



Exploration of marine red seaweed as a dietary fish meal replacement and its potentiality on growth, hematological, biochemical, and enzyme activity in freshwater fish *Labeo rohita*

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Abstract

The present study investigated the dietary fishmeal replacement by marine red seaweed (*Halymenia dilatata*) meal (RSM) on growth performance, feed utilization, chemical body composition, hematological constituents, digestive, antioxidant, and metabolic enzymes in freshwater fish *Labeo rohita* (Rohu) fingerlings. The fish were fed with RSM-free control diet (RSM0) and four experimental diets, which replaced fish meal (FM) with varying levels of RSM (25%, 50%, 75%, and 100%, represented as RSM25, RSM50, RSM75, and RSM100 respectively). After a 60-day feeding trial, the survival rate (SR), growth performance (length gain, weight gain, and specific growth rate), protein efficiency ratio, chemical body composition (protein, lipid, and ash), and digestive enzymes (amylase and protease) were significantly increased ($P < 0.05$) in the fish fed with RSM50 diet containing 39% protein level. The sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) revealed a higher staining intensity of muscle proteins in fish fed with the RSM50 diet. However, the hematological constituents (hemoglobin, hematocrit, red blood cell, white blood cell, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration), antioxidant enzyme - superoxide dismutase, and metabolic enzymes (glutamic oxaloacetic transaminase and glutamic pyruvic transaminase) were not significantly altered in RSM50 diet when compared to control. In contrast, hematological constituents were decreased ($P < 0.05$), and antioxidant and metabolic enzymes were increased in rohu fed with RSM75 and RSM100 ($P < 0.05$). Furthermore, these findings suggest that RSM might be adopted at a pace of 37% (estimated polynomial second-order regression) and is found to be beneficial for freshwater fish *L. rohita* diets that enhance growth and immune responses. The current study recommended substituting (50%) of marine red seaweed (*Halymenia dilatata*) for fish meal significantly improves the growth performance, chemical body composition, and digestive enzymes of *L. rohita* and this could be a valuable natural replacement for fishmeal to reduce the production cost of aquatic feed.

Keywords Fish nutrition · Weight gain · Feed efficiency · Polynomial regression · Protein profile

Introduction

Aquaculture contributes half of the total global biodiversity, and India shares 7.58% of global production next to China (Boyd et al. 2020). According to the

National Fisheries Development Board (NFDB), aquaculture is one of the food-producing industries in India that is growing rapidly. Fish are an important part of the aquatic fauna and have a significant potential for long-term economic growth (Pounds et al. 2022; Iitembu

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et al. 2022; Muñiz et al. 2022). Increased aquaculture production is required to meet the growing population's animal protein requirements in order to alleviate poverty and malnutrition and also ensure a sustainable livelihood (Madsen et al. 2022; Akegbejo-Samsoms, 2022). Globally, the production of freshwater fish *Labeo rohita* (rohu) was over 1.67 million tonnes in 2014 (FAO 2018). In Asia, the Indian major carp *L. rohita* contributes 35% of total aquaculture production, due to its economic significance, accelerated growth yield, controlled reproduction, and customer demand (Harikrishnan et al. 2021). However, recently, there is a demand for fish meal (FM), which is used as a protein source in commercial fish feeds (Naylor et al. 2009; FAO 2014; Maiolo et al. 2020). Therefore, research has already commenced finding alternative ingredients to replace FM in formulated feeds (Samaddar et al. 2015; Panase et al. 2018; Hassaan et al. 2019; Oliva-Teles et al. 2022).

Nowadays, seaweeds have been closely associated with human, animal, and fish life and have been used as a source of food, feed, and medicine (Demirel et al. 2009; Jimenez-Escrig et al. 2011; Rebours et al. 2014; García-Vaquero and Hayes 2016; Terriente-Palacios and Castellari, 2022). The edible seaweeds contain moderate concentrations of proteins, lipids, high levels of fiber, vitamins, minerals, omega-3 fatty acids, and antioxidant compounds like alkaloids, steroids, and terpenoids (Murata and Nakazoe 2001; Holdt and Kraan 2011; Rajapakse and Kim 2011; Afonso et al. 2021; Morais et al. 2020). The red seaweed *Porphyra* spp. and *Undaria* spp. have a higher protein when compared with brown (*Laminaria* spp. and *Undaria* spp.) and green (*Ulva* sp.) seaweeds (Murata and Nakazoe 2001; Marsham et al. 2007; Peñalver et al. 2020). In order to assess the potential of seaweed used as a nutritional aid, research is necessary to perform aquaculture nutrition-related studies, which are infrequent in fish (García-Ortega et al. 2016; Roleda and Hurd 2019). Previous reports suggest that the dietary inclusion of seaweed as the replacement for fishmeal, *Porphyra purpurea* (red seaweed) in the diet of *Chelon labrosus* (thicklip grey mullet) (Davies et al. 1997); *Oreochromis niloticus* (Nile tilapia) were fed with *Ulva rigida* (green seaweed) (Azaza et al. 2008); Incorporation of *Porphyra dioica* (red seaweed) to *Oncorhynchus mykiss* (Rainbow trout) (Soler-Vila et al. 2009); and *Pagrus major* (Red seabream) were fed with *Ascophyllum nodosum* (brown seaweed) (Nakagawa et al. 1997); whereas, Mustafa et al. (1995) had shown incorporation of all three seaweeds (*Ascophyllum nodosum*, *Porphyra yezoensis*, and *Ulva pertusa*) in the diet of *Pagrus major* (Red seabream). With these evidences, seaweeds are highly recommended to be included in the

fish feed that enhance the growth, physiological activity, and disease resistance of cultured fish.

Halymenia, a marine red seaweed (Family: Halymeniaceae), is found in various parts of the world (Guiry and Guiry 2020). Among them, the species *Halymenia dilatata* (Jainab et al. 2019), *Halymenia maculata* (Fantonalgo. 2018), *Halymenia durvillaei* (Fenoradosoa et al. 2009; Boominathan et al. 2022), *Halymenia formosa* (Hurtado et al. 2020), *Halymenia floresia* (Malairaj et al. 2016; das Neves Amorim et al. 2011), and *Halymenia palmata* (Deepak et al. 2019) are better sources of proteins, fatty acids, minerals (I, Cu, Ni, Cd, and Zn), pigments (carotene, chlorophyll a, chlorophyll d, lutein, phycocyanin, r-phycoerythrin, and zeaxanthin), and polysaccharides (sugars, carrageenan, floridean starch, funoran, furcellarin, and galactan) (Aldon 1998; Shah et al. 2022). They also act as antimicrobial, antioxidant, food additives, nematocide, pesticide, antiandrogenic, and flavors involving in anti-inflammatory activities (Balasubramanian et al. 2021; Fredrick Raja. 2022; Angulo et al. 2020). The present research is aimed to investigate fish meal replacement with a marine red seaweed *Halymenia dilatata* in the diet of *Labeo rohita*. The growth performance, digestive enzymes, chemical body composition, hematological parameters, antioxidant and metabolic enzymes were evaluated in this study. In addition, polynomial second-order (quadratic) regression model was adopted to estimate the optimal replacement of fishmeal with *H. dilatata*.

Materials and methods

Collection of marine red seaweed and feed ingredients

The marine red seaweed, *Halymenia dilatata*, was collected from Mandapam coastal area in the Gulf of Mannar (Lat. 09° 17.417'N; Long. 079° 08.558'E), Ramanathapuram district, Tamil Nadu, India. The seaweed was rinsed in pure water, allowed to air-dry then milled into a fine powder and kept in sterile, airtight containers. Feed ingredients such as fish meal (FM), groundnut oil cake (GOC), soybean meal (SBM), wheat bran (WB), rice bran (RB), and tapioca flour (TF) were purchased from the local market, and these ingredients were also powdered. The vitamins and minerals premixture was purchased from the medical shop at Tiruchirappalli, Tamil Nadu, India. All the feed ingredients were analyzed for proximate composition by the method of Castell and Tiews (1980), as given in AOAC (1995).

Diet preparation

Diet formulation was done to fulfill the basic requirement of protein (30–40%) and lipid (5–16%) for *L. rohita* fingerlings (Satpathy et al. 2003). The feed ingredients, protein (FM, GOC, and SBM), and carbohydrate (WB, RB, and TF) sources were combined, steam-cooked for 15 min at 60 °C, and then dried at 28 °C. The vitamin and mineral premixture was added to the experimental diets along with sunflower oil (Table 1). RSM-free control diet (RSM0) and four experimental diets, including varying levels of RSM (RSM25 = 25%, RSM50 = 50%, RSM75 = 75%, and RSM100 = 100%), were formulated. The dough was prepared with 10% hot water and pelletized separately. An air blower in a drying cabinet maintained at 38 °C was used to air-dry the experimental diets until the moisture content was around 10%. Followed by air-drying, the diets were chopped up into pellets sized (< 1 mm) and kept at – 20 °C until use. Proximate composition analyses of these diets were also performed, according to AOAC (1995). The results of the proximate composition of FM replaced with RSM included diets did not show much difference comparing to basic nutritional requirements of *L. rohita* fingerlings (Table 1).

Table 1 Formulation and proximate composition (g kg⁻¹) of fish meal replacement by red seaweed (*H. dilatata*) meal incorporated diets

Ingredients (g kg ⁻¹)	Experimental diets				
	RSM0	RSM25	RSM50	RSM75	RSM100
Fish meal	200	150	100	50	0
Red seaweed meal	0	50	100	150	200
Groundnut oil cake	200	200	200	200	200
Soybean meal	200	200	200	200	200
Rice bran	120	120	120	120	120
Wheat bran	120	120	120	120	120
Tapioca flour	120	120	120	120	120
Sunflower oil	20	20	20	20	20
Vitamin and mineral mix ^a	20	20	20	20	20
<i>Proximate composition (% of dry matter basis)</i>					
Moisture	8.58	8.53	8.49	8.35	8.41
Crude protein	41.67	40.32	38.55	37.09	36.16
Crude fiber	3.58	3.34	3.27	3.20	3.08
Ether extract	6.93	6.75	6.31	5.96	5.74
Ash	9.56	9.37	9.45	9.18	9.29
NFE	29.68	31.69	33.93	36.22	37.32
Gross energy (kJ g ⁻¹)	43.31	43.80	43.52	43.78	43.61

NFE, nitrogen-free extract

^aBecosules capsules: thiamine mononitrate (USP), 50 mg; riboflavin (USP), 25 mg; pyridoxine HCl (USP), 10 mg; cyanocobalamin (USP), 15 mcg; niacinamide (USP), 100 mg; calcium pantothenate (USP), 25 mg; folic acid (USP), 1 mg; ascorbic acid (BP), 150 mg

Collection and acclimatization of experimental fish

The freshwater fish, *L. rohita* fingerlings, were collected from Nathan fish farm in Thanjavur, Tamil Nadu, India. They were transported to the laboratory in well-oxygenated polythene bags, and they were stored in cement tanks with the following dimensions: height, 1.04 m; radius, 0.96 m, and volume, $\approx$ 3000 L. They were allowed to acclimatize to the laboratory conditions for 2 weeks. During acclimatization, the fish were fed with commercial feed ad libitum. To keep the fish in a healthy condition, the water was frequently changed (by 50%) each day, and the tanks were constantly aerated. The physicochemical properties were sustained as temperature, 29.14 ± 0.51 °C; pH, 7.20 ± 0.30 ; total dissolved solids, 0.102 ± 0.10 g L⁻¹; and dissolved oxygen, 6.15 ± 0.40 mg L⁻¹, throughout the experimental period (APHA 1998).

Feeding experiment

While maintaining and handling fish throughout the experiment, all applicable criteria for the care and use of animals provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, have been carefully followed. Twenty *L. rohita* fingerlings (3.35 ± 0.06 g; 6.47 ± 0.06 cm) (mean $\pm$ S.D) for each diet in triplicates were maintained in plastic troughs (height, 27 cm; radius, 21.5 cm) with 20 L of water. The experimental groups were fed with RSM0, RSM25, RSM50, RSM75, and RSM100 experimental diets. The feed quantity was adjusted every 10 days based on the 10% bodyweight of fingerlings. Throughout the 60-day experimental period, the daily feed was split in half and fed twice a day (at 09:00 and 18:00); moderate aeration was continually administered to maintain the ideal oxygen level (> 6.00 mg L⁻¹).

Growth parameters

After the feeding trial, survival rate (SR), growth parameters [final length (FL), length gain (LG), final weight (FW), weight gain (WG), specific growth rate (SGR)], and feed utilization [feed conversion ratio (FCR) and protein efficiency ratio (PER)] were determined according to García-Ortega et al. (2016) as follows:

Survival rate (%)

$$= 100 \times (\text{final number of fish} / \text{initial number of fish})$$

Length gain (cm) = FL – IL

where FL is the final length of individual fish (cm) and IL is the initial length of individual fish (cm)

$$\text{Weight gain (g)} = \text{FW} - \text{IW}$$

where FW is the final weight of individual fish (g) and IW is the initial weight of individual fish (g)

$$\text{Specific growth rate (\% day}^{-1}\text{)} = 100 \times (\log_{10} \text{FW} - \log_{10} \text{IW}) / \text{days}$$

Feed conversion ratio (g)

$$= \text{individual feed intake (g)} / \text{individual weight gain (g)}$$

$$\text{Protein efficiency ratio (g)} = (\text{FW} - \text{IW}) / \text{individual feed intake} \\ \times \text{crude protein g}^{-1} \text{ of diet}$$

Analysis of the proximate composition

Fish muscle tissues were analyzed in triplicates for proximate composition (moisture, protein, lipid, and ash) using the following standard methods. Protein was determined by the method of Lowry (1951) using trichloroacetic acid (TCA) precipitation. Lipid was assessed by the method of Barnes and Blackstock (1973) using chloroform–methanol extraction (Folch et al. 1957). The content of moisture and ash was determined, according to AOAC (1995).

Analysis of protein profile/sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

Fish muscle tissues were homogenized in phosphate buffer (pH 7.4) solution and centrifuged at 14,000 rpm at 4 °C for 15 min. The soluble protein content was determined according to the method of Bradford (1976). For the determination of the molecular weight of proteins, 10% SDS-PAGE (Laemmli 1970) was performed in a vertical gel electrophoresis system (Bio-Rad, Hercules, CA, USA). Thermo Fisher Scientific (Massachusetts, USA) 10–180-kDa protein molecular markers were used as a standard marker. The gel was stained by Coomassie blue (0.025%). Molecular weights of the polypeptide bands were determined by comparing the relative mobility of protein bands to the standard protein marker.

Analysis of hematological parameters

The cardiac puncture was done, and the blood samples were collected in a heparinized (Beparine^R heparin sodium, IP 1000 IU mL⁻¹, an anticoagulant) medical grade disposable readymade (26-gauge needle) syringe from the experimental fish after 60 days experimental trial. Then, the blood was transferred into heparinized plastic vials. The blood was analyzed for hematological parameters [hemoglobin (Hb), hematocrit (Hct), red blood cells (RBC), white blood cells

(WBC), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC)] using Mindray Auto Hematology Analyzer BC 2800 (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China).

Assay of digestive enzymes

The digestive tract of fish was carefully dissected out and homogenized [1:10 (w/v %) wet mass: volume ice-cold double distilled water] by a glass homogenizer and centrifuged at 9300 g for 20 min at 4 °C. The suspension of the supernatant was used as an enzyme source.

Amylase activity was determined according to the starch-hydrolysis method of Bernfeld (1955). Maltose was used as standard, and 1 unit of amylase activity (U) was determined as the amount of enzyme to produce 1 mg of maltose min⁻¹ mg⁻¹ protein at 25 °C.

Protease activity was assayed by the casein-hydrolysis method of Furne et al. (2005), L-tyrosine was used as standard, and 1 unit of protease enzyme activity (U) was determined as the amount of enzyme desired to liberate 1 µg of tyrosine min⁻¹ mg⁻¹ protein at 37 °C.

Assay of antioxidant enzyme (SOD) activity

Gills, liver, and muscle tissues of fish were individually homogenized (10% w/v) in ice-cold 50-mM Tris buffer (pH 7.4) and centrifuged at 9300 g for 20 min at 4 °C.

The supernatant was used to assay the SOD enzyme activity. Soluble tissue protein concentration was determined by the method of Lowry (1951). The SOD activity was measured using pyrogallol (10 mM) autooxidation in Tris buffer (50 mM, pH 7.0), as described by Marklund and Marklund (1974). The activity of SOD was expressed in U mg⁻¹ protein.

Assay of metabolic enzymes (GOT) and (GPT) activities

Fish gills, liver, and muscle tissues were individually homogenized in a solution of 0.25-M sucrose and centrifuged at 9300 g for 20 min at 4 °C. The supernatant was obtained and used as a source of enzymes. The glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were analyzed according to the method of Reitman and Frankel (1957) using the kit (Medox Biotech India PVT, Ltd).

For GOT analysis, the substrate solution, L-aspartic acid (500 µL; pH 7.4), was added to 100 µL of sample and incubated at 37 °C for 1 h. Further, 500 µL of 2,4-dinitrophenyl hydrazine was added and allowed to stand for 20 min at room temperature. The final mixture was added to 3 mL of freshly prepared 4-N sodium hydroxide solution. The colored solution was read at 505 nm using SynergyTM HTX Multi-Mode Microplate Reader (Biotek, Winooski, VT,

USA) within 15 min. Sodium pyruvate (160 U L^{-1}) was used as a calibrator. The activity of GOT was expressed as U L^{-1} .

For GPT analysis, buffered L-alanine and 2-oxoglutarate substrate ($500 \mu\text{L}$; pH 7.4) was added to $100 \mu\text{L}$ of sample and incubated at 37°C for 20 min. Then, $500 \mu\text{L}$ of 2,4-dinitrophenyl hydrazine was added and allowed to stand at 28°C for 30 min followed by the addition of 3 mL of freshly prepared 4-N sodium hydroxide solution. The colored solution was read at 505 nm using a Synergy™ HTX Multi-Mode Microplate Reader (Biotek) within 15 min. Sodium pyruvate (170 U L^{-1}) was used as a calibrator. The activity of GPT was expressed as U L^{-1} .

Data analysis

The polynomial second-order (quadratic) regression analysis was used to evaluate the effect of the increasing levels of dietary RSM on growth performance, proximate composition, digestive enzyme activity, hematology, and immune response. One-way analysis of variance (ANOVA) was used to differentiate among the diets, and significant ($P < 0.05$) differences among diet means were ranked by Duncan's multiple range test (DMRT). The data were expressed as mean $\pm$ SE (standard error of the mean).

Results

Survival and growth parameters

The results of SR, growth performance (IL, FL, IW, FW, WG, SGR), and feed utilization (FCR and PER) were shown in Table 2. Significantly increased values ($P < 0.05$)

in SR, FW, WG, SGR, and PER values were observed in *L. rohita* fed with the diets containing FM replacement with RSM50 followed by RSM25 as compared with RSM0. Meanwhile, the SR, FW, WG, SGR, and PER for both diet RSM75 and RSM100 were significantly decreased ($P < 0.05$) in comparison to those of other diet groups. Additionally, the dietary FM replacement by RSM35, RSM34, and RSM37 level promoted the most significant amount of WG, SGR, and PER, as estimated by the polynomial regression with strong positive correlation ($y = -0.000x^2 + 0.048x + 5.236$; $y = -7E - 05x^2 + 0.004x + 0.671$; $y = 0.000x^2 + 0.014x + 1.722$, respectively) (Table 2; Fig. 1a, b, and d).

The FCR in *L. rohita* was insignificantly decreased ($P > 0.05$) in groups fed RSM incorporated diets, especially at the level of RSM50 followed by RSM25 as compared with the control group. The diet RSM50 showed the lowest FCR values among the experimental diets (Table 2). Additionally, the polynomial regression of dietary RSM level and FCR was the fit regression modeling with high goodness ($y = 0.000x^2 - 0.008x + 1.392$) (Table 2; Fig. 1c). The significant value of FCR was estimated at the RSM25 diet according to the regression curve.

Chemical body composition

The protein, lipid, and ash contents were significantly elevated ($P < 0.05$) in *L. rohita* fed on dietary FM replacement by RSM50 and RSM25 diets when compared to other experimental diets, whereas in the moisture content, no significant ($P > 0.05$) change was detected among the diet groups, as shown in Table 3. However, the FM replacement by the RSM analysis showed that the dietary RSM level

Table 2 Survival, growth performance, and feed utilization of *L. rohita* fingerlings fed with experimental diets for 60 days

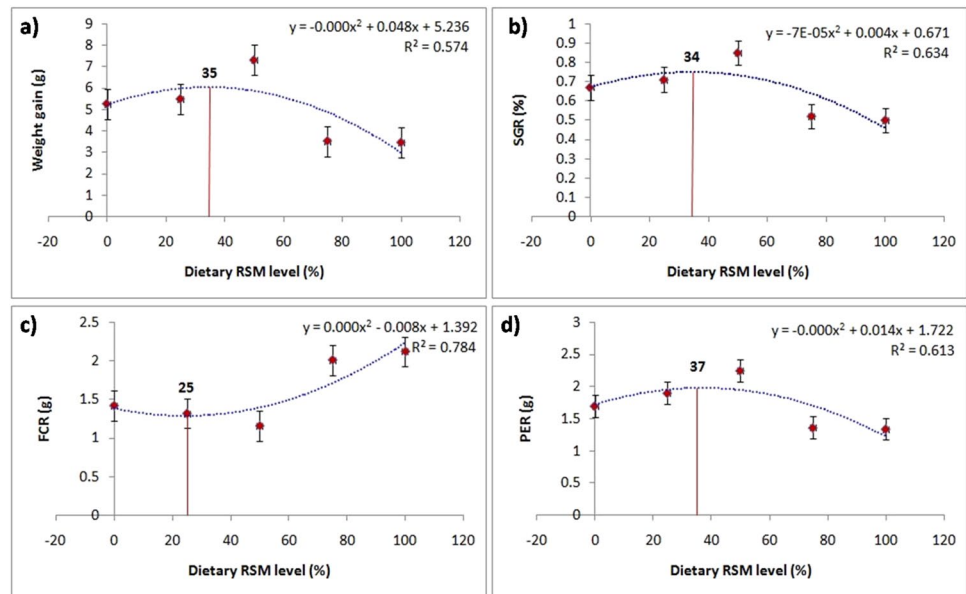
Parameters	Experimental diets					PSEM	Regression ²		ANOVA	
	RSM0	RSM25	RSM50	RSM75	RSM100		Equation	R ²	P value	
SR (%)	80.00 $\pm$ 5.00 ^{ab}	80.00 $\pm$ 5.00 ^{ab}	86.66 $\pm$ 2.88 ^a	71.66 $\pm$ 5.77 ^{bc}	66.66 $\pm$ 2.88 ^c	5.12	$79 + 0.221x - 0.003x^2$	0.787	0.002	
IL (cm)	6.57 $\pm$ 0.23 ^a	6.41 $\pm$ 0.17 ^a	6.49 $\pm$ 0.24 ^a	6.54 $\pm$ 0.25 ^a	6.52 $\pm$ 0.22 ^a	0.07				
FL (cm)	9.40 $\pm$ 0.45 ^{bc}	9.66 $\pm$ 0.50 ^b	10.90 $\pm$ 0.41 ^a	8.77 $\pm$ 0.35 ^{cd}	8.35 $\pm$ 0.46 ^d	0.81	$9.338 + 0.042x - 0.000x^2$	0.653	0.000	
LG (cm)	2.83 $\pm$ 0.33 ^{bc}	3.25 $\pm$ 0.52 ^b	4.41 $\pm$ 0.35 ^a	2.23 $\pm$ 0.22 ^{cd}	1.83 $\pm$ 0.57 ^d	0.81	$2.802 + 0.044x - 0.000x^2$	0.670	0.000	
IW (g)	3.41 $\pm$ 0.21 ^a	3.31 $\pm$ 0.15 ^a	3.29 $\pm$ 0.20 ^a	3.38 $\pm$ 0.24 ^a	3.42 $\pm$ 0.12 ^a	0.03				
FW (g)	8.67 $\pm$ 0.72 ^b	8.80 $\pm$ 0.85 ^b	10.60 $\pm$ 0.78 ^a	6.90 $\pm$ 0.73 ^c	6.87 $\pm$ 0.82 ^c	1.45	$8.636 + 0.044x - 0.000x^2$	0.563	0.001	
WG (g)	5.26 $\pm$ 0.76 ^b	5.49 $\pm$ 0.70 ^b	7.31 $\pm$ 0.88 ^a	3.52 $\pm$ 0.64 ^c	3.45 $\pm$ 0.64 ^c	1.47	$5.236 + 0.048x - 0.000x^2$	0.574	0.000	
SGR (%)	0.67 $\pm$ 0.08 ^b	0.71 $\pm$ 0.04 ^b	0.85 $\pm$ 0.08 ^a	0.52 $\pm$ 0.07 ^c	0.50 $\pm$ 0.08 ^c	0.12	$0.671 + 0.004x - E - 05x^2$	0.634	0.001	
FCR (g)	1.42 $\pm$ 0.16 ^b	1.32 $\pm$ 0.18 ^b	1.16 $\pm$ 0.14 ^b	2.01 $\pm$ 0.34 ^a	2.12 $\pm$ 0.41 ^a	0.28	$1.392 - 0.008x + 0.000x^2$	0.784	0.004	
PER (g)	1.70 $\pm$ 0.20 ^{bc}	1.90 $\pm$ 0.27 ^{ab}	2.25 $\pm$ 0.29 ^a	1.36 $\pm$ 0.21 ^c	1.34 $\pm$ 0.26 ^c	0.33	$1.722 + 0.014x - 0.000x^2$	0.613	0.006	

The data were expressed as mean $\pm$ standard error of the mean, $n = 3$ triplicates per treatments

Different superscript letters within a row indicates statistically significant ($P < 0.05$) differences as evaluated by Duncan multiple range test

PSEM, pooled standard error of the mean; RSM, red seaweed meal; SR, survival rate; FL, final length; LG, length gain; FW, final weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio

Fig. 1 Polynomial second-order (quadratic) regression of weight gain (a), specific growth rate (SGR) (b), feed conversion ratio (FCR) (c), and protein efficiency ratio (PER) (d) in *L. rohita* fingerlings fed increasing inclusions of RSM for 60 days



fits with protein and lipid are polynomial regression with a strong positive correlation ($R^2 = 0.679$ and 0.544 , respectively) (Table 3).

Protein profile

Polypeptide bands of molecular weights between 10 and 180 kDa were resolved in the muscle tissue of *L. rohita* fingerlings (Fig. 2). Seven Coomassie blue-stained protein bands (37, 30, 23, 17, 15, 13, and 10 kDa) were observed in FM replacement with RSM diets fed.

L. rohita, when compared with the standard molecular marker. Various polypeptide bands resolved in RSM25, RSM50, and RSM75 stained more intensely when compared with RSM0 and RSM100 diets. Mainly, 37, 23, 17, 15, 13, and 10 kDa polypeptide bands in diet RSM50-fed *L. rohita* were stained more intensely among the experimental groups.

Hematology

The highest level of hematological indices (Hb, Hct, RBC, WBC, MCV, MCH, and MCHC) was recorded in *L. rohita* fed on RSM50, RSM25, and RSM0, and the lowest level was recorded in *L. rohita* fed on diet containing RSM75 followed by RSM100 diet (Table 4). The hematological indices of *L. rohita* were significantly altered by RSM75 and RSM100 diets ($P > 0.05$), as demonstrated in Table 4. In addition, the polynomial regression of high-goodness dietary substitution of FM with RSM levels and Hb, Hct, RBC, WBC, MCV, MCH, and MCHC was an effective regression model ($R^2 = 0.734, 0.734, 0.709, 0.946, 0.887, 0.827$, and 0.777 respectively; Table 4).

Digestive enzymes

The amylase and protease activities were significantly enhanced ($P < 0.05$) in *L. rohita* fed with dietary FM replacement by

Table 3 Chemical body composition of *L. rohita* fed with experimental diets for 60 days

Parameters	Experimental diets					PSEM	Regression		ANOVA
	RSM0	RSM25	RSM50	RSM75	RSM100		Equation	R^2	P value
Moisture (%)	78.79 ± 1.24 ^a	78.90 ± 1.12 ^a	77.24 ± 1.40 ^a	79.36 ± 0.98 ^a	81.94 ± 1.63 ^a	3.32	$79.56 - 0.186x + 0.002x^2$	0.572	0.934
Protein (mg g ⁻¹ wet wt.)	125.17 ± 8.07 ^{bc}	129.60 ± 8.84 ^{ab}	139.18 ± 9.37 ^a	117.55 ± 2.92 ^{bc}	114.53 ± 5.02 ^c	7.88	$125.2 + 0.393x - 0.005x^2$	0.679	0.013
Lipid (mg g ⁻¹ wet wt.)	56.86 ± 4.45 ^b	58.43 ± 2.96 ^{ab}	66.27 ± 4.89 ^a	54.90 ± 5.30 ^b	53.72 ± 4.75 ^b	4.72	$56.46 + 0.243x - 0.002x^2$	0.544	0.047
Ash (%)	2.01 ± 0.06 ^{bc}	2.08 ± 0.06 ^{ab}	2.14 ± 0.07 ^a	1.99 ± 0.08 ^{bc}	1.94 ± 0.04 ^c	0.04	$2.013 + 0.004x - 5E-05x^2$	0.800	0.023

The data were expressed as mean ± standard error of the mean, $n = 3$ triplicates per treatments

Different superscript letters within a row indicate statistically significant ($P < 0.05$) differences as evaluated by Duncan multiple range test

PSEM, pooled standard error of the mean; RSM, red seaweed meal

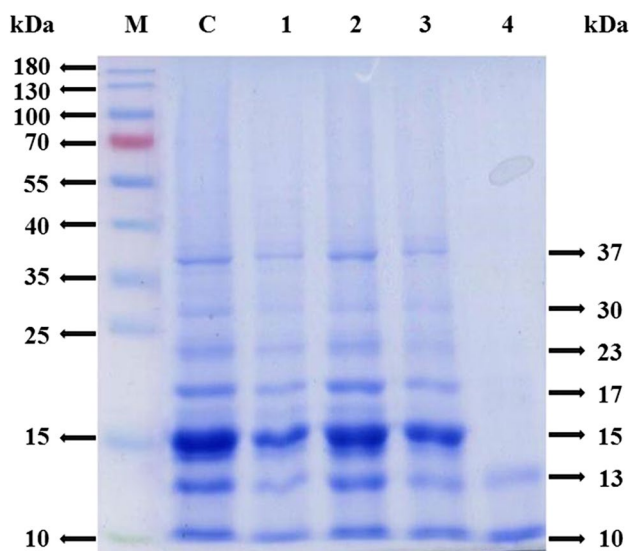


Fig. 2 Protein profile of the muscle tissue of *L. rohita* fingerlings fed with experimental diets for 60 days. Lane C, RSM0 diet-fed *L. rohita*; lane 1, RSM25 diet-fed *L. rohita*; lane 2, RSM50 diet-fed *L. rohita*; lane 3, RSM75 diet-fed *L. rohita*; lane 4, RSM100 diet-fed *L. rohita*; lane M, marker protein

RSM50 and RSM25 when compared to the other experimental diets (Table 5). Therefore, the polynomial regression of dietary RSM protease and amylase was the best regression model with high goodness ($R^2=0.298$ and 0.789) (Table 5).

Antioxidant (SOD) and metabolic enzyme (GOT and GPT) activities

Insignificant values ($P>0.05$) were observed in the SOD, GOT, and GPT enzyme levels in the gills, liver, and muscle of *L. rohita* fed on FM replacement by RSM50 and RSM25 diets when compared with that in the RSM0, RSM75, and RSM100 (Table 6). The antioxidant and metabolic enzymes

were increased significantly ($P<0.05$) in FM replaced with RSM75 and RSM100 (Table 6). Moreover, the polynomial regression of high-quality dietary replacement of FM with RSM levels and SOD, GOT, and GPT was an effective regression model (gills, $R^2=0.943$, 0.977 , and 0.995 ; liver, $R^2=0.957$, 0.982 , and 0.997 ; muscle: $R^2=0.958$, 0.938 , and 0.923) (Table 6).

Discussion

Marine seaweeds are increasingly used in biological applications, particularly in the pharmaceutical and feed formulation industries, because they contain a diverse range of bioactive and nutritional compounds and primary and secondary metabolites with potential therapeutic activity such as antimicrobial, anticancer, antiviral, anti-inflammatory, and immunomodulatory (Shah et al. 2022; Lomartire et al. 2021; Carpena et al. 2022; Ashkenazi et al. 2022). The marine seaweed-derived polysaccharides can be used for animal nutrition and improved animal intestinal integrity and enhanced immune responses (Leandro et al. 2019; Silva-Brito et al. 2022). Recently, many novel ingredients (insect meal, macro- and microalgae, yeast) have been widely used as an alternative and essential protein diet for fish growth (Aragão et al. 2022). Pradhan et al. (2020) stated that plant-based alternatives to fishmeal supplementation increase the plankton population and also enhance fish growth. In this present study, the marine red seaweed (*Halymenia dilatata*) meal has been used as an alternative source for fishmeal in terms of growth performance, hematological, antioxidant, and digestive enzyme activity in the freshwater fish *Labeo rohita*.

The SR, LG, WG, SGR, and PER were significantly increased in *L. rohita* fed diets containing FM replacement with RSM50 diet (39% protein level) followed by RSM25 as compared with RSM0. The RSM properties like protein levels, vitamin and mineral composition, pigments, and

Table 4 Hematological constituents of *L. rohita* fed with experimental diets for 60 days

Parameters	Experimental diets					PSEM	Regression		ANOVA	
	RSM0	RSM25	RSM50	RSM75	RSM100		Equation	R^2	P value	
Hb (g dL ⁻¹)	8.23 ± 0.61 ^a	8.58 ± 0.66 ^a	8.95 ± 0.59 ^a	5.02 ± 0.37 ^b	5.17 ± 0.46 ^b	1.40	8.454 + 0.015x - 0.000x ²	0.734	0.000	
Hct (%)	31.07 ± 1.24 ^a	31.92 ± 1.02 ^a	32.66 ± 1.05 ^a	20.11 ± 1.09 ^b	21.02 ± 1.02 ^b	4.53	31.85 + 0.022x - 0.001x ²	0.734	0.000	
RBC (× 10 ⁶ μL ⁻¹)	3.62 ± 0.19 ^a	3.69 ± 0.15 ^a	3.77 ± 0.14 ^a	2.45 ± 0.12 ^b	2.61 ± 0.08 ^b	0.48	3.705 + 0.009x - 0.000x ²	0.709	0.000	
WBC (× 10 ³ μL ⁻¹)	14.70 ± 1.67 ^a	15.30 ± 1.28 ^a	15.66 ± 1.83 ^a	9.70 ± 1.51 ^b	6.10 ± 1.59 ^c	1.37	14.74 + 0.007x - 0.001x ²	0.946	0.000	
MCV (fl)	85.86 ± 1.08 ^a	86.52 ± 0.75 ^a	86.84 ± 0.43 ^a	82.06 ± 0.42 ^b	80.51 ± 0.22 ^b	1.36	86.03 + 0.048x - 0.001x ²	0.887	0.000	
MCH (pg)	22.71 ± 0.49 ^a	23.22 ± 0.84 ^a	23.72 ± 0.68 ^a	20.47 ± 0.51 ^b	19.78 ± 0.65 ^b	1.02	22.82 + 0.035x - 0.000x ²	0.827	0.000	
MCHC (g dL ⁻¹)	26.46 ± 0.91 ^{ab}	26.85 ± 1.21 ^a	27.38 ± 0.92 ^a	24.94 ± 0.48 ^{bc}	24.58 ± 0.37 ^c	0.81	26.53 + 0.028x - 0.000x ²	0.777	0.008	

The data were expressed as mean ± standard error of the mean, $n=3$ triplicates per treatments

Different superscript letters within a row indicate statistically significant ($P<0.05$) differences as evaluated by Duncan multiple range test

PSEM, pooled standard error of the mean; RSM, red seaweed meal; Hb, hemoglobin; Hct, hematocrit; RBC, red blood cell; WBC, white blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration

Table 5 Digestive enzymes of *L. rohita* fingerlings fed with experimental diets for 60 days

Parameters	Experimental diets					PSEM	Regression		ANOVA	
	RSM0	RSM25	RSM50	RSM75	RSM100		Equation	R ²	P value	
Amylase (IU mg ⁻¹ protein)	3.08 ± 0.17 ^b	3.22 ± 0.16 ^b	3.56 ± 0.10 ^a	2.15 ± 0.11 ^d	2.76 ± 0.14 ^c	0.63	3.180 + 0.002x - 9E - 05x ²	0.298	0.000	
Protease (IU mg ⁻¹ protein)	0.21 ± 0.06 ^{ab}	0.23 ± 0.04 ^{ab}	0.27 ± 0.05 ^a	0.20 ± 0.04 ^{ab}	0.17 ± 0.03 ^b	0.02	0.208 + 0.002x - 2E - 05x ²	0.789	0.166	

The data were expressed as mean ± standard error of the mean, *n* = 3 triplicates per treatments

Different superscript letters within a row indicate statistically significant (*P* < 0.05) differences as evaluated by Duncan multiple range test

PSEM, pooled standard error of the mean; RSM, red seaweed meal

bioactive compounds enhanced fish growth (Saleh 2020). Our findings are in agreement with results obtained by Ergun (2009) who reported that lower-level inclusion of *Ulva* seaweed meal improves the growth of the fish through positive variations in the WG, PER, and FCR by the optimum level of lipids in the diets of seaweed meal. Marinho et al. (2013) investigated that up to 10% inclusion of *Ulva* meal doesn't affect the growth of the fish and it possessed higher PER and % of intake digestion which may be due to the presence of enormous amount of amino acids like methionine, phenylalanine, and histidine in red seaweed meal. Wassef (2001) reported that 20% dietary inclusion of *Ulva* seaweed meal was optimum for the growth of mullets because this seaweed has higher vitamin E content. Wan et al. (2016) suggested that 15% inclusion of *Palmaria palmata* (red seaweed) in Atlantic salmon enhances the growth and nutrient digestibility due to the absorption of nutrients

and efficient digestion. The sulfated polysaccharides present in the *Ulva* sp. (seaweed meal) enhance the growth of the fish, hematology parameters, and immune responses of the Indian edible carp *Labeo rohita* (Harikrishnan et al. 2021). The results obtained by Thepot et al. (2022) indicate that red seaweed meal inclusion in the diet increases the growth of fish by reducing the hemolytic and respiratory burst activity as well as immune system simultaneously. Moreover, the alternatives for fishmeal from plant and animal sources possessed optimum dietary minerals that could enhance the growth of the aquatic organism (Ramasamy et al., 2021). Our data showed that poor survival, feed intake, and growth recorded in RSM75 and RSM100 diet-fed *L. rohita* might be deleteriously associated with the excess amount of RSM in the diet. This could be due to the decline in the amino acid levels of the diets consisting of fish meal replaced with algae/plant-based sources (Dileep et al. 2021). Higher

Table 6 Antioxidant (SOD) and metabolic (GOT and GPT) enzymes of *L. rohita* fingerlings fed with experimental diets for 60 days

Parameters	Experimental diets					PSEM	Regression		ANOVA	
	RSM0	RSM25	RSM50	RSM75	RSM100		Equation	<i>R</i> ²	<i>P</i> value	
Gills										
SOD (IU mg ⁻¹ protein)	17.36 ± 1.48 ^c	17.45 ± 1.31 ^c	17.40 ± 1.42 ^c	24.09 ± 1.70 ^b	27.47 ± 2.09 ^a	1.59	17.28 − 0.044x + 0.001x ²	0.943	0.000	
GOT (IU L ⁻¹)	90.75 ± 4.77 ^b	91.54 ± 6.90 ^b	91.88 ± 5.11 ^b	99.82 ± 6.11 ^{ab}	106.34 ± 6.43 ^a	1.45	90.89 − 0.06x + 0.002x ²	0.977	0.035	
GPT (IU L ⁻¹)	127.40 ± 11.31 ^b	129.99 ± 10.66 ^b	132.22 ± 12.92 ^{ab}	138.69 ± 11.28 ^a	145.77 ± 13.33 ^a	0.72	127.6 + 0.030x + 0.001x ²	0.995	0.386	
Liver										
SOD (IU mg ⁻¹ protein)	23.33 ± 1.70 ^b	23.77 ± 2.17 ^b	23.91 ± 1.75 ^b	27.60 ± 2.17 ^a	29.63 ± 1.45 ^a	0.82	23.32 − 0.011x + 0.000x ²	0.957	0.007	
GOT (IU L ⁻¹)	115.81 ± 4.90 ^c	116.66 ± 5.11 ^{bc}	120.47 ± 4.28 ^{bc}	126.70 ± 6.57 ^{ab}	130.73 ± 5.64 ^a	1.20	115.3 + 0.059x + 0.001x ²	0.982	0.027	
GPT (IU L ⁻¹)	221.53 ± 20.09 ^b	225.68 ± 14.62 ^b	233.16 ± 22.9 ^b	240.85 ± 25.41 ^a	248.86 ± 27.73 ^a	0.72	221.1 + 0.188x + 0.000x ²	0.997	0.595	
Muscle										
SOD (IU mg ⁻¹ protein)	14.06 ± 1.27 ^c	14.87 ± 2.09 ^{bc}	14.91 ± 1.64 ^{bc}	17.92 ± 1.81 ^{ab}	19.65 ± 2.14 ^a	0.68	14.12 + 0.001x + 0.000x ²	0.958	0.016	
GOT (IU L ⁻¹)	73.32 ± 8.88 ^b	74.51 ± 6.81 ^b	72.32 ± 3.92 ^b	82.47 ± 4.69 ^{ab}	89.70 ± 6.75 ^a	2.62	73.80 − 0.116x + 0.002x ²	0.938	0.034	
GPT (IU L ⁻¹)	97.40 ± 5.31 ^b	97.88 ± 4.93 ^b	97.92 ± 6.94 ^b	108.14 ± 9.40 ^{ab}	111.85 ± 8.69 ^a	2.67	97.18 − 0.033x + 0.001x ²	0.923	0.096	

The data were expressed as mean ± standard error of the mean, *n* = 3 triplicates per treatments

Different superscript letters within a row indicate statistically significant (*P* < 0.05) differences as evaluated by Duncan multiple range test

PSEM, pooled standard error of the mean; RSM, red seaweed meal; SOD, superoxide dismutase; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase

inclusion levels of *U. rigida*, *C. barbata*, and *S. limacinum* incorporated diets had no significant effects on the growth and survival rate of *O. niloticus* and *Epinephelus lanceolatus* compared with fish fed with a control diet (Güroy et al. 2007; Azaza et al. 2008; García-Ortega et al. 2016). The decreased trend of FCR recorded up to RSM50 diet-fed fish indicates the fact that the formulated diets were superior in quality. Nonetheless, feed conversion efficiency (FCE) was similar in *O. mykiss* and *Sciaenops ocellatus*, fed with 50% inclusion of seaweed (*U. lactuca*, *Enteromorpha linza*, *S. limacinum*, and *Arthrospira* sp.) incorporated diets (Yildirim et al. 2009; Perez-Velazquez et al. 2018). Moreover, in this study, dietary FM replacement by RSM35, RSM34, and RSM37 inclusion promotes the most significant level of WG, SGR, and PER, as estimated by the polynomial regression equation. However, the RSM level that recorded the lowest FCR was RSM25. Several other studies indicated that the seaweed (*Gracilaria bursa-pastoris*, *U. rigida*, *Gracilaria cornea*, and *Gracilaria arcuata*) meals have essential amino acids, fatty acids, and other required compounds required for the growth and survival of European sea bass *D. labrax* and *O. niloticus* (Valente et al. 2006; Younis et al. 2018). According to Vazirzadeh et al. (2022), dietary inclusion of seaweeds (*Gracilariopsis persica*, *Hypnea flagelliformis*, and *Sargassum boveanum*) in *O. mykiss* had no adverse effects on fish growth and flesh quality. The seaweeds were used as a 10–15% replacement for soymeal and wheat flour in the fish diet. The study of Sáez et al. (2020) stated that low level (5%) inclusion of macroalgae *Ulva ohnoi* in diets of *Solea senegalensis* (Senegalese sole) improved the fillet quality.

The experimental fish fed with RSM50 in the diet had significantly increased the protein, lipid, and ash content when compared with control and other experimental diets suggesting that the optimum levels of dietary RSM compromised the nutritional quality of the experimental diets. López et al. (2012) demonstrated that FM replacement with 75% of *Arthrospira* meal and 25% of soybean meal mixture had significant results on the growth, feed utilization efficiency, and chemical composition of juvenile *O. mykiss*. Furthermore, in the present study, polypeptide bands at 10, 13, 15, 17, 23, and 37 kDa shown in SDS-PAGE were more intense in the muscle tissues of FM replaced with RSM50 diet-fed *L. rohita*. Red seaweed meal inclusion diets had influenced the protein synthesis due to the formation of ARA/EPA (arachidonic acid/eicosapentaenoic acid) ratio in the eicosanoid metabolism (Osmond et al. 2021). The protein bands were indicating the interactions of TOR (target of rapamycin) signalling which is actively involving in the protein synthesis, and it is influenced by the amino acids present in the RSM included diets (Qin et al. 2022). This recommends that the seaweeds can contribute to the absorption of dietary carbohydrates and protein as energy sources and stimulate

the assimilation of nutrients in the fish body (Olvera-Novoa et al. 1998; Kut-Güroy et al. 2007; Yildirim et al. 2009; Walker and Berlinsky 2011; Xuan et al. 2013; Peixoto et al. 2016; Younis et al. 2018; Liao et al. 2022). Carbohydrate levels were gradually increasing in diets from control to RSM100 because carbohydrate sources interchange the monomers involved in membrane transport modes through the molecule's metabolism thus increasing the absorption (de Souza et al., 2021). However, Stadlander et al. (2013) pointed out that during isocaloric (FM replacement with *Porphyra yezoensis* Ueda meal) diets, *Oreochromis niloticus* exhibited growth depression in response to excessive/moderate levels of dietary protein. Similarly, in this study, FM replacement with RSM75 and RSM100 (lower protein levels) diets depress the growth performance and chemical composition of *L. rohita*.

The use of active immunostimulant ingredients, seaweed meals, extracts, and isolated compounds was suitable for fish cultivation (Peixoto et al. 2016; Lafarga et al. 2020). Seaweed-based immunostimulants have the potential to improve the function of the fish head kidney directly (Thanigaivel et al. 2015; Araujo et al. 2016; Thepot et al. 2021) and also modulate the gut microbiota that enhances immunity indirectly (Guiry and Guiry. 2020). This may influence the hematological (Hb, Hct, RBC, and WBC) status of fish as observed in different studies, because these are the monitoring tool for the fish health status and oxidative stress-related responses (Kalla et al. 2008; Wan et al. 2016; Sotoudeh and Jafari 2017; Hassaan et al. 2019). Generally, algae are rich in phytic acid; it has a tendency to bind with cations as well as proteins and cause modifications in the blood parameters (Musa et al., 2021). The blood chemistry (glutamyl oxaloacetic transaminase, total bilirubin, glucose, blood urea nitrogen, and glutamic pyruvate transaminase) values were not significant when fed with *Eucheuma denticulatum* included diet-fed *Panaeolus olivaceus* (Ragaza et al. 2015). Results of hematological indices (Hb, Hct, RBC, WBC, MCV, MCH, and MCHC) in the current study were in general within the normal range when fed with FM replacement with RSM50 incorporated diet. However, hematological constituents were significantly decreased when dietary FM was replaced by RSM75 and RSM100. These were in agreement with the results obtained by Madibana et al. (2017), who reported that the blood parameters (Hct, neutrophil, monocyte, thrombocytes, eosinophil, and basophil) were a functional tool for assessing the enzyme ratio in the blood which plays a crucial role in cell damage to fish fed with seaweed *Ulva* sp. meal.

The changes in digestive enzyme activity in fishes indicate physiological responses to different nutritional (carbohydrates, lipids, and proteins) constituents of

experimental diets (Hidalgo et al. 1999; Fernandez et al. 2001; Hani et al. 2018). Amylase and protease enzyme levels were based on the effects of the diets fed by the fish related to the feed utilization (Pradhan et al. 2014). Our findings have shown that higher enzyme activities (amylase and protease) in FM replacement with RSM50 diet-fed fish contain around 39% of protein and 34% of carbohydrate levels. Higher digestive enzyme activity facilitates increased digestion by the diet, and gut microbiota coordinates the fish for digestion and absorption of nutrients (Kamunde et al. 2019). These results indicated that the incorporated RSM acted as a carbohydrate and protein source, and it was well utilized by *L. rohita*. Similarly, previous studies showed increased digestive enzymes (protease, amylase, and lipase) in fishes, *O. mykiss* and *O. niloticus*, fed with 30% seaweed meals of *Phytolacca dioica*, *Sargassum muticum*, *Spirodela polyrrhiza*, *Gracilaria vermiculophylla*, and *Ulva* spp. (Fasakin et al. 2001; Pereira et al. 2012; Silva et al. 2015; Tharaka et al. 2020).

In this study, the activities of antioxidant (SOD) and metabolic (GOT and GPT) enzymes were insignificant in gills, liver, and muscle tissues of fish fed with FM replacement up to RSM50 diet. Meanwhile, significant alterations were observed in antioxidants and metabolic enzyme activities in RSM75 and RSM100 incorporated diet-fed fish due to the activation of the antioxidant pathway by the red seaweed meal, which is an excellent source of antioxidant properties (Kiadaliri et al. 2020). Similarly, increased activities of two liver-bound enzymes, alkaline phosphatase (ALP) and alanine aminotransferase (ALT), were also observed in fish fed with *Ulva* meal-rich diets indicating increased antioxidants levels by seaweed components (Madibana et al. 2017). These enzymes are used to maintain the nutritional status of the blood vascular system and liver functions. Variations in these enzyme levels led to plasma membrane damage (Hassaan et al. 2019). Dietary antioxidants like red seaweed meal prevent oxidative stress in fish (Batista et al. 2020). The present study results are similar to that of the previous studies that demonstrate dietary red seaweed supplementation improves antioxidant capacity indirectly by modifying the activities of antioxidant defense mechanisms (Kim and Lee 2008; Luo et al. 2012; Queiroz et al. 2014; Thanigaivel et al. 2015).

Conclusion

The results of the present study indicate the fact that RSM incorporation in aquafeeds (39% protein level) could be an essential tool to increase the production of freshwater fish *L. rohita* fingerlings. These findings

offer an intriguing alternative not only to partially replacing fishmeal with RSM, but also to other dietary ingredients that have a direct effect on the growth performance and feed utilization of freshwater fish species. As a result of the current study, it was determined that the RSM incorporated experimental diets on freshwater *L. rohita* improved growth, antioxidant, and enzymatic activities. This study clearly showed that FM replaced with RSM37 (estimated polynomial second-order regression) has the potential alternative ingredient in the diet of *L. rohita* fingerlings. In addition, comparing the price of the seaweed to fish meal in the fish feed would be economically viable.

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Author contribution **Dinesh Babu Manikandan:** Formal analysis, investigation, resources, methodology, validation, visualization, and writing—original draft. **Srinivasan Veeran:** Formal analysis, investigation, resources, methodology, data curation, validation, visualization, writing—original draft, and writing—review and editing. **Subburaj Seenivasan:** Formal analysis, investigation, methodology, validation, visualization, and writing—original draft. **Arun Sridhar:** Investigation, methodology, data curation, and visualization. **Manikandan Arumugam:** Formal analysis, investigation, methodology, and visualization. **Zhou Yangen:** Formal analysis, validation, and writing—review and editing. **Thirumurugan Ramasamy:** Conceptualization, formal analysis, investigation, methodology, project administration, supervision, writing—original draft, and writing—review and editing.

Data availability All data generated or analyzed during this study are included in this published article.

Code availability Not applicable.

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

- Afonso, C., Correia, A.P., Freitas, M.V., Baptista, T., Neves, M. and Mouga, T., 2021. Seasonal changes in the nutritional composition of *Agarophyton vermiculophyllum* (Rhodophyta, gracilari-ales) from the center of Portugal. *Foods*, 10(5), p.1145. <https://doi.org/10.3390/foods10051145>
- Akegbejo-Samsons, Y., 2022. Aquaculture and Fisheries Production in Africa: Highlighting Potentials and Benefits for Food Security. In *Food Security for African Smallholder Farmers* (pp. 171–190). https://doi.org/10.1007/978-981-16-6771-8_11
- Aldon, E.T., 1998. Seaweeds: Utilization and product applications. *SEAFDEC Asian Aquaculture*, 20(1), pp.22–23. <http://hdl.handle.net/10862/1799>
- Angulo, C., Chavez-Infante, L., Reyes-Becerril, M., Angulo, M., Romero-Geraldo, R., Llinas-Cervantes, X. and Cepeda-Palacios, R., 2020. Immunostimulatory and antioxidant effects of supplemental feeding with macroalga *Sargassum* spp. on goat kids. *Tropical Animal Health and Production*, 52(4), pp.2023–2033. <https://doi.org/10.1007/s11250-020-02218-5>
- AOAC, 1995. Official methods of analysis of official analytical chemists international, 16th ed. AOAC, Arlington, VA, USA. AOAC International Publishers Arlington USA
- APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association (APHA) American Water Works Association (AWWA) and Water Environment Federation (WEF) Washington DC
- Aragão, C., Gonçalves, A.T., Costas, B., Azeredo, R., Xavier, M.J. and Engrola, S., 2022. Alternative Proteins for Fish Diets: Implications beyond Growth. *Animals*, 12(9), p.1211. <https://doi.org/10.3390/ani12091211>
- Araújo, M., Rema, P., Sousa-Pinto, I., Cunha, L.M., Peixoto, M.J., Pires, M.A., Seixas, F., Brotas, V., Beltrán, C. and Valente, L.M., 2016. Dietary inclusion of IMTA-cultivated *Gracilaria vermiculophylla* in rainbow trout (*Oncorhynchus mykiss*) diets: effects on growth, intestinal morphology, tissue pigmentation, and immunological response. *Journal of applied phycology*, 28(1), pp.679–689. <https://doi.org/10.1007/s10811-015-0591-8>
- Ashkenazi, D.Y., Segal, Y., Ben-Valid, S., Paz, G., Tsubery, M.N., Salomon, E., Abelson, A. and Israel, Á., 2022. Enrichment of nutritional compounds in seaweeds via abiotic stressors in integrated aquaculture. *Innovative Food Science & Emerging Technologies*, 80, p.103067. <https://doi.org/10.1016/j.ifset.2022.103067>
- Azaza, M.S., Mensi, F., Ksouri, J., Dhraief, M.N., Brini, B., Abdelmouleh, A. and Kraïem, M.M., 2008. Growth of Nile tilapia (*Oreochromis niloticus* L.) fed with diets containing graded levels of green algae ulva meal (*Ulva rigida*) reared in geothermal waters of southern Tunisia. *Journal of applied ichthyology*, 24(2), pp. 202–207. <https://doi.org/10.1111/j.1439-0426.2007.01017.x>
- Balasubramanian, B., Shanmugam, S., Park, S., Recharla, N., Koo, J.S., Andretta, I. and Kim, I.H., 2021. Supplemental impact of marine red seaweed (*Halymenia palmata*) on the growth performance, total tract nutrient digestibility, blood profiles, intestine histomorphology, meat quality, fecal gas emission, and microbial counts in broilers. *Animals*, 11(5), p. 1244. <https://doi.org/10.3390/ani11051244>
- Barnes, H. and Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for 'total' lipids. *Journal of experimental marine biology and ecology*, 12(1), pp.103–118. [https://doi.org/10.1016/0022-0981\(73\)90040-3](https://doi.org/10.1016/0022-0981(73)90040-3)
- Batista, S., Pereira, R., Oliveira, B., Baião, L.F., Jessen, F., Tulli, F., Messina, M., Silva, J.L., Abreu, H. and Valente, L.M., 2020. Exploring the potential of seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica*, single or blended, as natural dietary ingredients for European seabass *Dicentrarchus labrax*. *Journal of Applied Phycology*, 32(3), pp.2041–2059. <https://doi.org/10.1007/s10811-020-02118-z>
- Bernfeld, P., 1955. Amylase α and β . *Methods in Enzymology*, 1, pp.149–158. [https://doi.org/10.1016/0076-6879\(55\)01021-5](https://doi.org/10.1016/0076-6879(55)01021-5)
- Boominathan, R., Devanesan, S., AlSalhi, M.S., Balasubramanian, A., Alkhalid, I.Z., Paul, P. and Singh, A.R., 2022. Quorum quenching action of marine red alga *Halymenia durvillei* on biofilm forming Gram negative bacterial isolates from contact lens. *Algal Research*, 64, p.102693. <https://doi.org/10.1016/j.algal.2022.102693>
- Boyd, C.E., D'Abramo, L.R., Glencross, B.D., Huyben, D.C., Juarez, L.M., Lockwood, G.S., McNevin, A.A., Tacon, A.G., Teletchea, F., Tomasso Jr, J.R. and Tucker, C.S., 2020. Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. *Journal of the World Aquaculture Society*, 51(3), pp.578–633. <https://doi.org/10.1111/jwas.12714>
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1–2), pp.248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Carpene, M., Garcia-Perez, P., Garcia-Oliveira, P., Chamorro, F., Otero, P., Lourenço-Lopes, C., Cao, H., Simal-Gandara, J. and Prieto, M.A., 2022. Biological properties and potential of compounds extracted from red seaweeds. *Phytochemistry Reviews*, pp.1–32. <https://doi.org/10.1007/s11101-022-09826-z>
- Castell, J.D. and Tiews, K., 1980. Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research, Hamburg, Federal Republic of Germany, 21–23 March 1979. *Documents Techniques de la CECPI (FAO)*.
- das Neves Amorim, R.C., Rodrigues, J.A.G., Holanda, M.L., de Souza Mourão, P.A. and Benevides, N.M.B., 2011. Anticoagulant properties of a crude sulfated polysaccharide from the red marine alga *Halymenia floresia* (Clemente) C. Agardh. *Acta Scientiarum. Biological Sciences*, 33(3), pp.255–261. <https://doi.org/10.4025/actascibiolsci.v33i3.6402>
- Davies, S.J., Brown, M.T. and Camilleri, M., 1997. Preliminary assessment of the seaweed *Porphyra purpurea* in artificial diets for thick-lipped grey mullet (*Chelon labrosus*). *Aquaculture*, 152(1–4), pp.249–258. [https://doi.org/10.1016/S0044-8486\(96\)01513-X](https://doi.org/10.1016/S0044-8486(96)01513-X)
- de Souza, A.M., Campeche, D.F.B., Melo, J.F.B., dos Santos, A.T.S. and Vidal, L.V.O., 2021. Corn substitution by mesquite bean flour (*Prosopis juliflora*) maintains growth and improves protein metabolism of Nile tilapia juveniles (*Oreochromis niloticus*). *Tropical Animal Health and Production*, 53(4), pp.1–15. <https://doi.org/10.1007/s11250-021-02826-9>
- Deepak, P., Balamuralikrishnan, B., Park, S., Sowmiya, R., Balasubramani, G., Aiswarya, D., Amutha, V. and Perumal, P., 2019. Phytochemical profiling of marine red alga, *Halymenia palmata* and its bio-control effects against Dengue Vector, *Aedes aegypti*. *South African Journal of Botany*, 121, pp.257–266. <https://doi.org/10.1016/j.sajb.2018.11.011>
- Demirel, Z., Yilmaz-Koz, F.F., Karabay-Yavasoglu, U.N., Ozdemir, G. and Sukatar, A., 2009. Antimicrobial and antioxidant activity of brown algae from the Aegean Sea. *Journal of the Serbian Chemical Society*, 74(6), pp. 619–628. <http://www.doiserbia.nb.rs/img/doi/0352-5139/2009/0352-51390906619D.pdf>
- Dileep, N., Pradhan, C., Peter, N., Kaippilly, D., Sashidharan, A. and Sankar, T.V., 2021. Nutritive value of guar and copra meal after fermentation with yeast *Saccharomyces cerevisiae* in the diet of Nile tilapia, *Oreochromis niloticus*. *Tropical Animal Health and Production*, 53(4), pp.1–13. <https://doi.org/10.1007/s11250-021-02855-4>
- Ergun, S., Soyutürk, M., Güroy, B., Güroy, D. and Merrifield, D., 2009. Influence of Ulva meal on growth, feed utilization, and body

- composition of juvenile Nile tilapia (*Oreochromis niloticus*) at two levels of dietary lipid. *Aquaculture International*, 17(4), pp.355–361. <https://doi.org/10.1007/s10499-008-9207-5>
- Fantonalgo, R.N., 2018. Preliminary study on Biogeography and Diversity of Red alga *Halymenia* in Manila Bay, Philippines. *Annals of Geographical Studies*, 1(1), pp.1–10.
- FAO, 2014. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations Rome, 223. <http://www.fao.org/3/a-3720e.pdf>
- FAO, 2018. Cultured Aquatic Species Information Programme. *Labeo rohita*. Cultured Aquatic Species Information Programme, in: Jena, J.K. (Ed.), FAO Fisheries and Aquaculture Department [online], Rome. <http://www.fao.org/fishery/culturedspecies/>
- Fasakin, E.A., Balogun, A.M. and Fagbenro, O.A., 2001. Evaluation of Sun-dried water fern, *Azolla africana* and duckweed, *Spirodela polyrrhiza* in practical diets for Nile tilapia, *Oreochromis niloticus* fingerlings. *Journal of Applied Aquaculture*, 11(4), pp.83–92. <https://doi.org/10.1300/J028v11n0409>
- Fenoradosoa, T.A., Delattre, C., Laroche, C., Wadouachi, A., Dulong, V., Picton, L., Andriamadio, P. and Michaud, P., 2009. Highly sulphated galactan from *Halymenia durvillei* (Halymeniales, Rhodophyta), a red seaweed of Madagascar marine coasts. *International Journal of Biological Macromolecules*, 45(2), pp.140–145. <https://doi.org/10.1016/j.ijbiomac.2009.04.015>
- Fernandez, I., Moyano, F.J., Diaz, M. and Martinez, T., 2001. Characterization of α -amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). *Journal of Experimental Marine Biology and Ecology*, 262(1), pp.1–12. [https://doi.org/10.1016/S0022-0981\(01\)00228-3](https://doi.org/10.1016/S0022-0981(01)00228-3)
- Folch, J., Lees, M. and Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of biological Chemistry*, 226(1), pp. 497–509. <http://www.jbc.org/content/226/1/497.citation>
- Fredrick Raja, E., 2022. Growth Performance and Immune Response of *Oreochromis niloticus* L. with the Effect of Marine Macroalgal Fish Feed: *Halymenia Floresii* (Clemente) C. Ag. *Journal of Coastal Life Medicine*, 10, pp.28–39.
- Furne, M., Hidalgo, M.C., Lopez, A., Garcia-Gallego, M., Morales, A.E., Domezain, A., Domezaine, J. and Sanz, A., 2005. Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. *Aquaculture*, 250(1–2), pp.391–398. <https://doi.org/10.1016/j.aquaculture.2005.05.017>
- García-Ortega, A., Kissinger, K.R. and Trushenski, J.T., 2016. Evaluation of fish meal and fish oil replacement by soybean protein and algal meal from *Schizochytrium limacinum* in diets for giant grouper *Epinephelus lanceolatus*. *Aquaculture*, 452, pp.1–8. <https://doi.org/10.1016/j.aquaculture.2015.10.020>
- García-Vaquero, M. and Hayes, M., 2016. Red and green macroalgae for fish and animal feed and human functional food development. *Food Reviews International*, 32(1), pp.15–45. <https://doi.org/10.1080/87559129.2015.1041184>
- Guiry, M.D. and Guiry, G.M., 2020. AlgaeBase. World-wide electronic publication, National University of Ireland. Galway, Ireland. <http://www.algaebase.org>
- Güroy, B.K., Cirik, Ş.Ü.K.R.A.N., Güroy, D., Sanver, F. and Tekinay, A.A., 2007. Effects of *Ulva rigida* and *Cystoseira barbata* meals as a feed additive on growth performance, feed utilization, and body composition of Nile tilapia, *Oreochromis niloticus*. *Turkish Journal of Veterinary & Animal Sciences*, 31(2), pp.91–97. <https://journals.tubitak.gov.tr/veterinary/vol31/iss2/2>
- Hani, Y.M.I., Marchand, A., Turies, C., Kerambrun, E., Palluel, O., Bado-Nilles, A., Beaudouin, R., Porcher, J.M., Geffard, A. and Dedourge-Geffard, O., 2018. Digestive enzymes and gut morphometric parameters of threespine stickleback (*Gasterosteus aculeatus*): Influence of body size and temperature. *PLoS One*, 13(4), p.e0194932. <https://doi.org/10.1371/journal.pone.0194932>
- Harikrishnan, R., Devi, G., Van Doan, H., Balasundaram, C., Arockiaraj, J. and Jagruthi, C., 2021. Efficacy of ulvan on immune response and immuno-antioxidant gene modulation in *Labeo rohita* against columnaris disease. *Fish & Shellfish Immunology*, 117, pp.262–273. <https://doi.org/10.1016/j.fsi.2021.08.004>
- Hassaan, M.S., El-Sayed, A.I.M., Soltan, M.A., Iraqi, M.M., Goda, A.M., Davies, S.J., El-Haroun, E.R. and Ramadan, H.A., 2019. Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth, feed performance, hematological indices and associated gene expression markers (GH, IGF-I) for Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 503, pp.282–292
- Hidalgo, M.C., Urea, E. and Sanz, A., 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*, 170(3–4), pp.267–283. [https://doi.org/10.1016/S0044-8486\(98\)00413-X](https://doi.org/10.1016/S0044-8486(98)00413-X)
- Holdt, S.L. and Kraan, S., 2011. Bioactive compounds in seaweed: functional food applications and legislation. *Journal of applied phycology*, 23(3), pp. 543–597. <https://doi.org/10.1007/s10811-010-9632-5>
- Hurtado, A.Q., Magdugo, R. and Critchley, A.T., 2020. Harvesting and potential uses of selected red seaweeds in the Philippines with emerging high-value applications. In *Advances in Botanical Research*, 95, pp.19–56. <https://doi.org/10.1016/bs.abr.2019.12.004>
- Itembu, J.A., Gabriel, N.N., Tjipute, M., Asino, H. and Hamukwaya, J., 2022. The Governance of Aquaculture in Namibia as a Vehicle for Food Security and Economic Growth. In *Food Security for African Smallholder Farmers*, pp.391–403. https://doi.org/10.1007/978-981-16-6771-8_23
- Jainab, S.B., Azeez, A., Fathima, A. and Kumar, R.R., 2019. GC-MS analysis of the marine algae *Halymenia dilatata* Zanardini a potential source of fish feed in future. *Indian Hydrobiol*, 18(1–2), pp.164–169.
- Jiménez-Escrig, A., Gómez-Ordóñez, E. and Rupérez, P., 2011. Seaweed as a source of novel nutraceuticals: sulfated polysaccharides and peptides. *Advances in Food and Nutrition Research*, 64, pp. 325–337. <https://doi.org/10.1016/B978-0-12-387669-0.00026-0>
- Kalla, A., Yoshimatsu, T., Araki, T., Zhang, D.M., Yamamoto, T. and Sakamoto, S., 2008. Use of *Porphyra spheroplasts* as feed additive for red sea bream. *Fisheries Science*, 74(1), pp.104–108. <https://doi.org/10.1111/j.1444-2906.2007.01501.x>
- Kamunde, C., Sappal, R. and Melegy, T.M., 2019. Brown seaweed (AquaArom) supplementation increases food intake and improves growth, antioxidant status and resistance to temperature stress in Atlantic salmon, *Salmo salar*. *PLoS One*, 14(7), p.e0219792. <https://doi.org/10.1371/journal.pone.0219792>
- Kiadaliri, M., Firouzbakhsh, F. and Deldar, H., 2020. Effects of feeding with red algae (*Laurencia caspica*) hydroalcoholic extract on antioxidant defense, immune responses, and immune gene expression of kidney in rainbow trout (*Oncorhynchus mykiss*) infected with *Aeromonas hydrophila*. *Aquaculture*, 526, p.735361.
- Kim, S.S. and Lee, K.J., 2008. Effects of dietary kelp (*Ecklonia cava*) on growth and innate immunity in juvenile olive flounder *Paralichthys olivaceus* (Temminck et Schlegel). *Aquaculture research*, 39(15), pp.1687–1690. <https://doi.org/10.1111/j.1365-2109.2008.02046.x>
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685. <https://doi.org/10.1038/227680a0>

- Lafarga, T., Acién-Fernández, F.G. and Garcia-Vaquero, M., 2020. Bioactive peptides and carbohydrates from seaweed for food applications: Natural occurrence, isolation, purification, and identification. *Algal research*, 48, p.101909. <https://doi.org/10.1016/j.algal.2020.101909>
- Leandro, A., Pereira, L. and Gonçalves, A.M., 2019. Diverse applications of marine macroalgae. *Marine drugs*, 18(1), p.17. <https://doi.org/10.3390/md18010017>
- Liao, H., Liu, P., Deng, Y., Zhang, W., Pan, C., Jia, Y., Long, F. and Tang, H., 2022. Feeding effects of low-level fish meal replacement by algal meals of *Schizochytrium limacinum* and *Nannochloropsis salina* on largemouth bass (*Micropterus salmoides*). *Aquaculture*, 557, p.738311. <https://doi.org/10.1016/j.aquaculture.2022.738311>
- Lomartire, S., Marques, J.C. and Gonçalves, A.M., 2021. An overview to the health benefits of seaweeds consumption. *Marine Drugs*, 19(6), p.341. <https://doi.org/10.3390/md19060341>
- López, O.A., Araiza, M.A.F., Flores, G.H. and Hernández, L.H.H., 2012. Effects of total replacement of fishmeal with Spirulina powder and soybean meal on juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum). *Israeli Journal of Aquaculture-Bamidgeh*, 64, p.20612. <http://hdl.handle.net/10524/31828>
- Lowry, O.H., 1951. Protein measurement with the Folin phenol reagent. *Journal of biological Chemistry*, 193, pp.265–275. <http://www.jbc.org/content/193/1/265.citation>
- Luo, Z., Liu, C.X. and Wen, H., 2012. Effect of dietary fish meal replacement by canola meal on growth performance and hepatic intermediary metabolism of genetically improved farmed tilapia strain of Nile tilapia, *Oreochromis niloticus*, reared in fresh water. *Journal of the World Aquaculture Society*, 43(5), pp.670–678. <https://doi.org/10.1111/j.1749-7345.2012.00601.x>
- Madibana, M.J., Mlambo, V., Lewis, B. and Fouché, C., 2017. Effect of graded levels of dietary seaweed (*Ulva* sp.) on growth, hematological and serum biochemical parameters in dusky kob, *Argyrosomus japonicus*, sciaenidae. *The Egyptian Journal of Aquatic Research*, 43(3), pp.249–254. <https://doi.org/10.1016/j.ejar.2017.09.003>
- Madsen, H., Nguyen, H.M., Lanza, G.R. and Stauffer Jr, J.R., 2022. A One Health Approach Relative to Trematode-Caused Diseases of People and Animals Associated with Aquaculture. *Reviews in Fisheries Science & Aquaculture*, pp.1–25. <https://doi.org/10.1080/23308249.2022.2090830>
- Maiolo, S., Parisi, G., Biondi, N., Lunelli, F., Tibaldi, E. and Pastres, R., 2020. Fishmeal partial substitution within aquafeed formulations: Life cycle assessment of four alternative protein sources. *The International Journal of Life Cycle Assessment*, 25(8), pp.1455–1471. <https://doi.org/10.1007/s11367-020-01759-z>
- Malairaj, S., Muthu, S., Gopal, V.B., Perumal, P. and Ramasamy, R., 2016. Qualitative and quantitative determination of R-phycoerythrin from *Halymenia floresia* (Clemente) C. Agardh by polyacrylamide gel using electrophoretic elution technique. *Journal of Chromatography A*, 1454, pp.120–126. <https://doi.org/10.1016/j.chroma.2016.05.063>
- Marinho, G., Nunes, C., Sousa-Pinto, I., Pereira, R., Rema, P. and Valente, L.M., 2013. The IMTA-cultivated Chlorophyta *Ulva* spp. as a sustainable ingredient in Nile tilapia (*Oreochromis niloticus*) diets. *Journal of applied phycology*, 25(5), pp.1359–1367. <https://doi.org/10.1007/s10811-012-9965-3>
- Marklund, S. and Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of biochemistry*, 47(3), pp.469–474. <https://doi.org/10.1111/j.1432-1033.1974.tb03714.x>
- Marshall, S., Scott, G.W. and Tobin, M.L., 2007. Comparison of nutritive chemistry of a range of temperate seaweeds. *Food chemistry*, 100(4), pp.1331–1336. <https://doi.org/10.1016/j.foodchem.2005.11.029>
- Morais, T., Inácio, A., Coutinho, T., Ministro, M., Cotas, J., Pereira, L. and Bahcevandziev, K., 2020. Seaweed potential in the animal feed: A review. *Journal of Marine Science and Engineering*, 8(8), p.559. <https://doi.org/10.3390/jmse8080559>
- Muñiz, R.D.L.M.J., Jimber del Río, J.A., Jiménez Beltrán, F.J. and Vera Gilces, P., 2022. The fisheries and aquaculture sector in Latin America: Exports to East Asia and production. *PloS one*, 17(7), p.e0267862. <https://doi.org/10.1371/journal.pone.0267862>
- Murata, M. and Nakazoe, J.I., 2001. Production and use of marine algae in Japan. *Japan Agricultural Research Quarterly: JARQ*, 35(4), pp.281–290. <https://doi.org/10.6090/jarq.35.281>
- Musa, S.O., Okomoda, V.T., Tihamiyu, L.O., Solomon, S.G., Adeyemo, B.T., Alamanjo, C.C. and Abol-Munafi, A.B., 2021. Dietary implications of toasted *Jatropha curcas* kernel on the growth, haematology, and organ histology of *Clarias gariepinus* fingerlings. *Tropical Animal Health and Production*, 53(2), pp.1–11. <https://doi.org/10.1007/s11250-021-02678-3>
- Mustafa, G., Wakamatsu, S., Takeda, T.A., Umino, T. and Nakagawa, H., 1995. Effects of algae meal as feed additive on growth, feed efficiency, and body composition in red sea bream. *Fisheries Science*, 61(1), pp.25–28. <https://doi.org/10.2331/fishsci.61.25>
- Nakagawa, H., Umino, T. and Tasaka, Y., 1997. Usefulness of Ascophyllum meal as a feed additive for red sea bream, *Pagrus major*. *Aquaculture*, 151(1–4), pp.275–281. [https://doi.org/10.1016/S0044-8486\(96\)01488-3](https://doi.org/10.1016/S0044-8486(96)01488-3)
- Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K. and Nichols, P.D., 2009. Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences*, 106(36), pp.15103–15110. <https://doi.org/10.1073/pnas.0905235106>
- Nobrega, R.O., Batista, R.O., Corrêa, C.F., Mattioni, B., Filer, K., Pettigrew, J.E. and Fracalossi, D.M., 2019. Dietary supplementation of *Aurantiochytrium* sp. meal, a docosahexaenoic-acid source, promotes growth of Nile tilapia at a suboptimal low temperature. *Aquaculture*, 507, pp.500–509. <https://doi.org/10.1016/j.aquaculture.2019.04.030>
- Oliva-Teles, A., Enes, P., Couto, A. and Peres, H., 2022. Replacing fish meal and fish oil in industrial fish feeds. *Feed and Feeding Practices in Aquaculture*, pp.231–268. <https://doi.org/10.1016/B978-0-12-821598-2.00011-4>
- Olivera-Novoa, M.A., Domínguez-Cen, L.J., Olivera-Castillo, L. and Martínez-Palacios, C.A., 1998. Effect of the use of the microalga *Spirulina maxima* as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. *Aquaculture research*, 29(10), pp.709–715. <https://doi.org/10.1046/j.1365-2109.1998.29100709.x>
- Osmond, A.T., Arts, M.T., Hall, J.R., Rise, M.L., Bazinet, R.P., Armenta, R.E. and Colombo, S.M., 2021. *Schizochytrium* sp. (T18) Oil as a Fish Oil Replacement in Diets for Juvenile Rainbow Trout (*Oncorhynchus mykiss*): Effects on Growth Performance, Tissue Fatty Acid Content, and Lipid-Related Transcript Expression. *Animals*, 11(4), p.1185. <https://doi.org/10.3390/ani11041185>
- Panase, P., Uppapong, S., Tuncharoen, S., Tanitson, J., Soontornprasit, K. and Intawicha, P., 2018. Partial replacement of commercial fish meal with Amazon sailfin catfish *Pterygoplichthys pardalis* meal in diets for juvenile Mekong giant catfish *Pangasianodon gigas*. *Aquaculture Reports*, 12, pp.25–29. <https://doi.org/10.1016/j.aqrep.2018.08.005>
- Peixoto, M.J., Salas-Leitón, E., Pereira, L.F., Queiroz, A., Magalhães, F., Pereira, R., Abreu, H., Reis, P.A., Gonçalves, J.F.M. and de Almeida Ozório, R.O., 2016. Role of dietary seaweed

- supplementation on growth performance, digestive capacity and immune and stress responsiveness in European seabass (*Dicentrarchus labrax*). *Aquaculture Reports*, 3, pp.189–197. <https://doi.org/10.1016/j.aqrep.2016.03.005>
- Peñalver, R., Lorenzo, J.M., Ros, G., Amarowicz, R., Pateiro, M. and Nieto, G., 2020. Seaweeds as a functional ingredient for a healthy diet. *Marine Drugs*, 18(6), p.301. <https://doi.org/10.3390/md18060301>
- Pereira, R., Valente, L.M., Sousa-Pinto, I. and Rema, P., 2012. Apparent nutrient digestibility of seaweeds by rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*). *Algal Research*, 1(1), pp.77–82. <https://doi.org/10.1016/j.algal.2012.04.002>
- Perez-Velazquez, M., Gatlin III, D.M., González-Félix, M.L. and García-Ortega, A., 2018. Partial replacement of fishmeal and fish oil by algal meals in diets of red drum *Sciaenops ocellatus*. *Aquaculture*, 487, pp.41–50. <https://doi.org/10.1016/j.aquaculture.2018.01.001>
- Pounds, A., Kaminski, A.M., Budhathoki, M., Gudbrandsen, O., Kok, B., Horn, S., Malcorps, W., Mamun, A.A., McGoohan, A., Newton, R. and Ozretich, R., 2022. More Than Fish—Framing Aquatic Animals within Sustainable Food Systems. *Foods*, 11(10), p.1413. <https://doi.org/10.3390/foods11101413>
- Pradhan, C., Mohanty, S.N., Rath, S.C. and Giri, S.S., 2014. Influence of Feeding an All Plant Ingredients Containing Diet at Different Levels on Growth and Digestive Enzyme Activity of Pond Raised Indian Major Carps. *Animal Nutrition and Feed Technology*, 14(2), pp.251–262.
- Pradhan, C., Giri, S.S., Mohanty, T.K. and Mohanty, S.N., 2020. Influence of a diet containing plant ingredients at different levels on growth performance, carcass biochemical composition, and blood parameters in Indian major carps grown in polyculture earthen ponds. *Tropical Animal Health and Production*, 52(4), pp.1769–1777. <https://doi.org/10.1007/s11250-019-02184-7>
- Qin, Y., He, C., Geng, H., Wang, W., Yang, P., Mai, K. and Song, F., 2022. Muscle Nutritive Metabolism Changes after Dietary Fishmeal Replaced by Cottonseed Meal in Golden Pompano (*Trachinotus ovatus*). *Metabolites*, 12(7), p.576. <https://doi.org/10.3390/metabo12070576>
- Queiroz, A.C.S., Pereira, R., Domingues, A.F., Peixoto, M.J.D., Gonçalves, J.F.M. and Ozorio, R.O., 2014. Effect of seaweed supplementation on growth performance, immune and oxidative stress responses in gilthead seabream (*Sparus aurata*). In *IMMR International Meeting on Marine Research*. <https://doi.org/10.3389/conf.fmars.2014.02.00018>
- Ragaza, J.A., Koshio, S., Mamauag, R.E., Ishikawa, M., Yokoyama, S. and Villamor, S.S., 2015. Dietary supplemental effects of red seaweed *Eucheuma denticulatum* on growth performance, carcass composition and blood chemistry of juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture Research*, 46(3), pp.647–657. <https://doi.org/10.1111/are.12211>
- Rajapakse, N. and Kim, S.K., 2011. Nutritional and digestive health benefits of seaweed. *Advances in food and nutrition research*, 64, pp.17–28. <https://doi.org/10.1016/B978-0-12-387669-0.00002-8>
- Ramasamy, T., Veeran, S., Sridhar, A., Sekar, R.K., Manikandan, D.B., Arumugam, M. and Periasamy, S., 2021. Effects of graded levels of mineral mixtures (Aquamin® and Agrimin®) supplemented diets on growth, survival, proximate composition, and carcass mineralization of juvenile freshwater prawn, *Macrobrachium malcolmsonii* (H Milne-Edwards 1844). *Tropical Animal Health and Production*, 53(1), pp.1–13. <https://doi.org/10.1007/s11250-021-02558-w>
- Rebours, C., Marinho-Soriano, E., Zertuche-González, J.A., Hayashi, L., Vásquez, J.A., Kradolfer, P., Soriano, G., Ugarte, R., Abreu, M.H., Bay-Larsen, I. and Hovelsrud, G., 2014. Seaweeds: an opportunity for wealth and sustainable livelihood for coastal communities. *Journal of applied phycology*, 26(5), pp.1939–1951. <https://doi.org/10.1007/s10811-014-0304-8>
- Reitman, S. and Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, 28(1), pp.56–63. <https://doi.org/10.1093/ajcp/28.1.56>
- Roe, J.H., 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *Journal of Biological Chemistry*, 212, pp.335–343. <http://www.jbc.org/content/212/1/335.citation>
- Roleda, M.Y. and Hurd, C.L., 2019. Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. *Phycologia*, 58(5), pp.552–562. <https://doi.org/10.1080/00318884.2019.1622920>
- Saleh, H.H., 2020. Review on Using of Macro Algae (seaweeds) in Fish Nutrition. *Journal of Zoological Research*, 2(2). <https://doi.org/10.30564/jzr.v2i2.2054>
- Samaddar, A., Kaviraj, A. and Saha, S., 2015. Utilization of fermented animal by-product blend as fishmeal replacer in the diet of *Labeo rohita*. *Aquaculture Reports*, 1, pp.28–36. <https://doi.org/10.1016/j.aqrep.2015.03.004>
- Satpathy, B.B., Mukherjee, D. and Ray, A.K., 2003. Effects of dietary protein and lipid levels on growth, feed conversion and body composition in rohu, *Labeo rohita* (Hamilton), fingerlings. *Aquaculture Nutrition*, 9(1), pp.17–24. <https://doi.org/10.1046/j.1365-2095.2003.00223.x>
- Sáez, M.I., Vizcaíno, A., Galafat, A., Anguís, V., Fernández-Díaz, C., Balebona, M.C., Alarcón, F.J. and Martínez, T.F., 2020. Assessment of long-term effects of the macroalgae *Ulva ohnoi* included in diets on Senegalese sole (*Solea senegalensis*) fillet quality. *Algal Research*, 47, p.101885.
- Shah, M.D., Venmathi Maran, B.A., Shaleh, S.R.M., Zuldin, W.H., Gnanaraj, C. and Yong, Y.S., 2022. Therapeutic Potential and Nutraceutical Profiling of North Bornean Seaweeds: A Review. *Marine Drugs*, 20(2), p.101. <https://doi.org/10.3390/md20020101>
- Silva, D.M., Valente, L.M.P., Sousa-Pinto, I., Pereira, R., Pires, M.A., Seixas, F. and Rema, P., 2015. Evaluation of IMTA-produced seaweeds (*Gracilaria*, *Porphyra*, and *Ulva*) as dietary ingredients in Nile tilapia, *Oreochromis niloticus* L., juveniles. Effects on growth performance and gut histology. *Journal of Applied Phycology*, 27(4), pp.1671–1680. <https://doi.org/10.1007/s10811-014-0453-9>
- Silva-Brito, F., Cardoso, A., Machado, M., Ramos-Pinto, L., Hinzmann, M., Abreu, H., Costas, B. and Magnoni, L., 2022. Dietary supplementation with *Gracilaria gracilis* by-products modulates the immune status and oxidative stress response of gilthead seabream (*Sparus aurata*) stimulated with *Photobacterium damselae* subsp. piscicida. *Fish & Shellfish Immunology*. <https://doi.org/10.1016/j.fsi.2022.05.028>
- Soler-Vila, A., Coughlan, S., Guiry, M.D. and Kraan, S., 2009. The red alga *Porphyra dioica* as a fish-feed ingredient for rainbow trout (*Oncorhynchus mykiss*): effects on growth, feed efficiency, and carcass composition. *Journal of Applied Phycology*, 21(5), pp.617–624. <https://doi.org/10.1007/s10811-009-9423-z>
- Sotoudeh, E. and Jafari, M., 2017. Effects of dietary supplementation with red seaweed, *Gracilaria pygmaea*, on growth, carcass composition and hematology of juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquaculture International*, 25(5), pp.1857–1867. <https://doi.org/10.1007/s10499-017-0158-6>
- Stadtlander, T., Khalil, W.K.B., Focken, U. and Becker, K., 2013. Effects of low and medium levels of red alga *Nori* (*Porphyra yezoensis* Ueda) in the diets on growth, feed utilization and metabolism in intensively fed Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture Nutrition*, 19(1), pp.64–73. <https://doi.org/10.1111/j.1365-2095.2012.00940.x>

- Terriente-Palacios, C. and Castellari, M., 2022. Levels of taurine, hypotaurine and homotaurine, and amino acids profiles in selected commercial seaweeds, microalgae, and algae-enriched food products. *Food Chemistry*, 368, p.130770. <https://doi.org/10.1016/j.foodchem.2021.130770>
- Thanigaivel, S., Vidhya Hindu S., Vijayakumar S., Mukherjee A., Chandrasekaran N., Thomas J., 2015. Differential solvent extraction of two seaweeds and their efficacy in controlling *Aeromonas salmonicida* infection in *Oreochromis mossambicus*: A novel therapeutic approach. *Aquaculture*, 443, pp.56-64. <https://doi.org/10.1016/j.aquaculture.2015.03.010>
- Tharaka, K., Gunathilaka, B.E., Veille, A., Kim, M.G., Shin, J., Lim, H., Jeong, J.B., Meallet, V. and Lee, K.J., 2020. Algae-clay powder (sea lettuce, *Ulva lactuca* and red algae, *Solieria chordalis* in exfoliated micronized montmorillonite) supplementation in a fish meal-reduced diet for olive flounder (*Paralichthys olivaceus*). *Aquaculture Reports*, 18, p.100498. <https://doi.org/10.1016/j.aqrep.2020.100498>
- Thépot, V., Campbell, A.H., Rimmer, M.A. and Paul, N.A., 2021. Meta-analysis of the use of seaweeds and their extracts as immunostimulants for fish: a systematic review. *Reviews in Aquaculture*, 13(2), pp.907-933. <https://doi.org/10.1111/raq.12504>
- Thépot, V., Campbell, A.H., Rimmer, M.A., Jelocnik, M., Johnston, C., Evans, B. and Paul, N.A., 2022. Dietary inclusion of the red seaweed *Asparagopsis taxiformis* boosts production, stimulates immune response and modulates gut microbiota in Atlantic salmon, *Salmo salar*. *Aquaculture*, 546, p.737286. <https://doi.org/10.1016/j.aquaculture.2021.737286>
- Valente, L.M.P., Gouveia, A., Rema, P., Matos, J., Gomes, E.F. and Pinto, I.S., 2006. Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, 252(1), pp.85-91. <https://doi.org/10.1016/j.aquaculture.2005.11.052>
- Vazirzadeh, A., Marhamati, A. and Chisti, Y., 2022. Seaweed-based diets lead to normal growth, improved fillet color but a down-regulated expression of somatotrophic axis genes in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 554, p.738183. <https://doi.org/10.1016/j.aquaculture.2022.738183>
- Walker, A.B. and Berlinsky, D.L., 2011. Effects of partial replacement of fish meal protein by microalgae on growth, feed intake, and body composition of Atlantic cod. *North American Journal of Aquaculture*, 73(1), pp.76-83. <https://doi.org/10.1080/15222055.2010.549030>
- Wan, A.H., Soler-Vila, A., O'Keeffe, D., Casburn, P., Fitzgerald, R. and Johnson, M.P., 2016. The inclusion of *Palmaria palmata* macroalgae in Atlantic salmon (*Salmo salar*) diets: effects on growth, haematology, immunity and liver function. *Journal of Applied Phycology*, 28(5), pp.3091-3100. <https://doi.org/10.1007/s10811-016-0821-8>
- Wassef, E.A., El Masry, M.H. and Mikhail, F.R., 2001. Growth enhancement and muscle structure of striped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal-based diets. *Aquaculture Research*, 32, pp.315-322. <https://doi.org/10.1046/j.1355-557x.2001.00043.x>
- Xuan, X., Wen, X., Li, S., Zhu, D. and Li, Y., 2013. Potential use of macro-algae *Gracilaria lemaneiformis* in diets for the black sea bream, *Acanthopagrus schlegelii*, juvenile. *Aquaculture*, 412, pp.167-172. <https://doi.org/10.1016/j.aquaculture.2013.07.022>
- Yildirim, Ö., Ergün, S., Yaman, S. and Türker, A., 2009. Effects of two seaweeds (*Ulva lactuca* and *Enteromorpha linza*) as a feed additive in diets on growth performance, feed utilization, and body composition of rainbow trout (*Oncorhynchus mykiss*). 15(3), pp. 455-460 <https://doi.org/10.9775/kvfd.2009.042-A>
- Younis, E.S.M., Al-Quffail, A.S., Al-Asgah, N.A., Abdel-Warith, A.W.A. and Al-Hafedh, Y.S., 2018. Effect of dietary fish meal replacement by red algae, *Gracilaria arcuata*, on growth performance and body composition of Nile tilapia *Oreochromis niloticus*. *Saudi journal of biological sciences*, 25(2), pp.198-203. <https://doi.org/10.1016/j.sjbs.2017.06.012>

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