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Effects of different levels of macroalga *Gracilaria lemaneiformis* on growth performance and feed utilization on the red sea bream, *Pagrosomus major*

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Abstract
In the present study, diets prepared to contain 0% (D0), 3% (D3), 6% (D6), 9% (D9), 12% (D12), and 15% (D15) of the red alga *Gracilaria lemaneiformis* were used to investigate the effects of *G. lemaneiformis* on the growth, carcass composition, and activities of enzymes related to nutritional metabolism of juvenile red sea bream (*Pagrosomus major*). The weight gain (WG), specific growth rate (SGR), apparent digestibility coefficients (ADC), feed efficiency (FE), protein efficiency ratio (PER), and protein retention (PR) were significantly higher in D3 than in the other treatment groups ($P < 0.05$). Although the feed intake (FI) of D15 was greatly higher than that of the D0, the ADC of crude lipid, hepatic lipid level, and hepatosomatic index (HSI) of D15 ($P > 0.05$) were significantly reduced. Compared to the D0, hepatic glycogen and serum aspartate aminotransferase (AST) activity in the liver as well as serum total cholesterol (TC) of the fish on D3 and D6 were significantly higher ($P < 0.05$). Significantly higher activity of lipoprotein lipase (LPL) in the abdominal adipose tissues of fish fed on D3 was also observed ($P < 0.05$). The activities of lipase in anterior intestines appear decreased when the *G. lemaneiformis* supplementation level was over 12%. These results indicate that incorporate 3% *G. lemaneiformis* in diet could improve the growth performance and feed utilization of juvenile red sea bream. Incorporation of *G. lemaneiformis* at 15% level in the diet was also feasible for juvenile red sea bream, as it had no influence on growth performance.

Keywords *Gracilaria lemaneiformis* · *Pagrosomus major* · Rhodophyta · Growth · Digestive enzyme activity · Feed utilization · Lipid metabolism

Introduction

Prices for traditional aquacultural feed ingredients have been rising due to increasing demand (FAO 2016). Fish meal is the major dietary protein source for the aquaculture industry; hence, the price of fish meal directly affects the cost of feed. For sustainability of the aquaculture industry, it is crucial to find new or alternative aquaculture feed ingredients that are locally and readily available (Tschirner and Kloas 2017). In an intensive culture system composition of the cultured fish may be affected by high-fat artificial diets and with the composition and deposition of the lipid further influencing the nutritional value and organoleptic properties of the fish (Peres and Oliva-Teles 1999).

The beneficial effects of algae on the improvement of growth performance and lipid metabolism of fish have been demonstrated (Araújo et al. 2016; Lozano et al. 2016; Cian et al. 2018; Guerreiro et al. 2018; Moutinho et al. 2018). There is therefore an increasing interest in the use of algae sources for animal nutrition, although the mechanism of its benefits as a feed additive is not clear. Among the different species of macroalgae, red algae have relatively high protein content, which makes them most suitable feed source for animal nutrition. The red alga *Gracilaria lemaneiformis* is an important raw material for agar-production and possesses various bioactive functions such as anti-viral, antioxidant, anti-influenza, and immunomodulation effects (Chen et al. 2005a, 2005b). Due to its large-scale production in China, *G. lemaneiformis* could be used as a feed ingredient for commercially important animals from the economic point of view (Xuan et al. 2013).

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Adult red sea bream (*Pagrosomus major*) is a euryhaline and omnivorous bottom-dwelling fish which is known to ingest algae (Mustafa et al. 1995; Nakagawa 1997). In recent years, because of its market value and high demand, the red sea bream has become one of the important culture fish species in China (Dawood et al. 2015).

To the best of our knowledge, few studies have been carried out on the effects of algae on the nutritional metabolism of fish. The objective of the present study was to evaluate the feasibility of using *G. lemaneiformis* as a feed ingredient on the juvenile red sea bream by assessing its growth performance, body composition, serum biochemical indexes, and the activities of enzymes related to the digestive–absorptive function.

Materials and methods

Experimental diets

Fresh *Gracilaria lemaneiformis* was purchased from a seaweed farm (Zhanjiang, Guangdong Province, China). After being washed in seawater, *G. lemaneiformis* was air-dried in the shade for 2 weeks and then finely ground using a laboratory mill. Other dietary ingredients were purchased from a feed company (DoYoo Industrial Co., Ltd., Zhengzhou, China). Proximate analysis of major dietary ingredients was performed prior to formulation of the experimental diets (Table 1).

The diet without *G. lemaneiformis* served as control (D0) diet, while five isonitrogenous (49% crude protein) and isolipid (12% crude lipid) experimental *G. lemaneiformis* meal-based diets were formulated to contain 3% (D3), 6% (D6), 9% (D9), 12% (D12), and 15% (D15) *G. lemaneiformis* by substituting fish meal on an equal protein basis (Kader et al. 2012; Ragaza et al. 2012). Starch from wheat was used as the primary ingredient responsible for the binding properties without influencing the growth of the red sea bream (Wilson 1994).

The ingredients of the six diets were thoroughly mixed and pelleted by passing through a laboratory feed-pelletizer equipped with a 2-mm die (SLP-45; Fishery Mechanical Facility Research Institute, Shanghai, China) at 65 ± 5 °C. The pellets were then air-dried using a fan at room temperature. The dry pellets were packed in sealed plastic bags and stored at − 20 °C until used. About 20 g of each diet was sampled in triplicates for the analysis of biochemical composition.

Table 1 Formulation and proximate composition of the experimental diets (% dry matter)

Ingredient composition (%)	Diets					
	D0	D3	D6	D9	D12	D15
Fish meal[1]	52.64	51.85	51.07	50.28	49.49	48.70
Casein	5.00	5.00	5.00	5.00	5.00	5.00
Soy protein concentrate[1]	10.00	10.00	10.00	10.00	10.00	10.00
DGL[1]	0.00	3.00	6.00	9.00	12.00	15.00
Soybean oil	1.81	1.88	1.95	2.02	2.10	2.17
Pollack liver oil	4.00	4.00	4.00	4.00	4.00	4.00
Wheat starch	18.00	16.74	15.48	14.22	12.96	11.70
Cellulose	5.85	4.83	3.80	2.78	1.75	0.73
Vitamin premix[2]	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[3]	0.50	0.50	0.50	0.50	0.50	0.50
Monocalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20
Proximate composition (%)						
Moisture	9.87	9.67	10.12	9.77	10.21	10.09
Crude protein	49.25	48.97	49.16	48.83	49.22	49.01
Crude lipid	11.97	12.35	12.24	11.87	12.17	12.29
Ash	10.27	10.34	10.14	10.21	10.19	10.22

[1] Fish meal, crude protein 72% dry matter, crude lipid 11% dry matter; soybean protein concentrate, crude protein 66% dry matter, crude lipid 4% dry matter; DGL, dried *Gracilaria lemaneiformis*, crude protein 18.9% dry matter, crude lipid 0.5% dry matter

[2] Vitamin premix (IU or g kg^{-1} mixture): retinol, 3.6×10^6 IU; cholecalciferol, 1.5×10^6 IU; tocopherol, 7.5×10^3 IU; menadione, 10 g; thiamin, 12 g; riboflavin, 8 g; pyridoxine hydrochloride, 12 g; cyanocobalamin, 0.2 g; nicotinic acid, 35 g; Ca pantothenate, 25 g; folic acid, 2 g; biotin, 0.1 g; inositol, 60 g; ascorbic acid, 100 g

[3] Mineral premix (g kg^{-1} mixture): $MgSO_4$, 15 g; $FeSO_4$, 2.5 g; $CuSO_4$, 0.031 g; $MnSO_4$, 0.162 g; $ZnSO_4$, 0.353 g; KIO_3, 0.003 g; Na_2SeO_3, 0.003 g; $CoSO_4$, 0.001 g

Experimental fish and feeding conditions

Red sea bream was obtained from a commercial producer (Zhangzhou, China). After rearing in a net cage for 2 weeks in the sea, the fish were then acclimated in laboratory tanks for 2 weeks prior to use in the experiment. A 56-day growth trial was carried out from May to July 2018 in cylindrical tanks. Fish of homogenous size (initial average weight 7.86 ± 0.03 g) were randomly distributed into 18 plastic buckets (diameter by height 80 × 70 cm, 400 L) with 25 fish per bucket. Each diet was randomly assigned to triplicate buckets. Fish in each bucket were collectively weighed on the initial day after being fasted for 24 h and anesthetized with eugenol at a concentration of 40 mg L^{-1}. Fish were fed to visual apparent satiety, at 08:30 and 16:30 daily, and the amounts fed were recorded daily. Dead fish and uneaten feed were weighted and removed. The temperature was maintained at 27 ± 1.5 °C and the salinity, at 32 ± 1‰. Photoperiod was set at 12-h light:12-h dark. During the experimental period, half the aquarium water was changed twice a day (morning and evening) and water kept aerated.

Digestibility trial

The digestibility trial was conducted during the last 2 weeks of the feeding trial. Cr_2O_3 (1%) (99.9% trace metals basis, Shanghai Macklin Biochemical Co., Ltd.) was used as the external indicator in the control and five experimental diets. After a week acclimation of experimental diet, feces from each replicate were collected by siphoning 1–2 h after feeding. Briefly, once feces were observed, they were immediately collected by gently siphoning, dried for 5 h at 60 °C and stored at − 20 °C until analysis.

Sample collection

At the beginning of the feeding trial, 10 juvenile red sea bream were randomly weighed and stored at − 20 °C as the initial samples. At the end of the growth trial, fish were weighed after being anesthetized with eugenol and the total number of the fish in each tank was recorded after fasting for 24 h to calculate the weight gain (WG), survival, specific growth rate (SGR), feed efficiency (FE), feed intake (FI), protein efficiency ratio (PER), protein retention (PR), and the final mean weight. Five fish from each bucket were collected and stored frozen (− 20 °C) until being used for proximate analysis of the whole body. Another six fish were randomly collected from each bucket and sacrificed to determine hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor (CF). The muscles and livers were separated immediately and frozen at − 20 °C for proximate composition. Three fish per bucket were anesthetized using eugenol (40 mg L^{-1}), and the blood samples were immediately collected with needle and syringe through the caudal vein into tubes. Blood was separated in a 4 °C centrifuge (1500 rpm, 15 min), and the serum was stored at − 80 °C for biochemical assays. Another five fish were dissected; liver, stomach, adipose, and anterior intestine tissues were removed onto ice, were washed using distilled water, and then immediately dipped into liquid nitrogen to snap freeze before being stored at − 80 °C.

Biochemical analysis

The protein (984.13, A–D), crude lipid (920.39, A), ash content (942.05), and dry matter (934.01) of diets or fish samples were analyzed using established methods according to AOAC (2006). Protein contents of diets and fish samples were determined using a Kjeldahl Auto Sampler System 1035 Analyzer (Foss, Sweden), while crude lipid content was determined with Soxhlet extraction. Ash content was analyzed through combustion of samples in a muffle furnace at 550 °C for 8 h. Dry matter was determined by exposing samples to 105 °C temperature in a drying oven overnight.

Evaluation of growth performance

The parameters were calculated as shown as follows:

$$\mathrm{WG}\ (\%) = (\text{final body weight (g)} - \text{initial body weight (g)}) / \text{initial body weight (g)} \times 100.$$

$$\mathrm{SGR}\ (\%\ \mathrm{day}^{-1}) = (\ln \text{final body weight (g)} - \ln \text{initial body weight (g)}) / \text{days} \times 100.$$

$$\mathrm{FE} = \text{wet weight gain (g)} / \text{dry feed consumed (g)}.$$

$$\mathrm{Survival}\ (\%) = \text{final fish individuals} / \text{initial fish individual} \times 100.$$

$$\mathrm{FI}\ (\%\text{body weight day}^{-1}) = \text{total feed consumed (g)} / [\text{days} \times (\text{initial body weight (g)} + \text{final body weight (g)})/2] \times 100.$$

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

$$\mathrm{HSI}\ (\%) = \text{liver weight (g)} / \text{whole body weight (g)} \times 100.$$

$$\mathrm{VSI}\ (\%) = \text{viscera weight (g)} / \text{whole body weight (g)} \times 100.$$

$$\mathrm{IFR}\ (\%) = \text{intraperitoneal fat weight (g)} / \text{wet body weight (g)} \times 100.$$

$$\mathrm{CF} = \text{wet body weight (g)} / \text{total length}^3\ (\text{cm}) \times 100.$$

$$\mathrm{PR}\ (\%) = 100 \times (\text{final body weight (g)} \times N_f - \text{initial body weight (g)} \times N_i) / (I_d \times N_d)$$

where I_d is feed intake in dry matter; N_d, N_f, and N_i represent protein contents in diet, final, and initial fish body, respectively.

Measurement of enzymatic activities

The activities of pepsin and lipase in the stomach; trypsin, amylase, and lipase activities in the liver and anterior intestine; LPL activity in abdominal adipose tissue; the Na^+/K^+-ATPase, alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT), and creatine kinase (CK) activities in the anterior intestine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities in the liver and serum; total protein (TP) and total cholesterol (TC) levels in the serum; and liver glycogen level were determined with a spectrophotometry. The test kits (pepsin, trypsin, amylase, lipase, LPL, Na^+/K^+-ATPase, γ-glutamyl transferase, creatine kinase, transaminase, triglyceride, total cholesterol, and glycogen) for these assays were provided by Nanjing Jiancheng Bioengineering Institute.

Determination of apparent digestibility coefficients

The apparent digestibility coefficient (ADC) determination followed the method described by Cho and Kaushik (1990), with chromic oxide (1% dry basis) as the digestibility marker. The ADCs for dry matter, protein, and lipid for the different experimental diets were calculated using the following equations:

$$\text{ADC of dry matter (\%)} = (1 - \%\text{chromium in feed}/\%\text{chromium in feces}) \times 100.$$

$$\text{ADC of nutrient (\%)} = (1 - (\%\text{chromium in feed}/\%\text{chromium in feces}) \times (\%\text{nutrient in feces}/\%\text{nutrient in feed})) \times 100.$$

Statistical analysis

Results are presented as mean ± SE ($n = 3$). All data were subjected to one-way ANOVA. Where there were significant differences ($P < 0.05$), the group means were further compared with Tukey's multiple range test. All statistical analyses were performed using SPSS 20.0 (USA).

Results

Growth performance and feed utilization

Fish from all groups were fed actively on the experimental diets. The results of different growth parameters are presented in Table 2. The WG, SGR, PER, PR, and the FE of the fish fed with D3 diet improved significantly ($P < 0.05$) while the poorest growth performance was observed in fish that received D12 diet. FI of the fish on D15 diet was significantly higher than that of the other groups ($P < 0.05$).

The nutrient digestibility of the experimental diets is presented in Table 3. The ADC of the dry matter and the crude protein of fish from group D3 were significantly higher, while the ADC of the crude lipid of the group D15 was significantly lower than that of the control group ($P < 0.05$).

Fish body composition

Final body composition of the fish did not show significant variation among the test and control groups, except for that of fish fed with D15 diet, which exhibited significantly lower hepatic lipid content (Table 4), and their HSI lower than that of the control group ($P < 0.05$) (Table 2).

Digestive and absorptive enzymes activities

The activity of pepsin and lipase in stomach as well as the activities of the pancreatic enzymes including trypsin, lipase, and amylase in the liver and the anterior intestinal amylase activities of the fish were not significantly affected by different levels of dietary *G. lemaneiformis* supplementation ($P > 0.05$) (Table 5). The activity of lipase in anterior intestine decreased when the supplemented level of *G. lemaneiformis* was over 12% ($P < 0.05$) (Table 5). The activities of Na^+/K^+-ATPase, ALP, and γ-GT in anterior intestine of D3 group were significantly higher than that of D15 group ($P < 0.05$); however, the activity of CK in the anterior intestine of the fish was not significantly affected by the *G. lemaneiformis* experimental diets ($P > 0.05$) (Fig. 1).

Nutrient metabolism indices

The activity of LPL in the abdominal adipose tissue of fish in D3 group, AST activity in the liver, and the serum total cholesterol of fish that received diets D3 and D6 were greatly higher ($P < 0.05$) than those fed with control diets. The ALT activity in the liver and the TC and TG levels in serum of fish in the D3 group were greatly higher than that of D15 group ($P < 0.05$). Compared to the control group, the hepatic glycogen of fish in groups D3 and D6 was greatly increased (Table 6).

Discussion

An enhancement of growth performance in terms of WG and SGR was observed with 3% *G. lemaneiformis* supplementation in red sea bream *P. major*. The current results are comparable to those previously reported where diets of red sea bream (*P. major*) were supplemented with 3% *Porphyra yezoensis* or 5% *Ascophyllum nodosum* and *Undaria pinnatifida* (Mustafa

Table 2 Growth performance, feed utilization, and morphological measurements of juvenile red sea bream fed the experiment diets with different levels of GL

Items	Diets					
	D0	D3	D6	D9	D12	D15
Initial BW (g)	7.83 ± 0.07	7.77 ± 0.05	7.85 ± 0.10	7.88 ± 0.11	8.01 ± 0.08	7.81 ± 0.10
Final BW (g)	34.04 ± 0.48ab	36.70 ± 0.48c	35.73 ± 0.32bc	35.24 ± 0.53abc	34.03 ± 0.28ab	33.45 ± 0.43a
WG (%)	335.14 ± 3.87a	373.26 ± 6.89b	355.37 ± 5.29ab	347.09 ± 9.56ab	325.64 ± 7.40a	328.17 ± 3.02a
SGR (% day^{-1})	2.62 ± 0.01ab	2.77 ± 0.03c	2.71 ± 0.02bc	2.68 ± 0.04abc	2.58 ± 0.03a	2.60 ± 0.01ab
Survival (%)	97.33 ± 1.33	98.67 ± 1.33	97.33 ± 2.67	97.33 ± 2.67	98.67 ± 1.33	96.00 ± 2.31
FE (%)	0.73 ± 0.01ab	0.83 ± 0.03c	0.79 ± 0.01bc	0.79 ± 0.02bc	0.75 ± 0.02abc	0.67 ± 0.01a
PER	1.50 ± 0.02ab	1.69 ± 0.05c	1.63 ± 0.02bc	1.62 ± 0.03bc	1.53 ± 0.03b	1.38 ± 0.01a
PR (%)	27.76 ± 0.64ab	33.23 ± 0.92c	31.48 ± 1.26bc	29.67 ± 0.89bc	28.44 ± 0.91b	24.14 ± 0.21a
FI (%)	3.04 ± 0.03a	2.81 ± 0.08a	2.89 ± 0.03a	2.86 ± 0.04a	2.95 ± 0.07a	3.29 ± 0.02b
HSI (%)	2.24 ± 0.02b	2.13 ± 0.04ab	2.13 ± 0.03ab	2.18 ± 0.01ab	2.12 ± 0.01ab	2.07 ± 0.04a
VSI (%)	6.97 ± 0.06	6.92 ± 0.18	7.00 ± 0.05	6.98 ± 0.12	6.86 ± 0.06	6.69 ± 0.14
IFR (%)	1.81 ± 0.05	1.80 ± 0.10	1.77 ± 0.03	1.81 ± 0.04	1.79 ± 0.10	1.80 ± 0.06
CF (g cm^{-3})	1.55 ± 0.14	1.53 ± 0.04	1.55 ± 0.10	1.54 ± 0.02	1.52 ± 0.09	1.51 ± 0.07

Values represent mean ± SE ($n = 3$). Values in the same line with different lowercase letters are significantly different ($P < 0.05$)

et al. 1995; Mustafa and Nakagawa 1995). Feed utilization is generally considered one of the major factors that affect fish growth (Valente et al. 2006). In fact, fish fed with the lower *G. lemaneiformis* supplemented diets, especially at 3%, exhibited significantly higher FE and ADC of dry matter and crude protein than those of the control group, which implies inclusion of 3% *G. lemaneiformis* to fish diets might significantly improve efficiency in absorption and assimilation of dietary nutrients. At the same time, it is reasonable to assume that the presence of bioactive substances in *G. lemaneiformis*, in addition to macronutrients, such as proteins and lipids, might be responsible for stimulating the growth of the fish. For example, non-starch polysaccharides (NSPs) may serve as potential prebiotics (Jia et al. 2009; Xie et al. 2018) while phycobiliprotein has potential antioxidant activity (Zhang et al. 2005; Sfriso et al. 2018).

It has been reported that inclusion of different seaweeds, i.e. *Cystoseira barbata* (Azaza et al. 2008), *Ulva lactuca* (Güroy et al. 2007), *Ulva rigida* (Valente et al. 2006), and *Gracilaria cornea* (Wassef et al. 2005) decreased growth performance and feed utilization in fish at supplement level of 10%. In the present study, when the supplemented level of *G. lemaneiformis* was up to 12%, the growth performance was reduced slightly than that of the control group, as indicated by the WG index. The decrease in lipid digestibility and activity of the absorptive-related enzymes might be responsible for the decreased growth performance of the fish in D15 group, although the decrease in growth performance was not significant compared to the control group. This observation might be attributed to the fact that the red sea bream, which itself is capable of ingesting algae, hence, it has a good adaptability to *G. lemaneiformis* (Nakagawa 1997).

Lipid digestibility can be strongly affected by anti-nutrients (Samarakoon and Jeon 2012). In the present study, a reverse relationship was found between dietary *G. lemaneiformis* content and apparent digestibility of crude fat when the level of supplemented *G. lemaneiformis* was above 9%. The main reasons for the anti-nutritive effects might be that the soluble

Table 3 Apparent digestibility coefficients of dry matter, crude protein and crude lipid of juvenile red sea bream fed the experiment diets with different levels of GL (%)

Items	Diets					
	D0	D3	D6	D9	D12	D15
Dry matter (%)	66.26 ± 0.24ab	69.20 ± 0.33c	67.78 ± 0.52bc	64.70 ± 0.55a	65.35 ± 0.28a	65.12 ± 0.29a
Crude protein (%)	84.44 ± 0.47a	88.07 ± 0.51c	87.95 ± 0.21c	86.80 ± 0.46bc	84.94 ± 0.40ab	83.93 ± 0.62a
Crude lipid (%)	89.54 ± 0.59bc	90.26 ± 0.51c	90.36 ± 0.38c	89.16 ± 0.58abc	87.57 ± 0.67ab	86.84 ± 0.37a

Values represent mean ± SE ($n = 3$). Values in the same line with different lowercase letters are significantly different ($P < 0.05$)

Table 4 Proximate composition of the whole body, muscle and liver in juvenile red sea bream fed the experimental diet for 8 weeks (% wet weight)

Items	Diets					
	D0	D3	D6	D9	D12	D15
Whole body composition (% wet weight)						
Moisture	68.68 ± 0.38	67.53 ± 0.39	67.77 ± 0.55	68.68 ± 0.52	68.58 ± 0.51	68.91 ± 0.24
Protein	17.69 ± 0.20ab	18.67 ± 0.19b	18.31 ± 0.47ab	17.58 ± 0.35ab	17.72 ± 0.40ab	16.95 ± 0.25a
Lipid	10.02 ± 0.10ab	10.30 ± 0.06b	9.81 ± 0.13ab	9.80 ± 0.10ab	9.66 ± 0.10a	9.61 ± 0.12a
Ash	4.11 ± 0.03	4.19 ± 0.06	4.25 ± 0.04	4.17 ± 0.07	4.27 ± 0.07	4.29 ± 0.06
Muscle composition (% wet weight)						
Moisture	75.76 ± 0.14	76.03 ± 0.03	76.20 ± 0.12	76.23 ± 0.15	76.02 ± 0.11	76.03 ± 0.06
Protein	19.98 ± 0.28	20.17 ± 0.16	20.04 ± 0.32	19.83 ± 0.37	20.07 ± 0.22	19.92 ± 0.37
Lipid	1.65 ± 0.04	1.66 ± 0.04	1.56 ± 0.04	1.56 ± 0.04	1.53 ± 0.02	1.52 ± 0.01
Liver composition (% wet weight)						
Moisture	52.26 ± 0.53a	54.73 ± 0.54ab	56.14 ± 1.50ab	56.38 ± 1.18ab	56.58 ± 0.74ab	57.03 ± 0.69b
Protein	14.79 ± 0.31	13.32 ± 0.32	12.59 ± 0.51	12.87 ± 0.68	13.51 ± 0.42	13.78 ± 0.53
Lipid	26.74 ± 0.25c	25.55 ± 0.24bc	24.60 ± 0.65bc	24.13 ± 0.63ab	23.59 ± 0.50ab	22.26 ± 0.42a

Values represent mean ± SE ($n = 3$). Values in the same line with different lowercase letters are significantly different ($P < 0.05$)

NSPs of *G. lemaneiformis*, which have gelatinous properties, form a physical barrier that hinders the diffusion of dietary lipids into the intestinal mucosa cells thereby decreasing the rate of triglyceride hydrolysis (Pasquier et al. 1996). The negative effect of *G. lemaneiformis* on the apparent digestibility of crude fat by *Acanthopagrus schlegelii* fed with diet containing 20% *G. lemaneiformis* was demonstrated by Xuan et al. (2013).

Mustafa et al. (1995) found that feeding red algae *Porphyra yezoensis* increased lipid reserves, especially for muscle and intraperitoneal fat body ratio of red sea bream. In this study, the body composition of fish among the treatment groups was not greatly influenced by the *G. lemaneiformis* diets; however, the hepatic lipid content and the HSI of fish in the D15 group were significantly decreased, with little effect found on VSI and IFR. This observation is similar to the results reported by Xuan et al. (2013), who found that the effect of *G. lemaneiformis* diets on the lipid content of fish was apparently related more to the lipid content of the liver than to the adipose tissue around the intestine. It is believed that the effects of dietary algae on lipid metabolism might vary according to the algae species and the growth stage of the particular fish (Mustafa et al. 1995; Morshedi et al. 2018); thus, the reduced hepatic lipid content may partially mirror the decrease in lipid digestibility, which was mentioned above.

Table 5 The activities of the digestive enzymes in juvenile red sea bream after fed the experimental diets for 8 weeks

Items	Diets					
	D0	D3	D6	D9	D12	D15
Stomach (U mg^{-1}prot)						
Protease ($\times 10^{-3}$)	198.04 ± 3.26	188.34 ± 5.40	194.98 ± 6.23	185.36 ± 5.38	179.09 ± 4.58	177.66 ± 4.59
Lipase ($\times 10^{-3}$)	20.97 ± 0.92	21.59 ± 0.97	21.16 ± 0.96	20.70 ± 0.56	21.79 ± 0.78	19.35 ± 0.69
Liver (U mg $prot^{-1}$)						
Protease ($\times 10^{-3}$)	85.78 ± 3.11	92.19 ± 2.66	89.56 ± 2.94	92.68 ± 1.79	86.17 ± 3.71	90.06 ± 1.99
Lipase ($\times 10^{-1}$)	42.26 ± 1.19	44.06 ± 1.09	43.46 ± 1.08	44.56 ± 0.89	41.61 ± 0.68	44.02 ± 1.14
Amylase ($\times 10^{-2}$)	144.02 ± 2.72	144.63 ± 1.96	141.25 ± 2.23	144.11 ± 2.25	138.98 ± 2.41	140.63 ± 1.63
Anterior intestinal (U mg^{-1} prot)						
Protease ($\times 10$)	103.03 ± 1.35	106.18 ± 0.61	102.46 ± 1.91	100.92 ± 1.19	100.73 ± 1.95	100.26 ± 0.56
Lipase	63.68 ± 1.61b	63.14 ± 1.49b	64.25 ± 0.81b	61.48 ± 1.39ab	60.11 ± 1.54ab	56.63 ± 1.02a
Amylase ($\times 10^{-1}$)	209.18 ± 6.65	201.28 ± 4.99	203.31 ± 5.91	216.71 ± 3.91	210.77 ± 5.53	202.91 ± 5.65

Values represent mean ± SE ($n = 3$). Values in the same line with different lowercase letters are significantly different ($P < 0.05$)

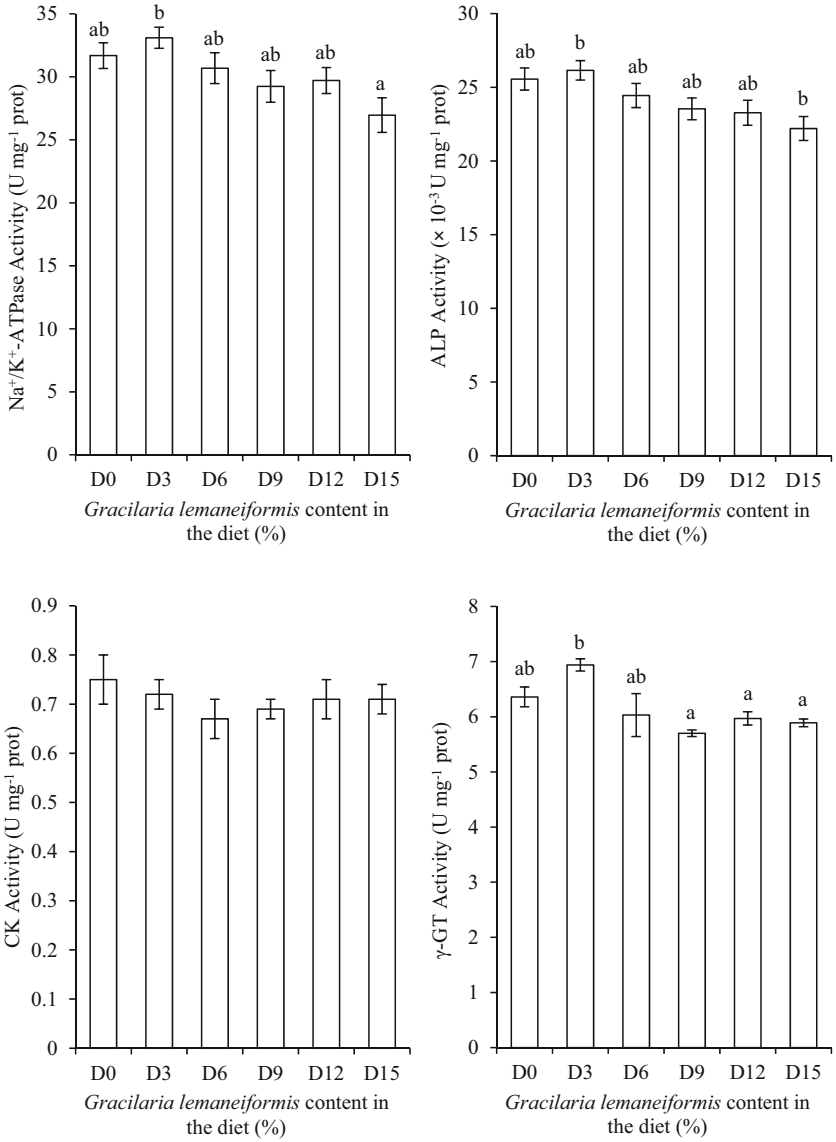

Fig. 1 The activities of absorptive enzyme (Na^+/K^+-ATPase sodium–potassium adenosine triphosphatase, ALP alkaline phosphatase, CK creatine kinase, γ-GT γ-glutamyl transferase) in anterior intestine of juvenile red sea bream fed with the experimental diets for 8 weeks. Different lowercase letters on the similar bars indicate significance limits ($P < 0.05$). Values represent mean ± SE ($n = 3$)

To the best of our knowledge, few studies have examined the enzymes activities related to the digestive–absorptive function of fish fed with seaweed-based diets (Xuan et al. 2013; Zhu et al. 2017; Wang et al. 2018). Digestive enzymes are mainly synthesized and secreted in the pancreas (Zambonino Infante and Cahu 2001). In the present study, the activities of pepsin and pancreatic enzymes in the liver of fish were not influenced by the *G. lemaneiformis* diets, which suggest that the secretion of digestive enzymes was not influenced by *G. lemaneiformis* supplementation. The digestive enzyme activity in the intestine is a comprehensive representation of a dynamic process involving secretion and turnover of enzymes (Schneeman and Gallaher 1980), so the decreased lipase activities in the intestine of the fish in the 12% and 15% groups could be associated with the dietary fiber content of *G. lemaneiformis*, which may reduce the activity of the digestive enzymes through special characteristics such as ionic interaction, matrix restriction, water and oil holding capacity (Schneeman 1978). However, data on anti-nutritional or anti-physiological factors of *G. lemaneiformis* are virtually non-existent at present, and the exact reason for the reduced lipase activity needs to be further examined.

The Na^+/K^+-ATPase which is localized in the basolateral membrane of enterocytes plays an important role in nutrient transport in the small intestine (Gal-Garber et al. 2003). CK catalyzes the transfer of phosphate to creatine in an ATP-dependent manner, and therefore play a role in energy transfer in tissues with high and fluctuating energy demands (Tang et al. 2009). In the present study, the activities of Na^+/K^+-ATPase were significantly decreased when the *G. lemaneiformis* supplementation level was above 12%, suggesting that the intestinal uptake of nutrients by active transport across the brush border of the intestine may be reduced, and the physiological mechanism needs to be further studied.

Table 6 Hematological characteristics and activities of metabolic related enzymes of red sea bream fed with the experimental diets for 8 weeks

Items	Diets					
	D0	D3	D6	D9	D12	D15
Adipose tissue						
LPL (U mg^{-1}prot)	3.93 ± 0.07a	4.38 ± 0.09b	4.06 ± 0.09ab	4.10 ± 0.13ab	3.94 ± 0.10ab	3.99 ± 0.07ab
Liver						
AST (U mg^{-1}prot)	56.72 ± 1.03a	65.10 ± 1.22b	63.67 ± 1.28b	60.99 ± 1.60ab	61.16 ± 0.82ab	57.67 ± 0.69a
ALT (U mg^{-1}prot)	24.49 ± 0.17ab	26.02 ± 0.50b	25.16 ± 0.48ab	24.86 ± 0.72ab	24.16 ± 0.31ab	23.37 ± 0.28a
LPL (U mg^{-1}prot)	1.25 ± 0.04	1.36 ± 0.03	1.29 ± 0.04	1.34 ± 0.04	1.28 ± 0.04	1.27 ± 0.03
Hepatic glycogen (mg g^{-1})	61.52 ± 1.18a	67.06 ± 0.64b	67.42 ± 1.00b	62.76 ± 1.21ab	62.48 ± 0.88ab	61.48 ± 1.30a
Serum						
TP (g prot dL^{-1})	4.01 ± 0.07	4.07 ± 0.09	3.87 ± 0.06	4.06 ± 0.11	3.93 ± 0.06	3.91 ± 0.10
TC (mg dL^{-1})	129.50 ± 1.50a	140.31 ± 1.91b	139.83 ± 1.29b	131.98 ± 1.80ab	130.91 ± 2.41a	127.62 ± 1.81a
TG (mg dL^{-1})	129.37 ± 2.18ab	129.64 ± 3.02b	122.67 ± 2.43ab	120.55 ± 1.91ab	120.30 ± 0.99ab	119.62 ± 1.16a
GLU (mg dL^{-1})	59.53 ± 1.01	61.11 ± 1.09	60.46 ± 0.81	58.85 ± 1.30	60.87 ± 1.12	59.37 ± 0.83
AST (U L^{-1})	182.40 ± 1.84	180.31 ± 3.07	176.91 ± 3.71	178.27 ± 3.94	177.72 ± 3.70	179.81 ± 2.50
ALT (U L^{-1})	56.98 ± 0.57	56.32 ± 0.96	56.26 ± 0.77	55.69 ± 1.23	57.18 ± 1.44	56.17 ± 0.78

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *TP* total protein, *TC* total cholesterol, *TG* triglyceride, *GLU* glucose, *LPL* lipoprotein lipase

Values represent mean ± SE ($n = 3$). Values in the same line with different lowercase letters are significantly different ($P < 0.05$)

The liver is the main organ that plays an important role in many metabolic processes of nutrients. Aminotransferases of the liver such as AST and ALT, which are important and critical for the Kreb's cycle, are often considered as an indicator for protein metabolism (Shakoori et al. 1994; Yousef et al. 2002). In this study, the activity of AST in the liver of fish fed with 3 and 6% *G. lemaneiformis* diets increased greatly. Although the activity of ALT was not significantly affected, the trends shown by the treatment groups were similar to those of AST activity. The higher PER and PR of the 3% group could at least in part be due to effective anabolism of proteins. It is well known that liver glycogen is a readily available source of energy. The hepatic glycogen in fish fed with 3 and 6% *G. lemaneiformis* diets were greatly increased, which seems to suggest that energy metabolism was stimulated in fish in the D3 and D6 groups. The growth performance of fish fed 3 and 6% *G. lemaneiformis* diets could partly be attribute to more efficient metabolism of nutrients and energy. Active ingredients such as phycobiliprotein and agar polysaccharide in *G. lemaneiformis* might be responsible for the effective stimulus of metabolism (Chen et al. 2005b).

It is known that lipid absorption and transportation in fish were similar to that of mammals (Ostos Garrido et al. 1993) and that LPL is a glycoprotein enzyme which hydrolyzes triglycerides, generating free fatty acids that serve as either direct energy source or for storage (Auwerx et al. 1992). In this study, the significantly elevated activity of LPL in the adipose tissue of the fish in D3 group implied an improvement in lipid metabolism. Thus, when taking the great increase in WG into account, it is plausible to conclude that addition of 3% *G. lemaneiformis* to diets is beneficial, as it promotes lipid metabolism for better growth performance.

In the present study, 3 and 6% *G. lemaneiformis* supplementation in diets markedly elevated the serum TC levels of fish. The elevated serum TC could be related with endogenous cholesterol biosynthesis and/or better assimilation of cholesterol (Nakagawa et al. 2007; Sathivel et al. 2008). Wong et al. (1999) have reported that some seaweed, such as *Ecklonia cava*, *Colpomenia sinuosa*, and *Sargassum hemiphyllum* elevated serum cholesterol levels of rats due to increase in endogenous synthesis of cholesterol in the liver. In view of the better WG of D3 and D6 groups, the increased plasma cholesterol levels might reflect an increased disease resistance capacity, as proposed by Nakagawa et al. (2007).

Conclusion

In conclusion, the present study revealed that the incorporation of *G. lemaneiformis* in diets at the level of 3% is beneficial for red sea bream, as it improved growth performance and physiological state. It is also possible to add up to 15% *G. lemaneiformis* to diets, as lipid deposition in the liver of fish would be reduced without adverse effects on growth performance in terms of WG. The incorporation of *G. lemaneiformis* in fish diets will reduce the consumption or reliance on fish meal, given the increasing scale in the culture of red sea bream. Since the dietary fiber of

G. lemaneiformis is the main anti-nutritional factors for fish, it remains to be determined if further processing on the *G. lemaneiformis* such as fermentation could be an alternative way to limit the negative effects of the algal fiber.

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