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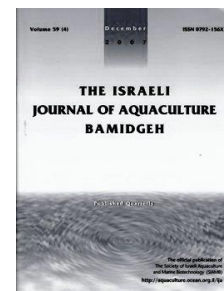
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Effect of Dietary Marine Red Yeast *Rhodotorula mucilaginosa* on the Growth Performance, and also Non-Specific Immune Responses of Juvenile Golden Pompano *Trachinotus Ovatus* when Challenged with *Vibrio Harveyi*

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Key words: *Trachinotus ovatus*; *Rhodotorula mucilaginosa*; Growth performance; Immune response; *Vibrio harveyi*

Abstract

The present study was conducted to investigate the effects of dietary marine red yeast *Rhodotorula mucilaginosa* supplementation on the growth performance, non-specific immune responses, and resistance to the pathogen *Vibrio harveyi* in Golden Pompano *Trachinotus ovatus*. A basal diet was supplemented with *R. mucilaginosa* at 0‰ (control), 1‰, 2‰, 3‰, 4‰, and 5‰ for 8 weeks. After the 8-week feeding trial, weight gain (WG) and specific growth rate (SGR) were significantly affected by the *R. mucilaginosa* levels, with the highest WG and SGR occurring at the 1‰ *R. mucilaginosa* level ($P < 0.05$). Compared to the control, the 4 and 5‰ *R. mucilaginosa* groups had significantly increased lysozyme (LYZ) and alkaline phosphatase (AKP) activity, but a decrease in nitric oxide synthase (NOS) was noted ($P < 0.05$). Compared to the control, the 2, 3 and 4‰ *R. mucilaginosa* groups had significantly increased hepatic superoxide dismutase (SOD) activity ($P < 0.05$), while hepatic malondialdehyde (MDA) content decreased significantly ($P < 0.05$). After challenge with *V. harveyi*, the group supplemented with 1‰ *R. mucilaginosa* had 100% survival rate. In addition, compared to the control group prior to challenge, the serum C3 level significantly increased in the group supplemented with 2‰ *R. Mucilaginosa* ($P < 0.05$). Compared to the control 12 h and 48h after challenge, serum C4 levels in the 4‰ *R. Mucilaginosa* group increased significantly ($P < 0.05$). Our results suggest that ingestion of a basal diet supplemented with 1-3‰ *R. mucilaginosa* in *T. ovatus* could enhance resistance against the pathogen *Vibrio harveyi*.

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Introduction

Golden pompano (*Trachinotus ovatus*) is an economically important warm-water marine fish species cultured in many parts of the world (Zheng et al., 2014; Sun et al., 2014). In recent years, golden pompano aquaculture has developed rapidly along the southern coast of China so too has culture density. Bacterial infectious diseases have also increased resulting in serious losses (Xia et al., 2012; Zhang et al., 2014). In *T. ovatus* aquaculture, chemicals are usually used for treatment and prophylactically to control diseases. However, increased use of chemicals in aquaculture raises health concerns related to chemical residues in aquatic environments and seafood, and the emergence of drug-resistant bacteria in sediments and water in aquaculture environments (Liu et al., 2012). Therefore, the development of the natural and non-chemical therapeutics has become a necessity leading to the use of probiotics, immuno-stimulants from plant sources (Ganguly et al., 2010; Zhou et al., 2015; Xia et al., 2013).

The purpose of the present study is to evaluate the effects of dietary *Rhodotorula mucilaginosa* on immunity and disease resistance of *T. ovatus*. Our results provide insight into the use of *R. mucilaginosa* as an immunostimulant and as a valuable alternative to the use of antibiotics and vaccines in the fight against infectious diseases in this fish species.

Materials and Methods

Experimental design and diets. *R. mucilaginosa* used in this study was provided by Xinhailisheng technology company (Guangzhou, China). It contained 9.3% crude protein, 4.2% total sugar, 1.3% β -glucan, 0.0014‰ β -carotene, 0.001‰ astaxanthin, 0.172‰ vitamin E. The proximate composition is given in table 1.

Table. 1 The basal diet and nutrition levels.

Ingredients (%)	Percentage dry weight
Fish meal ^a	25
Soy protein concentrate	20
Soybean meal	8
Peanut powder	6
Wheat flour	27
Soybean oil/ fish oil	10
Calcium biphosphate	1
Vitamin mixture ^b	1
Mineral mixture ^b	1
Choline choride (50%)	0.5
Lecithin	0.5
Nutrition levels	
Moisture	8.45
Crude protein	41.4
Crude fat	15.6
Crude ash	8.5

^a White fishmeal was north pacific white fishmeal and purchased from American Seafoods Company, Seattle, Washington, USA.

^b Vitamin and mineral mixture was based on Zhou et al. (2015).

Six experimental diets were formulated to contain approximately 41.4% crude protein and different levels of *R. mucilaginosa* at 0.0 (control), 1.0, 2.0, 3.0, 4.0 or 5.0 ‰. All ingredients were ground and sieved through a 40-mesh screen and thoroughly mixed in a Hobart-type mixer until a homogenous consistency was reached. 1-mm diameter pellets were wet-extruded with 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 used.

Experimental fish and experimental conditions. *T. ovatus* were obtained from Shenzhen Experimental Station of South China Sea Fisheries Research Institute of CAFS. Prior to the start of the 8 week feeding trial, fish were acclimated to a commercial diet (containing 42% crude protein) for 2 weeks and were fed twice daily to apparent satiation. At the beginning of the feeding trial, the fish were fasted for 24 h and weighed after being anesthetized with diluted eugenol (1: 10000; Shanghai Reagent Corp., China). Juvenile *T. ovatus* of similar size (initial weight 16.7±0.1g) were randomly

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allotted into 18 sea cages (1.0 m × 1.0 m × 1.5 m) each stocked with 25 fish. Each experimental diet was randomly assigned to three cages. *T. ovatus* were fed twice daily (08:00h and 16:00h). To prevent wastage of pellets, fish were slowly hand-fed to satiation, based on visual observation of their feeding behavior. Feed consumption was recorded per cage every day. During the experimental period, water temperature ranged from 21-29 °C, salinity from 14-17 g/L, pH from 7.5-8.0. Dissolved oxygen was not less than 5.0 mg/L and ammonia nitrogen was maintained lower than 0.1 mg/L. At the end of experiment, the fish were fasted for 24 h and fish in each cage were weighed.

Effect of R. mucilaginosa on survival of T. ovatus. After 8 weeks, a subsample of fish from the six groups (3 tanks/group, N=12 fish/group) were challenged with *Vibrio harveyi* provided by South China Sea Fisheries Research Institute, China. *V. harveyi* was activated twice and diluted by sterile normal saline. Initially the pathogenic isolates of *V. harveyi* were grown on nutrient broth for 24 h at 30 °C in an incubator and harvested by centrifuging the culture broth at 12000 g for 10 min at 4 °C (Alexander et al., 2011). The cells were then washed three times in sterile PBS (pH 8.0) and the final concentration was 3×10^8 CFU/mL. The bacterial suspension (1.0 ml, per 100 g body weight) was injected into the abdominal cavity and mortalities counted 0 h, 12 h, 24 h, 36h, 72 h, 96 h, 120 h and 168h post-challenge.

The effect of R. mucilaginosa on immune response of T. ovatus. A second subsample of fish (3 tanks/group, N=18 fish/group) was also challenged with *V. harveyi* (1.5×10^7 CFU/mL, 1.0 ml per 100 g) after 8 weeks following the procedure described above. Prior to the challenge, serum and liver samples from 9 individuals (3fish/tank) were collected at 6, 12, 24, and 48 h after the challenge.

Serum and liver sample collection and measurement. At each sampling point, fish were rapidly netted, and sedated with diluted eugenol (1: 10000). Serum from the caudal vein was collected with 2 ml heparinized syringes and stored at 4 °C for 1-2 h. The serum was then centrifuged for 10 min (4 °C, 3000×g), the supernatant removed and stored at -20 °C. The liver was excised, frozen in liquid nitrogen, and stored at -80 °C until analysis.

Serum lysozyme, alkaline phosphatase and nitric oxide synthase measurement. Lysozyme (LYZ) activity was measured with a turbidimetric assay (Muona and Soivio, 1992). Serum alkaline phosphatase (AKP) activity was tested by ROCHE-P800 automatic biochemical analyzer (Roche, Basel, Switzerland). Nitric oxide synthase (NOS) activity was measured with an assay kit (Nanjing Jiancheng Bioengineering Institute, China), with the modified Griess methods (Marzinzig et al., 1997). The detailed operation is described in the NOS kit instructions (Nanjing Jiancheng Bioengineering Insitute, China).

Serum complement 3 and complement 4 measurement. The plasma complement 3 (C3) and complement 4 (C4) levels were determined using immune turbidimetric method described by Sun et al. (2010), using commercial test kits (Nanjing Jiancheng Bioengineering Insitute, China).

Hepatic superoxide dismutase and malondialdehyde measurement. 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 superoxide dismutase (SOD), malondialdehyde (MDA). Hepatic SOD activity and MDA content were measured using a xanthine oxides (Marklund and Marklund 1974) and barbituric acid reaction chronometry (Drape et al., 1993), respectively. We measured the hepatic protein content using the Folin method (Lowry et al., 1951), with bovine serum albumin as the standard.

Statistical analysis. We used SPSS (19.0, SPSS Inc., Michigan Avenue, Chicago, IL, USA) software Duncan's multiple-range test to determine the differences between groups. $P < 0.05$ indicated that there were significant differences. All the results were expressed as the standard of the means (\pm SEM) of three replicates.

Results

The effect of R. mucilaginosa on the growth of T. ovatus. Growth, feed utilization, and biometric parameters of juvenile pompano fed different *R. mucilaginosa* levels are presented in Table 2.

Table 2. Effect of *R. mucilaginosa* on growth performance and feed utilization of *T. ovatus*

	<i>R. mucilaginosa</i> level (‰)					
	0	1	2	3	4	5
Initial body weight (g)	16.76±0.06	16.74±0.10	16.63±0.07	16.63±0.07	16.62±0.11	16.58±0.04
Final body weight (g)	68.93±2.46 ^a	85.41±5.36 ^b	73.64±1.88 ^a	74.23±1.18 ^a	71.56±3.83 ^a	70.28±3.73 ^a
WG (%)	5.77±0.28 ^a	7.62±0.50 ^b	6.35±0.23 ^a	6.42±0.16 ^a	6.13±0.47 ^a	6.00±0.42 ^a
SGR (%day ⁻¹)	2.62±0.07 ^a	3.02±0.10 ^b	2.75±0.05 ^a	2.77±0.04 ^a	2.80±0.09 ^a	2.77±0.03 ^a
FCR	1.19±0.07 ^{ab}	1.36±0.03 ^b	1.10±0.05 ^{ab}	0.95±0.06 ^a	1.05±0.09 ^{ab}	1.32±0.20 ^b
HSI	2.32±0.13 ^b	1.87±0.04 ^a	1.71±0.22 ^a	1.67±0.08 ^a	1.85±0.05 ^a	1.91±0.18 ^{ab}
VSI	6.54±0.30	6.20±0.03	6.33±0.34	5.95±0.09	6.51±0.11	6.35±0.25
CF	3.62±0.02	3.47±0.07	3.48±0.03	3.53±0.05	3.57±0.05	3.55±0.06

Values are means ± SEM of three replications. Means in the same line with different superscripts are significantly different ($P<0.05$). WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; HSI, hepatosomatic index; VSI, viserosomatic index; CF, condition factor

After the 8-week feeding trial, WG and SGR were significantly affected by the *R. mucilaginosa* levels, with the highest WG and SGR occurring at the 1‰ *R. mucilaginosa* level ($P<0.05$). There were no differences in WG among treatments, except for fish fed the 1‰ *R. mucilaginosa* diet, which was higher than those fed the other diets. A similar trend was observed in SGR ($P<0.05$). The FCR of pompano fed *R. mucilaginosa* levels of 1‰ and 5‰ were significantly higher than those of fish fed 3‰ *R. mucilaginosa* level, but not statistically different ($P>0.05$) from the FCR of the other treatments. There were no differences in the hepatosomatic index (HSI) among treatments, except for fish in the control group, which was higher than those in other treatments ($P<0.05$). There were no statistical differences in viserosomatic index (VSI) and condition factor (CF) ($P>0.05$) among all treatments.

The effect of *R. mucilaginosa* on hemolymph lysozyme, AKP and NOS of *T. ovatus*. Serum LYZ activity was significantly affected by *R. mucilaginosa* levels, with the highest LYZ activity occurring at the 4‰ and 5‰ *R. mucilaginosa* levels ($P<0.05$) (Table 3).

Table 3. Effect of *R. mucilaginosa* on haemolymph lysozyme, AKP and NOS of *T. ovatus*

<i>R. mucilaginosa</i> level (‰)	LYZ (U/mL)	AKP (U/g)	NOS (U/mL)
0	14.49±1.89 ^a	10.86±1.07 ^a	9.00±0.28 ^d
1	20.56±2.22 ^{ab}	13.11±0.42 ^{bc}	5.88±0.64 ^b
2	24.28±2.86 ^b	12.94±0.25 ^{bc}	8.74±0.47 ^d
3	27.26±0.37 ^{bc}	11.44±0.25 ^{ab}	6.60±0.35 ^{bc}
4	32.28±2.02 ^c	13.56±0.22 ^{cd}	8.12±0.47 ^c
5	32.98±2.59 ^c	14.96±0.44 ^d	3.31±0.35 ^a

Values are means ± SEM of three replications. Means in the same column with different superscripts are significantly different ($P<0.05$). LYZ, lysozyme; AKP, alkaline phosphatase; NOS, nitric oxide synthase.

Compared to the control, the 2, 3, 4 and 5‰ *R. mucilaginosa* groups had significantly increased LYZ activity ($P<0.05$). The serum AKP activity increased with increasing *R. mucilaginosa* levels up to 5‰ ($P<0.05$). Compared to the control, the 1, 2, 4 and 5‰ *R. mucilaginosa* groups had significantly increased alkaline phosphatase (AKP) activity ($P<0.05$). The 1, 3, 4 and 5‰ *R. mucilaginosa* groups had significantly decreased serum nitric oxide synthase (NOS) activity ($P<0.05$) compared to the control. In fish fed the highest *R. mucilaginosa* level serum NOS activity was significantly lower than those of fish in other groups ($P<0.05$).

The effect of *R. mucilaginosa* on hepatic superoxide dismutase and malondialdehyde activities of *T. ovatus*. The effect of *R. mucilaginosa* on hepatic superoxide dismutase and malondialdehyde activities is shown in Table 4.

Table 4. Effect of *R. mucilaginosa* on hepatic superoxide dismutase and malondialdehyde activities of *T. ovatus*

<i>R. mucilaginosa</i> level (‰)	SOD U/mgprot	MDA nmol/mL
0	94.00±1.06 ^{ab}	110.17±0.79 ^c
1	104.88±1.40 ^{bc}	138.13±0.78 ^d
2	124.80±3.10 ^{de}	79.69±2.71 ^b
3	113.55±4.78 ^{cd}	86.07±5.45 ^b
4	127.96±4.36 ^e	63.98±3.08 ^a
5	84.51±5.92 ^a	90.17±4.11 ^b

Values are means ± SEM of three replications. Means in the same column with different superscripts are significantly different ($P<0.05$). SOD, superoxide dismutase; MDA, malondialdehyde.

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Hepatic SOD activity increased with increasing *R. mucilaginosa* levels up to 4‰ and then declined ($P < 0.05$). Compared to the control, the 2, 3 and 4‰ *R. mucilaginosa* groups had significantly increased hepatic SOD activity ($P < 0.05$). Compared to the control, the 1‰ *R. mucilaginosa* groups had significantly increased hepatic MDA content ($P < 0.05$), however the group supplemented with 2, 3, 4 and 5‰ *R. mucilaginosa* had significantly decreased hepatic MDA content ($P < 0.05$). The hepatic MDA content of pompano fed *R. mucilaginosa* level of 4‰ was significantly lower than those of fish in other groups ($P < 0.05$).

Effect of R. mucilaginosa on survival in T. ovatus. After 8 weeks of being fed *R. mucilaginosa*, golden pompano were challenged with *V. harveyi*. Survival rate was calculated for seven days (Fig. 1). There were no significant differences among the survival rate in all groups at 0-24 h after *V. harveyi* challenge. But after 7 days of *V. harveyi* challenge, the survival rate in 1‰ *R. mucilaginosa* group was 100%, followed by 0‰, 2‰, 5‰, 4‰ and 3‰ *R. mucilaginosa* group.

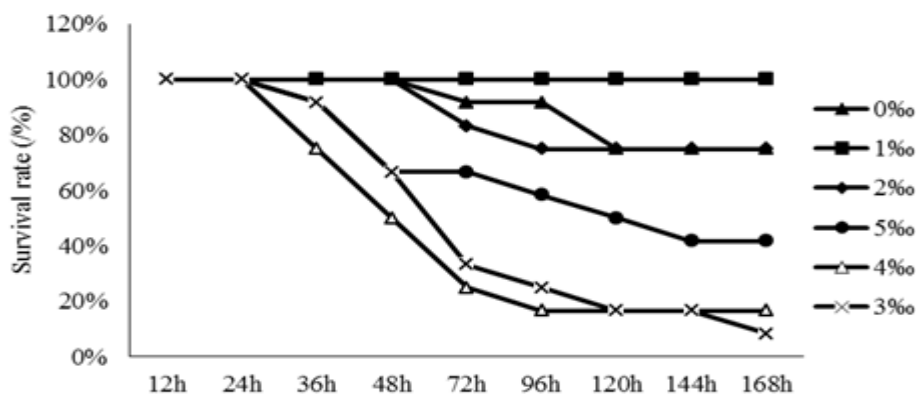
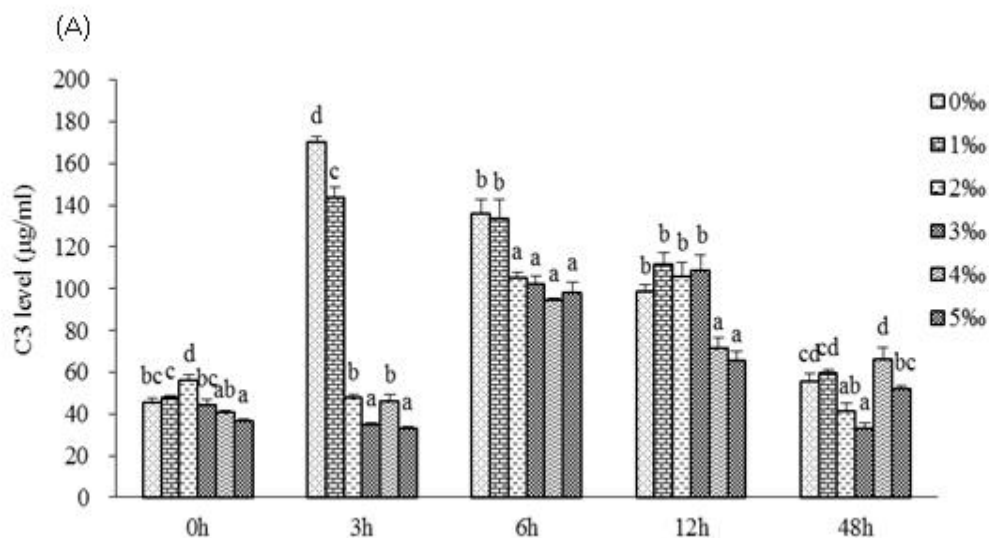


Fig.1. Effects of *R. mucilaginosa* on survival rate of *T. ovatus* after *V. harveyi* challenge.

The effect of R. mucilaginosa on hemolymph complement 3 (C3) and complement 4 (C4) of T. ovatus. Compared to the control group prior to challenge, the serum C3 level significantly increased in the group supplemented with 2‰ *R. Mucilaginosa*, the serum C3 level significantly decreased in 5‰ *R. Mucilaginosa* group ($P < 0.05$, Fig. 2 A).



The serum C3 levels were significantly lower in 2, 3, 4 and 5‰ *R. Mucilaginosa* groups than the control group 3 and 6 h after challenge. Compared to the control 12 h after challenge, the 4 and 5‰ *R. Mucilaginosa* groups had significantly decreased serum

C3 levels ($P < 0.05$). Compared to the control 48 h after challenge, the 2 and 3‰ *R. Mucilaginosa* groups had significantly decreased serum C3 levels ($P < 0.05$).

Compared to pre-challenge levels in the control group, the serum C4 levels significantly decreased in the treatment groups ($P < 0.05$, Fig. 2B).

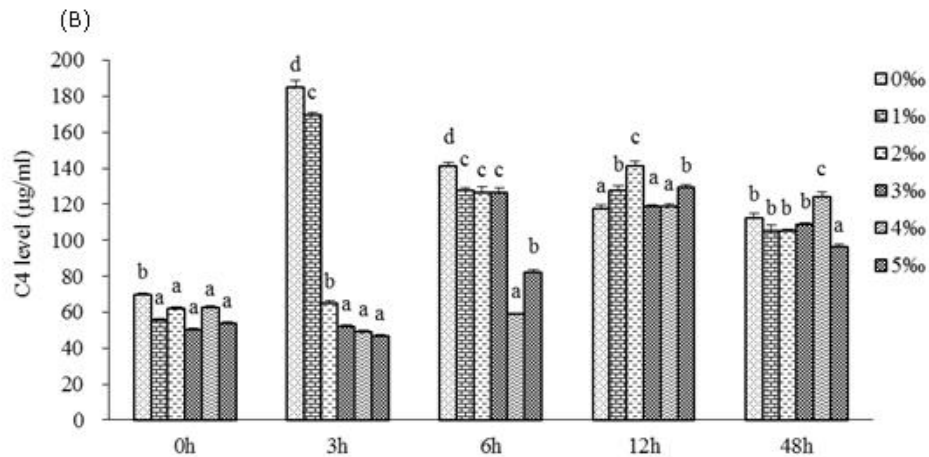


Fig.2. Change of serum C3 (A); C4 (B) levels of *T. ovatus* fed with different dietary *R. mucilaginosa* levels after *V. Harveyi* challenge.

The serum C4 levels were significantly lower in 1, 2, 3, 4 and 5‰ *R. Mucilaginosa* groups than the control group 3 and 6 h after challenge. Compared to the control 12 h after challenge, the 1, 4 and 5‰ *R. Mucilaginosa* groups had significantly increased serum C4 levels ($P < 0.05$). Compared to the control 48 h after challenge, the 4‰ *R. Mucilaginosa* groups had significantly increased serum C4 levels, the 5‰ *R. Mucilaginosa* groups had significantly decreased serum C4 levels ($P < 0.05$).

Discussion

The cost of supplemented *R. mucilaginosa* is reasonable and Marine red yeast has been found to have beneficial applicative potential for aquaculture (Yang et al., 2013; Yang et al., 2010; Zhang et al., 2013; Sun et al., 2015). In this present study, the WG and SGR of *T. ovatus* fed 1‰ *R. Mucilaginosa* diet were higher than the control group. Previous studies also showed that compared to the control group, WG and SGR of *Litopenaeus vannamei* fed diets of *R. paludigenum* supplementation increased significantly (Yang et al., 2010). Addition of 1‰ *Rhodotorula benthica* in brown fish meal significantly improved feeding rate, protein efficiency rate, and growth performance of turbot, similar to growth level from white fish meal (Zhang et al., 2013). The final mean weight of sea bass larvae in a group fed with 1.1% of marine yeast *D. hansenii* CBS8339 was twice that of the other groups (Tovar et al. 2004). These results suggest that marine red yeast produces many bioactive substances, such as protein, amino acids, fatty acid, polysaccharide and carotenoids, which could promote growth in aquatic animals.

LYZ is a bactericidal peptide, which is an important component of the immune defense system of marine fish species (Liu et al., 2012). It is responsible for breaking down the polysaccharide walls of many kinds of bacteria and thus provides some protection against pathologic infection (Hauge et al., 2002). The AKP is an important component of lysosomal enzymes that originate from hemocytes to destroy extracellular invaders (Cheng and Rodirick, 1975), suggesting that phagocytic competence and AKP activity are related to the quantity and quality of hemocytes. Similarly, in the present study, the *R. mucilaginosa* diets had significantly increased serum LYZ and AKP activities compared to the control. Nitric oxide produced by NOS is associated with diverse actions in neurotransmission, vascular systems, and immunity, including antimicrobial and antiviral activities by way of inhibiting DNA as well as protein and lipid synthesis (Howe et al., 2002). In this study, compared to other treatments, the group supplemented with 2‰ *R. mucilaginosa* had significantly increased serum NOS activity ($P < 0.05$). This agrees with the findings of Zhang et al. (2011), who found that immune parameters (LYZ activity and NOS activities) in shrimp (*Penaeus japonicus*) fed the diet with both *Bacillus*

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probiotics and IMO (T3) were significantly higher than the control group. These results suggest that oceanic red yeast may stimulate immune responses.

Increased free radicals in the liver have led to an increase in lipid peroxidation and peroxidation injury in fish (Liu et al., 2012). The breakdown of hepatic lipid peroxide yields large amounts of aldehydes, alcohols, and hydrocarbons such as MDA which is a strong toxic chemical and may lead to cellular metabolism, and even death. Antioxidant enzymes play a prominent role in resistance against lipid oxide damage (Lopes et al., 2001). Dietary supplementation with marine red yeast can significantly enhance antioxidant activity in aquatic animals (Li et al., 2004). Groups supplemented with 2-4‰ *R. mucilaginosa* had increased hepatic SOD activity compared to the control, whereas the hepatic MDA content in 2-5‰ *R. Mucilaginosa* groups decreased, especially the 4‰ *R. Mucilaginosa* group. The SOD activity of hepatopancreases from *L. vannamei* in groups fed with the live yeast diet and the dry yeast diet were significantly higher than that in the control group, whereas no statistical difference was found in MDA content of hepatopancreas (Yang et al., 2010). Our results suggest that supplementation of *R. Mucilaginosa* reduces the potential for oxidative damage in *T. Ovatus*.

Bacterial challenge tests have often been used as a final indicator of fish health status after nutrition trials (Zhou et al., 2015). Vibriosis caused by *V. harveyi* is a halophilic Gram-negative bacterium known to cause disease in fish, shrimp, and shellfish either in aquaculture or in wild aquatic environments (Sharma et al., 2012). In the present study, *T. ovatus* fed dietary *R. mucilaginosa* had an increased survival rate after a challenge with *V. Harveyi*. A previous study also showed that red yeast could colonize in the intestine of the European sea bass (*Dicentrarchus labrax*) fry, affect growth and accelerate the maturity of the digestive system, and improve survival rates of the fry when fed a diet supplemented with the red yeast (Gatesoupe 2007).

In this study, prior to challenge, the serum C3 level significantly increased in the group supplemented with *R. Mucilaginosa*, compared to the control group and after infection, the 1-3‰ *R. Mucilaginosa* groups had significantly increased serum C4 levels compared to the control. Complement components are the major humoral component of the innate immune responses and thus play an essential role in alerting the host immune system of the presence of potential pathogens as well as their clearance, which is initiated by one or a combination of three pathways, namely the classical, alternative, and lectin (Zhou et al., 2014). C3 is the central component of the complement system that is activated into its respective cleavage products C3a and C3b through any of these three pathways (Boshra et al., 2006). C4 plays an integral role in the activation of the classical and lectin pathways (Boshra et al., 2006). However, previous studies have shown that after challenge by *Aeromonas veronii*, there were no significant differences in complement 3 among all groups (Yu et al., 2014).

In conclusion, the results of the present study indicate that dietary *R. Mucilaginosa* supplementation could enhance growth, and significantly improve disease resistance by stimulating immunity in *T. ovatus*. The results suggest that oral administration of *R. Mucilaginosa* at 1‰ was better than higher doses. However, further research is needed to ascertain the mechanisms of *R. Mucilaginosa* with reduced length of administration period.

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References

- Alexander C., Sahu N.P., Pal A.K. and M.S. Akhtar**, 2011. Haemato-immunological and stress responses of *Labeo rohita* (Hamilton) fingerlings: effect of rearing temperature and dietary gelatinized carbohydrate. *J Anim Physiol Anim Nutr.*, 95: 653-663.
- Boshra H., Li J. and J.O. Sunyer**, 2006. Recent advances on the complement system of teleost fish. *Fish Shellfish Immunol.*, 20: 239-262.
- Cheng T.C. and G.E. Rodirick**, 1975. Lysosome and other enzymes in the haemolymph of *Crassostrea virginica* and *Mercenaria mercenaria*. *Comp. Biochem. Physiol.*, 52B: 443-447.
- Drape H.H., Squires E.J., Mahmoodi H., Wu J., Agarwal S. and M. Hadley**, 1993. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. *Free Radic Biol Med.*, 15: 353-363.
- Ganguly S., Paul I. and K.S. Mukhopadhyay**, 2010. Application and Effectiveness of Immunostimulants, Probiotics, and Prebiotics in Aquaculture: A Review. *Isr. J. Aquacult. - Bamidgeh*, 62(3): 130-138.
- Gatesoupe F.J.**, 2007. Live yeasts in the gut: Natural occurrence, dietary introduction, and their effects on fish health and development. *Aquaculture*, 267(1/4): 20-30.
- Hauge T., Kjuull A.K. and K. Stensvag**, 2002. Antibacterial activity in four marine crustacean decapods. *Fish Shellfish Immun.*, 12: 371-85.
- Howe D., Barrows L.F., Lindstrom N.M. and R.A. Heinzen**, 2002. Nitric oxide inhibits *Coxiella burnetii* replication and parasitophorous vacuole maturation. *Infect Immun.*, 70: 5140-5147.
- Li H. and K.S. Zhang**, 2004. The production of β -carotene by fermentation of red yeast. *Food Res Devel.*, 25(3): 58-60.
- Liu B., Ge X., Xie J., Xu P., He Y., Cui Y., Ming J.H., Zhou Q.L. and L.K. Pan**, 2012. Effects of anthraquinone extract from *rheum officinale* bail on the physiological responses and hsp70 gene expression of *megalobrama amblycephala* under *Aeromonas hydrophila* infection. *Fish Shellfish Immun.*, 32(1): 1-7.
- Lopes P.A., Pinheiro T., Santos M.S., Mathias M.D.L., Collares-Pereira M.J. and A.M. Viegas Crespo**, 2001. Response of antioxidant enzymes in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. *Sci Total Environ.*, 280: 153-63.
- Lowry O.H., Rosebrough N.J. and A.L. Farr**, 1951. Protein measurement with the Folin-phenol reagent. *J Biol Chem.*, 193: 265-275.
- Marklund S. and G. Marklund**, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem.*, 47: 469-474.
- Marzinzig M., Nussler A.K., Stadler J., Marzinzig E., Barthlen W., Nussler N.C., Beger H.G., Morris J.S.M. and U.B. Bruckner**, 1997. Improved methods to measure end products of nitric oxide in biological fluids: nitrite, nitrate, and S-nitrosothiols. *Nitric Oxide*, 1: 177-189.
- Muona M. and A. Soivio**, 1992. Changes in plasma lysozyme and blood leucocyte levels of hatchery-reared Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) during parr-smolt transformation. *Aquaculture*, 106: 75-87.
- Sharma K., Ramachandra S., Rathore G., Verma D.K., Sadhu N. and K.K. Philipose**, 2012. *Vibrio alginolyticus* infection in Asian seabass (*Lates calcarifer*, Bloch) reared in open sea floating cages in India. *Aquac Res.*, 44: 86-92.
- Sun J.N., Xie W.T., Liu Y. and C.H. Xu**, 2015. Research advance of the marine red yeast. *J Anhui Agric Sci.*, 43(4): 84-88.
- Sun L.Y., Guo H.Y., Zhu C.Y., Ma Z.H., Jiang S.G. and D.C. Zhang**, 2014. Genetic polymorphism of breeding populations of golden pompano (*Trachinotus ovatus*). *S China Fish Sci.*, 2: 67-71.
- Tovar D., Zambonino-Infante J., Cahu C., Gatesoupe F.J. and R. Vázquez-Juárez**, 2004. Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. *Aquaculture*, 234: 415-427.

- Xia D.M., Yang K., Li Z.J., Yang Y.Y., Wang Y., Wang J. and H.Z. Lin,** 2013. Effects of *Rhodotorula mucilaginosa* on growth performance and immunity of *Litopenaeus vannamei*. *Guangdong Agric Sci.*, 14: 133-137.
- Xia L.Q., Huang Y.C. and Y.S. Lu,** 2012. The diseases in *Trachinotus ovatus* culture and their research progress. *Anhui Agric Sci Bull.*, 18: 140-150.
- Yang S.P., Wu Z.H., Jian J.C.,** 2013. Effect of Marine Red Yeast *Rhodospiridium paludigenum* on Antioxidant-Related Gene Expression in *Litopenaeus vannamei*. [*Isr. J. Aquacult. - Bamiddgeh.*](#) 65.817, 6 pages.
- Yang S.P., Wu Z.H., Jian J.C. and X.Z. Zhang,** 2010. Effect of marine red yeast *Rhodospiridium paludigenum* on growth and antioxidant competence of *Litopenaeus vannamei*. *Aquaculture* 309: 62-65.
- Yu H.H., Xue M., Han F., Wang J., Zheng Y.H. and X.F. Wu,** 2014. A comparative study: Effect of several immunomodulators on growth performance, immunity and survival rate after bacterial challenge of Japanese seabass. *Chinese J Anim Nutr.*, 26(8): 1-11.
- Zhang Q., Tan B., Mai K., Zhang W., Ma H., Ai Q., Wang X.J. and Z. Liufu,** 2011. Dietary administration of bacillus (*B. licheniformis* and *B. subtilis*) and isomaltooligosaccharide influences the intestinal microflora, immunological parameters and resistance against *Vibrio alginolyticus* in shrimp, *Penaeus japonicus* (decapoda: penaeidae). *Aquacult Res.*, 42(7): 943-952.
- Zhang Q., Yu H.R., Tong T., Tong W.P., Dong L.F., Xu M.Z. and Z.C. Wang,** 2014. Dietary supplementation of *Bacillus subtilis* and fructooligosaccharide enhance the growth, non-specific immunity of juvenile ovate pompano, *Trachinotus ovatus* and its disease resistance against *Vibrio vulnificus*. *Fish Shellfish Immun.*, 38: 7-14.
- Zhang R.L., Niu S.J., Yang Z.C. and H.J. Zhang,** 2013. Effects of different amount of *Rhodotorula benthica* in diet on the feeding and growth of *Scophthalmus maximus* L. *J Hebei Normal University (Natural Science edition)*, 37(6): 618-621.
- Zheng P.L., Ma Z.H., Guo H.Y., Li Y.N., Zhang D.C. and S.G. Jiang,** 2014. Ontogenetic development of caudal skeletons in *Trachinotus ovatus* larvae. *S. China Fish Sci*, 5: 45-50.
- Zhou C.P., Ge X.P., Lin H.Z. and J. Niu,** 2014. Effect of dietary carbohydrate on non-specific immune response, hepatic antioxidative abilities and disease resistance of juvenile golden pompano (*Trachinotus ovatus*). *Fish Shellfish Immun.*, 41(2): 183-190.
- Zhou C., Lin H., Ge X., Niu J., Wang J., Wang Y., Huang Z., Yu W. and X.H. Tan,** 2015. The effects of dietary soybean isoflavones on growth, innate immune responses, hepatic antioxidant abilities and disease resistance of juvenile golden pompano *trachinotus ovatus*. *Fish Shellfish Immun.*, 43: 158-166.
IIC:63.2011.664 6 pages.