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Evaluation of the red alga *Gracilaria lemaneiformis* and brown alga *Sargassum horneri* as ingredients in diets for white spotted snapper *Lutjanus stellatus* Akazaki juveniles

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Abstract This study evaluated the red alga *Gracilaria lemaneiformis* and brown alga *Sargassum horneri* as ingredients to partially replace fishmeal in diets for white spotted snapper *Lutjanus stellatus* Akazaki (initial mass 12.0 ± 0.1 g). Nine test diets containing 0 (control), 5, 10, 15, and 20% *G. lemaneiformis* or *S. horneri* were prepared; each diet was assigned to triplicate groups of fish in a total of 27 floating sea cages (each cage contained 30 fish). After a 60-day feeding trail, significantly lower final body weight, weight gain, and specific growth ratio were found in fish fed 20% *G. lemaneiformis* diet ($P < 0.05$), whereas the poorest fish growth performance was obtained with the 20% *S. horneri* diet. Lipid content in muscle of fish fed 20% *G. lemaneiformis* diet was significantly lower than that of other groups ($P < 0.05$); however, body protein was higher than that in other groups. The lowest lipid and moisture contents in muscle were recorded in fish fed 5 and 15% *S. horneri* diets, and protein content in whole body of fish fed 20% *S. horneri* diet was significantly lower than that of control and 5% diets ($P < 0.05$). Activities of pepsin in the stomach and lipase and amylase in the intestine were significantly suppressed in fish fed 20% *G. lemaneiformis* diet compared with that of control ($P < 0.05$). The lowest pepsin activity in stomach and lipase activity in intestine were observed in fish fed 20% *S. horneri* diet. Based on a quadratic regression model of weight gain, the results suggested that the maximum incorporation of *G. lemaneiformis* and *S. horneri* in diets should be 16.44 and 15%, respectively, for juvenile white spotted snapper.

Keywords *Gracilaria lemaneiformis* · *Sargassum horneri* · *Lutjanus stellatus* · Growth performance · Biochemical composition · Enzyme activities

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Introduction

Fishmeal (FM) is known to be the best protein source in aquafeeds due to its high content of essential amino acids, fatty acids, high digestibility, and low levels of antinutritional factors. The increasing demand for FM accompanied by shortage in global supply has resulted in escalating FM prices during the past few years (Tacon et al. 2012). Therefore, intensive efforts have been made to maximize the use of available and less costly feed-grade ingredient protein sources to replace FM for aquaculture feed production (Hardy 2010; Borquez et al. 2011; Tacon et al. 2011).

Marine macro-algae are rich in proteins, dietary fibers, minerals, vitamins, antioxidants, phytochemicals, and polyunsaturated fatty acids, but are low in caloric value (Mohamed et al. 2012). Macro-algae have been harvested for many centuries for their nutritional and mineral content as part of human and animal food or for the functional properties of their polysaccharides (Fleurence 1999). Nakagawa et al. (1984) reported that physiological conditions of black sea bream *Acanthopagrus schlegelii* were effectively improved by 10% *Ulva pertusa* supplementary in diets. Several studies have evaluated the incorporation of various seaweed species in aquafeeds, such as *Monostroma nitidum* (Amano and Noda 1985), *Undaria pinnatifida*, and *Ascophyllum nodosum* (Yone et al. 1986); *Porphyra* (Davies et al. 1997); *Ulva* (Nakagawa et al. 1987; Wassef et al. 2001; Zhu et al. 2016); *Sargassum*

spp. (Casas-Valdez et al. 2006); *Gracilaria bursa-pastoris*, *G. cornea*, and *Ulva rigida* (Valente et al. 2006); *Palmaria palmata* (Wan et al. 2016); and *Gracilaria* and *Alaria* (Peixoto et al. 2017). Most of these studies reported promising results for the use of seaweed as partial replacement of fishmeal or protein hydrolisate in aquafeeds.

Gracilaria lemaneiformis is a common alga in tropical and subtropical waters. In the twentieth century, the increasing food demand has led to a rapid growth of the seaweed cultivation and industry in China (Fei et al. 1999). Large-scale cultivation of *G. lemaneiformis* has been encouraged in Chinese coastal waters in order to meet the demand of the agar industry and the feed of abalone culture industry (Fei et al. 1999). The contents of protein, carbohydrate, and crude fiber of dry weight (DW) of *G. lemaneiformis* cultivated in Shenao Bay, Nanao Island, are 19.1, 43.76, and 4.8%, respectively (Yu et al. 2006).

Sargassum horneri is an annual brown alga with a wide distribution ranging from the coast of Japan to the East and South China Sea, and crossing from mid-littoral to sublittoral zones (Hu et al. 2011). *Sargassum horneri* is rich in protein (22.38%, DW), carbohydrate (20%, DW) (Hossain et al. 2003), and lipid (0.47–1.43%, DW) (Nomura et al. 2013).

White spotted snapper, *Lutjanus stellatus* Akazaki, is a marine carnivore widely cultured in temperate West Pacific ranging from southern Japan to Taiwan and to the vicinity of Hong Kong because of its high economic value (Akazaki 1983; Shao et al. 2008). The inclusion of *Ulva lactuca* in diet for white-spotted snapper has been reported (Zhu et al. 2016); however, the inclusion of *G. lemaneiformis* and *S. horneri* in diet has not been studied.

The primary objective of this study was to evaluate the effects of using *G. lemaneiformis* and *S. horneri* as feed ingredients to partially replace fishmeal in diets for juvenile white spotted snapper. In the present study, growth performance, body composition, and activities of enzymes of the fish fed diets containing various levels of *G. lemaneiformis* and *S. horneri* were assessed.

Materials and methods

Experimental diets

Fresh *G. lemaneiformis* and *S. horneri* were obtained from Shenao Bay, Nanao Island, Marine Biology Station of Shantou University, Guangdong province, China. After being washed in seawater, the algae were sun-dried and further dried for 5 h at 60 °C, and then finely ground into powder using a laboratory mill. Other dietary ingredients were purchased from Yuequn feed company, Jieyang, Guangdong province, China. Proximate composition of major dietary ingredients is shown in Table 1.

Table 1 Proximate composition of dried fish meal, *G. lemaneiformis*, *S. horneri*, and soybean meal (%, dry matter)

Ingredients	DM	CP	CL	Ash	NFE
GLM[a]	87.22	19.13	0.54	19.74	60.59
SHM[b]	88.43	17.03	0.41	20.12	62.44
Fish meal[c]	93.63	69.25	8.92	13.14	8.69
Soybean meal[c]	90.35	43.52	1.36	5.74	49.38

DM dry matter, *CP* crude protein, *CL* crude lipid, *NFE* nitrogen-free extract = 100 − (CP + CL + ash)

[a] GLM, *G. lemaneiformis* meal, Shenao Bay, Guangdong Province, China

[b] SHM, *S. horneri* meal, Shenao Bay, Guangdong Province, China

[c] Yuequn feed company, Jieyang, Guangdong Province, China

The experimental diets with isonitrogenous (42% crude protein) and isolipid (8.7% crude lipid) were formulated (Table 2). A total of nine experimental diets, G1–G4 and S1–S4 diets containing 5, 10, 15, and 20% of *G. lemaneiformis* and *S. horneri*, respectively, and the control diet (Con) with no algae, were formulated.

Dry ingredients were finely ground into powder, passed through a 120-mesh sieve and then thoroughly mixed with soybean oil and water to produce stiff dough. Pellets were puffed by a laboratory feed pelletizer equipped with a 2-mm die (SLP-45; Fishery Mechanical Facility Research Institute, Shanghai, China) at 105 ± 5 °C. After being dried for about 12 h in a ventilated oven at 45 °C, pellets were packed in sealed plastic bags and stored at −20 °C until used. About 20 g of diet in triplicates was sampled for the analysis of biochemical composition.

Experimental fish and feeding trial

Fish used for the experiment were purchased from a local breeding base of fine *Haemulidae*, Raoping County, shipped with fresh seawater to the Marine Biology Station of Shantou University, then reared in a floating sea cage (2.0 × 2.0 × 2.0 m, L/W/H), and fed a commercial diet (Haiyi feed company, Zhuhai, Guangdong, China) for 2 weeks to adapt to the experimental culture conditions. Fish were starved for 24 h before being anesthetized with 40 mg L^{-1} eugenol and weighed individually (Iversen et al. 2003). Fish with similar size (mean 12.0 ± 0.1 g) were selected and randomly distributed into 27 sea cages (1 × 1 × 1.5 m, L/W/H), each cage with 30 fish in three triplicates per treatment.

Sea cages were anchored about 3.5 m underwater and 100 m away from the coast in a bay. Fish were held under natural photoperiod conditions throughout the feeding trial station, and hand-fed twice daily (8:00 a.m. and 17:00 p.m.) with diets of 4–6% body weight which was obtained biweekly for 60 days. During the experimental period, temperature ranged from 25 to 33 °C, salinity ranged from 27 to 33 ‰, and dissolved oxygen content was approximately 6.8 mg L^{-1}.

Table 2 Formulation and chemical composition of the diets (%, dry matter)

Ingredient	Diets								
	Con	G1	G2	G3	G4	S1	S2	S3	S4
Fish meal	50	48.6	47.2	45.8	44.4	48.8	47.6	46.4	45.2
Soybean meal	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5
Starch	19.5	15.9	12.2	9.5	5.8	15.7	11.8	8.9	5.0
Soybean oil	4.0	4.0	4.1	4.2	4.3	4.0	4.1	4.2	4.3
GLM	0	5.0	10.0	15.0	20.0	0	0	0	0
SHM	0	0	0	0	0	5.0	10.0	15.0	20.0
MCC[a]	4.0	4.0	4.0	3.0	3.0	4.0	4.0	3.0	3.0
Mineral premix[b]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix[c]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
CMC[d]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
CaH_2PO_4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
SLP[e]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Proximate composition									
Dry matter	89.45	89.15	88.74	89.49	88.86	90.16	88.54	88.95	87.99
Crude protein	42.05	41.99	42.08	42.04	42.03	41.71	41.27	41.14	41.48
Crude lipid	8.67	8.64	8.62	8.61	8.59	8.62	8.61	8.64	8.81
Crude ash	10.64	10.71	10.99	11.47	11.57	11.51	11.66	11.84	11.77

[a] MCC, microcrystalline cellulose, Sunhere Pharmaceutical Excipients Co., Ltd., Hefei, Anhui Province, China

[b] Per kilogram of mineral premix: NaF, 2 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; NaCl, 100 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; zoelite, 15.45 g. Formulated with cellulose as filling, Sintun Aqua-Tech Co., Ltd., Guangzhou, Guangdong Province, China

[c] Per kilogram of vitamins premix: A, 4×10^6 IU; D_3, 2×10^6 IU; E, 60 g; K_3, 6 g; B_1, 7.5 g; B_2, 16 g; B_6, 12 g; B_{12}, 100 mg; nicotinic acid, 88 g; pantothenic acid, 36 g; folic acid, 2 g; biotin, 100 mg; inositol, 100 g; C-monophospholipid compound, 200 g, Sintun Aqua-Tech Co., Ltd., Guangzhou, Guangdong Province, China

[d] CMC, carboxymethyl cellulose, Hongbo New Materials Co., Ltd., Hangzhou, Zhejiang Province, China

[e] SLP, squid liver paste, Yuequn feed company, Jieyang, Guangdong Province, China

Sample collection

At the termination of the experiment, fish were fasted for 24 h prior to sampling. Total number and mean body weight of fish in each cage were measured. Ten fish from each cage were randomly sampled and immediately euthanized with 40 mg L^{-1} eugenol (Iversen et al. 2003), and weighted individually. Five fish were used for biochemical analysis. The remaining fish were dissected for organ and tissue sampling. The dorsal muscles, stomach, and intestine were prepared and weighed separately. All samples were stored at −80 °C immediately before further analysis.

Evaluation of growth performance

Growth performance was evaluated by comparing weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) calculated as follows:

$$\mathrm{WG}\ (\%) = (\mathrm{W_2}-\mathrm{W_1})\,/\mathrm{W_1} \times 100$$
$$\mathrm{SGR}\ (\%/\mathrm{day}) = (\mathrm{LnW_2}-\mathrm{LnW_1})\,/\ \mathrm{t} \times 100$$
$$\mathrm{FCR} = \mathrm{dry}\ \mathit{feed}(\mathrm{g})\mathrm{fed}/(\mathrm{W_2}-\mathrm{W_1})$$
$$\mathit{Survival}\ (\%) = 100 \times \mathit{final\ fish\ number}/\mathit{initial\ fish\ number}$$

where W_2 is the final body weight (g) of fish, W_1 is the initial body weight (g), and t is the number of days for the feeding trial (Hardy and Barrows 2002).

Biochemical analysis

Protein, crude lipid, ash content, and dry matter of diets and fish samples were analyzed according to the methods described by the Association of Official Analytical Chemists (AOAC 2002). Protein contents were determined by using the Kjeldahl Autosampler System 1035 Analyzer (Foss, Sweden), and percent crude protein was then calculated as % N × 6.25. Crude lipid content was determined by Soxhlet extraction. Ash content was analyzed through combustion of samples in a muffle furnace at 550 °C for 6 h. Dry matter was determined by exposing diet samples in a drying oven at 105 °C for 4 h.

Measurement of enzyme activities

Fish stomach and intestine were separately homogenized in four volumes (*w*/*v*) of chilled Tris-HCl buffer solution (50 mM, pH 7.6) and then centrifuged (3000 rpm) at 4 °C for 5 min (Centrifuge 5417R, Eppendorf, Germany). After centrifugation, the floating top lipid layer was removed and the supernatant was divided into aliquots in 1.5-mL tubes. The samples were stored at −80 °C until analysis.

Total soluble protein of the homogenate was measured by using Folin phenol reagent. Activities of pepsin, trypsin,

Table 3 Growth performance of white spotted snapper fed with *G. lemaneiformis* diets

Parameters	Diets				
	Con	G1	G2	G3	G4
IBW (g)	11.98 ± 0.17	12.03 ± 0.14	11.90 ± 0.25	11.84 ± 0.17	12.11 ± 0.54
FBW (g)	37.81 ± 0.38b	38.36 ± 0.51ab	38.97 ± 0.56a	37.69 ± 0.45b	36.30 ± 0.23c
WG (%)	216 ± 4a	219 ± 5a	228 ± 8a	218 ± 5a	202 ± 4b
SGR (%/day)	1.92 ± 0.04ab	1.93 ± 0.04ab	1.98 ± 0.03a	1.93 ± 0.03ab	1.85 ± 0.05b
FCR (g/g)	2.15 ± 0.08ab	2.11 ± 0.07ab	2.08 ± 0.06b	2.25 ± 0.09ab	2.29 ± 0.06a
Survival (%)	93 ± 3	96 ± 2	98 ± 2	96 ± 3	94 ± 3

Means not sharing a common lowercase letter are significantly different ($P < 0.05$)

IBW initial body weight, *FBW* final body weight, *WG* weight gain, *SGR* specific growth rate, *FCR* feed conversion ratio

lipase, and amylase were determined by spectrophotometry using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu province, China) following the manufacturer's instructions. The units of the digestive enzymes activities were defined based on the method reported by Pan and Wang (1997) and the manual of detection kit. Briefly, 1 U of pepsin activity was calculated as 1 μg of tyrosine released per minute at 37 °C. Trypsin activity was expressed as the equivalent enzyme activity that was required to generate an optical density (OD) change of 0.003 at pH 8.0 and 37 °C. Amylase activity was calculated as the activity required to hydrolyze 10 mg of starch in 30 min at 37 °C. One unit of lipase activity was defined as the micromole of substrate hydrolyzed per minute at 37 °C. Enzyme activities were expressed as specific activity (U mg^{-1} protein).

Statistical analysis

Data were analyzed by using one-way ANOVA and are presented as mean ± SD. Tukey's multiple comparison tests were used to assess where significant differences occurred. Statistical analyses were performed using SPSS 20.0 (SPSS, USA). A quadratic regression model of average weight gain corresponding to dietary algae replacement level was established in Office Excel 2016 (Microsoft, USA).

Results

Growth performance

Growth parameters of white spotted snapper fed *G. lemaneiformis* diets are presented in Table 3. The survival was not significantly different among dietary treatments. FBW and WG of fish fed G4 diet were significantly lower than those of the control group ($P < 0.05$). Fish fed G4 diet showed significantly better FCR compared to those fed G2 diet ($P < 0.05$); the difference was not significant between fish fed G1, G3, and control diets ($P > 0.05$). Fish fed G4 diet showed significantly poorer SGR compared to those fed G2 diet ($P < 0.05$). According to the quadratic regression model of WG of fish fed *G. lemaneiformis* diets (Fig. 1), the maximal *G. lemaneiformis* replacement level was 16.44%, which would not reduce the growth performance of fish as compared to the control diet.

Data on growth performance and feed utilization of white spotted snapper fed *S. horneri* diets are shown in Table 4. The survival was not affected by dietary treatments. Fish fed S1 diet displayed significantly higher SGR compared to S4 diet ($P < 0.05$), but no significant difference was found between fish fed other diets. Moreover, fish fed S1 diet had the highest SGR, FBW, and WG, but the poorest FCR. The poorest fish growth performance was obtained with the S4 diet. The maximal *S. horneri* replacement level was 15%, which would not decrease the growth performance of fish as compared to the control diet (Fig. 2).

Body biochemical composition

The lipid and ash contents in muscle were significantly affected by dietary *G. lemaneiformis* level, and the lipid content in

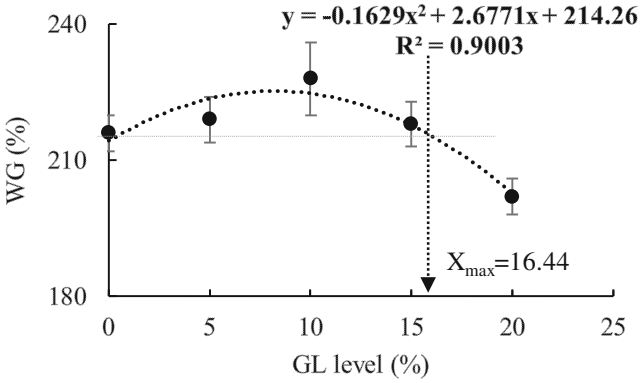

Fig. 1 A quadratic regression model was established on average body WG (*y-axis*) in response to fishmeal protein replacement level (*x-axis*) by *G. lemaneiformis*. According to the model, the maximal replacement level is 16.44%

Table 4 Growth performance of white spotted snapper fed with *S. horneri* diets

Parameters	Diets				
	Con	S1	S2	S3	S4
IBW (g)	11.98 ± 0.17	12.17 ± 0.29	11.94 ± 0.59	11.94 ± 0.68	12.14 ± 0.60
FBW (g)	37.81 ± 0.38b	40.49 ± 1.11a	38.79 ± 1.87ab	37.50 ± 0.29b	36.98 ± 0.72b
WG (%)	216 ± 4bc	233 ± 5a	225 ± 4ab	215 ± 16bc	205 ± 9c
SGR (%/day)	1.92 ± 0.04ab	2.00 ± 0.02a	1.96 ± 0.01ab	1.91 ± 0.08ab	1.85 ± 0.05b
FCR (g/g)	2.15 ± 0.08ab	2.14 ± 0.05a	2.27 ± 0.11ab	2.33 ± 0.03b	2.42 ± 0.06b
Survival (%)	93 ± 3	95 ± 4	93 ± 3	92 ± 4	91 ± 4

Means not sharing a common lowercase letter are significantly different ($P < 0.05$)

muscle of fish fed G4 diet was significantly lower than that of other groups ($P < 0.05$, Table 5). The protein content in whole body fed G4 and control diets was significantly higher than that of other groups ($P < 0.05$), but lipid, moisture, and ash contents in whole body of fish fed *G. lemaneiformis* diets were not changed significantly among the treatments ($P > 0.05$, Table 5).

The lipid and moisture contents in muscle, but not protein and ash contents, were significantly affected by dietary *S. horneri* level ($P < 0.05$, Table 6). The lowest lipid and moisture contents were recorded in muscle of fish fed S1 and S3 diets. No significant changes in moisture, lipid, and ash contents in whole body were observed among all treatments ($P > 0.05$), whereas protein content in whole body in fish fed S4 diet was significantly lower than that of control and S1 diets ($P < 0.05$, Table 6).

Enzyme activities

Effects of *G. lemaneiformis* on digestive enzyme activities in the stomach and intestine of the fish are shown in Table 7. Pepsin activity in stomach was significantly lower in fish fed G4 diet (44.55 U mg^{-1} protein), while lipase in stomach was not significantly affected by *G. lemaneiformis* levels ($P > 0.05$). Lipase and amylase activities in intestine were significantly lower in fish fed G4 diet (15.74×10^{-3} U mg^{-1} protein) ($P < 0.05$), while no effect of *G. lemaneiformis* was found on trypsin activities in the intestine ($P > 0.05$).

Pepsin and amylase in the stomach were significantly affected by dietary *S. horneri* levels (Table 8). The lowest pepsin activity in stomach was observed in fish fed S4 diet (49.41 U mg^{-1} protein), while lipase in stomach was not significantly affected by *S. horneri* levels ($P > 0.05$, Table 8). Pepsin in stomach of fish fed S3 and S4 diets decreased significantly ($P < 0.05$) as compared to that of fish fed control, S1, and S2 diets. Lipase and amylase in the intestine were significantly affected by dietary *S. horneri* level. The lowest lipase activity in intestine was observed in fish fed S4 diet (15.5710^{-3} U mg^{-1} protein). No effect of *S. horneri* was found on trypsin in the intestine.

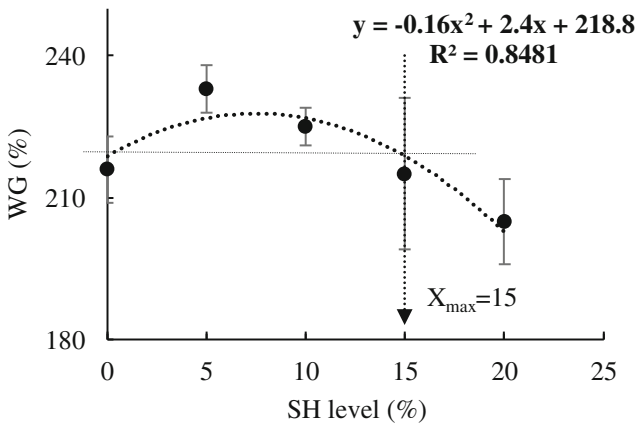

Fig. 2 A quadratic regression model was established on average body WG (*y-axis*) in response to fishmeal protein replacement level (*x-axis*) by *S. horneri*. According to the model; the maximal replacement level is 15%

Discussion

Seaweeds are receiving increasing attention as potential nutritional benefits (Rupérez and Saura-Calixto 2001) and as possible ingredients in fish diets (Wahbeh 1997). Studies suggested that omnivorous red sea bream *Pagrus major* (Mustafa et al. 1994, 1995; Nakagawa et al. 1997) may have potential ability to digest algae. Tacon et al. (1990) suggested that the maximum suitable algae supplement levels in diets may adapt to the feeding habits of fish and species of algae. In this experiment, all diets were readily accepted by fish, indicating that *G. lemaneiformis* and *S. horneri* could be used as possible ingredients in fish diets.

Previous reviews detailed that the supplementation of macro-algae meals enhanced growth and feed utilization (Mustafa and Nakagawa 1995; Mustafa et al. 1995). Diler et al. (2007) reported that the best and poorest growth performance of common carp *Cyprinus carpio* were fed 5 and 20% *U. rigida* diets, respectively. The supplementation of *U. rigida*, in the range from 5 to 15% in the diet, improved

Table 5 Proximate chemical composition of fish fed with *G. lemaneiformis* diets (fresh weight)

Composition	Diets				
	Con	G1	G2	G3	G4
Muscle (g kg^{-1})					
Protein	182.7 ± 4.4	180.3 ± 4.9	184.3 ± 6.4	183.1 ± 6.6	182.7 ± 6.8
Lipid	21.2 ± 0.6b	29.2 ± 1.8a	25.8 ± 2.4ab	21.3 ± 1.1b	15.6 ± 1.4c
Moisture	773.6 ± 5.5	762.8 ± 5.6	769.3 ± 13.7	780.1 ± 8.4	783.5 ± 9.2
Ash	13.5 ± 0.3b	15.6 ± 0.4a	15.4 ± 0.9a	13.8 ± 0.5ab	14.6 ± 0.6ab
Whole body (g kg^{-1})					
Protein	157.5 ± 4.6a	138.4 ± 4.1b	140.6 ± 2.6b	139.5 ± 2.9b	152.9 ± 3.7a
Lipid	53.0 ± 5.7	51.4 ± 3.3	53.7 ± 3.7	45.1 ± 5.5	48.6 ± 3.4
Moisture	738.4 ± 2.8	743.6 ± 29.0	742.7 ± 4.0	754.3 ± 15.2	738.7 ± 17.1
Ash	47.1 ± 1.3	44.2 ± 6.1	47.6 ± 1.0	50.9 ± 3.1	48.8 ± 2.9

Means not sharing a common lowercase letter are significantly different ($P < 0.05$)

not only the growth performance but also the quality of carp as a protein product. Therefore, the author suggested that the dietary *U. rigida* meal inclusion of 5 to 15% replacing wheat meal in carp diets could be acceptable. Xuan et al. (2013) reported that the WG and FER of the fish that received 20% *G. lemaneiformis* diet were significantly lower than those of the control group, and suggested that *G. lemaneiformis* in the diet at up to 15% level for black sea bream was feasible according to the growth performance and the physiological state. Shapawi et al. (2015) reported that supplementation of 6% cooked red seaweed *Kappaphycus alvarezii* achieved significantly higher WG and SGR in Asian seabass *Lates calcarifer* than other treatments. However, the survival (%) of experimental fish was significantly lower than that of other treatments when the dietary seaweed inclusion was at 22%. In this experiment, the results indicated that the FBW, WG, and SGR of fish fed with *G. lemaneiformis* and *S. horneri* did not decrease even at the inclusion level of 15% in diets. According to the quadratic regression models of WG in fish fed *G. lemaneiformis* and *S. horneri* diets, the maximal replacement levels were 16.44 and 15%, respectively.

Horie et al. (1995) reported that seaweeds rich in non-starch polysaccharides (NSPs) (e.g., xylans, agar, carrageenan, or alginates) are a type of antinutritional factors (ANFs) limiting the digestibility of nutrients of diets. Brinker (2009) reported that the soluble NSPs in diet form functional networks which reduce lipid, protein, and other nutrient digestibility and therefore decrease growth performance. In this experiment, *G. lemaneiformis* and *S. horneri* contained 60.6 and 62.4% nitrogen-free extract, respectively, and the results show that *G. lemaneiformis* or *S. horneri* inclusion level reached 20%; the poorest growth performance of the fish was observed. The most likely reason for the detrimental seaweed effects may be that algal soluble NSPs are generally viscous in nature, leading to increased viscosity of diet and the intestinal digesta. This stress will decrease protein and lipid

Table 6 Proximate chemical composition of fish fed with *S. horneri* diets (fresh weight)

Composition	Diets				
	Con	S1	S2	S3	S4
Muscle (g kg^{-1})					
Protein	182.7 ± 4.4	180.5 ± 2.3	187.8 ± 3.2	189.7 ± 5.3	185.5 ± 3.3
Lipid	21.2 ± 0.6b	15.4 ± 0.8a	16.9 ± 0.6a	20.8 ± 0.4b	22.6 ± 0.6b
Moisture	793.6 ± 5.5a	789.7 ± 2.8ab	781.1 ± 3.4ab	776.5 ± 5.2b	779.2 ± 3.9b
Ash	13.5 ± 0.3	13.1 ± 0.3	13.1 ± 0.2	13.6 ± 0.3	13.1 ± 0.3
Whole body (g kg^{-1})					
Protein	157.5 ± 4.6b	157.9 ± 3.9b	149.7 ± 1.2ab	147.7 ± 5.1ab	147.1 ± 3.3a
Lipid	53.0 ± 3.3	58.5 ± 2.9	57.5 ± 3.5	57.8 ± 1.4	58.8 ± 3.1
Moisture	738.4 ± 2.8	738.0 ± 5.2	743.9 ± 4.1	749.4 ± 7.7	739.9 ± 9.6
Ash	47.1 ± 1.3	48.2 ± 1.3	47.2 ± 0.9	47.4 ± 1.8	48.6 ± 2.3

Means not sharing a common lowercase letter are significantly different ($P < 0.05$)

Table 7 The enzyme activities of white spotted snapper fed with *G. lemaneiformis* diets

Parameters	Diets				
	Con	G1	G2	G3	G4
Stomach (U mg^{-1} $protein^{-1}$)					
Pepsin	78.60 ± 1.61a	75.11 ± 2.78a	75.86 ± 6.68a	51.36 ± 3.77b	44.55 ± 7.25b
Lipase ($\times10^{-3}$)	2.86 ± 0.30	3.02 ± 0.44	3.09 ± 0.17	2.36 ± 0.39	2.84 ± 0.40
Amylase ($\times10^{-3}$)	20.84 ± 2.41b	30.13 ± 2.72a	26.96 ± 2.65ab	24.21 ± 3.84ab	17.30 ± 3.29b
Intestine (U mg^{-1} $protein^{-1}$)					
Trypsin	21.51 ± 2.99	23.19 ± 2.43	22.27 ± 1.88	20.60 ± 4.87	19.83 ± 4.02
Lipase ($\times10^{-3}$)	19.26 ± 0.92b	19.87 ± 1.37b	19.12 ± 1.04ab	16.97 ± 1.55ab	15.74 ± 1.41a
Amylase ($\times10^{-2}$)	53.12 ± 4.58d	41.83 ± 3.87c	38.20 ± 4.47b	33.67 ± 2.77ab	27.21 ± 1.73a

Means not sharing a common lowercase letter are significantly different ($P < 0.05$)

digestibility and consequently growth performance. Meanwhile, the negative effects may be caused by the lower methionine in the seaweeds, which could limit the supplement of seaweeds in diets (Fleurence 2004).

Nakagawa et al. (1984) reported that 10% *U. pertusa* meal supplement to diet increased muscle lipid level in black sea bream. Norambuena et al. (2015) reported that the diets with 2.5 and 5% *Entomoneis* spp. and *Ulva ohnoi* inclusion, respectively, showed a partial (not statistically significant) reduction in total lipid content in whole body, but the diets with 2.5 and 5% *U. ohnoi* inclusion showed a partial increase (no significance) in lipid content in fillets of Atlantic Salmon (*Salmo salar*). The results showed significantly higher n-3/n-6 ratio in fillets of fish compared to other treatments due to the variation in n-6 and n-3 fatty acid in 5% *Entomoneis* spp. and 2.5% *Entomoneis* spp. + 2.5% *U. ohnoi* diets. Also, the results showed that the elongation of C18:3n-3 to C20:3n-3 was up-regulated in fish fed 2.5% *Entomoneis* spp. + 2.5% *U. ohnoi* diets compared to the other treatments. In the present experiments, lipid and ash contents in muscle and protein content in body were significantly affected by dietary *G. lemaneiformis* levels, and the lipid in muscle and protein in body were significantly affected by dietary *S. horneri* levels. The results were in agreement with the observations that addition of a small amount of algae in diets influences lipid synthesis and lipid mobilization in the body of fish (Nakagawa et al. 1997). On the other hand, increased lipid deposition with increasing seaweed inclusion levels could be due to a dietary essential amino acid imbalance. Such an imbalance may reduce utilization of protein for growth. This often occurs when fish meal is substituted by plant protein sources (Fournier et al. 2004; Goda et al. 2007).

Previous studies have shown that algal supplementation had effects on digestive enzyme activities both in vitro and in vivo (Indegaard and Minsaas 1991; Nandeesha et al. 1998; Xuan et al. 2013; Zhu et al. 2016). Horie et al. (1995) reported that the soluble dietary fiber fraction (SDF) or insoluble dietary fiber fraction (IDF) from brown seaweeds *Saccharina (Laminaria) japonica*, *U. pinnatifida*, and *Sargassum fusiforme* could inhibit pepsin activity in vitro, and the inhibition was significantly greater with SDFs than IDFs, and the greatest inhibition was observed with SDF of

Table 8 The enzyme activities of white spotted snapper fed with *S. horneri* diets

Parameters	Diets				
	Con	S1	S2	S3	S4
Stomach (U mg^{-1} $protein^{-1}$)					
Pepsin	78.60 ± 1.61a	78.28 ± 5.36a	72.46 ± 2.63a	63.83 ± 6.96b	49.41 ± 1.49c
Lipase ($\times10^{-3}$)	2.86 ± 0.30	3.21 ± 0.37	3.01 ± 0.29	2.68 ± 0.31	2.57 ± 0.56
Amylase ($\times10^{-3}$)	20.84 ± 2.41a	23.83 ± 2.01a	31.33 ± 3.34b	33.30 ± 3.53b	34.11 ± 3.35b
Intestine (U mg^{-1} $protein^{-1}$)					
Trypsin	21.51 ± 2.99	21.97 ± 4.96	21.36 ± 1.87	18.63 ± 1.44	20.32 ± 3.52
Lipase ($\times10^{-3}$)	19.26 ± 0.92a	20.21 ± 1.37a	17.46 ± 1.65ab	18.07 ± 2.15ab	15.57 ± 2.11b
Amylase ($\times10^{-2}$)	53.12 ± 4.58bc	54.93 ± 4.30bc	55.97 ± 1.63b	64.43 ± 4.75a	48.20 ± 5.19c

Means not sharing a common lowercase letter are significantly different ($P < 0.05$)

S. japonica. The results showed that the higher the concentration of SDF in the enzyme system, the greater its viscosity and pepsin activity inhibition. Consequently, the authors suggested that the inhibition of pepsin activity in vitro by SDF might be attributed to their viscous property. Nandeesha et al. (1998) reported that a reduction in intestinal protease and lipase activity of common carp was observed at higher levels of *Spirulina platensis* supplementation, and the hepatopancreatic protease activity of fish fed *Spirulina* was poorer compared to the control, while the amylase activity was better, and the intestinal amylase and hepatopancreatic lipase activity remained unaffected. In the present results, a significantly lower pepsin activity in the stomach was observed in fish fed 20% *G. lemaneiformis* or 20% *S. horneri* diets, and a significantly lower lipase activity in the intestine was observed in fish fed 20% *S. horneri* diet. The poorer lipase and pepsin activity observed in higher-level *G. lemaneiformis* and *S. horneri* diets may be a result of a combined effect of increasing NSPs, soluble or insoluble fibers of seaweeds, and the reduced digestive enzyme activities.

In conclusion, based on quadratic regression, it can be concluded that the maximal incorporation of red algae *G. lemaneiformis* and brown algae *S. horneri* in diets are 16.44 and 15%, respectively, for juvenile white spotted snapper. This study provides important information regarding the potential application of *G. lemaneiformis* and *S. horneri* as valuable alternative ingredient sources partially replacing fish meal for fish culture.

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