysis

Data were tested for normality and homogeneity of variances, considering the Kolmogorov-Smirnov and Levene's tests, respectively, and, if necessary, appropriately transformed. A two-way ANOVA was used to analyse data, considering the diet and stress as fixed factors, with the Statistica v13.5 (TIBCO Software Inc., Palo Alto, CA, USA) software. If significant effects were found, a pairwise multiple comparison test (Tukey HSD) was performed. The minimum level of significance was set at $P < 0.05$ for all analyses. Furthermore, Pearson's correlations were evaluated for the data and considered a two-tailed analysis of 0.05 and 0.01.

3. Results

3.1. Growth performance

A more detailed analysis of the impact of the hydrolysates on growth performances is available on Resende et al. (2022). Briefly, RNF did not differ from CTRL in the final fish weight (47.01 ± 7.30 vs. 48.93 ± 8.21, respectively; $P = 0.065$). AH displayed a significantly lower final body weight (46.62 ± 7.76; $P < 0.001$) than CTRL, but it was similar to RNF. Finally, RMF was the worst performing diet (33.37 ± 5.27, $P < 0.001$).

3.2. Plasma metabolites and innate immune markers analysis

Regarding the analysed plasma metabolites (Table 3), cortisol and lactate levels were significantly increased in stressed fish, thus validating our experimental design. However, no significant differences were found among diets. Glucose, cholesterol and triglycerides were unaffected by either diet or stress. NEFA was not affected by stress alone, but non-stressed fish fed AH displayed the highest levels.

Protein levels were not only reduced in stressed fish, but were also affected by diet since the RMF group displayed significantly lower protein levels than all other dietary treatments.

In terms of the analysed innate immune markers (Table 4), ACH50 and peroxidase were unaffected by either stress or dietary treatments. Lysozyme was not affected by the stress, but it was significantly reduced by the RMF diet.

3.3. Liver oxidative stress

LPO was significantly increased post-stress (Fig. 1). Moreover, while the non-stressed RMF-fed fish displayed the lowest LPO values, the stressed fish fed the same diet displayed the highest values. Prior to the stress, the AH diet displayed higher LPO than the RMF and RNF. However, after the stress, all diets were statistically similar. No differences among dietary treatments were found for carbonyl compounds levels, but stressed fish had lower levels compared to non-stressed. Regarding liver TAC, while both stressed and non-stressed fish fed CTRL display statistically similar levels, a significant decrease is seen when comparing stressed to non-stressed fish fed the BH diets.

Significant differences among stressed and non-stressed fish were observed in CAT and GPx, but dietary treatments did not have a significant impact. GR was highest for stressed AH-fed fish. The stressed AH group also showed an increase in GST activity, being significantly higher than the stressed control fish. RMF led to TG and SOD values that were significantly higher than all other diets.

3.4. Muscle antioxidant potential

Fig. 2 depicts the results from muscle antioxidant potential. When evaluated through ABTS, a significant increase was seen for stressed fish. However, this was not observed in the ORAC analysis, as no differences regarding stress or dietary treatments were found.

3.5. Pearson correlations

Some of the Pearson's are described in Table 5 and a full table is provided as supplementary material. Liver LPO displayed strong correlations with SOD activity and lactate, and weaker, but significant correlations with TAC, CAT and plasma cortisol. Furthermore, carbonyl levels were strongly but negatively correlated with TAC, CAT, cortisol and lactate. SOD was correlated with cortisol and CAT. Cortisol and lactate were also strongly correlated.

4. Discussion

As fish welfare becomes a major concern in aquaculture production, aquafeed development evolves to not only provide the basic nutrients, but to also enhance fish response to stressful situations. In this sense, we have hypothesized that functional feeds, low FM aquafeeds supplemented with blood hydrolysates, could improve the animals' response to stress conditions faced in farms.

Table 3
Plasma metabolite levels of fish prior to (non-stressed) or after (stressed) an air exposure.

	Diets								Two-way ANOVA (*P*-value)		
	Non-Stressed				Stressed						
	CTRL	RMF	RNF	AH	CTRL	RMF	RNF	AH	Diet	Stress	Diet × Stress
Cortisol (ng mL^{-1})	618 ± 101	589 ± 91	529 ± 47	652 ± 68	1014 ± 92	875 ± 91	859 ± 115	790 ± 74	0.420	< 0.001	0.419
Glucose (mmol L^{-1})	7.82 ± 0.89	7.17 ± 0.24	7.33 ± 0.36	9.58 ± 0.45	8.08 ± 0.48	8.57 ± 0.87	8.36 ± 0.39	8.84 ± 0.22	0.040 *	0.206	0.229
Lactate (mmol L^{-1})	3.69 ± 0.60	2.74 ± 0.33	2.75 ± 0.20	2.83 ± 0.15	3.84 ± 0.41	4.17 ± 0.42	4.01 ± 0.37	4.37 ± 0.35	0.797	< 0.001	0.873
Triglycerides (mmol L^{-1})	5.46 ± 1.08	5.49 ± 1.00	6.30 ± 1.11	5.86 ± 0.90	4.94 ± 0.63	4.46 ± 0.96	6.73 ± 1.43	4.90 ± 0.37	0.503	0.532	0.948
Cholesterol (mmol L^{-1})	7.92 ± 1.70	6.65 ± 1.04	8.66 ± 1.06	8.11 ± 1.15	7.27 ± 0.68	5.87 ± 1.26	7.01 ± 0.62	8.00 ± 0.66	0.380	0.310	0.919
NEFA (mmol L^{-1})	0.12 ± 0.01^{B}	0.14 ± 0.01^{B}	0.13 ± 0.01^{B}	0.20 ± 0.02 ^{A}	0.15 ± 0.01^{AB}	0.11 ± 0.01^{B}	0.12 ± 0.01^{B}	0.16 ± 0.01^{AB}	< 0.001	0.126	0.014
Protein (g dL^{-1})	4.23 ± 0.31^{a}	3.63 ± 0.26^{b}	4.12 ± 0.21^{a}	4.23 ± 0.19^{a}	3.94 ± 0.14^{a}	2.68 ± 0.27^{b}	3.72 ± 0.21^{a}	4.03 ± 0.20^{a}	< 0.001	0.007	0.348

Values are presented as mean ± SE. Different superscript lowercase letters denote significant differences among diets (P < 0.05), while different superscript uppercase letters indicate significant differences for Diet × Stress. * No significant differences were found after the post-hoc test.

Table 4
Humoral immune parameters of fish before (non-stressed) or after (stressed) air exposure.

	Diets								**Two-way ANOVA (*P*-value)**		
	Non-Stressed				**Stressed**						
	CTRL	RMF	RNF	AH	CTRL	RMF	RNF	AH	Diet	Stress	Diet × Stress
Lysozyme (μg mL^{-1})	15.74 ± 1.03^{a}	12.25 ± 0.76^{b}	14.41 ± 1.03^{a}	16.66 ± 0.68^{a}	15.99 ± 0.75^{a}	9.21 ± 0.49^{b}	15.49 ± 1.29^{a}	15.85 ± 1.19^{a}	<0.001	0.353	0.171
Peroxidase (EU mL^{-1})	33.8 ± 4.6	74.9 ± 18.0	33.8 ± 5.6	39.4 ± 5.1	62.0 ± 11.5	43.7 ± 7.5	55.0 ± 12.5	30.5 ± 3.4	0.146	0.749	0.034*
ACH50 (units mL^{-1})	167.6 ± 18.7	136.8 ± 31.1	111.6 ± 19.5	135.6 ± 30.6	127.1 ± 8.7	130.1 ± 7.8	126.1 ± 10.3	163.1 ± 9.4	0.217	0.901	0.279

Values are presented as mean ± SE. Different superscript lowercase letters denote significant differences among diets (P<0.05), while different superscript uppercase letters indicate significant differences for Diet × Stress. * No significant differences were found after the post-hoc test.

The evaluated stress challenge was an acute stress, with total duration of 1 min, followed by sampling after a recovery period of 6 h. The significantly increased responses on cortisol, lactate and liver lipid peroxidation in stressed fish validate the employed protocol, since these are typical indicators of acute stress (Barton, 2002; Cerqueira et al., 2021; Ciji and Akhtar, 2021). Cortisol is released by interrenal cells as a response to the secretion of adrenocorticotropin by the anterior pituitary, which had been previously stimulated by the corticotropin-releasing hormone produced by the hypothalamus. Thus, cortisol response is delayed a few minutes from stress recognition by the central nervous system (Barton, 2002; Ciji and Akhtar, 2021). While this is a common stress biomarker, it has a high degree of biological variability, even in individuals subjected to the same experimental conditions (Ciji and Akhtar, 2021; de Magalhães et al., 2020). Indeed, high standard error values were found in cortisol measurements, and while the impact of stress was visible, other more subtle differences caused by diets may be disguised by this high variability.

Cortisol promotes both glycogenolysis and gluconeogenesis, which may increase glucose levels, to yield the fish with the necessary energy to counteract the stressor (Martinez-Porchas et al., 2009). Fanouraki et al. (2011) provided useful information on the general patterns of the response of plasma cortisol, lactate, and glucose concentrations in seabass after acute stress. They observed that both glucose (12 mmol L^{-1}) and lactate (9 mmol L^{-1}) levels were considerably higher than in the present study (8.5 mmol L^{-1} and 4 mmol L^{-1}, respectively), even 8 h post-stress. Yet, they found that plasma glucose after 4 h dropped to basal levels for most of the species investigated. In our work, despite an absence of statistical differences, glucose levels 6 h post-stress are slightly higher for all groups except for fish fed AH. All these observations could provide evidence and support for the idea that glucose levels after 6 h may already have returned to basal levels. Rotllant and Tort (1997) reported that after an acute stress (net handling for 8 min), glucose levels of red porgy *Pagrus pagru* were elevated after a two-hour period, but not after 24 h. Davis and McEntire (2009) have described that glucose levels of sunshine bass (a hybrid *Morone chrysops X Morone saxatilis*) and white bass (*Morone chrysops*) only slightly rise after a stress (decrease in water volume in the tank) and are returned to normal after 6 h. Another hypothesis, stated by Martinez-Porchas et al. (2009) is that, while cortisol does increase circulating glucose levels, the stress may also increase the rate at which fish consume the energetic substrates. Moreover, in a stressful event, the animal's oxygen demand may increase, leading to cell hypoxia and anaerobic metabolism of glucose. This explains the increase in circulating lactate levels after the challenge and the absence of significant differences in glucose levels post-stress (Mirzargar et al., 2022).

Overall, the hydrolysates had a reduced impact on plasma stress indicators. Similarly, another work with swine blood by-products at 5% in diets for *Argyrosomus regius* also did not find any differences in glucose, lactate, protein or cortisol, although these biomarkers were evaluated on skin mucus rather than plasma, and without subjecting the animals to any challenge (Fernández-Alacid et al., 2021). It could be possible that further increased inclusion levels could have better outcomes, since the average value of cortisol was lower for BH diets, albeit not significantly.

Plasma protein levels can be an indicator of the nutritional status of fish (Pelusio et al., 2022). In this work, they decreased after the stress

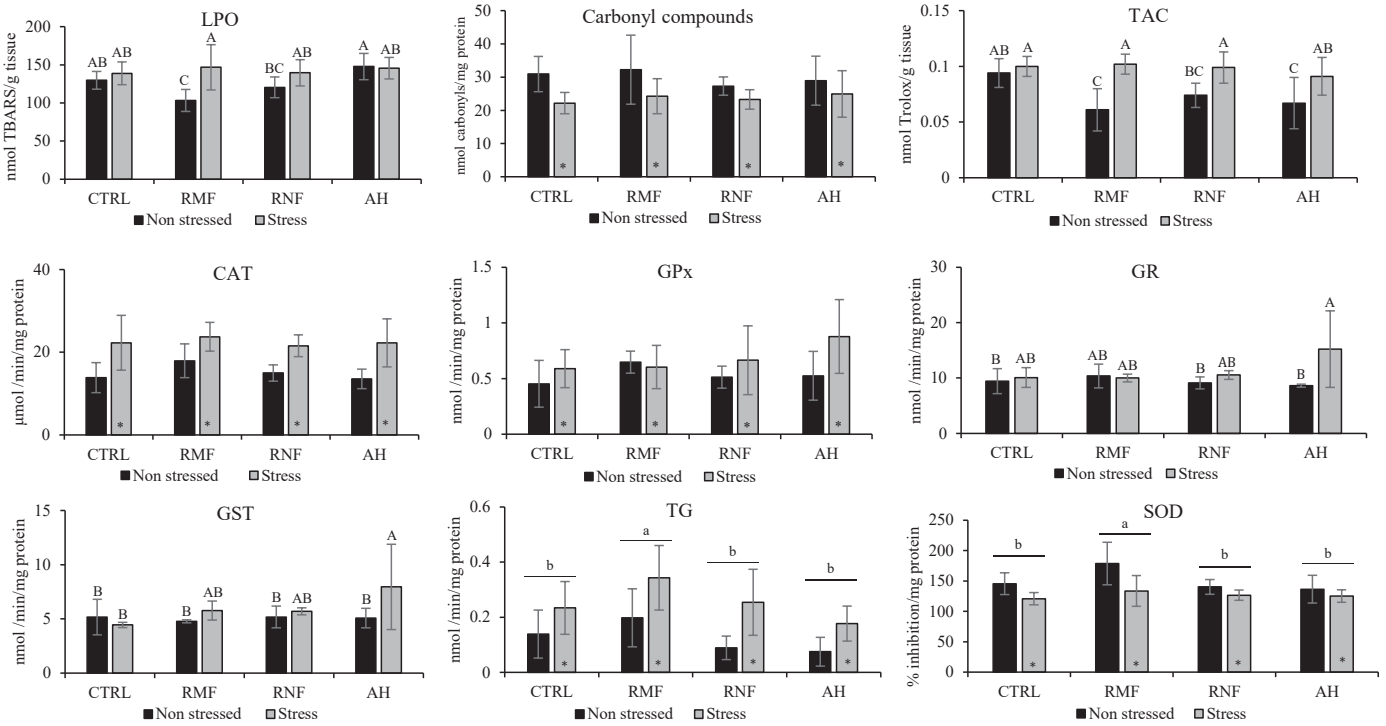

Fig. 1. Liver oxidative status and antioxidant enzymes' activity. LPO – lipid peroxidation; TAC – total non-enzymatic antioxidant activity; CAT – catalase; GPx – glutathione peroxidase; GR – glutathione reductase; GST – glutathione S-transferase; TG – total glutathione; SOD – superoxide dismutase. Values are presented as mean ± SE (n = 9). Different superscript lowercase letters denote significant differences among diets ($P < 0.05$), while different superscript uppercase letters indicate significant differences for Diet × Stress ($P < 0.05$). * indicates significant differences between stressed and non-stressed fish ($P < 0.05$).

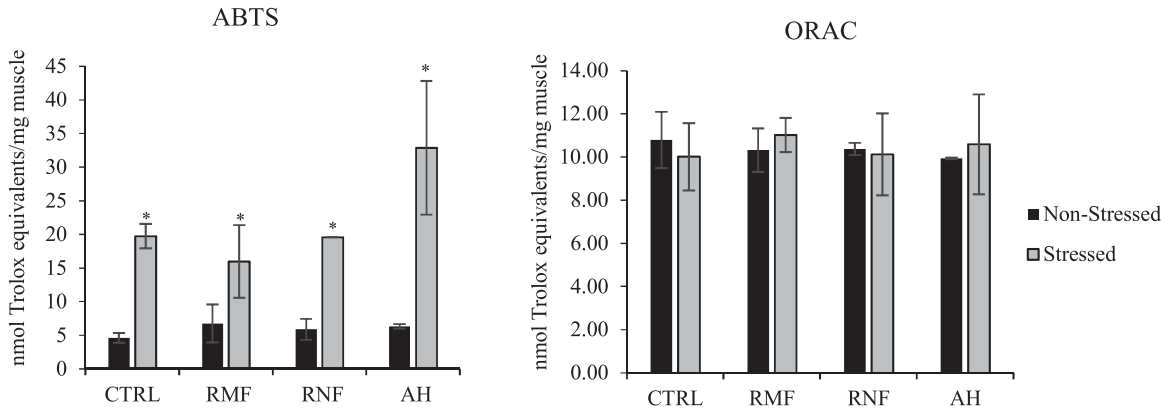

Fig. 2. Antioxidant potential of the muscle of fish fed the experimental diets, evaluated through 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) and oxygen radical absorbance capacity (ORAC) tests. Values are presented as mean ± SE (n = 9). Different superscript lowercase letters denote significant differences among diets ($P < 0.05$), while different superscript uppercase letters indicate significant differences for Diet × Stress ($P < 0.05$). Asterisk (*) indicates significant differences between stressed and non-stressed fish ($P < 0.05$).

challenge, in accordance with previous literature reports (Di Marco et al., 2008; Fernández-Alacid et al., 2019; Mommsen et al., 1999; Samaras et al., 2023). This decrease can be attributed to proteolysis derived from cortisol action (Di Marco et al., 2008; Mommsen et al., 1999; Pelusio et al., 2022). This proteolytic action has been claimed to be evident in white muscle (Mommsen et al., 1999) and can also explain the results from the ABTS tests, as an increased antioxidant potential was observed in muscle, probably derived from peptides released from this proteolysis. However, the ORAC test did not support these results. This is not unheard of, as the $ABTS^{\bullet+}$ is sterically-hindered stable, compared to the tested radical in the ORAC test, leading to the observed difference (Schaich et al., 2015). ORAC uses a biologically pertinent radical source (H_2O_2), meaning its results should be closer to an in vivo approach (Thaipong et al., 2006); yet this relates more to a possible benefit of fillets for consumers than to inferences regarding proteolysis of muscle. Additionally, the RMF diet led to lower plasmatic protein levels, which suggests an overall poorer nutritional status and possible higher protein oxidation. This is in agreement with its general worse growth performance (in terms of final weight), when compared to the remaining diets. Nevertheless, further analysis regarding amino acid and protein turnover should be performed to validate this evidence.

High values of NEFA in the plasma can be attributed to a mobilization of lipid reserves and their oxidation, caused by a higher metabolic demand (Di Marco et al., 2008). The AH diet led to the highest NEFA values, and also displayed high levels of liver LPO, particularly in non-stressed fish, which could be an indicator of impairment of lipid

Table 5
Pearson correlations between some evaluated parameters.

		LPO	Carbonyls	TAC	GPx	SOD	CAT	Cortisol	Lactate
LPO	Pearson correlation	1	-0.260*	0.295*	0.047	-0.503**	0.250*	0.284*	0.305**
	Sig (2-tailed)		0.027	0.034	0.697	< 0.001	0.034	0.016	0.009
Carbonyls	Pearson correlation		1	-0.327**	-0.035	0.179	-0.363**	-0.342**	-0.320**
	Sig (2-tailed)			0.005	0.771	0.133	0.002	0.003	0.006
TAC	Pearson correlation			1	-0.028	-0.560**	-0.364**	0.338**	0.413**
	Sig (2-tailed)				0.817	< 0.001	0.002	0.004	< 0.001
GPx	Pearson correlation				1	-0.045	0.192	0.157	0.334**
	Sig (2-tailed)					0.707	0.106	0.189	0.004
SOD	Pearson correlation					1	-0.241*	-0.286*	-0.223
	Sig (2-tailed)						0.042	0.015	0.060
CAT	Pearson correlation						1	0.355**	0.431**
	Sig (2-tailed)							0.002	< 0.001
Cortisol	Pearson correlation							1	0.468**
	Sig (2-tailed)								< 0.001
Lactate	Pearson correlation								1
	Sig (2-tailed)								

* significant at $p < 0.05$
** significant at $p < 0.01$

metabolism in fish fed this diet. In a previous work with these blood hydrolysates, a reduction of lipid digestibility was observed for this diet (Resende et al., 2022), which supports this hypothesis. However, despite alterations in NEFA, triglycerides and cholesterol were not affected, a similar situation to that reported by Di Marco et al. (2008). This could indicate that free fatty acids may derive from mesenteric fat or hepatic tissue, rather than from blood triglycerides, which suggests the activation of transcription factors that control metabolic pathways in a tissue-specific manner, regulating nutrient transport and modulating levels of plasma NEFA, triglycerides and cholesterol (Shearer et al., 2012).

Humoral immune parameters were not affected by stress in this report, despite the frequent association between both chronical and acute oxidative stress and impairments in the immune system (Dawood et al., 2022; Paray et al., 2021). It is, however, possible that acute stressors may not affect seriously the immune system, as other responses of the organism are occurring (Tort, 2011). This is in agreement with Machado et al. (2019a), who also found that a similar stress for European seabass did not affect some immune markers such as peroxidase and lysozyme. Additionally, the BH did not affect significantly the innate immune parameters in the plasma, compared to the CTRL. Diet RMF led to lower plasmatic lysozyme levels, possibly suggesting a poorer capacity to respond in the event of an infection. This hydrolysate possessed the lowest abundance of smaller sized peptides, which have been associated with beneficial effects on immunity (Resende et al., 2022; Siddik et al., 2021), and that could be the reason behind those values. In any case, the values observed for plasmatic lysozyme (Machado et al., 2019b), peroxidase (Campos et al., 2017) and ACH50 (Azeredo et al., 2017) are in agreement with previous reports for this species.

Other authors have found that marine hydrolysates in low FM diets increased the non-specific immunity biomarkers to values equal to or above to those induced by a non-supplemented FM-based diet (Costa et al., 2020; Gisbert et al., 2018). Yet, Leduc et al. (2018) claimed that a shrimp hydrolysate had stronger immunostimulant properties than a tilapia hydrolysate when supplemented at equal levels in European seabass diets. Thus, the raw material used to produce hydrolysates plays an important role in determining the outcomes, and this could be the reason behind the differences in our results, along with variations in inclusion levels of the hydrolysates. Other residual compounds, such as vitamins or minerals, eventually present in the hydrolysates, may also play an important role. In addition, it is suggested that, in future experiments, other immune markers, namely expression of immunoglobulins, antiproteases, or even inflammation markers, are assessed to obtain a clearer picture of the potential of hydrolysates to modulate immune responses.

Another concern regarding the impacts of stress and impaired fish welfare is related to the production of ROS, which then tend to accumulate in the liver (Awasthi et al., 2018). Indeed, liver LPO levels were increased after stress, similarly to previous literature reports on this species (Silva-Brito et al., 2019). Liver LPO pre-stress was lowest for the RMF diet, but this diet displayed the highest increase after the challenge. This could be partially due to the decrease in SOD levels after stress. SOD is an enzyme that catalyses the conversion of the superoxide radical into molecular oxygen and hydrogen peroxide, being an important antioxidant defence (Birben et al., 2012). Indeed, we have found a negative correlation between liver SOD activity and liver LPO ($p < 0.001$), as previously demonstrated by other authors in studies correlating higher SOD activity with lower LPO levels (Abdel-Tawwab et al., 2021; Passos et al., 2021).

Liver CAT activity was higher for stressed fish than for non-stressed ones and has a positive correlation with LPO. This suggests that once stressed, the increase in lipid peroxidation stimulates the activity of CAT, which aids in antioxidant defence by breaking down hydrogen peroxide (a pro-oxidant) into water and molecular oxygen (Moutinho et al., 2021). The results here presented for CAT are within the range described for this species (Islam et al., 2020).

GPx acts in a similar way to CAT, also converting hydrogen peroxide into water, and the range of its activity is within the range reported for this species (Lobo et al., 2018). Nevertheless, GPx is not significantly correlated with CAT in this work. GPx activity was higher for stressed fish in all diets apart from RMF, where it was decreased. This may also partially explain the increase of LPO in stressed fish fed this diet, due to the impairment of antioxidant enzymatic defences.

However, while the activity of SOD was significantly lower in all diets after the stress challenge, only the RMF diet displayed a significant increase in LPO (considering the two-way ANOVA results). Exogenous antioxidants present in the diet, such as bioactive peptides, can enhance non-enzymatic antioxidant response and minimize the need for enzymatic activity when facing oxidative stress (Batista et al., 2020; Moutinho et al., 2021; Pereira et al., 2022). The TAC values support this hypothesis, as a mobilization of non-enzymatic antioxidants seems evident in BH-diets post-stress. Furthermore, TAC has a negative correlation with SOD activity, which suggests that if non-enzymatic antioxidants are mobilized, the need for antioxidant enzymes decreases. It is possible that some of the bioactive peptides present in the BH could have an affinity for the superoxide radical in the liver of the stressed fish, minimizing the need for the SOD activity. However, further assessment of the mechanisms of action of such peptides are still needed to clarify this matter.

The AH diet required higher activities of GPx, GR, and GST, which

can suggest that this diet cannot modulate antioxidant defences, leading for higher needs of the enzymes to maintain homeostasis. This occurred despite the presence of low-sized (<13.7 kDa) peptides in this hydrolysate. A low bioavailability of such peptides could be the reason behind its inefficacy. Indeed, a previous work with this diet resulted in worse protein digestibility and higher faecal nitrogen losses (Resende et al., 2022), which would affect not only protein metabolism related to growth, but also reduce the availability of bioactive peptides. The RNF, which also displays small sized peptides, led to statistically similar GR and GST activities before and after the stress. As such, the bioactive peptides present in this potentially functional feed may be more available than those present in AH.

In oxidative stress events, ROS may directly or indirectly introduce carbonyl moieties at amino acid side chains. Higher amounts of carbonyl groups have been correlated with protein damage resulting from oxidative stress (Almroth et al., 2005). However, in this work, liver carbonyls are significantly decreased in stressed fish compared to non-stressed. Two reasons, previously stated in the literature, may aid explain this issue. Firstly, mildly oxidated proteins are more susceptible to proteolytic degradation, as a defence mechanism to prevent the spreading of oxidative damage (Passi et al., 2004). Moreover, the increase of lipid peroxidation products has been reported to act as an inhibitor for protein carbonyl formation (Almroth et al., 2005). Indeed, a negative correlation between carbonyls and LPO was observed. Therefore, both an increase and a decrease in carbonyls can be indicators of oxidative stress (Almroth et al., 2005); the RNF and AH diets, which led to the lowest difference between the non-stressed and stressed counterparts, could possibly be adequate to prevent an elevated impact of oxidative stress on proteins.

5. Conclusions

The tested diets seemed to affect the fish oxidative stress response in the liver. This could be due to their bioactive peptides, which aided in the non-enzymatic modulation of stress response. This seems to be confirmed by the TAC values in the liver. However, this impact was not sufficient to lower LPO levels compared to a control plant-based diet. In addition, plasmatic cortisol response was not affected by dietary treatments. Therefore, and while bioactive hydrolysates remain an option for improvement of farmed fish stress response, further research is needed. This includes optimizing inclusion levels, assessing other timepoints after an acute stress and verifying the impact of BH on chronical stress.

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CRediT authorship contribution statement

Daniela Resende: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ricardo Pereira:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **David Domínguez:** Investigation, Writing – review & editing. **Miguel Pereira:** Formal analysis, Investigation, Writing – review & editing. **Carlos Pereira:** Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. **Manuela Pintado:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Luísa M.P. Valente:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Cristina Velasco:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing – original draft, 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

Data will be made available on request.

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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.2023.101600.

References

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

Abdel-Tawwab, M., Khalil, R.H., Diab, A.M., Khallaf, M.A., Abdel-Razek, N., Abdel-Latif, H.M.R., Khalifa, E., 2021. Dietary garlic and chitosan enhanced the antioxidant capacity, immunity, and modulated the transcription of HSP70 and Cytokine genes in Zearalenone-intoxicated European seabass. Fish. Shellfish Immunol. 113, 35–41. https://doi.org/10.1016/j.fsi.2021.03.012.

Almroth, B.C., Sturve, J., Berglund, Å., Förlin, L., 2005. Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. Aquat. Toxicol. 73, 171–180. https://doi.org/10.1016/j.aquatox.2005.03.007.

Araújo-Rodrigues, H., Coscueta, E.R., Pereira, M.F., Cunha, S.A., Almeida, A., Rosa, A., Martins, R., Pereira, C.D., Pintado, M.E., 2022. Membrane fractionation of *Cynara cardunculus* swine blood hydrolysate: ingredients of high nutritional and nutraceutical value. Food Res. Int. 158, 111549 https://doi.org/10.1016/j.foodres.2022.111549.

Ashley, P.J., 2007. Fish welfare: current issues in aquaculture. Appl. Anim. Behav. Sci. 104, 199–235. https://doi.org/10.1016/j.applanim.2006.09.001.

Aubin, J., Callier, M., Rey-Valette, H., Mathé, S., Wilfart, A., Legendre, M., Slembrouck, J., Caruso, D., Chia, E., Masson, G., Blancheton, J.P., Ediwarman, Haryadi, J., Prihadi, T.H., de Matos Casaca, J., Tamassia, S.T.J., Tocqueville, A., Fontaine, P., 2019. Implementing ecological intensification in fish farming: definition and principles from contrasting experiences. Rev. Aquac. 11, 149–167. https://doi.org/10.1111/raq.12231.

Awasthi, Y., Ratn, A., Prasad, R., Kumar, M., Trivedi, S.P., 2018. An in vivo analysis of Cr^{6+} induced biochemical, genotoxicological and transcriptional profiling of genes related to oxidative stress, DNA damage and apoptosis in liver of fish, *Channa punctatus* (Bloch, 1793). Aquat. Toxicol. 200, 158–167. https://doi.org/10.1016/j.aquatox.2018.05.001.

Azeredo, R., Machado, M., Kreuz, E., Wuertz, S., Oliva-Teles, A., Enes, P., Costas, B., 2017. The European seabass (*Dicentrarchus labrax*) innate immunity and gut health are modulated by dietary plant-protein inclusion and prebiotic supplementation. Fish. Shellfish Immunol. 60, 78–87. https://doi.org/10.1016/j.fsi.2016.11.019.

Bah, C.S.F., Bekhit, A.E.-D.A., Carne, A., McConnell, M.A., 2013. Slaughterhouse blood: an emerging source of bioactive compounds. Compr. Rev. Food Sci. Food Saf. 12, 314–331. https://doi.org/10.1111/1541-4337.12013.

Baker, M.A., Cerniglia, G.J., Zaman, A., 1990. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Anal. Biochem. 190, 360–365. https://doi.org/10.1016/0003-2697(90)90208-Q.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42, 517–525. https://doi.org/10.1093/icb/42.3.517.

Batista, S., Pereira, R., Oliveira, B., Baião, L.F., Jessen, F., Tulli, F., Messina, M., Silva, J. L., Abreu, H., Valente, L.M.P., 2020. 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. 32, 2041–2059. https://doi.org/10.1007/s10811-020-02118-z.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative stress and antioxidant defense. World Allergy Organ. J. 5, 9–19. https://doi.org/10.1097/WOX.0b013e3182439613.

Bird, R.P., Draper, H.H., 1984. Comparative studies on different methods of malonaldehyde determination. Methods Enzym. 105, 299–305. https://doi.org/10.1016/s0076-6879(84)05038-2.

Bonga, S.E.W., 1997. The stress response in fish. Physiol. Rev. 77, 591–625. https://doi.org/10.1152/physrev.1997.77.3.591.

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

Bui, H.T.D., Khosravi, S., Fournier, V., Herault, M., Lee, K.-J., 2014. Growth performance, feed utilization, innate immunity, digestibility and disease resistance of juvenile red seabream (*Pagrus major*) fed diets supplemented with protein hydrolysates. Aquaculture 418–419, 11–16. https://doi.org/10.1016/j.aquaculture.2013.09.046.

Campos, I., Matos, E., Marques, A., Valente, L.M.P., 2017. Hydrolyzed feather meal as a partial fishmeal replacement in diets for European seabass (*Dicentrarchus labrax*) juveniles. Aquaculture 476, 152–159. https://doi.org/10.1016/j.aquaculture.2017.04.024.

Cerqueira, M., Millot, S., Silva, T., Félix, A.S., Castanheira, M.F., Rey, S., Mackenzie, S., Oliveira, G.A., Oliveira, C.C.V., Oliveira, R.F., 2021. Stressor controllability modulates the stress response in fish. BMC Neurosci. 22, 48. https://doi.org/10.1186/s12868-021-00653-0.

Chataigner, M., Mortessagne, P., Lucas, C., Pallet, V., Layé, S., Mehaignerie, A., Bouvret, E., Dinel, A.L., Joffre, C., 2021. Dietary fish hydrolysate supplementation containing n-3 LC-PUFAs and peptides prevents short-term memory and stress response deficits in aged mice. Brain Behav. Immun. 91, 716–730. https://doi.org/10.1016/j.bbi.2020.09.022.

Ciji, A., Akhtar, M.S., 2021. Stress management in aquaculture: a review of dietary interventions. Rev. Aquac. 13, 2190–2247. https://doi.org/10.1111/raq.12565.

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

Conde-Sieira, M., Gesto, M., Batista, S., Linares, F., Villanueva, J.L.R., Míguez, J.M., Soengas, J.L., Valente, L.M.P., 2018. Influence of vegetable diets on physiological and immune responses to thermal stress in Senegalese sole (*Solea senegalensis*). PLOS ONE 13, e0194353. https://doi.org/10.1371/journal.pone.0194353.

Costa, M., Costas, B., Machado, M., Teixeira, C., Fernández-Boo, S., Sá, T., Batista, S., Marques, A., Miranda, F., Valente, L.M.P., 2020. Anchovy and giant squid hydrolysates can enhance growth and the immune response of European seabass (*Dicentrarchus labrax*) fed plant-protein-based diets. Aquaculture 523, 735182. https://doi.org/10.1016/j.aquaculture.2020.735182.

Cribb, A.E., Leeder, J.S., Spielberg, S.P., 1989. Use of a microplate reader in an assay of glutathione reductase using 5,5′-dithiobis(2-nitrobenzoic acid). Anal. Biochem. 183, 195–196. https://doi.org/10.1016/0003-2697(89)90188-7.

Davis, K., McEntire, M., 2009. Comparison of the cortisol and glucose stress response to acute confinement among white bass, *Morone chrysops*, striped bass, *Morone saxatilis*, and sunshine bass, *Morone chrysops* X *Morone saxatilis*. J. World Aquac. Soc. 40, 567–572. https://doi.org/10.1111/j.1749-7345.2009.00275.x.

Dawood, M.A.O., Alkafafy, M., Sewilam, H., 2022. The antioxidant responses of gills, intestines and livers and blood immunity of common carp (*Cyprinus carpio*) exposed to salinity and temperature stressors. Fish. Physiol. Biochem. 48, 397–408. https://doi.org/10.1007/s10695-022-01052-w.

Di Marco, P., Priori, A., Finoia, M.G., Massari, A., Mandich, A., Marino, G., 2008. Physiological responses of European sea bass *Dicentrarchus labrax* to different stocking densities and acute stress challenge. Aquaculture 275, 319–328. https://doi.org/10.1016/j.aquaculture.2007.12.012.

Dinel, A.-L., Lucas, C., Le Faouder, J., Bouvret, E., Pallet, V., Layé, S., Joffre, C., 2021. Supplementation with low molecular weight peptides from fish protein hydrolysate reduces acute mild stress-induced corticosterone secretion and modulates stress responsive gene expression in mice. J. Funct. Foods 76, 104292. https://doi.org/10.1016/j.jff.2020.104292.

Encarnação, P., 2016. Functional feed additives in aquaculture feeds. In: Nates, S.F. (Ed.), Aquafeed Formulation. Academic Press, San Diego, CA, USA, pp. 217–237.

European Commission, 2021. Pigmeat statistics. Retrieved from https://ec.europa.eu/info/food-farming-fisheries/farming/facts-and-figures/markets/overviews/market-observatories/meat/pigmeat-statistics_en on 08–07-2021.

Fanouraki, E., Mylonas, C.C., Papandroulakis, N., Pavlidis, M., 2011. Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. Gen. Comp. Endocrinol. 173, 313–322. https://doi.org/10.1016/j.ygcen.2011.06.004.

Faustino, M., Veiga, M., Sousa, P., Costa, E.M., Silva, S., Pintado, M., 2019. Agro-food byproducts as a new source of natural food additives. Molecules 24, 1056. https://doi.org/10.3390/molecules24061056.

Fernández-Alacid, L., Sanahuja, I., Ordóñez-Grande, B., Sánchez-Nuño, S., Herrera, M., Ibarz, A., 2019. Skin mucus metabolites and cortisol in meagre fed acute stress-attenuating diets: correlations between plasma and mucus. Aquaculture 499, 185–194. https://doi.org/10.1016/j.aquaculture.2018.09.039.

Fernández-Alacid, L., Firmino, J.P., Sanahuja, I., Madrid, C., Polo, J., de Borba, M.R., Balsalobre, C., Gisbert, E., Ibarz, A., 2021. Impact of dietary porcine blood by-products in meagre (*Argyrosomus regius*) physiology, evaluated by welfare biomarkers and the antibacterial properties of the skin mucus. Fish. Shellfish Immunol. 118, 241–250. https://doi.org/10.1016/j.fsi.2021.09.011.

Gisbert, E., Fournier, V., Solovyev, M., Skalli, A., Andree, K.B., 2018. Diets containing shrimp protein hydrolysates provided protection to European sea bass (*Dicentrarchus labrax*) affected by a *Vibrio pelagius* natural infection outbreak. Aquaculture 495, 136–143. https://doi.org/10.1016/j.aquaculture.2018.04.051.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8.

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

Hutchinson, T.H., Manning, M.J., 1996. Seasonal trends in serum lysozyme activity and total protein concentration in dab (*Limanda limanda* L.) sampled from Lyme Bay, U. K. Fish. Shellfish Immunol. 6, 473–482. https://doi.org/10.1006/fsim.1996.0045.

Islam, M.J., Kunzmann, A., Thiele, R., Slater, M.J., 2020. Effects of extreme ambient temperature in European seabass, *Dicentrarchus labrax* acclimated at different salinities: growth performance, metabolic and molecular stress responses. Sci. Total Environ. 735, 139371 https://doi.org/10.1016/j.scitotenv.2020.139371.

Kır, M., Sunar, M.C., Gök, M.G., 2019. Acute ammonia toxicity and the interactive effects of ammonia and salinity on the standard metabolism of European sea bass (*Dicentrarchus labrax*). Aquaculture 511, 734273. https://doi.org/10.1016/j.aquaculture.2019.734273.

Ko, J.-Y., Kim, E.-A., Lee, J.-H., Kang, M.-C., Lee, J.-S., Kim, J.-S., Jung, W.-K., Jeon, Y.-J., 2014. Protective effect of aquacultured flounder fish-derived peptide against oxidative stress in zebrafish. Fish. Shellfish Immunol. 36, 320–323. https://doi.org/10.1016/j.fsi.2013.11.018.

Kurutas, E.B., 2016. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr. J. 15, 71. https://doi.org/10.1186/s12937-016-0186-5.

Leduc, A., Zatylny-Gaudin, C., Robert, M., Corre, E., Corguille, G.L., Castel, H., Lefevre-Scelles, A., Fournier, V., Gisbert, E., Andree, K.B., Henry, J., 2018. Dietary aquaculture by-product hydrolysates: impact on the transcriptomic response of the intestinal mucosa of European seabass (*Dicentrarchus labrax*) fed low fish meal diets. BMC Genom. 19, 396. https://doi.org/10.1186/s12864-018-4780-0.

Liang, M., Wang, J., Chang, Q., Mai, K., 2006. Effects of different levels of fish protein hydrolysate in the diet on the nonspecific immunity of Japanese sea bass, *Lateolabrax japonicus* (Cuvieret Valenciennes, 1828). Aquac. Res. 37, 102–106. https://doi.org/10.1111/j.1365-2109.2005.01392.x.

Lobo, G., Pereira, L.F., Gonçalves, J.F.M., Peixoto, M.J., Ozório, R.O.A., 2018. Effect of dietary seaweed supplementation on growth performance, antioxidant and immune responses in European seabass (*Dicentrarchus labrax*) subjected to rearing temperature and salinity oscillations. Int. Aquat. Res. 10, 321–331. https://doi.org/10.1007/s40071-018-0208-3.

Machado, M., Castro, C., Oliva-Teles, A., Costas, B., 2019a. Interactive effects of dietary vegetable oil and carbohydrate incorporation on the innate immune response of European seabass (*Dicentrarchus labrax*) juveniles subjected to acute stress. Aquaculture 498, 171–180. https://doi.org/10.1016/j.aquaculture.2018.08.050.

Machado, M., Azeredo, R., Domingues, A., Fernandez-Boo, S., Dias, J., Conceição, L.E.C., Costas, B., 2019b. Dietary tryptophan deficiency and its supplementation compromises inflammatory mechanisms and disease resistance in a teleost fish. Sci. Rep. 9, 7689. https://doi.org/10.1038/s41598-019-44205-3.

de Magalhães, C.S.F.R., Cerqueira, M.A.C., Schrama, D., Moreira, M.J.V., Boonanuntanasarn, S., Rodrigues, P.M.L., 2020. A Proteomics and other Omics approach in the context of farmed fish welfare and biomarker discovery. Rev. Aquac. 12, 122–144. https://doi.org/10.1111/raq.12308.

Manzoor, M., Singh, J., Gani, A., 2022. Exploration of bioactive peptides from various origin as promising nutraceutical treasures: *In vitro*, *in silico* and in vivo studies. Food Chem. 373, 131395 https://doi.org/10.1016/j.foodchem.2021.131395.

Martinez-Porchas, M., Martinez-Cordova, L.R., Ramos-Enriquez, R., 2009. Cortisol and glucose: reliable indicators of fish stress? Panam. J. Aquat. Sci. 4, 158–178.

Mirzargar, S.S., Taheri Mirghaed, A., Hoseini, S.M., Ghelichpour, M., shahbazi, M., Yousefi, M., 2022. Biochemical responses of common carp, *Cyprinus carpio*, to transportation in plastic bags using thymol as a sedative agent. Aquac. Res. 53, 191–198. https://doi.org/10.1111/are.15564.

Mohandas, J., Marshall, J.J., Duggin, G.G., Horvath, J.S., Tiller, D.J., 1984. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: possible implications in analgesic nephropathy. Biochem. Pharm. 33, 1801–1807. https://doi.org/10.1016/0006-2952(84)90353-8.

Mohapatra, S., Chakraborty, T., Kumar, V., DeBoeck, G., Mohanta, K.N., 2013. Aquaculture and stress management: a review of probiotic intervention. J. Anim. Physiol. Anim. Nutr. 97, 405–430. https://doi.org/10.1111/j.1439-0396.2012.01301.x.

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

Moutinho, S., Pedrosa, R., Magalhães, R., Oliva-Teles, A., Parisi, G., Peres, H., 2021. Black soldier fly (*Hermetia illucens*) pre-pupae larvae meal in diets for European

seabass (*Dicentrarchus labrax*) juveniles: effects on liver oxidative status and fillet quality traits during shelf-life. Aquaculture 533, 736080. https://doi.org/10.1016/j.aquaculture.2020.736080.

National Research Council, 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC.

Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Little, D. C., Lubchenco, J., Shumway, S.E., Troell, M., 2021. A 20-year retrospective review of global aquaculture. Nature 591, 551–563. https://doi.org/10.1038/s41586-021-03308-6.

Okoye, C.O., Ezeorba, T.P.C., Okeke, E.S., Okagu, I.U., 2022. Recent findings on the isolation, identification and quantification of bioactive peptides. Appl. Food Res. 2, 100065 https://doi.org/10.1016/j.afres.2022.100065.

Olmos-Soto, J., Paniagua-Michel, J., López, L., Ochoa, L., 2015. In: Kim, S.-K. (Ed.), Functional feeds in aquaculture. Springer Handbook of Marine Biotechnology: Part J - Industrial Applications, Berlin, Germany, pp. 1303–1319.

Paray, B.A., El-Basuini, M.F., Alagawany, M., Albeshr, M.F., Farah, M.A., Dawood, M.A. O., 2021. *Yucca schidigera* usage for healthy aquatic animals: potential roles for sustainability. Animals 11, 93. https://doi.org/10.3390/ani11010093.

Passi, S., Ricci, R., Cataudella, S., Ferrante, I., De Simone, F., Rastrelli, L., 2004. Fatty acid pattern, oxidation product development, and antioxidant loss in muscle tissue of rainbow trout and *Dicentrarchus labrax* during growth. J. Agric. Food Chem. 52, 2587–2592. https://doi.org/10.1021/jf030559t.

Passos, R., Correia, A.P., Pires, D., Pires, P., Ferreira, I., Simões, M., do Carmo, B., Santos, P., Pombo, A., Afonso, C., Baptista, T., 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.

Pelusio, N.F., Bonaldo, A., Gisbert, E., Andree, K.B., Esteban, M.A., Dondi, F., Sabetti, M. C., Gatta, P.P., Parma, L., 2022. Different fish meal and fish oil dietary levels in European sea bass: welfare implications after acute confinement stress. Front. Mar. Sci. 8, 779053 https://doi.org/10.3389/fmars.2021.779053.

Pereira, R., Costa, M., Velasco, C., Cunha, L.M., Lima, R.C., Baião, L.F., Batista, S., Marques, A., Sá, T., Campos, D.A., Pereira, M., Jesus, D., Fernández-Boo, S., Costas, B., Pintado, M., Valente, L.M.P., 2022. Comparative analysis between synthetic vitamin E and natural antioxidant sources from tomato, carrot and coriander in diets for market-sized *Dicentrarchus labrax*. Antioxidants 11, 636. https://doi.org/10.3390/antiox11040636.

Quade, M.J., Roth, J.A., 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet. Immunol. Immunopathol. 58, 239–248. https://doi.org/10.1016/s0165-2427(97)00048-2.

Resende, D., Costas, B., Sá, T., Golfetto, U., Machado, M., Pereira, M., Pereira, C., Marques, B., Rocha, C.M.R., Pintado, M., Valente, L.M.P., 2022. Innovative swine blood hydrolysates as promising ingredients for European seabass diets: impact on growth performance and resistance to *Tenacibaculum maritimum* infection. Aquaculture 561, 738657. https://doi.org/10.1016/j.aquaculture.2022.738657.

Ribeiro, T.B., Oliveira, A., Campos, D., Nunes, J., Vicente, A.A., Pintado, M., 2020. Simulated digestion of an olive pomace water-soluble ingredient: relationship between the bioaccessibility of compounds and their potential health benefits. Food Funct. 11, 2238–2254. https://doi.org/10.1039/C9FO03000J.

Rotllant, J., Tort, L., 1997. Cortisol and glucose responses after acute stress by net handling in the sparid red porgy previously subjected to crowding stress. J. Fish. Biol. 51, 21–28. https://doi.org/10.1111/j.1095-8649.1997.tb02510.x.

Sadoul, B., Alfonso, S., Cousin, X., Prunet, P., Bégout, M.-L., Leguen, I., 2021. Global assessment of the response to chronic stress in European sea bass. Aquaculture 544 737072 (737071–737072), 737017. https://doi.org/10.1016/j.aquaculture.2021.737072.

Samaras, A., Tsoukali, P., Katsika, L., Pavlidis, M., Papadakis, I.E., 2023. Chronic impact of exposure to low dissolved oxygen on the physiology of Dicentrarchus labrax and Sparus aurata and its effects on the acute stress response. Aquaculture 562, 738830. https://doi.org/10.1016/j.aquaculture.2022.738830.

Samuel-Fitwi, B., Meyer, S., Reckmann, K., Schroeder, J.P., Schulz, C., 2013. Aspiring for environmentally conscious aquafeed: comparative LCA of aquafeed manufacturing using different protein sources. J. Clean. Prod. 52, 225–233. https://doi.org/10.1016/j.jclepro.2013.02.031.

Schaich, K.M., Tian, X., Xie, J., 2015. Reprint of "Hurdles and pitfalls in measuring antioxidant efficacy: a critical evaluation of ABTS, DPPH, and ORAC assays". J. Funct. Foods 18, 782–796. https://doi.org/10.1016/j.jff.2015.05.024.

Shearer, G.C., Savinova, O.V., Harris, W.S., 2012. Fish oil — how does it reduce plasma triglycerides? Biochim. Biophys. Acta - Mol. Cell. Biol. Lipids 1821, 843–851. https://doi.org/10.1016/j.bbalip.2011.10.011.

Siddik, M.A.B., Chaklader, M.R., Foysal, M.J., Howieson, J., Fotedar, R., Gupta, S.K., 2020. Influence of fish protein hydrolysate produced from industrial residues on antioxidant activity, cytokine expression and gut microbial communities in juvenile barramundi Lates calcarifer. Fish. Shellfish Immunol. 97, 465–473. https://doi.org/10.1016/j.fsi.2019.12.057.

Siddik, M.A.B., Howieson, J., Fotedar, R., Partridge, G.J., 2021. Enzymatic fish protein hydrolysates in finfish aquaculture: a review. Rev. Aquac. 13, 406–430. https://doi.org/10.1111/raq.12481.

Silva-Brito, F., Timóteo, F., Esteves, Â., Peixoto, M.J., Ozorio, R., Magnoni, L., 2019. Impact of the replacement of dietary fish oil by animal fats and environmental salinity on the metabolic response of European Seabass (*Dicentrarchus labrax*). Comp. Biochem. Physiol. B 233, 46–59. https://doi.org/10.1016/j.cbpb.2019.04.004.

Sneddon, L.U., Wolfenden, D.C.C., Thomson, J.S., 2016. Stress Management and Welfare. In: Schreck, C.B., Tort, L., Farrell, A.P., Brauner, C.J. (Eds.), Fish Physiology. Academic Press, Cambridge, MA, USA, pp. 463–539.

Sunyer, J.O., Tort, L., 1995. Natural hemolytic and bactericidal activities of sea bream *Sparus aurata* serum are effected by the alternative complement pathway. Vet. Immunol. Immunopathol. 45, 333–345. https://doi.org/10.1016/0165-2427(94)05430-Z.

Tang, H.-g, Wu, T.-x, Zhao, Z.-y, Pan, X.-d, 2008. Effects of fish protein hydrolysate on growth performance and humoral immune response in large yellow croaker (*Pseudosciaena crocea* R.). J. Zhejiang Univ. Sci. B. 9, 684–690. https://doi.org/10.1631/jzus.B0820088.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Hawkins Byrne, D., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compos. Anal. 19, 669–675. https://doi.org/10.1016/j.jfca.2006.01.003.

Toldrà, M., Lynch, S.A., Couture, R., Álvarez, C., 2019. Chapter 5 - Blood Proteins as Functional Ingredients. In: Galanakis, C.M. (Ed.), Sustainable Meat Production and Processing. Academic Press, London, pp. 85–101.

Torrecillas, S., Caballero, M.J., Mompel, D., Montero, D., Zamorano, M.J., Robaina, L., Rivero-Ramírez, F., Karalazos, V., Kaushik, S., Izquierdo, M., 2017. Disease resistance and response against *Vibrio anguillarum* intestinal infection in European seabass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets. Fish. Shellfish Immunol. 67, 302–311. https://doi.org/10.1016/j.fsi.2017.06.022.

Tort, L., 2011. Stress and immune modulation in fish. Dev. Comp. Immunol. 35, 1366–1375. https://doi.org/10.1016/j.dci.2011.07.002.

Valente, L.M.P., Rema, P., Ferraro, V., Pintado, M., Sousa-Pinto, I., Cunha, L.M., Oliveira, M.B., Araújo, M., 2015. Iodine enrichment of rainbow trout flesh by dietary supplementation with the red seaweed *Gracilaria vermiculophylla*. Aquaculture 446, 132–139. https://doi.org/10.1016/j.aquaculture.2015.05.004.

Velasco, C., Conde-Sieira, M., Comesaña, S., Chivite, M., Míguez, J.M., Soengas, J.L., 2021. Role of the G protein-coupled receptors GPR84 and GPR119 in the central regulation of food intake in rainbow trout. J. Exp. Biol. (jeb242360), 224. https://doi.org/10.1242/jeb.242360.

Yin, B., Liu, H., Tan, B., Dong, X., Chi, S., Yang, Q., Zhang, S., 2020. Preliminary study of mechanisms of intestinal inflammation induced by plant proteins in juvenile hybrid groupers (♀*Epinephelus fuscoguttatus*×♂*E. lanceolatu*). Fish. Shellfish Immunol. 106, 341–356. https://doi.org/10.1016/j.fsi.2020.07.026.

Zheng, J.-L., Zeng, L., Xu, M.-Y., Shen, B., Wu, C.-W., 2017. Different effects of low- and high-dose waterborne zinc on Zn accumulation, ROS levels, oxidative damage and antioxidant responses in the liver of large yellow croaker *Pseudosciaena crocea*. Fish. Physiol. Biochem. 43, 153–163. https://doi.org/10.1007/s10695-016-0275-6.