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Nutritional evaluation of *Gracilaria pulvinata* as partial substitute with fish meal in practical diets of barramundi (*Lates calcarifer*)

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Abstract A 40-day feeding trial was carried out to investigate the potential of red algal (*Gracilaria pulvinata*) meal as a protein source in formulated diets for barramundi (*Lates calcarifer*) (initial mean body weight of 28.5 ± 0.5 g). Five practical diets were prepared using a fishmeal-based diet used as positive control (PC) and blends of soybean meal and fishmeal used as a negative control (NC), in which NC was supplemented with 3 (GL3%), 6 (GL6%), and 9% (GL9%) *G. pulvinata*. Each dietary treatment was replicated in triplicate. Results indicated that growth and feeding performance including specific growth rate, feed conversion, protein efficiency ratios, and feed intake in all treatments were not significantly different ($P > 0.05$). Crude protein, lipid, and ash contents of fish carcass were not markedly altered in all dietary treatments. Serum lysozyme, alternative complement activities, serum immunoglobulin, and total protein content significantly decreased in fish fed GL9 diets. Increasing dietary supplementation of *G. pulvinata* decreased the serum triglycerides and cholesterol when compared to the NC. *Gracilaria* inclusion levels did not affect intestinal total protease and amylase activities ($P < 0.05$); however, intestine lipase activity in fish fed GL6 diets was significantly higher than other groups ($P < 0.05$). The results of the present study recommend the inclusion of *Gracilaria* meal up to 3%, without significant negative effects on the growth performance, body composition, and health parameters of *L. calcarifer*.

Keywords Barramundi · Dietary macroalgae · Rhodophyta · Fish meal replacement · Digestive enzymes · Blood parameters

Introduction

Currently, aquaculture is the source of half of world fisheries production for human consumption and around 8–9% of the animal protein intake (FAO 2012). It is expected that aquaculture will supply more than 60% of fish destined for direct human consumption by 2030 (FAO 2014). The human population is projected to increase to more than 10,000 million by the end of the century, with most of the increase occurring during the next 40 years. Aquaculture production must supply the entire future increase in demand for fisheries products because the capture fisheries are not expected to increase and may possibly decline (Davis 2015). There is a growing tendency for intensification of production because land and water limited resources for aquaculture. In the most intensive aquaculture operations, feed is one of the highest recurring costs, accounting for over 50% of the production costs (Southgate and Lucas 2012).

In normal diet formulation, the ingredients providing proteins are usually the most expensive; with fish meal likely to be the most expensive of these ingredients. On the other hand, international fish meal price significantly increased in recent years due to increasing demand and limited supply; therefore, any saving on fish meal, though small, may greatly reduce the total cost of aquafeed production and increase returns. Numerous researches have established to seek effective ingredients that can either partially or totally replace fish meal and

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other ingredients as protein sources in aquaculture feed. Terrestrial plant-origin sources that are used to replace fish meal in feed formulations usually contain high amounts of fiber (Opstvedt et al. 2003) and a variety of antinutritional factors or toxicants (Krogdahl et al. 1994; Francis et al. 2001), as well as being generally nutritionally imbalanced in terms of essential amino acids (Floreto et al. 2000). Thus, excessive amounts of these ingredients in feed formulations can decrease nutrient digestibility, overall growth, and feed efficiency in many species (Refstie et al. 1998; Chou et al. 2004; Tantikitti et al. 2005; Deng et al. 2006; Sotoudeh et al. 2016b).

There is a growing interest to use the seaweeds in aquaculture feed because of their high nutritional content. The inclusion of seaweeds into aquaculture feed is not surprising as they contain essential fatty acid, pigments, antioxidants, and some polysaccharide components (mineral binding ability and important role as a colorants and preservatives); thereby providing better balanced nutrition and improved overall animal growth (Rajapakse and Kim 2011). The red seaweed, *Gracilaria*, is one of the algal genera that mainly serve as a raw material from which agar or carrageenan is extracted for use in food or for laboratory uses (Oliveira et al. 2009). *Gracilaria* also contains relatively high levels of essential amino acids, polyunsaturated fatty acids, and minerals (Tabarsa et al. 2012). Despite the high nutritional value of this seaweed, data on the potential use of *Gracilaria* in fish diets are scarce (Hashim and Saat 1992; Valente et al. 2006; Xuan et al. 2013; Vizcaíno et al. 2015; Peixoto et al. 2016). Many studies have explored the potential of seaweed as natural feed additives for fish feed formulation (Valente et al. 2006; Xu et al. 2011; Pereira et al. 2012; Stadtlander et al. 2013; Kumar et al. 2015; Ragaza et al. 2015; Shapawi et al. 2015; Sotoudeh and Jafari 2017). Seaweeds and their extracted compounds increased triglyceride and protein deposition in red sea bream (*Pagrus major*) muscle (Mustafa et al. 1995), better diet utilization and survival rate in striped mullet (*Mugil cephalus* L.) (Wassef et al. 2001), enhanced immunity and disease resistance against the pathogen in grouper (*Epinephelus fuscoguttatus*) (Cheng et al. 2008), and enhanced natural pigmentation in rainbow trout and Nile tilapia (*Oreochromis niloticus*) (Araújo et al. 2016; Valente et al. 2016).

Utilization of seaweed in aquaculture diets not only reduces the feed cost (Banerjee et al. 2010) by improving fish feed efficiency, but also affects water quality by improving diet texture. The inclusion of *Ulva fasciata*, *Spyridia insignis*, and *Sargassum wightii* seaweed in rohu fish (*Labeo rohita*) diet increased food assimilation efficiency and nutrient digestibility (Van Alstyne et al. 2001). The supplementation of different levels (3, 6, and 9%) of *Eucheuma denticulatum*, a red seaweed, resulted in improved growth performance and feed utilization efficiency in juvenile Japanese flounder, *Paralichthys olivaceus* (Ragaza et al. 2015). Valente et al. (2006) identified that seaweeds such as *Gracilaria bursa-pastoris*, *Gracilaria cornea*, and *Ulva rigida* have great potential as alternative ingredients in diets for European sea bass (*Dicentrarchus labrax*) juveniles at dietary inclusion levels up to 10% for *G. bursa-pastoris* and *U. rigida* and up to 5% for *G. cornea*.

Barramundi (also known as Asian sea bass) sea bass is an economically important marine carnivorous fish in the Persian Gulf, throughout Southeast Asia, India, northern Australia, Papua New Guinea, and the western Pacific (Jerry 2013). This fish is considered to have a high degree of farming potential because of its excellent meat quality, its adaptive capacity, and its capability to adapt to varying salinity (Boonyaratpalin et al. 1998; Singh 2000). In this study, we partly replaced fish meal with red seaweed, *Gracilaria pulvinata*, meal to evaluate this alga as a fish meal substitute and to assess its effect on growth and feeding performance, carcass and blood biochemical composition, and digestive enzyme activity of Asian sea bass.

Materials and methods

Fish and experimental design

The Asian sea bass (*Lates calcarifer*) were purchased from Ramoz Company (Bushehr, Iran). The fish were transported to the laboratory of the Aquatic Research, Persian Gulf University. They were acclimated to laboratory conditions for 2 weeks in two 4000-L tanks and fed on the commercial diet (Biza, Iran) containing 47% crude protein, 17% crude fat, 2% crude fiber, and 14% ash. At the end of the acclimation period, fish with an average weight of approximately 28.45 ± 0.52 g were randomly selected and stocked in fifteen 250-L tanks (triplicate groups per dietary treatment) at a density of ten fish per tank. Fish were fed by hand to apparent satiation (visual observation of first feed refusal) two times per day (at *h* 10 and 17) for 40 days (Azodi et al. 2016). Salinity was monitored at about 48 ppt, pH 8 and 70–80% saturation dissolved oxygen was maintained using electrical blowers and air stones. The photoperiod was left under natural conditions during the feeding trial. During this period, water in the holding tanks was changed daily (approximately 60–80%). All seawater used during the rearing process was collected from the Persian Gulf and was filtered and held in a 4000-L aerated tank.

Test diets

The red seaweed, *Gracilaria pulvinata*, used in the present study was identified based on previous studies and collected from the Persian Gulf coast in the south of Iran, on April 2015 (Børgesen 1939). It was thoroughly washed with sea water, dried at 60 °C for 48 h and fine-milled with a laboratory blender to produce raw *Gracilaria* meal. The basal diet was formulated to contain 46% crude protein and 17% crude lipid (Table 1). This diet satisfied crude protein and crude lipid requirements of Asian sea bass (NRC 2011). Fish meal (FM) with approximately 61.8% crude protein, soybean meal, gluten meal and wheat meal were used as protein sources. A positive control test diet was designated, which contained fish meal, gluten meal and wheat meal as primary protein source. A negative control (basal diet) was designated, which contained soybean meal, fish meal, gluten meal, and wheat meal. The other three test diets were formulated by adding increasing levels of *G. pulvinata* to the basal diets. Fish meal was replaced at a level of 3% (GL3), 6% (GL6) and 9% (GL9) by dried *G. pulvinata*. The dietary ingredients were first ground to a uniform particle size (<1 mm), then all the ingredients were thoroughly mixed with fish oil and soybean oil, and water was added to produce a stiff dough. The dough was then extruded by a pellet feed maker through a 3-mm-diameter die. The moist pellets were dried in a forced air oven at 60 °C for about 12 h and then stored at −20 °C until used (Nafisi and Soltani 2008; Sotoudeh et al. 2016a). All diets were calculated to be approximately iso-nitrogenous and iso-energetic. Formulation and chemical composition of the experimental diets is displayed in Table 1.

Table 1 Ingredients and chemical composition of the experimental diets (g (100 g)$^{-1}$)

Ingredient	Diets				
	PC	NC	GL3	GL6	GL9
Fish meal[1]	54.00	44.00	41.00	38.00	35.00
Soybean meal	0.00	14.30	15.00	16.35	19.30
Wheat gluten	11.90	11.90	12.00	12.00	12.00
Wheat meal	10.00	5.35	4.20	2.50	0.00
Fish oil[2]	6.25	6.70	6.80	6.80	6.80
DGP[3]	0.00	0.00	3.00	6.00	9.00
Soybean oil[4]	6.25	6.70	6.80	6.80	6.80
Vitamin premix[5]	1.50	1.50	1.50	1.50	1.50
Mineral premix[6]	1.50	1.50	1.50	1.50	1.50
Squid meal	1.50	1.50	1.50	1.50	1.50
Antioxidant	0.20	0.20	0.20	0.20	0.20
Gelatin	5.00	5.00	5.00	5.00	5.00
Filler	1.5	1.5	1.5	1.5	1.5
Proximate analysis (% dry diet)					
Crude protein	47.98	47.60	46.64	45.44	45.50
Crude fat	17.76	17.94	17.90	17.67	17.45
Ash	15.90	14.60	19.00	21.00	19.00
Moisture	10.19	9.66	9.66	9.96	9.53
Fiber	0.83	1.43	1.46	1.51	1.63
NFE[5]	17.53	18.43	15.0	14.38	16.87

[1] Pars kilka (Mazandaran, Iran)

[2] Havorash (Bushehr, Iran)

[3] Dried *Gracilaria pygmaea*—moisture, 8.1; protein, 16.68; lipid, 1; fiber, 1.2; ash, 29.5 (% dry matter)

[4] Product of Kesht Va Sanat Shomal Vegetable oil Factories Complex (Neca, Iran)

[5] Vitamin and mineral premix (supplied by Beyza Feed Mill, Fars, Iran) and covered known requirements for Asian sea bass

[6] Nitrogen-free extracts (NFE) = 100 - (crude protein + crude lipid +fiber + ash)

Growth measurement

At the end of the growth trial, the fish in each of the 15 tanks were individually weighed and growth performance was evaluated by condition factor (CF), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival as follows (Morshedi et al. 2013):

$$\mathrm{CF} = 100 \times \left[\text{wet } weight(\mathrm{g}) \middle/ total\ length(\mathrm{cm})^3\right]$$

$$\mathrm{SGR}\left(\%\mathrm{day}^{-1}\right) = 100 \times \left[\mathrm{Ln}(W_{\mathrm{f}}) - \mathrm{Ln}(W_{\mathrm{i}})\right] \big/ t$$

$$\mathrm{WG}\ (\%) = 100\ \left[(W_{\mathrm{f}} - W_{\mathrm{i}}) \middle/ W_{\mathrm{i}}\right]$$

where W_i is the mean initial body weight, W_f the final body weight, and t = time (days).

$$\mathrm{FCR} = \text{dry } feed\ intake(\mathrm{g}) \big/ \text{wet } weight\ gain(\mathrm{g})$$

$$\mathrm{PER}\ (\%) = \text{wet } weight\ gain(\mathrm{g}) \big/ protein\ intake(\mathrm{g})$$

$$Survival = 100 \times final\ fish\ number \big/ initial\ fish\ number$$

Chemical analysis

After 6 weeks, all the fish were fasted for 24 h. Three fish from each replicate tank were randomly collected and taken for whole-body proximate analysis. The analyses of proximate composition of feed ingredients, experimental diets, and fish were performed using the standard methods of AOAC (1995). Briefly, dry matter was measured gravimetrically after oven drying of homogenized samples for 24 h at 105 °C. Crude protein ($N \times 6.25$) was determined by the Kjeldahl procedure using an automatic Kjeldahl system. Crude lipid was determined by ether extraction using Soxhlet and ash content was determined after incineration in a muffle furnace at 550 °C for 6 h.

Blood sampling and serum analysis

At the end of feeding trial, three fish per tank (nine per treatment) were randomly sampled and anesthetized (2-phenoxyethanol at 0.5 mL L^{-1}) and individually weighed. The blood samples for serum biochemical assays were drawn from the caudal vein of the individual fish. The whole blood was collected in a syringe, allowed to clot in microtubes at room temperature, stored in a refrigerator (4 h at 4 °C), and then serum was harvested by centrifuging at 3000×*g* for 10 min at 4 °C. All serum samples were preserved at −20 °C prior to analysis.

Serum lysozyme activity was determined by a turbidimetric assay according to the method described by Ellis (1990) based on the lysis of the lysozyme sensitive Gram positive bacterium *Micrococcus lysodeikticus* (Sigma, USA). Hen egg white (in 0.1 M phosphate citrate buffer, pH = 5.8) was used for the preparation of the standard curve. The optical density was measured after 15 and 180 s, using a spectrophotometer (Hitachi 220A, Japan) at 670 nm. The results of lysozyme activity are given as units per milliliter. The alternative complement pathway hemolytic activity (ACH50) was estimated following the procedure of Tort et al. (1996) and the volume yielding 50% hemolysis was determined and used for calculating the complement activity of the sample.

The concentration of serum total immunoglobulin was measured as described in Siwicki and Anderson (1993). Serum biochemical parameters were analyzed using commercial clinical investigation kits. Serum total protein was determined according to the Biuret method, using a diagnostic kit (ZiestChem, Iran). The albumin content was estimated by bromocresol green binding method (Dumas et al. 1997). The absorbance of standard and test were measured against blank in a spectrophotometer (Hitachi 220A, Japan) at 630 nm, using a diagnostic kit (ZiestChem, Iran). Serum triglycerides, albumin, cholesterol, and glucose were analyzed using an auto analyzer, with commercial clinical investigation kits (Pars Azmoon, Iran).

Digestive enzyme assays

At the end of the experiment (after 24 h starvation), the fish were collected for digestive enzyme analyses. Samples of the fish intestines (three per replicate) were homogenized immediately in 100 mM Tris–HCl buffer with 0.1 mM EDTA and 0.1% Triton X-100, pH 7.8, followed by centrifugation (30,000×*g*; 12 min at 4 °C). After centrifugation, the supernatant was collected and frozen at −80 °C (Furné et al. 2008).

Total protease, lipase, and amylase were assayed according to the methods described below. The specific activity of lipase was performed by the enzymatic photometric method using lipase kit (Bionik, Canada). It was based on 1,2-o-dilauryl-rac-3glutaric acid (6-methyresorufin) ester as a substrate that was broken down into 6-methyresorufin and glutaric acid 6-ethylresorufin-ester by lipase. Specific activity of amylase was measured using the enzymatic photometric method using amylase kit (Bionik, Canada). It was based on 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-alpha-D-maltoheptaoside (EPS-G7) as a substrate. Total protease activity quantification followed according to the method published by Anson (1938). In this method, casein was used as substrate. The reaction mixture containing 1 mL of 1.5% casein solution, pH 7.0, was placed at 37 °C and then 1 mL of supernatant sample was added. The reaction was incubated for 10 min before the addition of 2 mL of 0.4 M trichloroacetic acid. The solution was filtered and 2.5 mL of 0.4 M Na_2CO_3 and 0.5 mL of Folin reagent were added. Finally, the absorbance was measured at 660 nm. Total soluble protein was measured by the Bradford (1976) method using bovine serum albumin as a standard. Enzyme activity is expressed as specific activity per milligram protein.

Statistical method

All data were analyzed using SPSS 16.0 (SPSS Inc., USA). Normality and homogeneity of variances were tested initially using the Kolmogorov–Smirnov and Levene tests, respectively. Differences between the dietary groups were tested using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data are presented as means ± standard error (n = 3) and differences were considered to be significant at $P < 0.05$.

Results

Growth performance

Growth performance and feed utilization were assessed using Asian sea bass final body weight (FBW), specific growth rate (SGR), weight gain (WG), feed conversion ratio (FCR), condition factor (CF), and protein efficiency ratio (PER) (Table 2). FBW, SGR, WG, FCR, CF, and PER were not significantly affected by the fish meal replacement level ($P > 0.05$). Although not significant, GL3 fed fish exhibited higher FBW and SGR when compared with those of fish fed PC and NC. Increasing supplementation level (GL6 and GL9) resulted in depression of growth of fish compared with that of fish fed PC and NC. PER of fish fed GL3 was also improved ($P > 0.05$) when compared with those of fish fed NC. While, fish fed GL6 and GL9 showed a reduced PER when compared with those of fish fed GL3, NC, and PC. Daily dry-feed intake and total feed intake were not significantly different among dietary treatments ($P > 0.05$). There was no mortality recorded in the duration of the 40-day feeding period.

Body composition

Proximate compositions of fish fed the various diets are presented in Table 3. No statistical difference was observed over the 40 days of the experimental period in chemical composition (crude protein, lipid, ash, and moisture) ($P > 0.05$) in fish fed with different levels of *G. pulvinata*.

Table 2 Growth performance and feed intake of Asian sea bass fed diets with different levels of *G. pulvinata* for 40 days

Parameters[1]	Dietary treatments					SEM	*P* value
	PC	NC	GL3	GL6	GL9		
Initial weight (g)	28.46	28.00	29.20	28.16	28.43	0.56	0.409
FBW (g)	83.36	83.44	85.07	74.53	79.36	6.89	0.574
WG (%)	192.69	195.76	191.06	164.86	179.37	23.15	0.678
CF	0.65	0.73	0.65	0.62	0.59	0.08	0.509
SGR (% day^{-1})	2.68	2.70	2.64	2.42	2.56	0.20	0.668
FCR	1.08	1.07	0.93	1.23	1.14	0.09	0.101
PER	2.04	2.08	2.39	1.82	1.93	0.17	0.079
Daily feed intake (g)	16.78	16.28	16.05	16.81	15.95	1.10	0.894
Survival (%)	100	100	100	100	100	–	–

S.E.M. standard error of the means

[1] CF = 100 × [wet weight (g)/total length (cm)^3], SGR = 100 × [Ln (Mean final body weight) − Ln (Mean initial body weight)]/time (days), FCR = dry feed intake (g)/wet weight gain (g), PER = wet weight gain (g)/ protein intake (g), daily feeding intake (g) = g feed/day, Survival = 100 × final fish number/initial fish number

However, whole-body protein contents of fish fed 3% *Gracilaria* diets were higher than that of fish fed other diets ($P > 0.05$).

Hemato-immunological parameters

The hemato-immunological parameters of Asian sea bass fed the experimental diets are displayed in Table 4. Serum lysozyme activity was significantly higher ($P < 0.05$) in Asian sea bass fed the GL3 an NC diets compared to serum lysozyme activity in fish fed the PC, GL6, and GL9 treatments. Serum alternative complement (ACH50) activity was significantly lower in fish fed the GL9 diet, followed by fish fed diets with 3, 6% GL and NC, and highest in fish fed diets with PC diet. Increasing *G. pulvinata* level (GL3% and GL9%) generally decreased the immunoglobulin content, which were significantly lower in fish fed the GL9 diets compared to fish fed the NC and PC diets ($P < 0.05$).

Serum albumin and glucose contents among all the treatments did not change significantly ($P > 0.05$). Serum total protein, triglycerides, and cholesterol were significantly affected by the level of dietary ($P < 0.05$). Increasing dietary supplementation generally decreased the serum triglycerides and cholesterol, which were significantly higher in fish fed the GL3 diets compared to fish fed the GL6 and GL9 diets.

Table 3 Proximate analysis of whole body (percent of wet weight) of Asian sea bass fed diets with different levels of *G. pulvinata* for 40 days

Parameters	Dietary treatments					SEM	*P* value
	PC	NC	GL3	GL6	GL9		
Crude protein (%)	17.41	18.08	18.21	17.29	17.59	0.85	0.767
Crude lipid (%)	6.63	7.12	6.44	6.66	7.34	0.38	0.177
Ash (%)	4.53	4.88	4.70	4.15	4.07	0.39	0.267
Moisture (%)	68.90	68.06	69.79	69.66	68.96	0.73	0.205

S.E.M. standard error of the means

Digestive enzyme activity

The activities of the digestive enzymes total protease, amylase, and lipase of Asian sea bass fed diets with different levels of *G. pulvinata* are presented in Table 5. Digestive enzymes activities were significantly different among the dietary treatments ($P < 0.05$); however, *G. pulvinata* inclusion level did not coincide with the activities of total protease and amylase. Fish fed PC diet had the significantly higher total protease and amylase activity among all treatments ($P < 0.05$). The lipase activity in fish fed GL6 diets was significantly higher than that in fish fed other diets ($P < 0.05$).

Discussion

A number of studies have been conducted on dietary macroalgal meal in several fish species which have confirmed that macroalgae had no adverse effect on growth performance, feed utilization or apparent nutrient digestibility and carcass quality (Güroy et al. 2013; Pereira et al. 2012; Walker et al. 2009; Wassef et al. 2001). For this reason, there has been an increased interest in the substitution of fish meal by macroalgae in cultured fish nutrition. Several publications have addressed the effects of *Gracilaria* and other macroalgae on the health and performance of fish. The results of this study indicate that replacing 9% fish meal with *G. pulvinata* did not have negative effects on growth performance (in terms of SGR and CF) or feed utilization (in terms of FCR and PER) in Asian sea bass. These results indicate good palatability and

Table 4 Hemato-immunological and biochemical parameters of Asian sea bass fed diets with different levels of *G. pulvinata* for 40 days

Parameters	Dietary treatments					SEM	*P* value
	PC	NC	GL3	GL6	GL9		
Total protein (g dL^{-1})	3.40^{c}	4.03^{a}	3.76^{abc}	3.80^{ab}	3.43^{bc}	0.12	0.002
Lysozyme (U mL^{-1})	30.00^{b}	33.66^{a}	34.00^{a}	25.66^{c}	24.33^{c}	1.09	0.000
ACH50 (U mL^{-1})	150.33^{a}	135.00^{bc}	139.33^{b}	126.67^{cd}	123.67^{d}	3.31	0.000
Immunoglobulin (g dL^{-1})	19.50^{a}	20.00^{a}	17.60^{ab}	17.46^{ab}	15.66^{b}	0.78	0.002
Glucose (mg dL^{-1})	64.33	69.66	70.33	80.00	64.00	6.36	0.160
Triglycerides (mg dL^{-1})	68.33^{b}	99.33^{a}	87.00^{c}	85.33^{c}	84.00^{c}	3.61	0.000
Cholesterol (mg dL^{-1})	287.00^{bc}	327.67^{a}	317.00^{ab}	303.33^{abc}	275.33^{c}	9.44	0.001
Albumin (g dL^{-1})	1.90	2.03	2.03	2.00	1.83	0.06	0.054

Values in the same row not sharing a common superscript are significantly different ($P < 0.05$)

S.E.M. standard error of the means

nutritional effects of diets containing *G. pulvinata* for a carnivorous fish like Asian sea bass. Previous works with European sea bass (*Dicentrarchus labrax*) juveniles showed that the incorporation of *G. bursa-pastoris* up to 10% and up to 5% inclusion level for *G. cornea* had no negative effect on growth performance and feed utilization efficiency, and increasing incorporation of both *G. bursa-pastoris* and *U. rigida*, from 5 to 10%, did not affect growth performance of this fish (Valente et al. 2006). The authors concluded that these two macroalgae have great potential as alternative ingredients for European sea bass. Xuan et al. (2013) investigated the feasibility of *G. lemaneiformis* (5, 10, 15, and 20%) as a feed ingredient for juvenile black sea bream (*Acanthopagrus schlegelii*). Their results showed that growth performance in terms of WG and FER of the juvenile black sea bream receiving the *G. lemaneiformis* based diets did not decrease even at the inclusion level of 15%. Moreover, El-Sayed (1994) reported that *Spirulina* could successfully replace up to 75% of the fish meal for silver sea bream (*Rhabdosargus sarba*) fingerlings without any adverse effects on growth performance and feed efficiency. Positive effects of dietary macroalgae on growth performance and feed utilization of fish may be related to their vitamin and trace elements content, lipid mobilization, and improved absorption and assimilation efficiency (Dy Peñaflorida and Golez 1996). In addition, it has been postulated that growth improvement effects of seaweed could be associated with activation of lipid metabolism such as accumulation and mobilization (Nakagawa 1997) and assimilation of dietary protein (Yone et al. 1986).

In this study, body composition parameters were not affected by the dietary treatments. There are contradictory data in the literature on the effect of macroalgae on chemical composition of fish. For example, inclusion of dried *Gracilaria lemaneiformis* at a level up to 33% did not modify the chemical composition of *Siganus canaliculatus* (Xu et al. 2011). Peixoto et al. (2016) described that dietary addition of *Gracilaria* spp., *Ulva* spp., or *Fucus* spp. at 2.5 or 7.5% levels, plus an additional diet with a blend of the three seaweeds, did not affect the whole-body composition of European seabass juveniles. A similar effect also reported by Valente et al. (2006) in *D. labrax* juvenile fed macroalgae *G. bursa-pastoris*, *U. rigida*, and *G. cornea*. In contrast, body lipid content in *Sparus aurata* juveniles fed different levels of macroalgae were negatively correlated with *G. cornea* and *U. rigida* levels (Vizcaíno et al. 2015). Similarly, carcass lipid deposition in *O. niloticus* was significantly affected by the percentage of *U. rigida* in the diet, and there was an overall trend of decreasing carcass lipid content with increasing inclusion levels of *U. rigida* (Azaza et al. 2008). Based on the available data and the foregoing discussion, it may therefore be concluded that the impact of dietary macroalgae on chemical composition of fish seems to depend on the algae species, its inclusion level, and also on the species of fish where the macroalga is tested.

Table 5 Specific activity of total protease, α-amylase and lipase in Asian sea bass fed diets with different levels of *G. pulvinata* for 40 days

Parameters	Dietary treatments					SEM	*P* value
	PC	NC	GL3	GL6	GL9		
Total protease (U mg protein^{-1})	2.06^{a}	0.91^{c}	1.20^{bc}	1.73^{ab}	1.40^{bc}	0.19	0.001
Lipase (U mg protein^{-1})	7.33^{b}	5.66^{b}	7.33^{b}	9.66^{a}	6.33^{b}	0.69	0.002
Amylase (U mg protein^{-1})	38.66^{a}	23.66^{c}	26.33^{bc}	28.33^{bc}	29.00^{b}	1.60	0.000

Values in the same row not sharing a common superscript are significantly different ($P < 0.05$)

S.E.M. standard error of the means

Lysozyme is an important key component of fish innate immune system, which is important in mediating protection against microbial invasion and in most cases, is positively correlated with disease resistance (Fevolden et al. 1994; Saurabh and Sahoo 2008). ACH50 activity is also commonly used as suitable indicators of the humoral nonspecific immune response in fish (Montero et al. 1998; Obach et al. 1993; Tort et al. 1996) and strong action against Gram-negative bacteria (Yano 1996). Several factors such as nutrition, feed additive, stress, and temperature can affect it (Boshra et al. 2006; Montero et al. 1998). In the present work, the dietary inclusion of *G. pulvinata* in diets for Asian sea bass had significant effect on hemato-immunological parameters. Seaweed polysaccharides such as carrageenan, alginates, β-glucans, and sodium alginate have been demonstrated to show great stimulatory effects on immunity and the protection against infectious diseases in fish (Castro et al. 2004; Fujiki et al. 1994; Gabrielsen and Austreng 1998). Agar is the main polysaccharide in *Gracilaria* spp. with similar structural and functional proprieties to carrageenan (Araújo et al. 2016). Previous studies show low molecular weight agar enhanced the nonspecific immune resistance of basa (*Pangasius bocourti*) against *Aeromonas hydrophila* (Van Doan et al. 2014). The exact mechanism of immune-enhancing effects is unclear but it appears that some low molecular weight polysaccharides derived from agar-bearing seaweeds were fermented by gut bacteria and exhibited potential to be used a source of prebiotics (Ramnani et al. 2012). In our experiment, fish fed the GL6 and GL9 diets showed a reduced lysozyme, alternative complement (ACH50) activity, and total immunoglobulin when compared to the GL3 and the PC diet, suggesting a dose-dependent response in these immune parameters. These results are similar to Peixoto et al. (2016) who reported that European seabass fed 7.5% *Ulva* spp. supplemented diets had a significant decrease in the lysozyme activity level, when compared to fish fed control or 2.5% *Ulva*-supplemented diets. However, the same authors found that fish fed supplemented either with *Gracilaria* spp. or *Fucus* spp. had lysozyme results similar to control diet. Valente et al. (2016) also reported that adding different levels of *Ulva* spp. meal to the diets of Nile tilapia *Oreochromis niloticus* had no beneficial effect on lysozyme or peroxidase activities. Recent results observed in tilapia (*O. mossambicus*) have demonstrated that ethanol and aqueous extracts of *Padina gymnospora* were found to be effective against gram-negative fish pathogen *Pseudomonas aeruginosa* (Thanigaivel et al. 2015). Since both lysozyme (Saurabh and Sahoo 2008) and immunoglobulin are potential activators of the complement system, it was possible to conclude that a general decrease observed in serum complement in fish fed diets supplemented with *G. pulvinata* may be a result of decreased total immunoglobulin and lysozyme activity. Several factors such as species and size of fish, differences in diet formulation, and environmental factors may account for the discrepancies that observed among research results.

Blood biochemical parameters are important tools for the indication of the physiological response as well as the general health status of fish. Serum glucose level is considered as an effective indicator of stress (Barton and Iwama 1991). In the present study, serum glucose concentration was not significantly affected by dietary *G. pulvinata* inclusion. This observation is similar to previous findings on serum glucose of juvenile Japanese flounder fed red seaweed, *Eucheuma denticulatum* (Ragaza et al. 2015). Seaweeds, however, have been shown to induce a decrease in serum glucose in *S. aurata* juveniles (Vizcaíno et al. 2015). These differences may be attributed to differences in capacity of fish to use carbohydrates or differences in dietary fiber composition of macroalgae. Inclusion of *G. pulvinata* in the diets lowered serum triglyceride and cholesterol levels in the fish. This is in agreement with data from earlier studies in juvenile Japanese flounder fed diets supplemented with *Chlorella* (Kim et al. 2002) and *E. denticulatum* (Ragaza et al. 2015). Moreover, *Porphyridium* spp. reduced serum triglyceride and cholesterol levels in rat (Dvir et al. 2009). The mechanism for lowering serum triglyceride and cholesterol by *G. pulvinata* may be explained by increased dietary fiber which is naturally present in seaweeds. Dietary soluble fibers, such as pectin, are known to have hypocholesterolemic effects (Castro et al. 2005). It has been reported that dietary soluble fibers hinder digestion and absorption of dietary fats, resulting in lower cholesterol delivery to the liver by cyclo micron remnants (Matanjun et al. 2010). Other compound that may contribute to the cholesterol and triglyceride-lowering effect of seaweeds are dietary n-3 fatty acids (Skulas-Ray et al. 2008), which are found in high amount in red seaweeds (Matanjun et al. 2009).

Serum proteins such as albumin are absolutely essential for maintaining a healthy immune system (Kumar et al. 2005). Evidence shows that increase in the serum proteins associated with stronger nonspecific immune response in fish (Wiegertjes et al. 1996). In our study, there were no significant differences in the albumin content among the different experimental groups.

Digestive enzyme activities were used as an indicator of nutrient digestibility and utilization state in order to allow for appropriate diet formulation in several fish species (Peixoto et al. 2016; Vizcaíno et al. 2015; Zambonino Infante and Cahu 2007). In this study, *G. pulvinata* inclusion level did not affect total protease and amylase activity. A similar effect has also been reported in the level of total alkaline protease activity in intestinal extracts of juvenile *S. aurata* fed on diets supplemented with *G. cornea* or *U. rigida* (Vizcaíno et al. 2015). Moreover, seaweed supplementation (*Gracilaria* spp., *Ulva* spp. and *Fucus* spp.) in practical diets for European seabass juveniles have no significant impact on digestive enzyme activities (Peixoto et al. 2016). The higher amylase and total proteolytic activity in fish fed the PC compared to the fish fed the NC diet might be due to the presence of antinutritional factors in soybean such as protease inhibitors or soybean lectins. A study by Krogdahl et al. (2003) indicates that exocrine pancreatic production and/or secretion in Atlantic salmon (*Salmo salar* L.) is stimulated by dietary soybean meal. Dietary soybeans have also been reported to induce pancreatic growth and hypersecretion of pancreatic enzymes in rats (Grant et al. 2000). This eventual antinutritional effect associated with soybean is not so severe that causes growth impairment and is apparently compensated by the presence of *G. pulvinata* in *Gracilaria*-supplemented diets. In present study protease and amylase activities were significantly lower in fish fed GL9 diet when compared to PC. The observed negative effect of *G. pulvinata* over protease and amylase activities could be attributed to fiber present in seaweeds (Oliveira et al. 2009). In fact, dietary high content of fiber may result in more rapid passage of food through fish digestive tract (Blender 1967) and reducing the time available for digestion, possibly adversely affecting digestive enzyme activity. In addition, seaweeds contain several antinutrients such as lectins and proteinase inhibitors (Dvir et al. 2009) that interfere with digestion and feed utilization processes (Francis et al. 2001). The lipase activity in Asian sea bass fed GL6 diets was significantly higher than those fed the other diets, but there were no clear trend with *G. pulvinata* inclusion level.

In conclusion, all diets were readily accepted by fish, indicating that *G. pulvinata* based diets did not cause any change in the palatability to the experimental fish. The present study also evidences that dietary inclusion of this macroalga had no negative effect on growth performance and feed utilization of *L. calcarifer*. Indeed, the inclusion of *G. pulvinata* had significant effect on hemato-immunological parameters but it did not affect the carcass composition of this fish. Based on the results of growth performance, body composition and health parameters, diet containing 3% GL showed better effect in Asian sea bass compared to other treatments. The results of the present investigation suggest that further investigations are required to evaluate the optimum dietary inclusion level of *G. pulvinata* in Asian sea bass diets.

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References

Anson ML (1938) The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. J Gen Physiol 22:79–89

AOAC (1995) Official methods of analysis of Official Analytical Chemists International, 16th edn. Association of Official Analytical Chemists, Arlington

Araújo M, Rema P, Sousa-Pinto I, Cunha LM, Peixoto MJ, Pires MA, Seixas F, Brotas V, Beltrán C, Valente LMP (2016) Dietary inclusion of IMTA-cultivated *Gracilaria vermiculophylla* in rainbow trout (*Oncorhynchus mykiss*) diets: effects on growth, intestinal morphology, tissue pigmentation, and immunological response. J Appl Phycol 28:679–689

Azaza MS, Mensi F, Ksouri J, Dhraief MN, Brini B, Abdelmouleh A, Kraïem MM (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. J Appl Ichthyol 24:202–207

Azodi M, Nafisi M, Morshedi V, Modarresi M, Faghih-Ahmadani A (2016) Effects of intermittent feeding on compensatory growth, feed intake and body composition in Asian sea bass (*Lates calcarifer*). Iran J Fish Sci 15(1):144–156

Banerjee K, Mitra A, Mondal K (2010) Cost-effective and eco-friendly shrimp feed from red seaweed *Catenella repens* (Gigartinales: Rhodophyta). Curr Biot 4:23–43

Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 1:3–26

Børgesen F (1939) Marine algae from the Iranian Gulf especially from the innermost part near Bushehr and Khark. In: Jessen K, Spärck R (eds) Danish scientific investigations in Iran. Part I. Munksgaard, Copenhagen, pp 47–141

Blender AE (1967) Dietetic foods. Leonard Hill Books, London, p 211

Boonyaratpalin M, Suraneiranat P, Tunpibal T (1998) Replacement of fishmeal with various type of soybean products in diets for Asian seabass, *Lates calcarifer*. Aquaculture 161:67–78

Boshra H, Li J, Sunyer JO (2006) Recent advances on the complement system of teleost fish. Fish Shellfish Immunol 20:239–262

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Castro IA, Barroso LP, Sinnecker P (2005) Functional foods for coronary heart disease risk reduction: a meta-analysis using a multivariate approach. Am J Clin Nutr 82:32–40

Castro R, Zarra I, Lamas J (2004) Water-soluble seaweed extracts modulate the respiratory burst activity of turbot phagocytes. Aquaculture 229:67–78

Cheng AC, Chen YY, Chen JC (2008) Dietary administration of sodium alginate and k-carrageenan enhances the innate immune response of brown-marbled grouper *Epinephelus fuscoguttatus* and its resistance against *Vibrio alginolyticus*. Vet Immunol Immunopathol 121:206–215

Chou RL, Her BY, Su MS, Hwang G, Wu YH, Chen HY (2004) Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. Aquaculture 229:325–333

Davis DA (2015) Feed and feeding practices in aquaculture. Woodhead Publishing, Cambridge

Deng J, Mai K, Ai Q, Zhang W, Wang X, Xu W, Liufu Z (2006) Effects of replacing fish meal with soy protein concentrate on feed intake and growth of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture 258:503–513

Dumas BT, Watson WA, Biggs HG (1997) Albumin standards and the measurement of serum albumin with bromcresol green. 1971. Clin Chim Acta 258:21–30

Dvir I, Stark AH, Chayoth R, Madar Z, Arad SM (2009) Hypocholesterolemic effects of nutraceuticals produced from the red microalga *Porphyridium* sp. in rats. Nutrients 1:156–167

Dy Peñaflorida V, Golez NV (1996) Use of seaweed meals from *Kappaphycus alvarezii* and *Gracilaria heteroclada* as binders in diets for juvenile shrimp *Penaeus monodon*. Aquaculture 143: 393–401

Ellis A (1990) Lysozyme assays. In: Stolen JS, Fletcher TC, Anderson DP, Roberson BS, van Muiswinkel WB (eds) Techniques in fish immunology. SOS Publications, New Jersey, pp 101–103

El-Sayed A-FM (1994) Evaluation of soybean meal, spirulina meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. Aquaculture 127:169–176

FAO (2014) The State of World Fisheries and Aquaculture 2014 (SOFIA). Rome

FAO (2012) The State of World Fisheries and Aquaculture. Rome

Fevolden SE, Røed KH, Gjerde B (1994) Genetic components of post-stress cortisol and lysozyme activity in Atlantic salmon; correlations to disease resistance. Fish Shellfish Immunol 4:507–519

Floreto EAT, Bayer RC, Brown PB (2000) The effects of soybean-based diets, with and without amino acid supplementation, on growth and biochemical composition of juvenile American lobster, *Homarus americanus*. Aquaculture 189:211–235

Francis G, Makkar HPS, Becker K (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197–227

Fujiki K, Matsuyama H, Yano T (1994) Protective effect of sodium alginates against bacterial infection in common carp, *Cyprinus carpio* L. J Fish Dis 17:349–355

Furné M, García-Gallego M, Hidalgo MC, Morales AE, Domezain A, Domezain J, Sanz A (2008) Effect of starvation and refeeding on digestive enzyme activities in sturgeon (*Acipenser naccarii*) and trout (*Oncorhynchus mykiss*). Comp Biochem Physiol A 149:420–425

Gabrielsen BO, Austreng E (1998) Growth, product quality and immune status of Atlantic salmon, *Salmo salar* L., fed wet feed with alginate. Aquac Res 29:397–401

Grant G, Alonso R, Edwards JE, Murray S (2000) Dietary soya beans and kidney beans stimulate secretion of cholecystokinin and pancreatic digestive enzymes in 400-day-old hooded-*Lister rats* but only soya beans induce growth of the pancreas. Pancreas 20:305–312

Güroy B, Ergün S, Merrifield DL, Güroy D (2013) Effect of autoclaved Ulva meal on growth performance, nutrient utilization and fatty acid profile of rainbow trout, *Oncorhynchus mykiss*. Aquac Int 21:605–615

Hashim R, Saat MAM (1992) The utilization of seaweed meals as binding agents in pelleted feeds for snakehead (*Channa striatus*) fry and their effects on growth. Aquaculture 108:299–308

Jerry DR (2013) Biology and culture of Asian seabass *Lates calcarifer*. CRC Press

Kim KW, Bai SC, Koo JW, Wang X, Kim SK (2002) Effects of dietary *Chlorella ellipsoidea* supplementation on growth, blood characteristics, and whole-body composition in juvenile japanese flounder *Paralichthys olivaceus*. J World Aquac Soc 33:425–431

Krogdahl A, Bakke-McKellep AM, Baeverfjord G (2003) Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.) Aquac Nutr 9:361–371

Krogdahl Å, Lea TB, Olli JJ (1994) Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol A 107:215–219

Kumar S, Sahoo D, Levine I (2015) Assessment of nutritional value in a brown seaweed *Sargassum wightii* and their seasonal variations. Algal Res 9:117–125

Kumar S, Sahu N, Pal A, Choudhury D, Yengkokpam S, Mukherjee S (2005) Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in *L. rohita* juveniles. Fish Shellfish Immunol 19:331–344

Matanjun P, Mohamed S, Muhammad K, Mustapha NM (2010) Comparison of cardiovascular protective effects of tropical seaweeds, *Kappaphycus alvarezii*, *Caulerpa lentillifera*, and *Sargassum polycystum*, on high-cholesterol/high-fat diet in rats. J Med Food 13:792–800

Matanjun P, Mohamed S, Mustapha NM, Muhammad K (2009) Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. J Appl Phycol 21:75–80

Montero D, Tort L, Izquierdo MS, Robaina L, Vergara JM (1998) Depletion of serum alternative complement pathway activity in gilthead seabream caused by α-tocopherol and n-3 HUFA dietary deficiencies. Fish Physiol Biochem 18:399–407

Morshedi V, Kochanian P, Bahmani M, Yazdani-Sadati MA, Pourali HR, Ashouri G, Pasha-Zanoosi H, Azodi M (2013) Compensatory growth in sub-yearling Siberian sturgeon, *Acipenser baerii* Brandt, 1869: effects of starvation and refeeding on growth, feed utilization and body composition. J Appl Ichthyol 29:978–983

Mustafa GM, Wakamatsu S, Takeda T, Umino T, Nakagawa H (1995) Effects of algae meal as feed additive on growth, feed efficiency and body composition in red sea bream. Fish Sci 61:25–28

Nafisi M, Soltani M (2008) Effect of different dietary energy levels and feeding rates on growth and body composition of fingerling rainbow trout, *Oncorhynchus mykiss*. Iran J Fish Sci 7:171–186

Nakagawa H (1997) Effect of dietary algae on improvement of lipid metabolism in fish. Biomed Pharmacother 51:345–348

NRC (2011) Nutrient requirements of fish and shrimp, 1st edn. National Academies Press, Washington, DC

Obach A, Quentel C, Baudin Laurencin F (1993) Effects of alpha-tocopherol and dietary oxidized fish oil on the immune response of sea bass *Dicentrarchus labrax*. Dis Aquat Org 15:175–185

Oliveira MN, Ponte-Freitas AL, Urano-Carvalho AF, Taveres-Sampaio TM, Farias DF, Alves-Teixera DI, Gouveia ST, Gomes-Pereira J, Castro-Catanho de Sena MM (2009) Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceara. Brazil. Food Chem 11:254–259

Opstvedt J, Nygård E, Samuelsen TA, Venturini G, Luzzana U, Mundheim H (2003) Effect on protein digestibility of different processing conditions in the production of fish meal and fish feed. J Sci Food Agric 83:775–782

Peixoto MJ, Salas-Leitón E, Pereira LF, Queiroz A, Magalhães F, Pereira R, Abreu H, Reis PA, Gonçalves JFM, de Ozório ROA (2016) Role of dietary seaweed supplementation on growth performance, digestive capacity and immune and stress responsiveness in European seabass (*Dicentrarchus labrax*). Aquac Reports 3:189–197

Pereira R, Valente LMP, Sousa-Pinto I, Rema P (2012) Apparent nutrient digestibility of seaweeds by rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*). Algal Res 1:77–82

Ragaza JA, Koshio S, Mamauag RE, Ishikawa M, Yokoyama S, Villamor SS (2015) Dietary supplemental effects of red seaweed *Eucheuma denticulatum* on growth performance, carcass composition and blood chemistry of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquac Res 46:647–657

Rajapakse N, Kim SK (2011) Nutritional and digestive health benefits of seaweed. Adv Food Nutr Res 64:17–28

Ramnani P, Chitarrari R, Tuohy K, Grant J, Hotchkiss S, Philp K, Campbell R, Gill C, Rowland I (2012) In vitro fermentation and prebiotic potential of novel low molecular weight polysaccharides derived from agar and alginate seaweeds. Anaerobe 18:1–6

Refstie S, Storebakken T, Roem AJ (1998) Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture 162:301–312

Saurabh S, Sahoo PK (2008) Lysozyme: an important defence molecule of fish innate immune system. Aquac Res 39:223–239

Shapawi R, Safiin NSZ, Senoo S (2015) Improving dietary red seaweed *Kappaphycus alvarezii* (Doty) Doty ex P. Silva meal utilization in Asian seabass *Lates calcarifer*. J Appl Phycol 27:1681–1688

Singh RK (2000) Growth, survival and production of *Lates calcarifer* in a seasonal rain-fed coastal pond of the Konkan region. Aquaculture 8: 55–60

Siwicki AK, Anderson DP (1993) Nonspecific defence mechanisms assay in fish II; potential killing activity of neutrophils and manocytes, lysozyme activity in serum and organs and total immunoglobulin (Ig) level in serum. In: Siwicki AK, Anderson DP, Waluga J (eds) Wydawnictwo Instytutu Rybactwa Strodladowego, Olsztyn, pp 105–111

Skulas-Ray AC, West SG, Davidson MH, Kris-Etherton PM (2008) Omega-3 fatty acid concentrates in the treatment of moderate hypertriglyceridemia. Expert Opin Pharmacother 9:1237–1248

Sotoudeh E, Abedian Kenari A, Khodabandeh S, Khajeh K (2016a) Combination effects of dietary EPA and DHA plus alpha-tocopherol: effects on performance and physiological status of Caspian brown trout (*Salmo trutta caspius*) fry. Aquac Nutr 22:1101–1115

Sotoudeh E, Amiri Moghaddam J, Shahhosseini G, Aramli MS (2016b) Effect of dietary gamma-irradiated and fermented soybean meal on the growth performance, body composition, and digestive enzymes activity of caspian brown trout, *Salmo trutta caspius*, juvenile. J World Aquacult Soc 47:830–842

Sotoudeh E, Jafari M (2017). Effects of dietary supplementation with red seaweed, *Gracilaria pygmaea*, on growth, carcass composition and hematology of juvenile rainbow trout, *Oncorhynchus mykiss*. Aquac Int. doi:10.1007/s10499-017-0158-6

Southgate P, Lucas J (2012) Reproduction, life cycles and growth. In: Lucas JS, Southgate PC (eds) Aquaculture: farming aquatic animals and plants. Wiley-Blackwell Publishing Ltd, Oxford, pp 126–137

Stadtlander T, Khalil WKB, Focken U, 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.) Aquac Nutr 19:64–73

Tabarsa M, Rezaei M, Ramezanpour Z, Waaland JR (2012) Chemical compositions of the marine algae *Gracilaria salicornia* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) as a potential food source. J Sci Food Agric 92:2500–2506

Tantikitti C, Sangpong W, Chiavareesajja S (2005) Effects of defatted soybean protein levels on growth performance and nitrogen and phosphorus excretion in Asian seabass (*Lates calcarifer*). Aquaculture 248:41–50

Thanigaivel S, Chandrasekaran N, Mukherjee A, Thomas J (2015) Investigation of seaweed extracts as a source of treatment against bacterial fish pathogen. Aquaculture 448:82–86

Tort L, Gomez E, Montero D, Sunyer JO (1996) Serum haemolytic and agglutinating activity as indicators of fish immunocompetence: their suitability in stress and dietary studies. Aquac Int 4:31–41

Valente LMP, Araújo M, Batista S, Peixoto MJ, Sousa-Pinto I, Brotas V, Cunha LM, Rema P (2016) Carotenoid deposition, flesh quality and immunological response of Nile tilapia fed increasing levels of IMTA-cultivated *Ulva* spp. J Appl Phycol 28:691–701

Valente LMP, Gouveia A, Rema P, Matos J, Gomes EF, Pinto IS (2006) Evaluation of three seaweeds *Gracilaria bursa-pastoris, Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 252:85–91

Van Alstyne K, Wolfe G, Freidenburg T, Neill A, Hicken C (2001) Activated defense systems in marine macroalgae: evidence for an ecological role for DMSP cleavage. Mar Ecol Prog Ser 213:53–65

Van Doan H, Doolgindachbaporn S, Suksri A (2014) Effects of low molecular weight agar and Lactobacillus plantarum on growth performance, immunity, and disease resistance of basa fish (*Pangasius bocourti*, Sauvage 1880). Fish Shellfish Immunol 41:340–345

Vizcaíno AJ, Mendes SI, Varela JL, Ruiz-Jarabo I, Rico R, Figueroa FL, Abdala R, Moriñigo MÁ, Mancera JM, Alarcón FJ (2015) Growth, tissue metabolites and digestive functionality in *Sparus aurata* juveniles fed different levels of macroalgae, *Gracilaria cornea* and *Ulva rigida*. Aquac Res 47:3224–3238

Walker AB, Fournier HR, Neefus CD, Nardi GC, Berlinsky DL (2009) Partial replacement of fish meal with laver *Porphyra* spp. in diets for Atlantic cod. N Am J Aquac 71:37–41

Wassef EA, Masry MHE, Mikhail FR (2001) Growth enhancement and muscle structure of striped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal-based diets. Aquac Res 32:315–322

Wiegertjes GF, Stet RM, Parmentier HK, van Muiswinkel WB (1996) Immunogenetics of disease resistance in fish: a comparative approach. Dev Comp Immunol 20:365–381

Xu S, Zhang L, Wu Q, Liu X, Wang S, You C, Li Y (2011) Evaluation of dried seaweed *Gracilaria lemaneiformis* as an ingredient in diets for teleost fish *Siganus canaliculatus*. Aquac Int 19:1007–1018

Xuan X, Wen X, Li S, Zhu D, Li Y (2013) Potential use of macro-algae *Gracilaria lemaneiformis* in diets for the black sea bream, *Acanthopagrus schlegelii*, juvenile. Aquaculture 412–413:167–172

Yano T (1996) The non-specific immune system: humoral defense. In: Iwama G, Nakanishi T (eds) The fish immune system: organism, pathogen, and environment. Academic Press, New York, pp 105–157

Yone Y, Furuichi M, Urano K (1986) Effects of wakame Undaria pinnatifida and *Ascophyllum nodosum* on absorption of dietary nutrients, and blood sugar and plasma free amino-N levels of red sea bream. Nippon Suisan Gakkaishi 52:1817–1819

Zambonino Infante JL, Cahu CL (2007) Dietary modulation of some digestive enzymes and metabolic processes in developing marine fish: applications to diet formulation. Aquaculture 268:98–105