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Research Article

Evaluation of Green Alga *Ulva Pertusa* as a Dietary Ingredient for Rabbitfish *Siganus Canaliculatus* Juveniles

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Abstract

The green alga *Ulva pertusa* has not been well used and their decomposition may result in heavy environmental pollution along the coast. This study aimed at evaluating the feasibility of using *U. pertusa* as a dietary ingredient for rabbitfish *Siganus canaliculatus*. Six formulated feeds with 32% of total protein and 8% of lipid but different levels of dried *U. pertusa* meal were prepared. Diet 1 without seaweed was used as control diet. Diets 2-4 contained 5%, 10% or 15% seaweed, respectively. Diets 5 and 6 contained 10% or 15% seaweed supplemented with 0.2% non-starch polysaccharide enzymes (NSP enzymes). After the rabbitfish juveniles were fed the six diets for 8 weeks, the growth performance and feed utilization efficiency of fish fed diets containing seaweed were inferior to fish fed the control diet, especially in fish fed diet with 15% seaweed. However, there was no difference in growth when comparing fish fed diet with 10% seaweed plus NSP enzymes with the control. Moreover, there was no significant difference in survival rate or whole body composition among the groups. The liver DHA and n-3 PUFA contents increased when the algal inclusion level was 10% or 15%. In addition, the liver superoxide dismutase (SOD) and catalase (CAT) activities in fish fed diet 3 increased whereas the liver malondialdehyde (MDA) contents in fish fed diets 2-4 and 6 decreased. These results indicate that the dietary inclusion of *U. pertusa* at levels up to 10% may be used, without significant negative effects on growth performance and body composition. The incorporation of seaweed in diets may also improve the antioxidant capacity of rabbitfish.

Keywords: *Siganus canaliculatus*; *Ulva Pertusa*; NSP Enzymes; Antioxidant Capacity

Introduction

Macroalgae including Florideophyceae, Phaeophyceae and Chlorophyceae are characterised by high contents of carbohydrate (generally 50-60%), a certain amount of protein (generally less than 20%), low fat level (generally less than 5%), various free amino acids, polyunsaturated fatty acids (PUFA), vitamins, minerals and unknown growth hormones [1-2]. They are the sources of raw materials for foods, pharmaceuticals and feeds [1, 3-4]. As a dietary ingredient for aquatic animals, macroalgae can enhance immunity and anti-virus capability, promote growth, improve meat quality and attract feeding [2, 5-6]. There are abundant resources of macroalgae in China. At present, only a few species of the red and brown algae are commonly used as human foods or for extracting industrial agar [3-4, 7]. The low-value green algae like *Enteromorpha* spp., *Ulva* spp. and *Chaetomorpha linum* and the rejected parts of the red algae *Gracilaria lemaneiformis* and *Porphyra haitanensis* have not been well utilized, leading to the waste of natural resources and serious environmental pollution due to their decomposition [8]. It is necessary to find a new way of fully utilizing these macroalgal resources.

Currently, carnivorous fishes are the main species of marine fishes farmed in the world. These fishes are highly dependent on dietary animal protein to grow. Their diets are mainly composed of chilled trash fish and a small amount of formulated feed with high levels of fish meal and fish oil. This poses certain disadvantages including destruction of natural fishery resources, environment pollution, high cultivation cost, etc. On the contrary, herbivorous fishes may directly utilize plant protein and are little dependent on animal protein than carnivorous fishes in diets, and thus can use macroalgae as food or as a dietary ingredient. In this way, a large amount of low-value and rejected macroalgal resources can be effectively utilized, and farming costs will be greatly reduced, rendering fish farming to be conducive to improving and protecting the environment. The Food and Agriculture Organization of the United Nations also highly recommends the development of farming herbivorous fishes [1,9].

The herbivorous rabbitfish *Siganus canaliculatus* (alias *Siganus oramin*) inhabits widely the coral reefs of Indo-Pacific region and mainly consumes algae and seagrasses. It is tender in meat and rich in PUFA [10], thus making this fish becoming an increasingly important culture species in southeastern Asia including the coast of southeast China in recent years [11].

Ulva pertusa belongs to the *Ulva* genus, Ulvaceae family in the Chlorophyta phylum, abundant in the coastal areas of China [12]. It contains rich carbohydrates, proteins, PUFA, minerals, vitamins and other nutrients [12]. Fresh *U. pertusa* is often used to feed abalone and other aquatic animals directly in China. But it is limited to small consumption and seasonal supply. While *U. pertusa* could be developed as a dietary ingredient for fish, its resource can be more effectively and comprehensively utilized. However, the majority of carbohydrate in seaweeds is algal polysaccharides, which is mainly non-starch polysaccharide (NSP) and is difficult to be digested by animals, and thus the inclusion level of seaweed in diets is limited, generally no more than 15% for fish [1]. Enzyme preparations such as non-starch polysaccharide enzymes (NSP enzymes) are usually used as a feed additive, to degrade the antinutritional factor of algal polysaccharides and increase digestive efficiency of feed for aquatic animals [13-14].

In this study, six diets with different inclusion levels of macroalgae meal (*U. pertusa*) (5%-15%) were prepared for rabbitfish, and one commercial product of NSP enzymes was additionally added to enhance the effect of rabbitfish digesting seaweed feeds. We compared the growth performance as well as the physiological and biochemical composition of various groups in order to evaluate the feasibility of utilizing *U. pertusa* as a dietary ingredient for rabbitfish. The results highlight an effective way of utilizing green alga *U. pertusa* resources and provide a basis for the development of a low-cost formulated feed for rabbitfish.

Materials and methods

Experimental diets

According to our previous study [15-16], six isonitrogenous (32% protein) and isolipid (8% lipid) formulated feeds with different inclusion levels of *U. pertusa* meal were made. Diet 1 without seaweed was used as control. Diets 2-4 were supplemented with 5%, 10% or 15% seaweed, respectively. Diets 5 and 6 were supplemented with 10% or 15% seaweed plus 0.2% NSP enzymes (Guangdong Zhaoqing Huafen Feed Enzymes Co., China.), respectively, and the dose of NSP enzymes was determined according to Chen (2009) [14], to aid improvement of algal digestive efficiency. The formulation and proximate composition of the six experimental feeds are shown in Table 1. Fresh *U. pertusa* was collected from the coastal waters near Nan Ao Island (23°23'33"-23°29'11"N, 116°56'24"-117°08'59"E) in eastern Guangdong Province, China. After washing with filtered seawater, they were sun-dried, finely ground and sieved through 60 mesh with a small mill. The alga meal was then thoroughly mixed with the other ingredients. The moistened mixture was pelleted through a laboratory pelletizer equipped with a 2mm die (model SLP-45, Fishery Machinery and Instrument Research Institute, Chinese Academy of Fishery Sciences), and the extruder temperature was controlled no more than 80°C during this process. After natural drying, the pellets were packaged and stored at -20°C until use. About 20g of each diet was sampled for biochemical composition analysis.

Experimental fish and feeding conditions

Juvenile rabbitfish *Siganus canaliculatus* were captured from the sea area near Nan Ao Marine Biology Station (NAMBS) of Shantou University. Before starting the experiment, fish were acclimatized to laboratory conditions with self-prepared feeds for 3 weeks. The feeding experiment was carried out in 200 L cylindrical tanks (70 cm Φ) equipped with a continuous recirculating seawater system in NAMBS for a period of 8 weeks from December 22, 2011 to February 16, 2012. The initial fish with an approximately average body weight of 15g were selected and randomly allocated into eighteen tanks (13 fish per tank). Each treatment was conducted in triplicate tanks. Fish were fed to apparent satiation two times a day (respectively at 8:30a.m. and 16:30p.m.). During the experimental period, water temperature was maintained at 22±0.5 ºC with constant aeration and natural photoperiod. Every morning, half of the aquarium water was replaced, and the faecal matter was removed with an auto-discharge device.

Evaluation of growth performance and sample collection

At the beginning and the end of the growth experiment, all fish were starved for 24h and weighed after anaesthetization with 0.01% 2-phenoxyethanol (Sigma-Aldrich, St. Louis, MO, USA) for evaluating growth performance and feed utilization efficiency according to the following formulae:

$$\text{Weight gain rate (WGR) (\%)} = \frac{W_t - W_0}{W_0} \times 100$$

$$\text{Specific growth rate (SGR) (\%)} = \frac{LnW_t - LnW_0}{n} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{total amount of diet}}{W_t - W_0}$$

$$\text{Protein efficiency rate (PER)} = \frac{W_t - W_0}{\text{total amount of diet} \times \text{protein content in diet}}$$

$$\text{Survival rate (SUR) (\%)} = \frac{\text{final fish quantity}}{\text{initial fish quantity}} \times 100$$

Where, W_0 and W_t indicate the initial and final weight of the fish in the growth experiment (g) and n indicates the number of experimental days.

After the final fish weight was determined, two fish were sampled from each tank for whole body composition analysis. Liver and muscle tissues were also obtained from two additional fish from each tank and stored at -80°C until use for determination of fatty acid composition and antioxidant indexes.

Determination of biochemical indexes

Proximate composition analysis on feed ingredients, experimental feeds and fish body were performed according to the method by Xu et al. (2012) [16]. The content of dry matter or moisture was determined by drying at 105°C overnight in a drying oven. The protein content was expressed as 6.25 times of total nitrogen content as determined by the Kjeldahl method. Crude lipid content was measured by Soxhlet's extraction. Ash content of the dry samples was determined by combusting in a muffle furnace at 550°C for 16h to constant weight.

For fatty acid composition analysis, lipids were extracted from diets and liver and muscle tissues of fish by the chloroform-methanol method as previously described [10,16]. The lipid fraction was subsequently methylated with boron trifluoromethanol (Sigma-Aldrich, St. Louis, MO, USA). Fatty acid methyl esters (FAME) were determined with a gas chromatograph (GC-17A; Shimadzu, Kyoto, Japan) equipped with an auto-sampler and a hydrogen flame ionization detector (FID). Individual fatty acids were identified by comparison with known commercial standards (Sigma, USA) and quantified with a CLASS-GC10 GC workstation (Shimadzu, Kyoto, Japan).

Antioxidant indexes in liver including superoxide dismutase (SOD) and catalase (CAT) activities and malondialdehyde (MDA) content were determined with kits purchased from Nanjing Jiancheng Bioengineering Institute, China.

Statistical analysis

All data were presented as mean values ± SEM (n=3 or 6), One-way ANOVA in combination with Tukey's test was used to find the difference among different treatments using the software *Origin* 7.0 (OriginLab, Northampton, MA). Statistical significance was accepted when P<0.05.

Results

Survival and growth performance

After an 8-week feeding experiment, data on survival and growth performance of rabbitfish fed the six diets are shown in Table 2. The supplementation of *U. pertusa* meal in the diets did not affect survival. However, weight gain rate (WGR) and specific growth rate (SGR) were significantly reduced only when the inclusion level of *U. pertusa* was up to 15% (P<0.05). The WGR in fish fed diet with 15% seaweed reduced by over 30%, whereas an almost equal WGR with the control group was observed in fish fed diet containing a lower inclusion level of 10% seaweed in the presence of 0.2% NSP enzymes (P>0.05). The fish fed 10% seaweed (diet 3 and diet 5) showed the lowest feed conversion ratio (FCR) which was not significantly different with that of the control group (P>0.05), whereas the fish fed 15% seaweed (diet 4 and diet 6) showed a higher FCR (P<0.05), regardless of presence of NSP enzymes. The protein efficiency rate (PER) only in fish fed 10% seaweed (diet 3 and diet 5) displayed no significant difference with the control group (P>0.05).

Table 2. Growth performance of S. canaliculatus fed the six experimental diets for 8 weeks*

	Dietary groups (supplementation level of seaweed)					
	Diet1 (0%)	Diet 2 (5%)	Diet 3 (10%)	Diet 4 (15%)	Diet 5 (10%+enzyme)	Diet 6 (15%+enzyme)
Initial weight (g)	15.9 ± 0.2	15.9 ± 0.2	16.0 ± 0.1	16.0 ± 0.2	15.8 ± 0.1	16.0 ± 0.1
Final weight (g)	43.3 ± 0.5^{a}	40.4 ± 1.5^{ab}	42.0 ± 1.9^{ab}	36.3 ± 0.9^{b}	42.8 ± 2.6^{ab}	38.2 ± 0.8^{ab}
WGR (%)	172 ± 2.1^{a}	154 ± 9.5^{ab}	164 ± 10.9^{ab}	127 ± 6.5^{b}	171 ± 13.4^{a}	139 ± 6.7^{ab}
SGR (%)	1.8 ± 0^{a}	1.7 ± 0.1^{ab}	1.7 ± 0.1^{ab}	1.5 ± 0.1^{b}	1.8 ± 0.1^{a}	1.6 ± 0.1^{ab}
FCR	1.3 ± 0.0^{b}	1.5 ± 0^{ab}	1.4 ± 0^{ab}	1.6 ± 0.1^{a}	1.4 ± 0^{ab}	1.5 ± 0.1^{a}
PER	2.3 ±0^{a}	2.0 ± 0^{b}	2.1 ± 0^{ab}	1.9 ± 0.1^{b}	2.1 ± 0.1^{ab}	2.0 ± 0.1^{b}
SUR (%)	97.4 ± 4.4	87.2 ± 4.4	94.9 ± 8.9	82.1 ± 11.8	87.2 ± 22.2	92.3 ± 13.3

*Data are mean ± SEM (n=3), which in the same row without sharing a common superscript indicate a significant difference (P<0.05).

WGR, weight gain rate; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency rate; SUR, survival rate; SEM, standard error of means.

Whole-body composition

The inclusion of *U. pertusa* in diets showed no great influence on biochemical composition of rabbitfish. As shown in Table 3, moisture, crude protein, crude lipid and ash showed no significant difference among different treatments (P>0.05).

	Dietary groups (supplementation level of seaweed)					
	Diet 1 (0%)	Diet 2 (5%)	Diet 3 (10%)	Diet 4 (15%)	Diet 5 (10%+enzyme)	Diet 6 (15%+enzyme)
Body composition (%)*						
Moisture	71.8 ± 1.0	71.8 ± 0.5	71.8 ± 1.3	71.3 ± 0.8	71.8 ± 0.6	71.5 ± 1.1
Crude protein	16.2 ± 0.5	16.9 ± 0.3	16.5 ± 0.4	16.1 ± 0.4	16.0 ± 0.1	16.4 ± 0.1
Crude lipid	7.7 ± 0.7	7.5 ± 0.7	7.4 ± 1.1	7.4 ± 1.2	7.7 ± 0.6	7.5 ± 0.9
Crude ash	3.7 ± 0	3.7 ± 0.1	3.9 ± 0.2	3.5 ± 0.1	3.7 ± 0.2	3.5 ± 0.1

Fatty acid composition of the tissues

Dietary inclusion of *U. pertusa* exerted some impacts on fatty acid composition in the liver and muscle of rabbitfish. As shown in Tables 4 and 5, the saturated fatty acids (SFA) were mainly 16:0, the monounsaturated fatty acids (MUFA) were mainly 18:1n-9, the n-6 polyunsaturated fatty acids (n-6 PUFA) were mainly composed of 18:2n-6, and docosahexaenoic acid (DHA) accounted for the main part of n-3 polyunsaturated fatty acids (n-3 PUFA). In the liver, fish fed diet 3 (10% seaweed) and diet 4 (15% seaweed) had higher DHA and n-3 PUFA contents than the control group (P<0.05). Moreover, there was no significant difference in muscle fatty acid composition between treatments, although a slightly higher DHA content was observed in seaweed groups (diets 2-6) (P>0.05).

Table 4. Main fatty acids in liver of *S. canaliculatus* fed the six experimental diets for 8 weeks (%area)*

Main fatty acids (%)	Dietary groups (supplementation level of seaweed)					
	Diet 1 (0%)	Diet 2 (5%)	Diet 3 (10%)	Diet 4 (15%)	Diet 5 (10%+enzyme	Diet 6 (15%+enzyme)
14:0	2.1 ± 0	2.1 ± 0.1	2.5 ± 0.2	2.0 ± 0.1	1.9 ± 0	2.1 ± 0
16:0	37.9 ± 0.7	39.5 ± 2.2	36.7 ± 1.7	35.0 ± 2.6	34.3 ± 2.0	34.6 ± 0.3
18:0	8.6 ± 0.5	8.5 ± 0.9	6.1 ± 0.6	7.5 ± 0.4	7.4 ± 0.2	7.3 ± 0.3
∑SFA	49.3 ± 0.2	50.3 ± 2.9	45.8 ± 1.2	44.6 ± 2.8	44.1 ± 1.4	44.6 ± 0.6
16:1n-9	8.8 ± 0.4	8.6 ± 1.1	10.9 ± 2.1	7.0 ± 0	9.0 ± 0.7	9.1 ± 0.1
18:1n-9	26.7 ± 0.4	27.3 ± 1.9	26.7 ± 0.4	27.3 ± 0.1	29.0 ± 1.4	29.0 ± 0.5
18:1n-7	4.1 ± 0.1	4.3 ± 0.4	4.2 ± 0.3	3.6 ± 0.1	4.9 ± 0	4.5 ± 0.2
20:1n-9	0.8 ± 0	1.0 ± 0.1	0.6 ± 0.1	1.4 ± 0.1	1.1 ± 0.3	1.1 ± 0.1
24:1n-9	1.2 ± 0.2	2.2 ± 0.4	1.5 ± 0.3	2.8 ± 0.9	1.4 ± 0.3	1.2 ± 0.2
∑MUFA	37.4 ± 0.6	39.1 ± 2.7	39.6 ± 1.7	38.5 ± 0.7	40.5 ± 0.7	40.4 ± 0.4
18:2n-6	4.7 ± 0.4^{b}	5.6 ± 0.8^{b}	6.6 ± 0.4^{b}	9.1 ± 0.6^{a}	5.6 ± 0.7^{b}	5.6 ± 0.1^{b}
18:3n-6	1.3 ± 0.2	1.5 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	1.0 ± 0.1	1.1 ± 0.2
20:2n-6	0.5 ± 0	0.6 ± 0	0.6 ± 0	0.6 ± 0	0.6 ± 0	0.6 ± 0
20:3n-6	0.8 ± 0.1	1.3 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.1 ± 0	1.2 ± 0
20:4n-6	0.6 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0
∑n-6 PUFA	8.0 ± 0.6^{b}	9.8 ± 0.8^{ab}	10.2 ± 0.4^{ab}	12.9 ± 0.6^{a}	9.3 ± 0.7^{ab}	9.3 ± 0.2^{ab}
20:5n-3 (EPA)	0.1 ± 0	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0	0.2 ± 0	0.2 ± 0
22:5n-3	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0
22:6n-3 (DHA)	2.2 ± 0.10^{c}	3.0 ± 0.1^{bc}	3.9 ± 0.5^{ab}	5.4 ± 0.1^{a}	3.0 ± 0.2^{bc}	3.4 ± 0.2^{bc}
∑n-3 PUFA	2.8 ± 0.2^{c}	3.8 ± 0^{b}	5.0 ± 0.7^{ab}	6.6 ± 0^{a}	3.9 ± 0.3^{bc}	4.4 ± 0,2^{bc}

* Data are mean ± SEM (n = 6), which in the same row without sharing a common superscript indicate a significant difference (P<0.05).

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic; DHA, docosahexaenoic acid.

Table 5. Main fatty acids in muscle of *S. canaliculatus* fed the six experimental diets for 8 weeks (% area)*

Main fatty acids (%)	Dietary groups (supplementation level of seaweed)					
	Diet 1 (0%)	Diet 2 (5%)	Diet 3 (10%)	Diet 4 (15%)	Diet 5 (10%+enzyme)	Diet 6 (15%+enzyme)
14:0	2.3 ± 0.1	2.3 ± 0.1	2.1 ± 0	2.5 ± 0	2.3 ± 0.1	2.2 ± 0.2
16:0	27.3 ± 1.0	27.0 ± 0.8	26.3 ± 0.4	27.3 ± 0.2	27.3 ± 0.5	24.9 ± 0.9
18:0	6.8 ± 0.2	6.6 ± 0.3	6.8 ± 0.2	6.5 ± 0.5	6.7 ± 0.3	6.6 ± 0.6
22:0	0.5 ± 0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0	0.5 ± 0.2	0.4 ± 0.2
∑SFA	38.0 ± 1.3	37.2 ± 1.0	36.8 ± 0.5	36.9 ± 0.3	37.5 ± 0.3	36.8 ± 1.6
16:1n-9	5.8 ± 0.5	6.5 ± 0.2	5.4 ± 0.4	6.3 ± 0.7	6.1 ± 0.3	5.7 ± 0.8
18:1n-9	20.4 ± 0.9	18.9 ± 0.4	19.4 ± 1.2	20.6 ± 1.4	20.1 ± 0.7	19.4 ± 1.5
18:1n-7	3.2 ± 0.2	3.0 ± 0.2	3.1 ± 0.1	3.4 ± 0.2	3.3 ± 0.1	3.2 ± 0.3
20:1n-9	0.9 ± 0	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0	0.9 ± 0	0.9 ± 0.1
24:1n-9	0.9 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	1.0 ± 0.2	0.8 ± 0.2	0.8 ± 0.1
∑MUFA	31.2 ± 2.0	30.0 ± 0.5	30.0 ± 0.7	32.3 ± 1.3	31.3 ± 1.4	30.0 ± 3.4
18:2n-6	18.2 ± 0.8	17.4 ± 1.2	17.5 ± 0.8	17.7 ± 0.9	17.3 ± 0.6	17.6 ± 0.4
18:3n-6	1.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
20:2n-6	0.8 ± 0	0.7 ± 0	0.7 ± 0	0.6 ± 0	0.7± 0.1	0.8 ± 0.1
20:3n-6	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.7 ± 0	0.8 ± 0.1	0.8 ± 0
20:4n-6	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	1.0 ± 0.3
∑n-6 PUFA	21.9 ± 0.7	21.0 ± 0.9	21.1 ± 0.8	20.7 ± 1.1	20.7 ± 0.6	21.0 ± 0.6
18:3n-3	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0	1.9 ± 0.1
20:5n-3 (EPA)	0.9 ± 0	1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.2
22:5n-3	2.3 ± 0	2.6 ± 0.1	2.6 ± 0.1	2.5 ± 0.2	2.6 ± 0.1	2.6 ± 0.2
22:6n-3 (DHA)	4.3 ± 0.2	5.1 ± 0.4	4.7 ± 0.1	4.4 ± 0.6	4.5 ± 0.4	5.2 ± 0.6
∑n-3 PUFA	9.2 ± 0.1	10.5 ± 0.5	10.1 ± 0.2	9.7 ± 0.9	10.0 ± 0.4	10.8 ± 1.0

* Data are mean ± SEM (n = 6), which in the same row without sharing a common superscript indicate a significant difference (P<0.05).

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic; DHA, docosahexaenoic acid.

Table 6. Antioxidant indexes in liver of *S. canaliculatus* fed the six experimental diets for 8 weeks*

	Dietary groups (supplementation level of seaweed)					
	Diet 1 (0%)	Diet 2 (5%)	Diet 3 (10%)	Diet 4 (15%)	Diet 5 (10%+enzyme)	Diet 6 (15%+enzyme)
SOD	16.2 ± 0.6^{b}	18.2± 1.1^{ab}	22.1 ± 0.9^{a}	15.4 ± 1.6^{b}	19.5 ± 0.8^{ab}	17.5 ± 0.4^{ab}
CAT	62.5 ± 23.6^{bc}	55.9 ± 3.0^{bc}	133.6 ± 10.3^{a}	43.6 ± 18.9^{c}	125 ± 10.8^{ab}	159 ± 3.3^{a}
MDA	16.2 ± 0.7^{a}	3.3 ± 1.4^{b}	1.8 ± 0.1^{b}	2.5 ± 0^{b}	13.6 ± 0.3^{a}	3.1 ± 0.8^{b}

* Data are mean ± SEM (n = 6), which in the same row without sharing a common superscript indicate a significant difference (P<0.05).

SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde. The units of SOD and CAT are U mg protein $^{-1}$, and the unit of MDA is nmol mg protein $^{-1}$.

Antioxidant indexes of the tissues

The effects of dietary alga on liver antioxidant indexes of rabbitfish are shown in Table 6. The SOD activity in fish fed diet 3 (10% seaweed) was significantly higher than that of the control group (P<0.05). The CAT activity in fish fed diet 6 (15% seaweed plus NSP enzymes) displayed the highest value followed by that in fish fed diet 3 (10% seaweed). These two groups showed higher CAT activities than the control group (P<0.05). The MDA content in fish fed diet 2 (5% seaweed), diet 3 (10% seaweed), diet 4 (15% seaweed) and diet 6 (15% seaweed plus NSP enzymes) showed a lower level than the control group (P<0.05). These results indicated that the diet with 10% seaweed increased the SOD and CAT activities and decreased the MDA content in the liver of rabbitfish.

Discussion

As macroalgae contain abundant carbohydrates, proteins, PUFA, vitamins, minerals, etc, they can be used as a feed additive or dietary ingredient source for aquatic animals [1-2]. Many studies have shown that incorporation of 1%-5% macroalgae in the feeds can promote growth, improve body color and enhance disease resistance and anti-stress capability of aquatic animals [1,17]. However, due to the high contents of crude fiber and ash in macroalgae (ash content of 32.66% and crude fiber content of 4.66% in *U. pertusa* in Table 1), the inclusion of macroalgae in the feeds may lead to poor palatability and decreased digestibility. It is suggested not to exceed 10% -15% in the diets of fish [1]. In this study, the supplementation of *U. pertusa* in the diets of rabbitfish made a few increment in the ash content among them (Table 1), triggering a higher FCR than the control group without *U. pertusa*, especially when the inclusion level is up to 15% (Table 2). Moreover, the supplementation of 15% of *U. pertusa* in the diet made an obvious decrease in WGR and SGR, whereas a lower inclusion level of *U. pertusa* up to 10% showed no significant difference with the control group, even nearly equal growth performance was obtained when 0.2% NSP enzymes were additionally added (Table 2). So, the suggested inclusion level of *U. pertusa* in the diets of rabbitfish is about 10%.

Theoretically, herbivorous fish should have a stronger capacity of digesting algal feed than carnivorous or omnivorous fish. El-Tawil (2010) [18] reported that the inclusion level of *Ulva spp*. in the diets of the red tilapia (*Oreochromis niloticus)* could be up to 15%. Moreover, it was reported that the supplementation of *U. rigida* in the diets of rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*) could reach 10%, respectively [19-20]. In this study, the inclusion level of *Ulva* meal in the diets of herbivorous rabbitfish *S. canaliculatus* was not higher than those of the carnivorous rainbow trout and European sea bass, maybe due to the differences in the feed formulae used in the different assays. For example, the addition of fish meal in the formulated feed of rainbow trout was up to 50% [19] and it was only 29% for rabbitfish in the present study, and other ingredients were also different. Nutrients, especially amino acid balance, may affect growth performance. In further studies, it is necessary to optimize the feed formula of rabbitfish for increasing the inclusion level of *U. pertusa* in the formulated feed.

Table 1. Ingredients and composition of experimental diets for *Siganus canaliculatus*

	Diet (supplementation level of seaweed)					
	Diet 1 (0%)	Diet 2 (5%)	Diet 3 (10%)	Diet 4 (15%)	Diet 5 (10%+enzyme)	Diet 6 (15%+enzyme)
Ingredients (g/100 g)						
Fish meal [a]	29	29	29	29	29	29
Soybean meal [b]	26	24	22	20	22	20
Fish oil	1.7	1.7	1.7	1.7	1.7	1.7
Soybean oil	3.4	3.4	3.4	3.4	3.4	3.4
α-Starch	5	5	5	5	5	5
Wheat starch	22.8	19.8	16.8	13.8	16.8	13.8
U. pertusa [c]	0	5	10	15	10	15
Vitamin mixture [d]	1	1	1	1	1	1
Mineral mixture [e]	1	1	1	1	1	1
Cellulose	9	9	9	9	8.8	8.8
Choline chloride	0.08	0.08	0.08	0.08	0.08	0.08
Vitamin C	0.02	0.02	0.02	0.02	0.02	0.02
NSP enzymes [f]	0	0	0	0	0.2	0.2
DL-Methionine	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (%, dry matter basis)						
Dry matter	94	93	93	93	92	92
Crude protein	34	33	32	34	34	34
Crude lipid	9	9	8	9	8	9
Ash content	11	13	15	15	14	15
Main fatty acids (% area)						
14:0	2.9	3.1	2.9	2.9	2.8	3.0
16:0	18.6	20.2	20.1	20.0	19.9	20.3
18:0	5.3	6.0	6.5	7.0	6.6	6.6
16:1n-9	3.4	4.2	3.3	3.3	3.3	3.4
18:1n-9	19.9	20.2	21.3	20.7	21.1	21.4
18:1n-7	2.4	2.5	2.6	2.4	2.5	2.7
18:2n-6	32.7	29.9	30.0	30.6	30.1	29.6
18:3n-3	4.1	3.7	3.8	3.9	3.7	3.8
20:5n-3（EPA）	5.1	4.7	4.6	4.8	4.3	4.6
22:6n-3（DHA）	5.1	4.9	5.1	4.9	5.6	5.0
∑SFA	27.1	29.3	29.6	29.8	29.4	29.9
∑MUFA	27.2	26.7	27.0	26.1	27.0	27.1
∑n–3 PUFA	14.3	14.2	13.5	13.6	13.6	13.4
∑n–6 PUFA	32.7	29.2	30.0	30.6	30.1	29.6

a The crude protein and lipid content of fish meal are 66% and 10%;

b The crude protein content of soybean meal is 49%;

c The dry matter, crude protein, lipid, fiber and ash content of *U. pertusa* are 17%, 18%, 0.4%, 5% and 3 respectively;

d The amounts of following vitamins in per kg of premix were: A, 1×10^{6} IU; D$_{3}$, 3×10^{5}IU; E, 5,000 IU; 1,040mg; B$_{1}$, 1,500mg; B$_{2}$, 2,400mg; B$_{6}$, 1,200mg; B$_{12}$, 5mg; nicotinic acid, 8,000mg; D-calcium pantothen 3,200mg; folic acid, 400mg; biotin, 10mg; inositol, 12,000 mg; C-monophopholipid, 16,000mg

e The amounts of following ingredients in per kg of premix were: iron, 10g; zinc, 3.2g; manganese, 3g; cob
52mg; iodine, 65mg; selenium, 15mg

f The main ingredients of NSP enzyme are cellulase, beta dextranase and xylanase, and their relat
proportions are not provided in the description.

EPA, eicosapentaenoic; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fa
acids; PUFA, polyunsaturated fatty acids.

As an efficient biological catalyst, NSP enzymes can improve feed utilization, eliminate anti-nutritional factors and promote animal growth, and have been applied in aquaculture [21]. Algal polysaccharides are main non-starch polysaccharide (NSP) and are not digestible by fish [22]. The supplementation of NSP enzymes in the feed can not only help to decompose the soluble NSP, reducing its anti-nutritional characteristics, but also contribute to destroy plant cell wall, promote the release of protein, fat and other nutrients wrapped in the cell wall and increase the full contact between intestinal digestive enzymes and the nutrients [23]. In the present study, it was shown that the addition of 0.2% NSP enzymes could promote the utilization of *U. pertusa* by rabbitfish. For example, fish fed diet 5 (10% seaweed plus NSP enzymes) and diet 6 (15% seaweed plus NSP enzymes) showed slightly higher WGR and SGR than fish fed diet 3 (10% seaweed) and diet 4 (15% seaweed), respectively. Especially, almost equal WGR and SGR with those of the control group were obtained when the supplementation of *U. pertusa* was 10% in the presence of NSP enzymes. So, the additional supplementation of 0.2% NSP enzymes in the diet containing 10% of *U. pertusa* for rabbitfish is better accepted.
In this study, although the fish fed diets 2, 3, 4, 5 and 6 had slightly higher protein content and slightly lower lipid content than the control group, the analysis of body composition exhibited no significant difference among the different dietary treatments (Table 3). This response is as anticipated because all test diets were formulated to be isonitrogenous and isolipid. These results are also in agreement with our previous results [24].

Although macroalgae contain a low lipid level, they are an important source of PUFA [25]. Dietary algae can activate lipid metabolism especially lipolysis by accelerating the assimilation of ascorbic acid [26-27], leading to the release of free fatty acids and thus an increase of PUFA in fish fillet. Walker et al. (2009) [28] observed that addition of 30% macroalgae *porphyra* spp. in the diets of cod (*Gadus morhua*) could significantly increase the muscle arachidonic acid (ARA) content. Güroy et al. (2013) [19] reported that addition of 10% of *U. rigida* in the diets of rainbow trout could significantly increase the DHA and EPA contents in the muscle. In the present study, the diets with *U. pertusa* made an increment in the contents of EPA (except diet 4), DHA and n-3PUFA in the liver and muscle of rabbitfish, especially the liver DHA and n-3PUFA contents significantly increased when the inclusion lever of *U. pertusa* was 10% or 15% (Tables 4 and 5). As macroalgae *Ulva* are rich in PUFA especially in 18C PUFA [29-30], the high liver DHA content in fish fed diets with the higher inclusion level of *U. pertusa* (10% or 15%) indirectly proves that rabbitfish *S. canaliculatus* has a capacity of converting 18C PUFA into 20-22C PUFA (HUFA) [10]. As HUFA are mainly biosynthesized in the liver, they are then transferred to other organs to be further utilized. The fish muscle DHA content did not exhibit a significant increase as expected (Table 5), maybe due to lacking enough high DHA content in liver. After adding 0.2% NSP enzymes, the liver DHA and n-3 PUFA contents showed a significant decrease in the fish with 15% dietary algae (Table 4), but displayed no significant difference with the control group. These results indicate that the dietary inclusion of *U. pertusa* up to 10%-15% may have no negative effects on tissue fatty acid composition for rabbitfish.

Normal physiological and biochemical processes in organisms will generate many unstable reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydroxyl radicals (•OH) and hydrogenperoxide (H_2O_2), etc. However, if these excessive oxygen free radicals cannot be removed in time, they will react rapidly with the unsaturated acid chains of cell membrane and lead to lipid peroxidation, resulting in an increase of membrane permeability and cell or tissue damage ultimately [31-32]. The defensive system including CAT and SOD in the body can timely remove excessive oxygen free radicals and keep body healthy, and MDA is the final product of lipid peroxidation. So, their levels may reflect the antioxidant capacity of animals on a centain extent.

Macroalgae contain many natural antioxidant substances such as polyphenlics, carotenoid, vitamin E and C, and polysaccharides, etc [33], especially algal polysaccharides which are the major ingredient of carbohydrate can effectively scavenge superoxide and hydroxyl radicals and prevent oxidative damage [34]. In this study, the SOD and CAT activities in fish fed diet with 10% of *U. pertusa* were significantly higher than those in the control group, and the MDA contents in them reduced significantly (Table 6). These results indicate that the addition of 10% of *U. pertusa* in diets will improve the capability of resistance to oxidative damage for rabbitfish. However, the MDA contents increased abnormally after supplementation of 0.2% NSP enzymes (Table 6). This result shows no accordance with the previous reports which indicated that the low molecular weight ulvan would have stronger antioxidant activity after degradation [35-36]. The reasons remain unknown.

In general, taking into account the growth performance and biochemical indexes of rabbitfish, the addition of *U. pertusa* in the formulated feed of rabbitfish may reach 10%, and the additional supplementation of 0.2% NSP enzymes contributes to improve growth performance. The addition of *U. pertusa* in the feed can improve the antioxidant capacity and increase the DHA content in the liver of rabbitfish. This study can promote comprehensive utilization of the resources of low-value green alga *U. pertusa* and are conducive to the development of effective and low-cost formulated feeds for promoting the cultiva-

tion of herbivorous fish including rabbitfish.

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