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Study on *Schizochytrium sp.* improving the growth performance and non-specific immunity of golden pompano (*Trachinotus ovatus*) while not affecting the antioxidant capacity

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ABSTRACT

A feeding experiment was conducted to determine the effects of *Schizochytrium sp.* on growth performance, antioxidant capacity and non-specific immunity in golden pompano (*Trachinotus ovatus*).Two diets were formulated with or without *Schizochytrium sp.* supplemented (D1:0% and D2: 3%) to feed fish for 8 weeks. Results showed that growth performance, feed intake and survival rate increased significantly with *Schizochytrium sp.* supplemented ($P < 0.05$). Feed coefficient rate (FCR) of golden pompano fed the diet supplemented with *Schizochytrium sp.* was significantly lower than that of fish fed the control diet ($P < 0.05$). No significant differences were found in antioxidant capacity both in transcriptional level, including nclear factor erythroid-2-related factor-2 (Nrf2), Kelch-like-ECH-associated protein (keap1), catalase (CAT), glutathione peroxidase (GSH-PX) and heme oxygenase 1 (HO-1) and enzyme activity, such as total antioxidant capacity (T-AOC), malondialdehyde (MDA) and superoxide dismutase (SOD) ($P > 0.05$). Gut amylase and lipase were significantly higher in dietary *Schizochytrium sp.* supplemented treatment than that in control group ($P < 0.05$). The relative peroxisome proliferator-activated receptor-α (PPARα) expression level in liver was significantly higher in *Schizochytrium sp* supplemented treatment than that in control one ($P < 0.05$). The mRNA expression of myeloid differentiation factor 88 (MyD88), IL-1R-associated kinases 4 (IRAK4), interferon regulating Factor 3 (IRF3), interferon regulating Factor 3(IRF7) and heat shock protein 70 (HSP70) were significantly lower in *Schizochytrium sp.* supplemented treatment than that in control one ($P < 0.05$). In *Schizochytrium sp.* supplemented diet, golden pompano had significantly longer villi length than that in control diet ($P < 0.05$); muscle thickness in *Schizochytrium sp.* supplemented diet was thicker than that in control one ($P < 0.05$) and there were more goblet cells in *Schizochytrium sp.* treatment ($P < 0.05$). After the rearing trial, an air exposure trial was conducted. Results showed that the air-exposure mortality (AEM) and mRNA expression level of Nrf2, keap1, CAT, GSH-PX and HO-1 showed no significant difference ($P > 0.05$). These results indicated that dietary *Schizochytrium sp.* improved the growth performance and non-specific immunity of golden pompano while made no difference to antioxidant capacity.

1. Introduction

As is known, fish oil is the major source of long-chain highly unsaturated fatty acids (PUFA), such as eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic (22:6n-3; DHA) acids in aquafeeds. These kinds of fatty acids are important to promote optimal growth and health of farmed marine fish, involving in a variety of physiological processes [1], and associated with positive effects on brain function, immunity and inflammation [2]. Nowadays, the demand for fish oil has been rising sharply while the global supply remained stagnant, which led to the investigation of alternative, sustainable sources [3].

Research has shown that marine algaes are promising alternative sources [4], which are primary producers of long-chain polyunsaturated fatty acids (LC-PUFAs), such as DHA and EPA and considered a sustainable source of these essential fatty acids (EFAs) [5]. Except for EFAs, microalgae is thought to contain high content of proteins (28 and 71%), lipids(10–20%),carbohydrate(5–15%),mineral, pigments and antioxidant substances [6–9]. Previous studies have shown that a number of different microalgae species is able to improve the growth performance of various fish species, such as *Scenedesmus*

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almeriensis [10], *Scenedesmus obliqus* [11], *Tisochrysis lutea* and *Tetraselmis suecica* [12]. Among all this microalgae, the thraustochytrids have been preferred due to their ease for large-scale heterotrophic cultivation under controlled conditions to produce a high lipid product rich in n−3 LC-PUFA [13]. *Schizochytrium sp*. is a fast growing thraustochytrid microalgae [14], which contains high level of lipids (55–75% of dry matter), with as much as 49% docosahexaenoic acid (DHA) [15].

Golden pompano (*T. ovatus*) belongs to family carangidae, genus Trachinotus. It is a warm-water species (25–32 °C) and a carnivorous fish that preys mainly on zooplankton, small crustaceans, shellfish and small fish [16]. Recently, pompano is widely farmed owing to its high price in the market and resilience to salinity and temperature ranges [17]. The aim of the present study was to evaluate the *Schizochytrium sp*. as dietary ingredients on growth performance, immune response and antioxidant capacity.

2. Methods

2.1. Diet preparation and dietary treatments

In this study, two isonitrogenous and isoenergetic practical diets were formulated supplementing with or without dried *Schizochytrium sp*. (D1: 0%; D2: 3%) (Table 1). The method of diet preparation was the same as described by Niu et al. [18]. Briefly, all dry ingredients were finely ground, weighed, mixed manually for 5 min and then transferred to a Hobart mixer (A-200T Mixer Bench Model unit, Resell Food Equipment Ltd., Ottawa, Canada) for another 15-min mixing. Soya lecithin was added to a pre-weighed fish oil, and mixed until homogenous. The oil mix was then added to the Hobart mixer slowly while mixing was still continuing. All ingredients were mixed for another 10 min. Then distilled water (about 30–35%, v/w) was added to the mixture to form a dough. A dough of even consistency was passed through a pelletizer with a 1.2-mm-diameter die (Institute of Chemical Engineering, South China University of Technology, Guangzhou, PR China). The diets were air dried until the moisture was reduced to less than 10%. The dry pellets were placed in plastic bags and stored −20 °C until fed.

Table 1
Ingredients and proximate composition of the two experimental diets (%).

Ingredients	Diet 1	Diet 2
Fish meal	28	28
Soybean mea	20	20
Soy protein concentrate	17	17
Wheat flour	20	17
Fish oil	8	8
Soya lecithin	2	2
Ca(H2PO4)2	2	2
Pre-vitamin[a]	1	1
Pre-mineral[b]	1	1
Choline	0.5	0.5
DL-Met	0.4	0.4
Lys-HCL (78%)	0.1	0.1
Schizochytrium sp.	0	3
Sum	100	100
Nutrient levels		
Moisture	9.01	9.76
Crude protein	37.51	38.04
Crude fat	10.67	10.98
Ash	10.37	11.07

1: Pre-vitamine (mg or g kg−1 diet): thiamin, 25 mg; riboflavin,45 mg; pyridoxine HCl, 20 mg; vitamin B12, 0.1 mg; vitamin K3,10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid,200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinal acetate, 32 mg;cholecalciferol, 5 mg; a-tocopherol, 120 mg; ascorbic acid,2000 mg; choline chloride, 2500 mg; ethoxyquin 150 mg; wheatmiddling, 14.012 g [54].
2: Pre-mineral (mg or g kg−1 diet): NaF, 2 mg; KI, 0.8 mg;CoCl2.6H2O (1%), 50 mg; CuSO4.5H2O, 10 mg; FeSO4.H2O, 80 mg;ZnSO4.H2O, 50 mg; MnSO4.H2O, 60 mg; MgSO4.7H2O, 1200 mg; Ca(H2PO4)2.H2O, 3000 mg; NaCl, 100 mg; zoelite,15.447 g [54].

2.2. Animal rearing and experimental procedures

The feeding trial was conducted at an experimental station of South China Sea Fisheries Research Institute of Chinese Academy of Fishery Sciences (Sanya, Hainan). Prior to the start of the trial, juvenile *T. ovatus* were acclimated to a commercial diet for 2 weeks and were fed twice daily to apparent satiation. At the beginning of the feeding trial, the fish were starved for 24 h, weighed, and then fish with similar size (initial body weight 7.65 ± 0.07 g) were randomly allotted to 8 sea cages (1.0 m × 1.0 m × 1.5 m; four cages per diet treatment); each cage was stocked with 20 fish. Each experimental diet was randomly assigned to four cages. The feeding frequency was twice daily at 8:00 and 16:00 and lasted for 8 weeks. To prevent the waste of pellets, fish were slowly hand-fed to satiation based on visual observation of their feeding behavior. Feed consumption was recorded for each cage every day.

2.3. Sample collection

At the end of the feeding trial, fish were starved for 24 h and then weighed and counted the total number. Eight fish from each cage were randomly collected for sampling: two for analysis of whole-body composition and six were used to obtain weights of individual whole body, viscera, and liver. The livers were rapidly removed and frozen in the liquid nitrogen separately for analysis of lipid peroxidation and antioxidant status. The foreguts were collected for analysis of digestive enzyme activity and intestinal morphology in liquid nitrogen and Bouin's solution, respectively.

2.4. Air-exposure trial

After blood and tissue sampling, 8 fish were randomly collected from each cage, exposed to air at an ambient temperature of approximately 28 °C for 12min and then put back into the water according to the method described by Wang [19]. Accumulated mortality during the subsequent 20min was monitored and was defined as air-exposure mortality (AEM). Samples were collected for further analysis.

2.5. Biochemical analysis

Samples from the diets and the whole fish were frozen dried and then grounded. Moisture, crude protein, crude lipid and crude ash of the diets and fish were determined using standard methods [20]. Moisture was determined by oven drying at 105 °C for 24 h, and ash was determined using a muffle furnace at 550 °C for 24 h. Crude protein was analyzed by the Kjeldahl method after acid digestion (1030-Auto-analyzer, Tecator, Sweden). Crude lipid was determined by the ether-extraction method by Soxtec System HT (Soxtec System HT6, Tecator).

2.6. Antioxidant capacity analysis and digestive enzymes 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 15 min (4 °C, 3500 rmp), and aliquots of the supernatant were used to quantify hepatic antioxidant status and digestive enzymes analysis. All indices were measured with commercial assay kits (Nanjing Jiancheng Bioengineering Institute Nanjing, China) in accordance with the instructions of the manufacturer.

2.7. Quantitative reverse transcriptase PCR analysis

Total RNA was extracted from hepatic using Trizol® reagent (Invitrogen, USA). Agarose gel electrophoresis and spectrophotometric

Table 2
Sequences of primers used in this study.

Primers	forward/reverse (5′–3′)
chymotrypsinogen	F: GCCTCCTCCAGCACCAACATC
	R: CACTCCAGAAGCACCAGCACAG
PPARα	F: AATCTCAGCGTGTCGTCTT
	R: GGAAATGCTTCGGATACTTG
C-Lyz	F: GGAGTCTGGTGTTTCTGCTCTTTG
	R: GGTGGCTCTAGTGTTGTAGTTCG
MyD88	F: AATACCTTGACAGCGATGCCTG
	R: GTGCAAGGCCTGGTGTAATCA
IRF3	F: GCTGGGATCCGCTTAGTCTACA
	R: GCCCAGCTTGTCCAGGATG
IRF7	F: CGAATACACCAACCGCATCCT
	R: AGCTTTCTTGGGTCTGGGTCA
IRAK4	F: CTGGCGACAGAGATGCTTGATA
	R: CCGCCATCATGGTTGTTCA
CAT	F: GGATGGACAGCCTTCAAGTTCTCG
	R: TGGACCGTTACAACAGTGCAGATG
GSH-PX	F: GCTGAGAGGCTGGTGCAAGTG
	R: TTCAAGCGTTACAGCAGGAGGTTC
HO-1	F: AGAAGATTCAGACAGCAGCAGAACAG
	R: TCATACAGCGAGCACAGGAGGAG
Keap1	F: CAGATAGACAGCGTGGTGAAGGC
	R: GACAGTGAGACAGGTTGAAGAACTCC
Nrf2	F: TTGCCTGGACACAACTGCTGTTAC
	R: TCTGTGACGGTGGCAGTGGAC
β-actin	F: TACGAGCTGCCTGACGGACA
	R: GGCTGTGATCTCCTTCTGCA

analysis (A260:A280 nm) were used to assess RNA quality and concentration. The cDNA was synthesized using a PrimeScriptTM RT reagent kit with gDNA Eraser (Takara, Japan), according to the manufacturer's instructions. Briefly, oligo dT primers and Random 6 mers were used to reverse transcribe 800 ng RNA in the presence of PrimeScriptTM RT enzyme Mix I, 5 × PrimeScriptTM buffer, and RNase-free dH2O at 37 °C for 15 min, following inactivation at 85 °C for 5 s.

Real-time PCR for the target genes were performed using a SYBR® Premix Ex Taq™ II (Takara, Japan) and quantified on the LightCycler 480 (Roche Applied Science, Basel Switzerland) using the following program: 400 nM of forward and reverse specific primers, 20 ng of cDNA template and nuclease free water to final volume of 10 μl, denaturation step at 95 °C for 1 min, followed by 40 amplification cycles of 5 s denaturation at 95 °C, 15 s annealing at 60 °C and 20 s extension at 72 °C, followed by a melt-curve analysis and cooling to 4 °C. The primers were showed in Table 2.

2.8. Intestinal morphology

Samples fixed in Bouin solution were dehydrated in ethanol, equilibrated in xylene and embedded in paraffin according to the method described by Krogdahl et al. [21]. The paraffin blocks was sectioned (5 μm) in serial sagittal section using a Leica RM 2135 rotary microtome and stained with haematoxylin and eosin (H & E). The sections were examined using a light microscope with villi length and muscle thickness measured. Photographs were taken with an Olympus digital camera attached to the microscope. 10 random villi from each segment were measured.

2.9. Calculations and statistical analysis

The following variables were calculated:

Final body weight (FBW, g) = final body weight / final number of fish;

Weight gain rate (WG, %) = 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) = dry diet fed /wet weight gain;

Feed intake (FI, g/100 gBW/d) = diet intake × 100/ ((final body weight + initial body weight)/2 × t)

Survival rate (%) = 100 × (final number of fish) / (initial number of fish);

Viscerosomatic index (VSI, %) = 100 × (viscera weight, g) / (whole bodyweight, g);

Hepatosomatic index (HSI, %) = 100 × (liver weight, g) / (whole body weight, g)

Condition factor (CF, g/cm3) = 100 × (body weight, g) / (body length, cm3);

All data are presented as means ± S.E.M. and subjected to independent-sample *t*-test to test the effects of experimental diets using the software of the SPSS for windows (ver 16.0, U.A.S). Statistical significance was examined at $P < 0.05$ unless otherwise noted.

3. Results

3.1. Growth performance and air-exposure mortality

Growth performance, feed utilization and biometric parameters of juvenile pompano fed different dietary *Schizochytrium sp.* levels were shown in Table 3. Results showed that growth performance, including final body weight, weight gain and specific growth rate increased significantly with *Schizochytrium sp.* supplemented ($P < 0.05$). Feed intake and survival rate showed the same trend as the growth performance with significant difference ($P < 0.05$). FCR of *T. ovatus* fed the diets supplemented with *Schizochytrium sp.* was significantly lower than that of fish fed the control diet ($P < 0.05$). There was no significant difference among hepatosomatic indices (HSI), visceral somatic indices (VSI) and condition factor (CF) between the two different diet treatments. After the air exposure, there was no significant difference of AEM between the two treatments ($P < 0.05$).

3.2. Whole body composition

Whole body composition of golden pompano fed different dietary *Schizochytrium sp.* levels were shown in Table 4. There were no significant differences in whole body composition of fish between the two diet treatments ($P > 0.05$).

Table 3
Growth performance and biometric parameters of golden pompano fed diets with and without supplementation of *Schizochytrium sp.*

	D1	D2
FBW (g)	28.37 ± 3.55a	55.76 ± 5.50b
WG (%)	272.15 ± 46.83a	626.24 ± 71.53b
SGR (%/day)	2.22 ± 0.23a	3.39 ± 0.19b
SR (%)	76.25 ± 1.25a	92.50 ± 2.50b
FCR	1.28 ± 0.07a	1.05 ± 0.02b
FI (g/100gBW/d)	3.51 ± 0.15a	3.72 ± 0.45b
HSI (%)	1.00 ± 0.07	0.90 ± 0.08
VSI (%)	5.57 ± 0.18	5.55 ± 0.20
CF (g/cm3)	3.41 ± 0.06	3.48 ± 0.05
AEM	100.00 ± 0.00	90.63 ± 5.98

[1]Values are mean ± SEM of four replicates, and values in the same row with different letters are significant different ($P < 0.05$).

Table 4
Whole-body compositions (% dry weight) of golden pompano fed diets with and without supplementation of *Schizochytrium sp.*

	D1	D2
Moisture (%)	70.24 ± 0.89	68.69 ± 0.69
Crude protein (%)	57.21 ± 2.45	57.80 ± 0.70
Crude lipid (%)	27.28 ± 4.30	26.41 ± 2.17
Ash (%)	12.93 ± 0.88	13.14 ± 0.22

Values are mean ± SEM of four replicates, and values in the same row with different letters are significant different ($P < 0.05$).

Table 5
Hepatic antioxidant statuses of golden pompano fed diets with and without supplementation of *Schizochytrium sp.*

	D1	D2
MDA (nmol/ml)	0.26 ± 0.05	0.29 ± 0.04
SOD (U/mgprot)	188.44 ± 10.66	186.87 ± 4.83
T-AOC (U/mg protein)	0.15 ± 0.01	0.20 ± 0.04

Values are mean ± SEM of four replicates, and values in the same row with different letters are significant different ($P < 0.05$).

3.3. Hepatic antioxidant statuses and gut digestive enzymes analysis

Hepatic antioxidant statuses of golden pompano fed different dietary *Schizochytrium sp.* levels were shown in Table 5. There were no significant differences in TAOC, SOD and MDA of fish between the two diet treatments ($P > 0.05$).

Gut amylase and lipase were significantly higher in dietary *Schizochytrium sp.* supplemented treatment than that in control group ($P < 0.05$) (see Table 6).

3.4. Hepatic digestion-related gene expression level

The relative digestion-related gene expression level of golden pompano fed diets with different levels of *Schizochytrium sp.* was showed in Table 7. The relative expression of chymotrypsinogen in liver showed no significant difference between diets with or without *Schizochytrium sp.* supplemented ($P > 0.05$) while the relative expression of PPARα was significantly higher in *Schizochytrium sp* supplemented treatment than that in control one ($P < 0.05$).

3.5. Hepatic non-specific immunology gene expression level

The relative non-specific immunology-related gene expression level of golden pompano fed diets with different levels of *Schizochytrium sp.* was showed in Table 8. Results showed that there was no significantly difference among two diets for mRNA expression of C-Lyz ($P > 0.05$) while the mRNA expression of MyD88, IRAK4, IRF3, IRF7 and HSP70 were significantly lower in *Schizochytrium sp.* supplemented treatment than that in control ones ($P < 0.05$).

Table 6
Intestinal digestive enzyme activity of golden pompano fed diets with and without supplementation of *Schizochytrium sp.*

	D1	D2
Amylase (U/mgprot)	1.80 ± 0.07a	2.47 ± 0.25b
Lipase (U/mgprot)	93.03 ± 7.65a	133.67 ± 11.80b

Values are mean ± SEM of four replicates, and values in the same row with different letters are significant different ($P < 0.05$).

Table 7
The relative digestion-related genes expression of golden pompano fed diets with different levels of *Schizochytrium sp.*

	D1	D2
chymotrypsinogen	1.02 ± 0.21	1.27 ± 0.75
PPARα	1.00 ± 0.04a	1.95 ± 0.13b

Values are mean ± SEM of four replicates, and values in the same row with different letters are significant different ($P < 0.05$).

Table 8
The relative non-specific immunology-related genes expression level of golden pompano fed diets with different levels of *Schizochytrium sp.*

	D1	D2
C-Lyz	0.99 ± 0.04	0.86 ± 0.09
HSP70	1.02 ± 0.12a	0.54 ± 0.06b
MyD88	1.14 ± 0.32a	0.36 ± 0.09b
IRF3	1.01 ± 0.08a	0.54 ± 0.07b
IRF7	1.02 ± 0.11a	0.63 ± 0.08b
IRAK4	1.06 ± 0.20a	0.43 ± 0.07b

Values are mean ± SEM of four replicates, and values in the same row with different letters are significant different ($P < 0.05$).

3.6. Hepatic antioxidation-related genes expression level in rearing and air exposure trial

The relative antioxidation-related genes expression of golden pompano fed diets with different levels of *Schizochytrium sp.* in rearing and air-exposure trial were showed in Table 9. There was no significant difference between the two treatments in all antioxidation related genes ($P > 0.05$).

3.7. Intestinal morphology

Intestinal morphology was presented in Table 10 and Fig. 1. Results showed that gut morphology was significantly changed by the dietary *Schizochytrium sp.* With *Schizochytrium sp.* supplemented in diet, villi length were significantly longer than that in control diets ($P < 0.05$); muscle thickness in *Schizochytrium sp.* supplemented diets were thicker than that in control ones ($P < 0.05$) and there were more goblet cells in *Schizochytrium sp.* treatment ($P < 0.05$).

4. Discussion

The present results indicated that *T. ovatus* fed diet with *Schizochytrium sp.* supplemented showed better growth performance than the control treatment. This result was in agreement with those in previous studies on channel catfish [22], Nile Tilapia (*Oreochromis niloticus*) [23] and Atlantic salmon (*Salmo salar L.*) [24]. *Schizochytrium sp.* was low in carbohydrate and ash and high in essential fatty acid levels, mainly DHA, which is advantageous in aquatic animals with higher requirements for DHA [25]. Studied showed that there was high

Table 9
The relative antioxidation-related genes expression of golden pompano fed diets with different levels of *Schizochytrium sp.* in rearing and air-exposure trial.

	Rearing trial		Air-exposure trial	
	D1	D2	D1	D2
CAT	1.05 ± 0.21	0.62 ± 0.04	1.01 ± 0.06	0.84 ± 0.03
GSH-PX	1.08 ± 0.25	0.84 ± 0.32	1.03 ± 0.15	0.86 ± 0.04
HO-1	1.05 ± 0.20	0.65 ± 0.18	1.05 ± 0.21	0.85 ± 0.14
Keap1	1.01 ± 0.09	1.54 ± 0.29	1.03 ± 0.15	0.97 ± 0.28
Nrf2	1.02 ± 0.11	1.18 ± 0.32	1.02 ± 0.12	1.06 ± 0.39

Table 10
Gut morphology of golden pompano fed diets with and without supplementation of *Schizochytrium sp.*

	D1	D2
goblet cells	116.90 ± 4.78a	162.40 ± 9.37b
muscle thickness	133.24 ± 5.24a	165.84 ± 4.56b
villi length	604.62 ± 27.09a	804.28 ± 32.46b

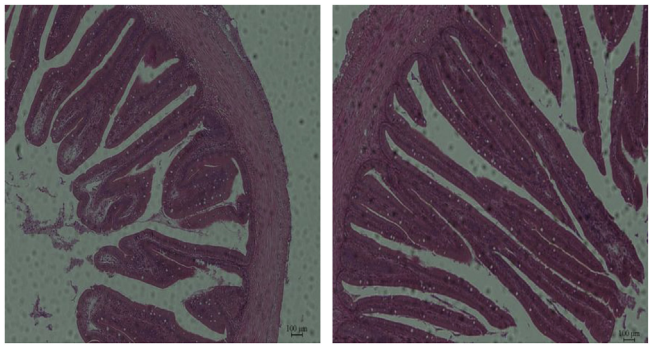

Fig. 1. Comparison of gut morphology between golden pompano fed diets with and without supplementation of *Schizochytrium sp.* (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

digestibility of lipid and all unsaturated fatty acid fractions of *Schizochytrium sp.* [26]. Besides, the algal derived DHA can also increase the feed efficiency rate [22] and stimulate growth performance of aquatic animals [27,28]. In addition, the *Schizochytrium sp.* may contain high levels of a micronutrient, such as the caroteniod and bioactive compounds, which may result in better weight gain [23,29].

Except for improving the feed utilization, *Schizochytrium sp.* supplemented in feed also increased feed intake of fish, which may be attributed to microalgae's fishlike flavor, which is highly palatable for fish [30], contributing to better growth performance. This result was consistent with the previous, such as Nile Tilapia [23] and gilthead sea bream (*Sparus aurata*) [31]. However, whether dietary incorporation of microalgaes improved feed intake and nutrient utilization were determined by several factors, such as fish and microalgae species, the inclusion level, and the nutritional composition of algae themselves, as pointed out by several studies [32,33].

With dietary supplementation with microalgaes, the proportion of PUFA in fish body composition increased in channel catfish (*Ictalurus punctatus*) [22], Nile Tilapia (*Oreochromis niloticus*) [23] and Atlantic salmon (*Salmo salar L.*) [24]. However, PUFA are prone to oxidation, generating various metabolites, inducing oxidative stress, eventually increasing lipid peroxidation content [34]. To evaluate the antioxidant capacity, the experiment measured the relevant index in rearing trail and air exposure trail. T-AOC is an overall indicator of the antioxidant status of an individual, representing the level of enzyme and non-enzyme original antioxidant in the body [35]. T-AOC andMDA, a product of lipid peroxidation, were slightly higher in *Schizochytrium sp.* supplemented diets than that in the control group, but showed no significant difference between the two treatments, which indicated that *T. ovatus* did not suffer from oxidative stress.

SOD, CAT, GSH-PX and HO-1 are the common antioxidases, which is regulated by Nrf2-ARE signal pathway. Normally, under basal conditions Nrf2 is bound to Keap1 in the cytoplasm due to an interaction between a single Nrf2 protein and a Keap1 dimer which controls the Nrf2 ubiquitin-dependent degradation [36,37]. Exposure to a number of stressors leads to dissociation of Nrf2 from Keap1, thereby rescuing Nrf2 from proteasomal degradation and allowing for entry into the nucleus [38]. Once inside the nucleus, Nrf2 dimerizes with small Maf proteins leading to binding of Nrf2 to the antioxidant response element present in the promoter of Nrf2-target genes and transcriptional activation of these genes [39]. The present results showed that the antioxidases were slightly lower in the *Schizochytrium sp.* treatment than that in the control treatment, which was in accordance with the observation of significantly lower AEM of fish but without significant difference, indicating that *Schizochytrium sp.* would not increase the risk of oxidative stress in *T. ovatus*.

The ability of fish to use the ingested nutrients depends on the presence of an adequate set of digestive enzymes, which can be considered as indicators of the fish digestive and absorptive capacity [40]. In the present study, fish fed diets with *Schizochytrium sp.* supplemented had higher amylase and lipase ability. Peroxisome proliferator-activated receptor-α (PPAR-α) functions as a lipid sensor in liver and regulates the transcriptional expression of genes involved in lipid metabolism [41]. *Schizochytrium sp.* supplemented diets up-regulated the lipid metabolism in the present study. Increasing activities of digestive enzymes may have effects on the improved growth performance of fish in *Schizochytrium sp.* supplemented treatment.

Except for digestive enzyme activities, the structure and morphology of the intestine is crucial for nutrient absorption and the maintenance of normal intestinal functions [10,42]. The villi length in a way reflects the function of the intestinal wall [43],led to better nutrient absorption and better growth performance [44]. Muscle thickness as well plays a role in intestinal digestion and absorption. Increased muscle thickness may improve intestinal digestion and absorption ability [45]. Goblet cells play a crucial role in intestinal homeostasis, which could synthesize and secrete mucin glycoproteins covering the surface of gastrointestinal epithelium to protect intestine from infection [46]. In the present study, fish fed diet with *Schizochytrium sp.* supplemented got better intestinal structure and morphology, increased villus length, muscle thickness and the number of goblet cells in the intestine of *T. ovatus*. This suggested that dietary supplementation with *Schizochytrium sp.* not only promoted the early intestinal development of golden pompano to improve intestinal digestion and absorption ability but also improved intestinal homeostasis and protecting barrier to resist the pathogen microflora by increasing goblet cells.

Innate immunity is the first defensive line against invading pathogens, which is based on pattern recognition receptors to recognize conserved pathogen-associated molecular patterns and trigger downstream signals in fish [47]. One of the well-studied pattern recognition receptors was the Toll-like receptor (TLR) family [48], which trigger downstream signal pathway by myeloid differentiation primary MyD88-and toll-interleukin 1 receptor domain-containing adapter inducing interferon-β (TRIF)-dependent pathways [49]. As the TLR downstream protein [50], MyD88 interacts with the corresponding IL-1R-associated kinases (IRAKs) [51], while the TRIF adapter activates transcription factor and cascade of a signaling pathway, such as IRF3, leading to the induction of type I IFNs and inflammatory cytokines [52] and had a major impact on the understanding of the molecular mechanism of the pathogen induced innate antiviral response [53]. After intaking diets supplemented with *Schizochytrium sp., T. ovatus* had relative lower mRNA expression of MyD88, IRAK4, IRF3, IRF7 compared to the control group, indicating that inclusion of *Schizochytrium sp.* in diet might have an effect on resistance to pathogen and increased the innate antiviral capacity and innate immunity by MyD88-and TRIF-dependent pathways. To sum up, *Schizochytrium sp.* diet made significant difference to *T. ovatus*.

5. Conclusion

In conclusion, dietary *Schizochytrium sp.* supplementation improved the growth performance of *T. ovatus* by enhancing feed intake and feed conversion rate and non-specific immunity, while made no difference to antioxidant capacity.

Declaration of competing interest

The authors declare that they have no competing interests.

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List of abbreviations

FBW	final body wet weight
WG	weight gain
SGR	specific growth rate
FCR	feed coefficient ratio
SR	survival rate
T-AOC	total antioxidant capacity
MDA	Malondialdehyde
SOD	superoxide dismutase
FI	feed intake
VSI	viscerosomatic index
HSI	hepatosomatic index
CF	condition factor
AEM	air-exposure mortality; C-Lyz, c-type lysozyme
IRAK4	IL-1R-associated kinases 4
IRF3	interferon regulating Factor 3
PPARα	Peroxisome proliferator-activated receptor-α
MyD88	myeloid differentiation factor 88
GSH-PX	glutathione peroxidase
CAT	catalase
HO-1	heme oxygenase 1

Authors' contributions

The authors thank the participants who gave their time to the trial. Jin Niu, Yong-Jian Liu and Li-Xia Tian designed the study. Jia-Jun Xie, Hao-Hang Fang and Shi-Yu Liao carried out the rearing work. Jia-Jun Xie, and Jin Niu analyzed the results and Jia-Jun Xie wrote the paper with contributions from the other authors. There are no conflicts of interest.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in Sun Yat-sen University, Guangzhou, China, and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

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