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Growth performance, digestive enzyme activities and serum nonspecific immunity of the red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) fed diets supplemented with ultrafine powder of *Enteromopha prolifera**

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Abstract The present study evaluated effects of ultrafine powder of the green macroalgae *Enteromopha prolifera* as dietary supplement on growth performance, digestive enzyme activities and serum nonspecific immune responses of the red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*). The red tilapia were fed five diets supplemented with different levels of *E. prolifera* ultrafine powder as well as a control diet containing no *E. prolifera* for seven weeks (Diets 0–6 contained 0 (control), 10, 20, 30, 40 and 50 g/kg of *E. prolifera* ultrafine powder, respectively). The results showed that diets supplemented with *E. prolifera* ultrafine powder generally improved growth, immunity and digestive enzyme activities of the red tilapia. In particular, the fish fed the diet incorporated 50 g/kg (5%) *E. prolifera* ultrafine powder (Diet 5) achieved the highest percentage weight gain, specific growth rate and the condition factor (increased by 15.4%, 8.0% and 5.7%, respectively when compared to the control). Feeding the diet also led to significantly increases (P<0.05) in serum nonspecific immune responses, including total superoxide dismutase, acid phosphatase, alkaline phosphatase, lysozyme activities and serum total protein (increased by 19.4%, 48.1%, 29.5%, 30.3% and 8.7%, respectively) as well as digestive enzyme activities of erepsin, gastric amylase, gastric lipase, pepsin, intestinal amylase and gastric lipase (increased by 15.7%, 33.3%, 16.3%, 21.3%, 52.3% and 28.2%, respectively) than those of the control. Based on these results, it is recommended that the inclusion level of *E. prolifera* ultrafine powder in the diet of the red tilapia should be 50 g/kg (or 5%).

Keyword: *Enteromopha prolifera*; ultrafine powder; red tilapia; growth performance; digestive enzyme activities; serum nonspecific immunity

1 INTRODUCTION

The hybrid red tilapia, *Oreochromis mossambicus* × *Oreochromis niloticus*, is a popular aquaculture species in China. The species exhibits excellent characteristics for aquaculture, such as high tolerance to harsh environmental conditions, easy reproduction, fast growth and high quality meat (Chiu et al., 2013). The red tilapia is considered having high potentials for further production expansion because of its strong market demands and high adaptive capacity (Romana-Eguia and Eguia, 1999). Past studies on the red tilapia have focused more on the strain variations in growth and other important traits, such as fecundity, feed conversion efficiency, red color inheritance, and reproduction under high salinity or temperature conditions (e.g. Koren et al., 1994; Richter et al., 2002) and there was relatively few studies investigated nutrition requirement, digestive physiology and immune responses of the red tilapia.

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Enteromorpha prolifera is a large green macroalgae belonging to the family Ulvaceae (Zeng et al., 1962; Hiraoka et al., 2003; Yao, 2011). It is a commercial important species (Aguilera-Morales et al., 2005) and distributes globally on rock and gravel between the intertidal to the upper subtidal zones (Hiraoka et al., 2003). In recent years, marine eutrophication in China has often led to blooms of *E. prolifera* in summer (as known as green tides), blanketing coastal surface. The algal blooms threatens marine environment and the local governments had to organize costly clean up. Although when forms green tides, *E. prolifera* is a threat to the environment, the algae actually contains high protein and dietary fiber, as well as rich in polysaccharide and minerals (Aguilera-Morales et al., 2005) and has been proved as a good feed supplement for livestock (Michalak and Chojnaka, 2009).

Additionally, given that the sub-therapeutic antibiotics were forbidden as growth-promoters in the European Union in 2006 (Regulation1831/2003/EC), consumer demands on eco-friendly farming practices and safe food products have increased significantly (Rawling et al., 2009). As the result, additive alternative to traditional antibiotics in aquaculture feeds, particularly those sourced from plants, have received much attention recently (Bricknell and Dalmo, 2005). Research have indicated high potential of phytogenic substances in fish diets as they can act as the alternatives for antibiotics and growth promoters (Jian and Wu, 2003) as well as enhance storage quality and antioxidant properties of fish fillet (Gatlin et al., 1992). In fact, in recent years, the rapid expansion of intensive aquaculture of tilapia in China and Southeast Asia has led to frequent outbreak of diseases, consequently antibiotics are often used for disease prevention and control. However, it has been questioned that resistant bacterial populations could be induced from the use of antibiotics, and more serious is the unpredictable long-term effects on public health. Hence, green feed additives, such as marine macroalgae, have been viewed as potential dietary alternatives for improving the immune responses, disease resistance and growth performance of cultured fish (Wassef et al., 2009, 2013).

There is limited information available on the application of *E. prolifera* as feed ingredient or supplement for feeding fish (Asino et al., 2011; Yang et al., 2016). As most marine macroalgae, *E. prolifera* contains relatively high fibre and this may limit its effective utilization by fish (Buddington, 1987); *E. prolifera* in the form of ultrafine powder might help fish digestion. As dry power, it is also much more convenience for storage and transport, as well as for feed manufacture as it is easier to mix with other feed ingredients. To the best of our knowledge, no information is currently available on *E. prolifera* being proceeded as ultrafine powder form and used as feed additive for feeding fish; the present study was hence carried out to investigate whether ultrafine powder of *E. prolifera* could be used as effective feed supplement for the red tilapia.

2 MATERIAL AND METHOD

All animals used in this study have been approved by the Animal Ethical Committee of Jimei University and experiments were carried out in accordance with the approved guidelines of the university.

2.1 Production of *E. prolifera* ultrafine powder and the experimental diets

Enteromorpha prolifera was harvested from a man-made canal for dragon boat racing in Xiamen, China during March to April 2012. After rinsing with clean seawater, the harvested *E. prolifera* was transported to a laboratory of Jimei University and oven-dried at 60°C for 4 h before being pulverized for 30 s by a low temperature pulverizer at 10°C. The resultant power was then sieved through a mesh screen (74 μm) and only those passed through the screen were collected and stored in plastic bags for late use.

Six experimental diets were formulated to be isonitrogenous and isoenergetic, the ingredients and results of proximate analysis of these diets are shown in Table 1 (the proximate analysis was carried out according to AOAC, 1995). To make the experimental diets, ultrafine powder of *E. prolifera* was thoroughly mixed with other feed ingredients (purchased from Yingxiang feed Co. Ltd., Xiamen, China) at the designated ratio of 0 g/kg or 0% (Diet 0; control), 10 g/kg or 1% (Diet 1), 20 g/kg or 2% (Diet 2), 30 g/kg or 3% (Diet 3), 40 g/kg or 4% (Diet 4) and 50 g/kg or 5% (Diet 5), respectively. Afterwards, they were squeezed to produce 2.5 mm pellets using a moist pelleting machine (CD4XITX, South China University of Technology, Guangzhou, China). The above products were then dried at room temperature and stored at -25°C until used.

2.2 Experimental design and setup

The feeding trial (2012.7–2012.8) was performed at the fisheries experimental station of Fisheries

Table 1 Formulation and proximate composition of the experimental diets

Ingredients (g/kg)	Experimental diet (supplementation level, g/kg)					
	Diet 0 (0%)	Diet 1 (1%)	Diet 2 (2%)	Diet 3 (3%)	Diet 4 (4%)	Diet 5 (5%)
E. prolifera ultrafine powder	0	10	20	30	40	50
Rapeseed meal	300	300	300	295	290	285
Soybean meal	270	270	270	270	270	270
Wheat meal	150	140	130	125	120	115
DDGS	150	150	150	150	150	150
Rice bran	60	60	60	60	60	60
Soybean oil	40	40	40	40	40	40
$Ca(H_2PO_4)_2$	15	15	15	15	15	15
Vitamin mixture[a]	6.0	6.0	6.0	6.0	6.0	6.0
Mineral mixture[b]	6.0	6.0	6.0	6.0	6.0	6.0
Choline chloride	1.5	1.5	1.5	1.5	1.5	1.5
Ethoxy quinoline	1.0	1.0	1.0	1.0	1.0	1.0
Calcium propinnate	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (%)						
Crude protein	29.97	29.93	29.90	29.76	29.63	29.49
Crude lipid	10.11	10.12	10.13	10.11	10.10	10.09
Crude fiber	6.65	6.68	6.71	6.69	6.67	6.65
Ash	7.20	7.32	7.45	7.54	7.64	7.74

Note: All of the ingredients were provided by Yingxiang feed company limited (Xiamen, China). Rapeseed meal: 35.7% protein, 7.4% lipid; Soybean meal, 44.2% protein, 1.9% lipid; wheat meal: 15.4% protein, 2.2% lipid; DDGS: distillers dried grains with soluble, 28.3% protein, 13.7% lipid; rice bran: 12.8% protein, 16.5% lipid. [a] Vitamin mixture (per kg of mixture): thiamin: 25 mg; riboflavin: 12 mg; pyridoxine HCL: 15 mg; vitamin B12: 0.12 mg; vitamin K3: 12 mg; inositol: 200 mg; pantothenic acid: 60 mg; niacin acid 70 mg; folic acid: 4 mg; biotin: 0.12 mg; retinol acetate: 50 mg; cholecalciferol: 12 mg; alpha-tocopherol: 100 mg; ascorbic acid: 1000 mg; ethoxyquin: 350 mg. [b] Mineral mixture (per kg of mixture): KCl: 800 mg; KI: 240 mg; $CoCl_2.6H_2O$: 30 mg; $CuSO_4.5H_2O$: 50 mg; $FeSO_4.H_2O$: 400 mg; $ZnSO_4.H_2O$: 600 mg; $MnSO_4.H_2O$: 300 mg; $Na_2SeO_3.5H_2O$ (1%): 200 mg; $MgSO_4.7H_2O$: 3 000 mg; $Ca(H_2PO_4)_2.H_2O$: 40 g; NaCl: 500 mg.

College, Jimei University, Xiamen, China. The red tilapia fingerlings were fed the control diet (Diet 0) for 2 weeks and after the acclimatization period, health individuals (weight: 4.68±0.48 g; length: 5.16±0.63 cm) were randomly selected and transferred to 18 fiberglass tanks containing 120 L water. Each of the tanks connected to a closed recirculating system (water temperature: 30±4°C, dissolved oxygen: 5.7–8.0 mg/L, and pH: 6.5–7.9, water quality parameters were measured using HACH/HQ30D portable digital multiparameter analyzer). Each treatment was triplicated and each tank stocked 30 individuals. The fish were fed the correspondent experimentally diets to satiation twice daily at 09:00 and 17:00. After each feeding, uneaten feed was recovered and dried before being weighed to estimate the amount of feed consumed.

The feeding experiment lasted for 7 weeks. At the end of the experiment, all surviving fish were not fed 24 h and batch weighted and counted from each tank. The calculation formulas are as follows:

percentage weight gain: $PWG\ (\%)=100\times(W_t-W_0)/W_0$;

specific growth rate: $SGR=100\times(\ln W_t-\ln W_0)/t$;

feed conversion ratio: $FCR=100\times FI/(W_t-W_0)$;

condition factor: $CF=100\times \text{weight}/\text{length}^3$;

hepatosomatic index: HI=100×liver mass/body mass;

survival rate: $SR\ (\%)=100\times N_t/N_0$,

where W_0 and W_t are the initial and the final body weight, respectively; t is the duration of experiment (days); FI is feed intake; N_0 and N_t are the initial and the final number of fish in each tank, respectively.

For serum immune parameters analysis, 6 fish from each replicate tank were randomly selected and euthanatized. Blood was extracted from each fish and centrifuged (3 000 r/min for 10 min, 4°C) to collect serum. Serum samples from three fish were pooled and therefore six serum samples from each treatment were obtained and kept at - 80°C until analysis.

For calculating hepatosomatic index (HI) and digestive enzyme analysis, 2 individuals from each tank (six samples from each treatment) were

Table 2 The growth performance and survival rates of the red tilapia fed the experimental diets containing different levels of *E. prolifera* ultrafine power

	E. prolifera ultrafine power inclusion level					
	Diet 0 (0%)	Diet 1 (1%)	Diet 2 (2%)	Diet 3 (3%)	Diet 4 (4%)	Diet 5 (5%)
Final weight (g)	21.14±0.78^{a}	21.93±0.28^{a}	22.22±0.81^{b}	22.91±0.26^{bc}	23.58±0.66^{c}	23.69±0.20^{c}
FI (g)	47.46±0.04^{a}	47.93±0.02^{b}	47.92±0.04^{b}	48.05±0.07^{bc}	48.15±0.07^{bc}	49.09±0.07^{c}
PWG (%)	350.9±16.54^{a}	367.9±5.97^{bc}	374.1±17.22^{cd}	388.9±5.53^{cd}	403.2±14^{d}	405.5±4.37^{d}
SGR	3.50±0.09^{a}	3.64±0.02^{ac}	3.63±0.16^{ab}	3.68±0.06^{bc}	3.79±0.04^{bc}	3.78±0.05^{bc}
FCR	2.89±0.13^{a}	2.78±0.04^{ac}	2.74±0.13^{ab}	2.63±0.04^{bcd}	2.55±0.09^{d}	2.58±0.02^{bd}
CF	4.01±0.51^{a}	4.02±0.54^{a}	4.05±0.26^{a}	4.10±0.23^{a}	4.15±0.23^{a}	4.24±0.26^{a}
HI	1.37±0.40^{a}	1.33±0.48^{a}	1.38±0.65^{a}	1.48±0.64^{a}	1.31±0.48^{a}	1.55±0.42^{a}
Survival (%)	100	100	100	100	100	100

FI: feed intake; PWG: percentage weight gain; SGR: specific growth rate; FCR: feed conversion ratio; CF: condition factor; HI: hepatosomatic index. Values are presented as mean±SD (n=3); different superscripts in a same row denote significant differences (P<0.05). The uppercase letters (a, b, c and d) have no specific meaning, and the diets with completely different letters label have significant difference.

euthanatized and dissected, and then intestine, stomach and liver were weighted and quickly treated by liquid nitrogen flash freezer for digestive enzyme analysis.

2.3 Digestive enzyme analysis

For digestive enzyme analysis, the intestine and stomach were rinsed with chilled distilled water, homogenized and centrifuged (2 500 r/min for 10 min). The resultant supernatants were then quickly collected and kept at -20°C. The pepsin, erepsin, gastric amylase, intestinal amylase, gastric lipase and intestinal lipase activities were analyzed according to the manufacture's instruction of respective test kits (Jiancheng technology Co. Ltd., Nanjing, China).

2.4 Serum nonspecific immune enzymes determination

Serum lysozyme activity was measured by a turbidometric assay using lyophilized *Micrococcus lysodeikticus* (Jiancheng technology Co. Ltd., Nanjing, Jiangsu, China) (Ellis 1990). A volume of 950 μL *M. lysodeikticus* at a concentration of 200 mg/mL (w/v) in 0.05 mol/L PBS (pH 6.2) was added to 50 μL of serum sample. The decrease in OD was recorded at 530 nm using a spectrophotometer (UV-2802S, Shimadzu, Kyoto, Japan) after 1 and 6 min at 25°C. A unit of lysozyme activity was defined as the amount of serum causing a reduction in absorbance of 0.001 units per min.

The total superoxide dismutase (T-SOD) activity was determined by an enzymatic assay method using a reagent kit (Jiancheng technology Co. Ltd., Nanjing, Jiangsu, China) as described by Sun et al. (2010). The alkaline phosphatase (AKP) and acid phosphatase (ACP) activities were assayed using AKP and ACP kits (Jiancheng technology Co. Ltd., Nanjing, Jiangsu, China) according to manufacturer's instruction.

2.5 Statistical analysis

Data are presented as mean±standard deviation (SD). One-way analysis of variance (ANONA) was employed for analysis of the data. Where significant differences (P<0.05) were detected, the least significant difference (LSD) was used to separate the differences among treatment means. The software used for statistical analysis was SPSS, Version 19.0 (Chicago, IL, USA).

3 RESULT

3.1 Growth performance

The growth performance of the red tilapia is shown in Table 2. The percentage weight gain (PWG), specific growth rate (SGR), condition factor (CF) and hepatosomatic index (HI) showed an overall trend of increasing with increased inclusion level of *E. prolifera* powder. In particular, the highest *E. prolifera* powder inclusion level of 5% (Diet 5) led to the highest increases in PWG (15.4%), SGR (8.0%) and HI (5.7%), respectively when compared to the control. Meanwhile, compared to that of the control, FCR decreased by 11.7% and 10.7% respectively when the fish were fed the diets with *E. prolifera* inclusion level at 4% (Diet 4) and 5% (Diet 5). Statistics analysis showed that PWG, SGR and FCR

Table 3 Activities of digestive enzymes (U/mg protein) of the red tilapia fed the experimental diets containing different levels of *E. prolifera* ultrafine power

Indicators	*Enteromopha prolifera* ultrafine power inclusion level					
	Diet 0 (0%)	Diet 1 (1%)	Diet 2 (2%)	Diet 3 (3%)	Diet 4 (4%)	Diet 5 (5%)
Pepsin	18.96±0.69^{a}	19.31±0.85^{a}	20.86±1.85^{b}	21.95±0.87^{b}	23.13±1.62^{b}	23.34±1.47^{b}
Erepsin	1509.3±150.0^{a}	1511.1±64.2^{a}	1520.0±96.2^{a}	1553.0±173.7^{a}	1562.1±185.0^{a}	1746.6±83.3^{a}
Gastric amylase	2.04±0.02^{a}	2.17±0.07^{a}	2.27±0.10^{ab}	2.30±0.31^{ab}	2.33±0.38^{b}	2.72±0.53^{bc}
Intestinal amylase	2.22±0.09^{a}	2.25±0.32^{ab}	2.76±0.20^{bc}	2.84±0.36^{c}	3.26±0.43^{cd}	3.38±0.31^{d}
Gastric lipase	198.14±2.53^{a}	203.38±18.24^{ab}	207.74±18.38^{ab}	221.24±13.49^{b}	219.77±20.04^{b}	230.35±25.0^{c}
Intestinal lipase	135.6±5.67^{a}	142.1±1.72^{a}	149.0±1.44^{a}	153.1±5.14^{ab}	165.5±11.7^{bc}	173.8±21.7^{c}

Values are presented as mean±SD (n=6); different superscripts in a same row denote significant differences (P<0.05).

Table 4 The serum total superoxide dismutase, lysozyme, acid and alkaline phosphatase activities and serum total protein levels of the red tilapia fed the experimental diets containing different levels of *E. prolifera* ultrafine power

Indicators	*E. prolifera* ultrafine power inclusion level					
	Diet 0 (0%)	Diet 1 (1%)	Diet 2 (2%)	Diet 3 (3%)	Diet 4 (4%)	Diet 5 (5%)
T-SOD (U/mg protein)	85.88±4.11^{a}	86.00±6.70^{a}	90.75±6.70^{ab}	95.38±11.71^{ab}	97.85±4.80^{ab}	102.56±2.57^{c}
ACP (U/mg protein)	9.13±0.52^{a}	10.03±0.85^{ab}	12.14±1.95^{ab}	12.74±2.08^{bc}	13.52±2.31^{c}	13.33±2.07^{bc}
AKP (U/mg protein)	3.83±0.68^{a}	4.45±0.51^{ab}	4.42±0.53^{ab}	4.64±0.26^{b}	4.90±0.43^{c}	4.96±0.61^{c}
Lysozyme (U/mg protein)	4.00±0.46^{a}	4.12±0.38^{a}	4.26±0.26^{a}	4.44±0.66^{ab}	4.70±0.11^{b}	5.21±0.49^{c}
Total protein (mg/mL)	34.41±1.68^{a}	34.94±1.41^{a}	35.56±0.80^{ab}	35.41±2.15^{ab}	36.46±1.08^{bc}	37.42±0.35^{c}

T-SOD: total superoxide dismutase; ACP: acid phosphatase; AKP: alkaline phosphatase. Values are presented as mean±SD (n=6); different superscripts in a same row denote significant differences (P<0.05).

of the treatments in which the fish were fed the diets with *E. prolifera* powder inclusion level between 2% (Diet 2) and 5% (Diet 5) were all significantly better than the control (P<0.05), however no significant difference in CF, HI and SR were detected for any experimental diets (P>0.05) (Table 2).

3.2 Digestive enzyme activities

The activities of gastrointestinal digestive enzymes are shown in Table 3. The results shown that when the red tilapia were fed the diets with increasing inclusion levels of *E. prolifera* ultrafine powder, the protease, amylase and lipase activities all showed upward trends, achieving peaks for the feeding treatment with the highest 5% *E. prolifera* inclusion level (Diet 5). Compared with the control, the pepsin and erepsin increased by 23.1% and 15.7%, respectively, the gastric amylase and the intestinal amylase increased by 33.3% and 53.3%, respectively, and the gastric lipase and the intestinal lipase increased by 16.3% and 28.2%, respectively, when the fish were fed this diet and the differences were statistically significant (P<0.05).

3.3 Serum nonspecific immunity

As shown in Table 4, incorporating *E. prolifera* ultrafine powder in the diets of the red tilapia enhanced serum non-specific immunity and an overall trend of improving with increasing *E. prolifera* inclusion level was shown. As the result, the T-SOD, ACP and AKP activities of the fish fed both the diets with *E. prolifera* inclusion level at 4% (Diet 4) and 5% (Diet 5) were significantly higher than the control (P<0.05). Significantly improved lysozyme activities and serum total protein were also observed in the fish fed the diet with *E. prolifera* incorporated at 5% (Diet 5) (P<0.05), which increased by 19.4%, 29.5%, 30.3% and 8.7%, respectively, as compared to the control (Table 4).

4 DISCUSSION

It is well known that *E. prolifera* is not only rich in major nutrients, but also contains high levels of various active ingredients—phenols, bioactive peptides, polysaccharide and terpenoids, for example (Chapman and Chapman, 1980; Oohusa, 1993; Zhou et al., 1995; Aguilera-Morales et al., 2005; Harnedy and FitzGerald, 2011). There have been several

reports on effects of *E. prolifera* incorporated as a minor component of practical feeds on the growth of marine fish. For example, Asino et al. (2011) found that adding *E. prolifera* at 100 (10%) and 150 g/kg (15%) to the diets of juvenile yellow croaker, *Pseudosciaena crocea*, positive effects on growth performance and feed efficiency were shown. On the other hand, Yousif et al. (2004) showed that the feed efficiency and growth performance of the rabbitfish *Siganus canaliculatus* was decreased by a dietary 30 g/kg (3%) inclusion level of dehydrated *Enteromorpha* sp., however, the inclusion of 10 (1%) and 20 g/kg (2%) did not have significantly effect. Likewise, dietary inclusion at 10 (1%), 20 (2%) and 30 g/kg (3%) of *Enteromorpha* sp. showed no significant effects on growth performance of the bluespot grey mullet *Valamugil seheli* fry (Yousif, 2012). Besides, Yang et al. (2016) have reported that diets supplemented with 20, 30 and 40 g/kg fermented *E. prolifera* can similarly improve the growth performance, feed efficiency, digestive enzyme activities and non-specific immune response of red tilapia and the recommended dose of fermented *E. prolifera* is 37–41 g/kg in the diet of red tilapia.

In the present study, the PWG and SGR showed a clear trend of improvement with increasing supplementation level of *E. prolifera* ultrafine powder. A possible explanation for this consistent positive result could be that processing of *E. prolifera* as superfine power in our study enhanced the incorporation of positive components of *E. prolifera* into the diets, the solubility of such components was likely significantly increased, which led to better digestion and absorption by the fish. Tkacova and Stevulova (1998) reported that superfine grinding technology substantially enhanced dispensability and solubility of the materials.

Other reasons that likely contributed to the improved growth performance observed in this study include the enhanced digestive ability and nonspecific immunity of the red tilapia fed the diets incorporating *E. prolifera* ultrafine powder. Our results showed that the digestion ability of the red tilapia increased with the increase of inclusion level of *E. prolifera* ultrafine powder. There are reports that fish digestive enzyme activities are partially correlated to the changes in diet compositions (Glencross, 2009; Booth et al., 2013). Different dietary ingredients with key bioactive chemicals could lead to very different results (Stone et al., 2003). *Enteromorpha Prolifera* is known to contain many bioactive substances, such as saccharicterpenin, xylooligosaccharide and rich in dietary fiber, which may contribute to the enhanced secretion of digestive enzymes. The different structural units of polysaccharides has been reported to concentrate trace elements for forming functional substances, which could stimulate the gastrointestinal tissue and have biological protective effects on the enzymes from degradation (Kolkovski, 2001). *Enteromopha prolifera* ultrafine powder may also acted as a feed attractant, not only improved diet ingestion, but also stimulated the digestion of the feeds by the fish.

In aquatic animals, the specific immune mechanisms are diverse and could be immature while the nonspecific immune responses develop earlier and play an important role in the immune defense (Dalmo et al., 1997; Magnadóttir, 2006). It has been confirmed that marine macroalgae as feed additive can promote the stress resistance, immune responses and survival in fish, for instance, *Enteromorpha* sp., *Ulva rigida* and *Chondrus crispus* reportedly improved the respiratory activity of turbot phagocytes (Castro et al., 2004; Wassef et al., 2009, 2013). In this research, all nonspecific immune parameters measured was significantly higher in fish fed the diet containing 50 g/kg of *E. prolifera* powder (Diet 5) while the T-SOD, ACP and AKP activities were also significantly higher in fish fed the diet containing 40 g/kg of *E. prolifera* powder (Diet 4), suggesting enhanced nonspecific immunoregulation in the fish (Saurabh and Sahoo, 2008; Van Muiswinkel and Nakao, 2014). Although the specific mechanisms need further investigation, the polysaccharides from *E. prolifera* maybe play a vital role in immunostimulation (Cho et al., 2010; Kim et al., 2011). In fact, it has been reported that polysaccharide of *E. prolifera* was able to significantly increase the immune activity of *Chlamys nobilis* in an in vitro study (Xu et al., 2005). Castro et al. (2004) reported that polysaccharides from *E. prolifera* can boost immunity of turbot by enhancing the vitality of neutrophils and macrophages. Besides, polysaccharides from *E. prolifera* can promote the humoral immunity, cellular immunity and mononuclear phagocytic system of mice Wei et al. (2014). Wei et al. (2015) has also recently reported that polysaccharide from *E. prolifera* enhanced non-specific immune responses and protection against *Vibrio splendidus* infection in sea cucumber.

5 CONCULUSION

Based on the results from this study, at 5% inclusion

level, *E. prolifera* ultrafine powder was shown to significantly improve the growth, immunity and digestive enzyme activities in the red tilapia with most of the measured parameters reached their peaks among different treatments. It is therefore recommended that the dose of *E. prolifera* ultrafine powder to be incorporated in the diets of the red tilapia should be 5% or 50 g/kg.

6 DATE AVAILABILITY STATEMENT

The datasets and materials supporting the conclusions of this article are included within the article.

7 ACKNOWLEDGMENT

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