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Effects of dietary *Spirulina platensis* on growth performance, hematological and serum biochemical parameters, hepatic antioxidant status, immune responses and disease resistance of Coral trout *Plectropomus leopardus* (Lacepede, 1802)

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ABSTRACT

The present study investigated the effects of dietary *Spirulina platensis* supplementation on growth performance, hematological and serum biochemical parameters, hepatic antioxidant status, immune responses and resistance to the pathogen infection in Coral trout *Plectropomus leopardus*. The fish were fed for 8-week with diets containing different levels of *S. platensis*: 0% (C), 2% (SP2), 4% (SP4), 6% (SP6), 8% (SP8) and 10% (SP10) as treatment groups, followed by a *Vibrio harveyi* infection test for 14 d. The study indicated that dietary supplementation with *Spirulina platensis* could significantly improve growth performance, and the highest weight gain rate (WGR) and specific growth rate (SGR) were observed in group SP10 ($P < .05$). Red cell count (RBC), white cell count (WBC), hemoglobin (Hb) and total antioxidant capacity (T-AOC) in the *S. platensis* supplemented groups were significantly higher than those of group C ($P < .05$). However, the levels of cholesterol, triglyceride and malondialdehyde (MDA) contents, and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities decreased with the increasing of dietary *S. platensis* levels. Compared with group C, the lysozyme (LYZ) and respiratory burst activities (RBA), and immunoglobulin (Ig) and complement contents in group SP4, SP6, SP8 and SP10 increased significantly than those of group C respectively ($P < .05$). After challenge with *V. harveyi*, the survival rate in group SP4, SP6, SP8 and SP10 was significantly higher than that of group C, and the highest survival rate was in group SP10 ($P < .05$). These results indicated that *P. leopardus* fed a diet supplemented with *S. platensis* (especially at 10%) could significantly promote its growth performance, improve its hepatic antioxidant status, and enhance its immune ability and resistance to *V. harveyi* infection.

1. Introduction

Coral trout *Plectropomus leopardus* is an important economical marine fish species in China [1]. Recent years, due to its successful development of technique for fry artificial breeding, *P. leopardus* aquaculture has developed rapidly and widely along the southern coast of China in tropical and subtropical climates [2]. However, when farmed *P. leopardus* suffers from stress conditions such as high rearing density and poor water quality, it is more susceptible to several pathogens than its wild counterpart. Diseases occurred more frequently resulting in serious economic losses to Province Hainan, especially between July and September every year [3,4]. Therefore, antibiotics, vaccines and some immunostimulants have been used to prevent disease outbreaks in *P. leopardus* aquaculture [5]. However, the intensive use of antibiotics to control the bacterial diseases in aquaculture has led to an increase in antibiotic resistance which is a threat to human health, and the application of vaccines is quite expensive [6,7]. Additionally, antibiotics can kill beneficial intestinal microbial flora and interfere the innate ecosystem which affects the physiology and immunity of fish [8,9]. To avoid such adverse situation, ecofriendly immunostimulants

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such as microalgae and probiotics have been studied to improve the growth performance, immune responses and disease resistance of aquatic animals [10–12].

Spirulina platensis is a helicoidal, unbranched and filamentous cyanobacterium that composes of various nutritional components, such as antioxidant pigments, essential amino acids, essential fatty acids, vitamins and minerals [13,14]. In previous studies, many researchers have confirmed that supplementation of *S. platensis* has positive effects on growth performance, skin pigmentation and body composition in different fish species, such as Guppy *Poecilia reticulata* [15], African Sharptooth Catfish *Clarias gariepinus* [16], Golden Pompano *Trachinotus ovatus* [17] and Queen loach *Botia dario* [18]. In recent years, *S. platensis* received more attention mainly as it can improve immunity and disease resistance of Nile Tilapia *Oreochromis niloticus* [19,20], Great Sturgeon *Huso huso* [21] and Olive Flounder *Paralichthys olivaceus* [22].

However, little is known about the effects of *S. platensis* on growth performance, antioxidant abilities, immunity and disease resistance for *P. leopardus*. Therefore, the purpose of the present study was to evaluate the effects of dietary *S. platensis* on growth performance, hematological and serum biochemical parameters, hepatic antioxidant status, immune responses and disease resistance to the pathogen infection of *P. leopardus*, which would encourage its use as a potential immunostimulant to feed additive.

2. Materials and methods

2.1. Experimental diets

Since the crude protein level of *S. platensis* and soy protein concentrate was nearly similar [17], the dietary soy protein concentrate was substituted for the alga. Six diets were formulated to contain 0% (C), 2% (SP2), 4% (SP4), 6% (SP6), 8% (SP8) and 10% (SP10) *S. platensis* (Yuequn Ocean Biological Research Development Co., Guangdong, China). Ingredients and proximate compositions of the experimental diets were presented in Table 1. Ingredients were weighed according to the feed formulation, and were thoroughly mixed for 10 min. Fish oil was then added to the other ingredients, and then sieved through a 425 μm sieve. All ingredients containing fish oil were mixed for another 10 min then distilled water was added and stirred for 5 min. The 2.0 mm and 2.5 mm diameter pellets were wet-extruded by a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China), and then air-dried, sealed in plastic bags and stored frozen at −20 °C until experiment started.

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

Items	C	SP2	SP4	SP6	SP8	SP10
Ingredients						
Fish meal	46	46	46	46	46	46
Soy protein concentrate	16	14	12	10	8	6
Peanut meal	11.5	11.5	11.5	11.5	11.5	11.5
Wheat flour	15	15	15	15	15	15
Brewers yeast	5	5	5	5	5	5
Fish oil	3	3	3	3	3	3
Soy lecithin	1	1	1	1	1	1
Mineral premix[a]	1	1	1	1	1	1
Vitamin premix[b]	1	1	1	1	1	1
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
S. platensis	0	2	4	6	8	10
Total	100	100	100	100	100	100
Proximate composition (% dry matter)						
Crude protein	54.55	54.51	54.44	54.33	54.19	54.13
Crude lipid	7.92	7.98	8.05	8.09	8.11	8.15
Ash	7.14	7.03	7.17	7.12	7.17	7.34
Moisture	9.42	9.48	9.39	9.41	9.33	9.45

[a] Mineral premix provides the following per kg of diet: NaF 4 mg, KI 1.6 mg, $CoCl_2{\cdot}6H_2O$ (1%) 100 mg, $CuSO_4{\cdot}5H_2O$ 20 mg, $FeSO_4{\cdot}H_2O$ 160 mg, $ZnSO_4{\cdot}H_2O$ 100 mg, $MnSO_4{\cdot}H_2O$ 120 mg, $MgSO_4{\cdot}7H_2O$ 2.4 g, $Ca(H_2PO_4)_2{\cdot}H_2O$ 6.0 g, NaCl 200 mg, and zeolite powder 30.90 g.

[b] Vitamin premix provides the following per kg of diet: Vitamin B_1 25 mg, Vitamin B_2 45 mg, Vitamin B_6 20 mg, Vitamin B_{12} 0.1 mg, Vitamin K_3 10 mg, inositol 800 mg, pantothenic acid 60 mg, nicotinic acid 200 mg, folic acid 1.2 mg, biotin 32 mg, Vitamin D_3 5 mg, Vitamin E 120 mg, Vitamin C 2.0 g, choline chloride 2.0 g, ethoxyquin 150 mg, and manna-croup 14.52 g.

2.2. Fish rearing

Fish were obtained from a commercial hatchery located in Lingshui, Hainan Province, P. R. China. Prior to the feeding trial, fish were acclimated to the experimental conditions for two weeks, and fed with control diet during this period. At the beginning of the trial, fish were starved for 24 h, and then weighed after being anesthetized with 10 mg L^{-1} eugenol (1:10,000; Shanghai Reagent Corp., China). A total of 540 healthy fish with an initial body weight of 18.0 ± 2.0 g were chosen from 1000 fish and then randomly stocked into eighteen 150 L fiberglass tanks at a density of 30 fish per tank. Fish were fed by hand to an apparent satiation twice a day (08:00 and 17:00) for eight weeks. During feeding period, the number and weight of dead fish and the amount of feed consumed by fish were recorded. During the experimental period, water temperature ranged from 25 to 29 °C, salinity was 28–30‰, pH was 7.2–7.6, ammonia nitrogen was 0.08–0.15 mg L^{-1}, nitrite was 0.15–0.32 mg L^{-1}, and the dissolved oxygen was maintained at 6.4–8.6 mg L^{-1}.

2.3. Sample collection

At the end of the feeding period, fish were starved for 24 h, then anesthetized with 10 mg L^{-1} eugenol (1:10,000; Shanghai Reagent Corp., China). All fish populations and mean body weight in each cage were determined. The blood from three fish randomly sampled from each tank (9 fish per treatment) was sampled from the caudal vein using 2 ml heparinized syringe. Blood samples were immediately divided into two half parts. One half was transferred to a tube containing anti-coagulant (heparin) to study the respiratory burst assay and make the hematological analysis, while the other half was transferred to non-heparinized tubes for biochemical and immunological studies. Sera samples were obtained by blood centrifugation (3000 × *g* at 4 °C for 10 min) and stored at −80 °C until use [21]. The liver was excised, frozen in liquid nitrogen, and stored at −80 °C until analysis.

2.4. Growth performance

The parameters were calculated as per following formulae:

Weight gain rate (WGR, %) = 100 × (final body weight - initial body weight)/initial body weight; Specific growth rate (SGR, % day^{-1}) = 100 × [(ln final individual weight - ln initial individual weight)/number of days; Feed conversion ratio (FCR) = total diet fed/total wet weight gain; Condition factor (CF, g/cm^3) = 100 × (fish body weight)/(fish body length)^3; Survival rate (SR, %) = 100 × (final fish number/initial fish number).

2.5. Hematological and serum biochemical parameters analysis

Red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb) and hematocrit (Ht) were determined with the method described by Li et al. [23]. Serum triglyceride, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cortisol and glucose were analyzed using commercial assay kits (Jiancheng, Ltd., Nanjing, China), according to the manufacturer instructions.

2.6. Antioxidant capacity analysis

Hepatic samples were homogenized in ice-cold phosphate buffer

(1:10 dilution) (phosphate buffer: 0.064 M, pH 6.4). The homogenate was then centrifuged for 20 min (4 °C, 3000 × *g*) and aliquots of the supernatant were used to quantify hepatic total antioxidative capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activity and malondialdehyde (MDA) content. T-AOC was measured by the method described in the other study using commercial assay kits (Jiancheng, Ltd., Nanjing, China) [24]. SOD activity was determined by using a xanthine oxides [25]. One unit of SOD activity was calculated using the amount of superoxide dismutase required to inhibit the reduction of nitroblue tetrazolium by 50%. CAT activity was determined by measuring the decrease of H_2O_2 concentration [26]. One unit of CAT activity was defined as the amount of CAT required to transform 1 μmoL of H_2O_2 min^{-1}. GPX activity was determined following the method described by Flohé et al. [27]. Lipid-peroxidation levels were determined based on the MDA level generated by oxidizing fatty acids. In the presence of thiobarbituric acid, malondialdehyde started producing colored thiobarbituric-acid-reacting substances (TBARS) were measured at 532 nm [28].

2.7. Immunological assays

2.7.1. Lysozyme (LYZ) and respiratory burst activity (RBA) assays

The LYZ activity was determined using the turbidimetric assay [29] with commercial assay kits (Jiancheng, Ltd., Nanjing, China). The RBA of phagocytes was tested following the nitro-bluetetrazolium (NBT; Sigma, USA) assay described by Anderson et al. [30] with some modifications by Kumari and Sahoo [31]. Dimethylformamide was used as the blank, and the optical density of supernatant was measured at 540 nm.

2.7.2. Immunoglobulin (Ig) and complement assays

Ig and complement contents were determined using a commercial assay kits (Jiancheng, Ltd., Nanjing, China). The methods were described by Wu et al. [32] for activity analysis including the measurement of turbidity increase after the immunity response and the increase of its antibody.

2.8. Challenge test with Vibrio harveyi

Vibrio harveyi was obtained from South China Sea Fisheries Research Institute (Guangzhou, China). Bacteria were inoculated into 10 ml of liquid trypticase soy broth (TSB, Sigma) and cultured at 28 °C for 48 h, then centrifuged at 1000 rpm for 15 min. Supernatant was removed and the pelleted bacteria were washed twice in sterile phosphate buffered saline (PBS) solution. The concentration of bacteria was adjusted to 1.6×10^5 CFU ml^{-1} using a spectrophotometer. After 2 days of initial sampling, fish (20 per cage) were injected intraperitoneally with 0.1 ml bacterial suspension using sterile medical syringe [4]. All fish were fed with the assigned diets and kept under observation for 14 days to record any abnormal behavior and mortality. At the end of the challenge test, the survival rate (%) was calculated.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 19.0 software (SPSS Inc., Michigan Avenue, Chicago, IL, USA). Data were expressed as mean ± SD and subjected to a one-way ANOVA followed by Duncan's multiple range test. Significant differences were set $P < .05$.

3. Results

3.1. Effects of dietary S. platensis on growth performance in P. leopardus

Growth performance of fish fed with different experimental diets was shown in Table 2. Both WGR and SGR were apeared a dose-dependent increase, and the highest WGR and SGR were observed in 10% *S. platensis* enriched diet ($P < .05$). On the other hand, dose related decreasing was observed in FCR, and the highest decrease was detected in SP10 group. The CF ranged from 2.42 to 2.65, also the SR fluctuated from 95.65% to 97.41%. No significant variations were detected on the CF and SR among all treatments ($P > .05$).

3.2. Effects of dietary S. platensis on hematological and serum biochemical parameters in P. leopardus

As shown in Table 3, RBC, WBC and Hb increased as a consequence of dietary administration of *S. platensis* to *P. leopardus*. More concretely, the levels of RBC, WBC and Hb increased significantly in group SP4, SP6, SP8 and SP10 in contrast to group C ($P < .05$). Maximum values of RBC, WBC, and Hb were observed in group SP10. However, no significant differences were observed in Ht levels among all treatments ($P > .05$).

Serum biochemical parameters of fish fed with different experimental diets were shown in Table 4. Fish fed the control diet had the highest levels of cholesterol and triglyceride, and those levels decreased with the increasing of dietary *S. platensis* levels ($P < .05$). ALT, AST, cortisol and glucose were not affected by experimental diets ($P > .05$).

3.3. Effects of dietary S. platensis on hepatic antioxidant status in P. leopardus

Hepatic antioxidant status of fish fed with different experimental diets were shown in Table 5. T-AOC level increased with the increasing of dietary *S. platensis* levels, and the *S. platensis* supplemented groups was significantly higher than that of C group ($P < .05$). On the contrary, the levels of CAT, SOD, GPX and MDA decreased with the increasing of dietary *S. platensis* levels. The level of SOD in group C was higher than that of group SP6, SP8 and SP10 ($P < .05$). Compared with group C, the levels of CAT and GPX in group S8 and S10 decreased significantly ($P < .05$). Similarly, the level of MDA in group C was higher than that of group SP4, SP6, SP8 and SP10 ($P < .05$).

3.4. Effects of dietary S. platensis on LYZ, RBA, Ig and complement in P. leopardus

LYZ activity increased with the increasing of *S. platensis* levels, and the lowest was in group C ($P < .05$) (Fig. 1A). RBA levels in group SP4, SP6, SP8 and SP10 were higher than those of group C and SP2 ($P < .05$). However, no significant differences were observed among group SP6, SP8 and SP10 ($P > .05$) (Fig. 1B). Ig contents increased with dietary *S. platensis* level, with no significant differences among the treatments with over 4% *S. platensis*. Compared with group C, Ig contents significantly increased in group SP6, SP8 and SP10 ($P < .05$) (Fig. 1C). Complement contents increased with the increasing levels of *S. platensis*, and the highest increment was observed in group SP10 ($P < .05$) (Fig. 1D).

3.5. Effects of dietary S. platensis on resistance against V. harveyi infection in P. leopardus

The challenge test showed that the survival rate of fish increased with the increasing of dietary *S. platensis* levels. As shown in Fig. 2, the survival rates in group SP4, SP6, SP8 and SP10 were significantly higher than that of group C, and the highest was found in group SP10 ($P < .05$), but no significant differences between group C and SP2 ($P > .05$).

4. Discussion

During the feeding trial, all the experimental diets containing the *S. platensis* were well accepted by the experimental fish. The growth of fish showed increasing trends with 2–10% of the *S. platensis* supplemented,

Table 2
Effects of dietary *S. platensis* on growth performance in *P. leopardus*.

Growth parameters	Treatments					
	C	SP2	SP4	SP6	SP8	SP10
IBW(g)	18.21 ± 0.12	18.26 ± 0.22	18.19 ± 0.31	18.22 ± 0.34	18.15 ± 0.35	18.20 ± 0.28
FBW(g)	42.27 ± 3.84^{b}	42.52 ± 3.83^{b}	43.52 ± 2.18^{ab}	44.92 ± 4.56^{ab}	46.22 ± 2.45^{a}	48.72 ± 2.94^{a}
WGR(%)	132.13 ± 4.1^{b}	132.85 ± 3.20^{b}	139.25 ± 2.01^{b}	146.54 ± 3.51^{ab}	154.66 ± 4.22^{a}	167.69 ± 3.65^{a}
SGR(%/d)	1.40 ± 0.03^{b}	1.41 ± 0.02^{b}	1.45 ± 0.08^{ab}	1.52 ± 0.05^{ab}	1.58 ± 0.06^{a}	1.69 ± 0.05^{a}
FCR	1.64 ± 0.18^{b}	1.61 ± 0.09^{b}	1.55 ± 0.10^{ab}	1.41 ± 0.15^{ab}	1.42 ± 0.12^{ab}	1.35 ± 0.06^{a}
CF(%)	2.56 ± 0.21	2.64 ± 0.22	2.53 ± 0.23	2.42 ± 0.17	2.59 ± 0.21	2.65 ± 0.17
SR(%)	96.42 ± 1.21	95.65 ± 1.42	96.35 ± 2.01	96.52 ± 1.58	96.33 ± 1.63	97.41 ± 1.89

Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($P < .05$). IBW: initial body weight; FBW: final body weight; WGR: weight gain rate; SGR: specific growth rate; FCR: feed conversion ratio; CF: condition factor; SR: survival rate.

Table 3
Effects of dietary *S. platensis* on hematological parameters in *P. leopardus*.

Parameters	Treatments					
	C	SP2	SP4	SP6	SP8	SP10
RBC (10^9 mL^{-1})	1.83 ± 0.12^{a}	1.86 ± 0.22^{a}	1.97 ± 0.31^{b}	2.22 ± 0.34^{bc}	2.45 ± 0.35^{c}	2.50 ± 0.28^{c}
WBC (10^6 mL^{-1})	9.28 ± 0.14^{a}	9.32 ± 0.23^{a}	9.55 ± 0.18^{b}	9.62 ± 0.16^{bc}	9.82 ± 0.15^{c}	10.13 ± 0.21^{d}
Hb (g 100 mL^{-1})	5.51 ± 0.11^{a}	5.55 ± 0.15^{a}	5.64 ± 0.05^{b}	5.78 ± 0.21^{c}	5.83 ± 0.12^{cd}	5.96 ± 0.21^{d}
Ht (%)	43.22 ± 1.21	42.85 ± 1.42	43.35 ± 1.01	43.22 ± 1.58	43.41 ± 1.63	43.42 ± 1.89

Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($P < .05$). RBC: red blood cell; WBC: white blood cell; Hb: hemoglobin; Ht:: hematocrit.

Table 4
Effects of dietary *S. platensis* on serum biochemical parameters in *P. leopardus*.

Parameters	Treatments					
	C	SP2	SP4	SP6	SP8	SP10
Triglyceride (mmol L^{-1})	4.83 ± 0.12^{c}	4.66 ± 0.15^{bc}	4.17 ± 0.21^{b}	3.82 ± 0.22^{ab}	3.65 ± 0.15^{a}	3.53 ± 0.18^{a}
Cholesterol (mmol L^{-1})	4.51 ± 0.11^{c}	4.45 ± 0.15^{c}	4.24 ± 0.08^{b}	3.98 ± 0.21^{ab}	3.73 ± 0.13^{a}	3.66 ± 0.21^{a}
ALT (U L^{-1})	38.21 ± 2.11	37.82 ± 1.62	38.31 ± 1.21	39.24 ± 1.34	40.33 ± 1.23	41.12 ± 1.31
AST (U L^{-1})	53.22 ± 2.21	52.85 ± 3.42	54.35 ± 1.01	51.22 ± 1.58	54.41 ± 1.63	55.42 ± 1.89
Cortisol (ng ml^{-1})	68.35 ± 1.25	67.13 ± 2.26	68.43 ± 2.35	69.54 ± 2.43	70.81 ± 1.09	67.82 ± 1.71
Glucose (mg dL^{-1})	73.12 ± 2.67	71.95 ± 3.15	73.24 ± 2.16	74.35 ± 2.76	73.65 ± 2.35	71.11 ± 2.14

Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($P < .05$). ALT: alanine aminotransferase; AST: aspartate aminotransferase.

and significant differences were observed in WGR and SGR among SP10, SP8 and C groups ($P < .05$). In the present study, we observed a significant increase of WGR and SGR in fish fed diets supplemented with 10% *S. platensis* for 8 weeks. The result revealed that the fish improved growth performance when diet was supplemented with *S. platensis*, which was similar with previous studies in sturgeon *Huso huso* [21] and queen loach *Botia dario* [18]. *S. platensis* can enhance growth because it is a good source of protein for animal feed, being containing high amounts of essential fatty acids, vitamins and minerals [19]. Also, *S. platensis* can improve intestinal flora, digestive enzymes activity, breakdown of indigestible components, which also may be the responsible of the growth-enhancing effects [12].

The effects of *S. platensis* on hematological parameters of *P. leopardus* were surveyed in the present study. Regarding the levels of RBC, WBC and Hb, they were observed a dose-dependent increase, being found the highest increments in those fish fed 10% *S. platensis*. Similar to our results, the administration of *S. platensis* in diet significantly increased RBC and Hb of Great Sturgeon *Huso huso* [21]. *S. platensis* contains lots of iron and it has significant effects on erythropoiesis in anemic rats by improving the levels of RBC and Hb [33]. Thus the

Table 5
Effects of dietary *S. platensis* on hepatic T-AOC, SOD, CAT, GPX activities and MDA contents in *P. leopardus*.

Parameters	Treatments					
	C	SP2	SP4	SP6	SP8	SP10
T-AOC (U/mg)	0.52 ± 0.03^{a}	0.61 ± 0.02^{b}	0.68 ± 0.04^{bc}	0.74 ± 0.02^{c}	0.82 ± 0.04^{cd}	0.91 ± 0.03^{d}
SOD (U/mg)	82.21 ± 2.12^{c}	79.26 ± 1.22^{c}	73.19 ± 1.31^{bc}	66.22 ± 2.34^{b}	61.15 ± 1.35^{a}	55.20 ± 1.28^{a}
CAT (U/mg)	32.27 ± 1.14^{b}	29.52 ± 0.83^{b}	27.52 ± 0.48^{ab}	26.92 ± 0.56^{ab}	25.22 ± 0.45^{a}	24.72 ± 0.34^{a}
GPX (U/mg)	161.71 ± 4.1^{b}	142.22 ± 3.20^{b}	124.31 ± 2.01^{b}	115.67 ± 3.51^{ab}	107.67 ± 2.22^{a}	98.15 ± 2.65^{a}
MDA (nmol/mg)	9.42 ± 1.21^{c}	8.85 ± 1.42^{bc}	8.05 ± 2.01^{b}	7.52 ± 1.58^{ab}	6.83 ± 1.63^{a}	6.21 ± 1.89^{a}

Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($P < .05$). T-AOC: hepatic total antioxidative capacity; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase.

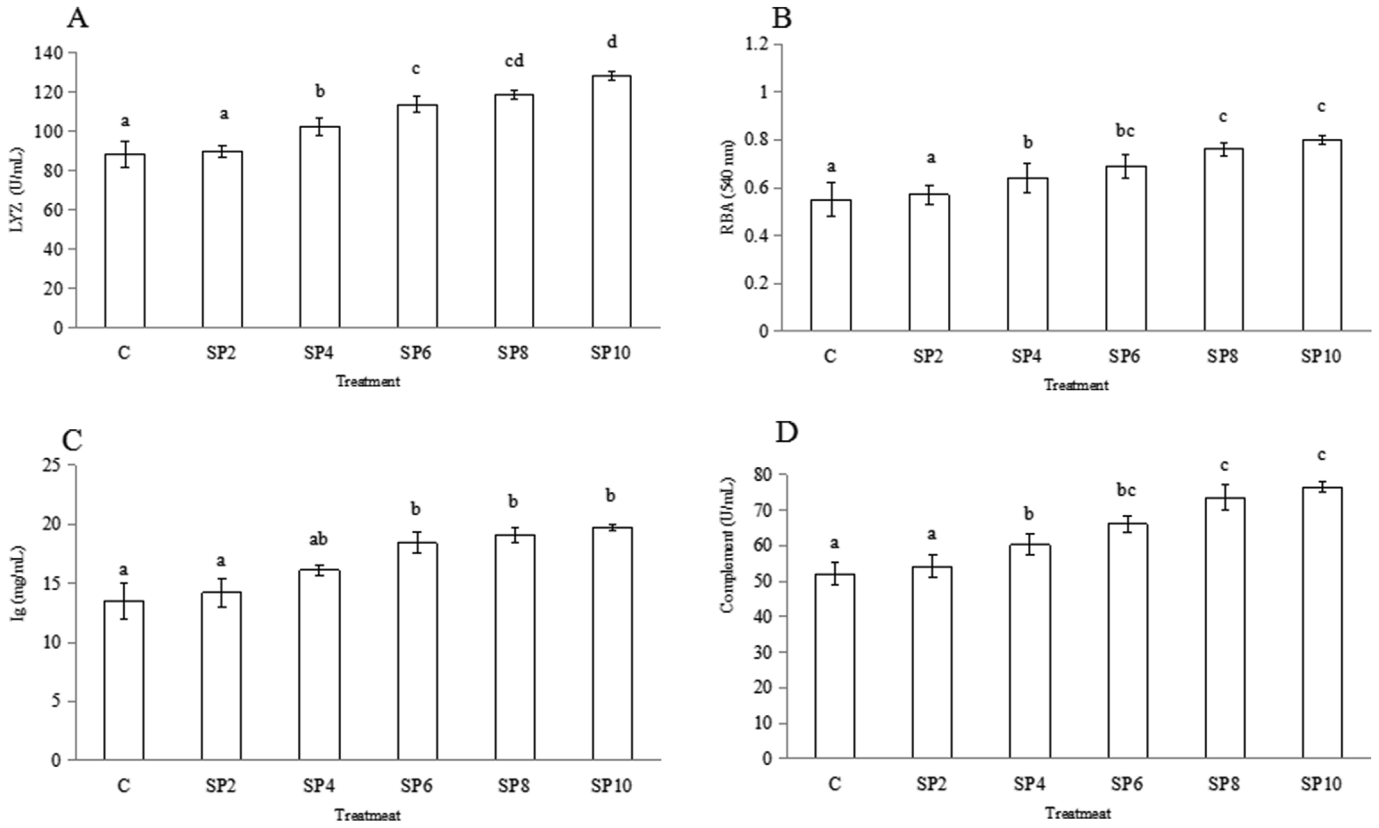

Fig. 1. Effects of dietary *S. platensis* on LYZ (A), RBA (B), Ig (C) and complement (D) levels in *P. leopardus*. Data expressed as mean ± SD. Different superscripts show significant differences ($P < .05$) in different groups.

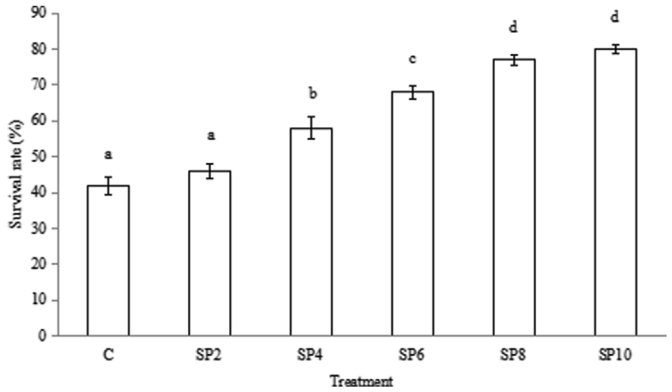

Fig. 2. Effects of dietary *S. platensis* on the survival rate of *P. leopardus* after *V. harveyi* (1.6×10^6 CFU ml^{-1}) infection at the fourteenth day. Data expressed as mean ± SD. Different superscripts show significant differences ($P < .05$) in different groups.

increase of RBC and Hb in our study might be ascribed to the presence of iron element in *S. platensis*. Additionlly, in the present study, dietary supplementation of *S. platensis* exhibited higher levels of WBC when compared with the group C, which was in line with the results in large yellow croaker *Pseudosciaena crocea* [23] and Carp *Cyprinus carpio* [34]. Higher WBC levels in fish fed *S. platensis* supplemented diets might be related to the presence of a polypeptide-phycocyanin, which was found to be an important factor for enhancing WBC in mice [35].

A previous study has suggested that the inclusion of dietary *S. platensis* can make a positive effect on serum biochemical parameters of fish [36]. In the present study, treatment groups exhibited decreased levels of cholesterol and triglyceride when compared with the group C. This result was similar to that reported by Lü et al. [37], who found that the dietary *S. platensis* had the potential to reduce levels of cholesterol and triglyceride in *Cranoglanis bouderius multiradiatus*. *S. platensis* contains α-linolenic acid, linoleic acid and β-carotene, which can decrease the levels of cholesterol and triglyceride and prevent the hypercholesterolemia and atherosclerosis [38,39]. Fish exhibit an increase in serum cortisol concentrations in response to stressors, such as confinement, poor water quality and pathogen infection [40,41]. Higher levels of cortisol are generally followed by an elevation in serum glucose levels [42,43]. In this study, no significant differences were found in levels of cortisol and glucose in all experimental groups, which indicated that *S. platensis* supplementation did not cause stress response to *P. leopardus*.

Earlier studies have suggested that dietary *S. platensis* can improve T-AOC level of several fish species, including *Cranoglanis bouderius multiradiatus* and Nile tilapia *Oreochromis niloticus* [37,44]. In the present study, T-AOC levels were higher in the *S. platensis* supplemented groups, indicating that *S. platensis* could improve the antioxidant status of *P. leopardus*. *S. platensis* is a rich source of antioxidant compounds such as β-carotene, phycocyanin, tocopherols and zeaxanthin that have significant effects on scavenging free radicals [13,45].

CAT, SOD and GPX are closely relevant to scavenge free radicals and protect the important cellular macromolecules and organelles from oxidative damage [46,47]. High levels of dietary fat-soluble antioxidants can reduce need for endogenous antioxidant enzymes such as SOD, CAT and GPX [48]. Trenzado et al. [49] reported that excessive activation of antioxidant enzyme systems due to hormesis of reactive oxygen species may be mitigated by supplementation of dietary antioxidant compounds. Previous studies showed that dietary supplementation of *S. platensis* reduced the activities of antioxidant enzymes in Carp *Cyprinus carpio* [50], Nile tilapia *Oreochromis niloticus* [44] and Goldfish *Carassius auratus* [51]. In this study, fish fed the control diet led to excessive activation of antioxidant enzyme systems, while the activities of CAT, SOD and GPX decreased with the increasing of dietary *S. platensis* levels. These results indicated *S. platensis* possess single oxygen quenching properties and may serve as an antioxidant in system

to quench free radicals [52,53]. Carotenoids and phycocyanin from *S. platensis* are thought to be responsible for the antioxidant properties [54].

MDA is the final product of lipid peroxidation which leads to cell toxicity and accelerates the damage of cells and tissues [28,55]. MDA contents can reflect the extent of lipid peroxidation [56]. In this study, dietary *S. platensis* significantly decreased the MDA contents, which was in agreement with the previous studies in Golden Pompano *Trachinotus ovatus* [17], Nile tilapia *Oreochromis niloticus* [44] and Carp *Cyprinus carpiovar* [57]. Antioxidant enzymes and antioxidants play an important role in resisting damage of lipid peroxidation [55]. Carotenoids from *S. platensis* may serve as an antioxidant in systems containing unsaturated fatty acids to eliminate free radicals [53]. The result in current study showed that the accumulation of lipid peroxides could be effectively reduced by *S. platensis.*

In fish, the humoural components including LYZ, Ig and complement are important participant in both specific and non-specific immunity [58]. LYZ is an important index of innate immunity of fish that can defense against bacterial pathogens by attacking the β-1, 4 glycosidic bonds of bacterial cell walls [59–61]. Ig is the essential immunoglobulin that plays an important role in host defense and can be used as a considerable biomarker for immune response in fish [62]. Complement plays an essential role in the innate immune response of fish, which is activated by one or a combination of three pathways, namely, the classical, lectin and alternative [5]. *S. platensis* has been demonstrated to increase LYZ activity and complement content in a number of fish species such as great sturgeon *Huso huso* [21], Carp *Cyprinus carpio* [34] and Nile tilapia *Oreochromis niloticus* [63]. Similarly, the present study also suggested that dietary supplementation with *S. platensis* could significantly increase LYZ activity, complement and Ig content of *P. leopardus*. Additionally, RBA has been widely used to assess the defense ability against pathogens [61]. Previous studies have revealed that dietary supplementation of *S. platensis* significantly improved RBA of Great Sturgeon *Huso huso* [21], Nile tilapia *Oreochromis niloticus* [64] and Olive Flounder *Paralichthys olivaceus* [65]. In the present study, it was clear that *S. platensis* had an enhancing effect on RBA of *P. leopardus*. These results indicated that *S. platensis* could improve the specific and non-specific immunity of fish. To date, exact explanations on how *S. platensis* works to increase LYZ activity, RBA, complement and Ig content in fish have not been illustrated. Thus, further studies are necessary to explain the mechanism of the effects of *S. platensis* on LYZ activity, RBA, complement and Ig content of fish.

Bacterial challenge tests have often been used as a final indicator of fish health status after nutrition trials [5,66,67]. *V. harveyi* is known to cause disease to *P. leopardus* in intensive culture system [4,68]. In the present study, dietary supplementation of *S. platensis* significantly increased the survival rate of *P. leopardus* challenged with *V. harveyi*. This was in line with studies by Hany et al. [44] and Mohsen et al. [69], who found dietary *S. platensis* improved the survival rate of Nile tilapia *Oreochromis niloticus* against challenged with *Vibrio alginolyticus* and *Aeromonas hydrophila.* The result in present study demonstrated that dietary *S. platensis* had a significant effect on *P. leopardus* with the capability of enhancing disease resistance against *V. harveyi.*

5. Conclusion

In conclusion, our study indicated that *P. leopardus* fed a diet supplemented with *S. platensis* (especially at 10% of fed supplement) for 8 weeks improved its growth performance; promoted its hepatic antioxidant status by increasing hepatic T-AOC; enhanced immune ability by increasing LYZ activity, RBA, Ig content, complement content and the levels of hematological parameters (RBC, WBC and Hb); decreased the levels of cholesterol and triglyceride. Additionally, dietary supplementation of *S. platensis* significantly increased the survival rate of *P. leopardus* challenged with *V. harveyi*. *S. platensis* can be supplemented in the diet as an immunostimulant and growth promoter to enhance hepatic antioxidant status and immune ability of fish.

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