



# *Isaria cicadae* Miquel Improves the Growth Performance, Physiological Response and Meat Quality of Giant Freshwater Prawn, *Macrobrachium rosenbergii*

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## ABSTRACT

A 9-week feeding trial was conducted to investigate the effects of *Isaria cicadae* Miquel (*I. cicadae*) on growth performance and meat quality of giant freshwater prawn (*Macrobrachium rosenbergii*). The prawns were fed with five isonitrogenous (40.54% crude protein) and isolipid (6.16% crude lipid) diets with different *I. cicadae* levels (0, 100, 200, 400, and 800 mg Kg<sup>-1</sup>). Results revealed that *I. cicadae* significantly increased specific growth ratio (SGR) and protein efficiency ratio (PER), while decreased feed intake (FI) and feed conversion ratio (FCR). Meanwhile, *I. cicadae* significantly increased plasma ALT activity, urea nitrogen, total protein, albumin and globulin contents, while significantly decreased AST activity in 400 mg Kg<sup>-1</sup> group. Haemolymph SOD and GSH-PX activity was enhanced significantly in 400 and 800 mg Kg<sup>-1</sup> group, respectively, while MDA content was significantly decrease in these two groups. *I. cicadae* did not affect the muscle cooking loss, but significantly increased the compression loss. Moreover, *I. cicadae* significantly increased the muscle texture profile including gumminess, hardness and chewiness, and decreased the muscle sensory evaluations including whiteness and succulency. Therefore, 100 mg Kg<sup>-1</sup> *I. cicadae* could improve growth performance and meat quality of giant freshwater prawn, reduce the damage of oxidative stress.

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## Authors' Contribution

WH, BL conceived and designed the research. CS, HZ conducted the experiment. WH, QZ, BL analyzed the data and wrote the paper. CS revised the paper.

## Key words

*Macrobrachium rosenbergii*, *Isaria cicadae* Miquel, Growth performance, Non-specific immunity, Muscle quality

## INTRODUCTION

Aquaculture has progressed as one of the fastest food producing segments subsequently to agriculture, contributing significantly to global food security. Crustacean is one of the major parts of the global aquaculture production (Asaikkutti *et al.*, 2018). The giant freshwater prawn, *Macrobrachium rosenbergii*, is a famous economically species among crustaceans, which distributed from the south of China to the East of China, due to its faster growth, better tolerance to the environment and delicious meat quality (Mohan *et al.*, 2016). There were lots of reports on the nutrient requirement of giant freshwater prawn, such as protein level (Teshima *et al.*, 2006), amino acid (Dong *et al.*, 2018), lipid (Muralisankar *et al.*, 2014), vitamins (Asaikkutti *et al.*, 2016, 2018), etc. There were also some reports related the use of plant

minerals (Tavabe *et al.*, 2013; Muralisankar *et al.*, 2015), extraction in the diet of giant freshwater prawn (Liu *et al.*, 2010a, 2010b; Sriket *et al.*, 2011; Chang *et al.*, 2013; Rattanavichai and Cheng, 2015). However, most of them were limited on the improvement of immunity. A little information is available on the role of the meat quality of giant freshwater prawn.

*Isaria cicadae* Miquel is a traditional Chinese medicine, which is widely used as a tonic for nourishment as well as a functional food, for it possesses remarkable biological activity including immunomodulatory activity, antioxidation, anti-aging, antitumor, anti-inflammation and amelioration in renal function (Xu *et al.*, 2018). It has been found that the main active components are adenosine and polysaccharide. Polysaccharides are prebiotic substance which is widely accepted as a dietary ingredient for regulating growth and health status of organisms including humans (Bohn and BeMiller, 1995; Zekovic *et al.*, 2005). In the aquaculture industry, dietary supplementation of polysaccharides (k-carrageenan, laminaran,  $\beta$ -glucan, chitosan, chitin, fucoidan, mannan oligosaccharide) can

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regulate the survival, growth and immune performance in fish and crustaceans (Chang *et al.*, 2000; Murthy *et al.*, 2009; Rodregeuz *et al.*, 2007; Meshram *et al.*, 2014; Sivagnanavelmurugan *et al.*, 2014; Kumar *et al.*, 2006). It was also reported that the culture medium after culturing *I. cicadae* could improve the growth, decrease the feed conversion ratio, and increase the immunity of chicken (Liu *et al.*, 2012). However, no reports could find about the effects of *I. cicadae* on the crustaceans and fish. Hence, the current study was performed to evaluate the effect of dietary *I. cicadae* on the growth performance and feed utilization of giant freshwater prawn, to investigate the plasma biochemistry factors related the protein metabolism and to find the changes on the muscle quality of prawn so as to provide the theoretical basis for prophylaxis of shrimp diseases.

## MATERIALS AND METHODS

### Ethics statement

The experiments were performed in accordance with the guidelines on the care and use of animals for scientific purpose set by the Institutional Animal Care and Use Committee (IACUC) of the Chinese Academy of Fishery Science (CAFS). This study was specifically approved by the Committee on the Ethics of Animal Experiments of Freshwater Fisheries Research Center at Chinese Academy of Fishery Science. All efforts were made to minimize the suffering of the animals.

### Diet preparation

According to Wang *et al.* (2004), the basal diet was formulated with 40.54% crude protein and 7.16% crude lipid (Table I). The protein ingredients of basal diet included fishmeal, shrimp meal, soybean meal, and the lipid was fish oil and soybean oil. The fruiting bodies of *I. cicadae* was added into the basal diet with the levels as 0 (D1, control diet), 100 mg Kg<sup>-1</sup> (D2), 200 mg Kg<sup>-1</sup> (D3), 400 mg Kg<sup>-1</sup> (D4) and 800 mg Kg<sup>-1</sup> (D5). All ingredients except fish oil and soybean oil were ground into powder through 60 mesh sieve, mixed with fish oil, soybean oil and water, then forced through a pelletizer (die diameter 1.0 and 2.0 mm, South China University of Technology, Guangzhou China) and dried in a ventilated oven at 45°C. The diameter of diet was 1mm. After drying, all diets were sealed in bags and stored at -15°C until used.

### The powder of fruiting bodies of *I. cicadae*

The fruiting bodies of *I. cicadae* was commercial product supplied by Zhejiang Bio Asia Biomedical Co., Ltd., China. It contained the doses of 130 mg/g adenosine and 25 mg/g polysaccharide. Firstly, the *I. cicadae* Miquel

strain was activated for 7 days in 24°C in culture medium and pre-amplified for 10-15 days in 24°C for cicadae expansion. Secondly, the expanded cicadae was subjected into the solid culture medium, which was prepared with wheat and water (both sterilized) with a ratio of 1:1.3. Lastly, after 3-5 days dark culture incubation (22-24°C) and 20-23 days light incubation (20-22°C, light intensity 100-200 lux), the fermentation products were finally dried, inactivated to harvest the sporophores and crushed into 80 meshes for further study.

**Table I. Formulation and proximate composition of basal diet.**

Ingredients	Basal diet (%)	Proximate composition	(%)
Fish meal <sup>1</sup>	35.00	Crude protein	40.54
Squid visceral ointmen <sup>1</sup>	3.00	Crude lipid	6.16
Shrimp meal <sup>1</sup>	8.00	Ash	13.41
Soybean meal <sup>1</sup>	20.00	Lys	2.71
Canola meal <sup>1</sup>	10.00	Met+cys	1.39
α-starch	13.95		
Soyean oil <sup>2</sup>	3.00		
Soybean lecithin <sup>3</sup>	1.00		
Cholesterol <sup>4</sup>	0.30		
Phagostimulant <sup>5</sup>	0.05		
Ecdysone <sup>5</sup>	0.20		
Choline chloride <sup>6</sup>	1.00		
Vitamin premix <sup>7</sup>	1.00		
Mineral premix <sup>8</sup>	1.00		
Bentonite <sup>6</sup>	0.50		
Monocalcium phosphate <sup>6</sup>	2.00		

<sup>1</sup>Provided by Tongwei Co., Ltd (Wuxi, China).

<sup>2</sup>Oil was mixed with fish oil and soybean oil with the equal volum.

<sup>3</sup>Provided by Da Bei Nong Group (Huaian, China) with concentration 50%.

<sup>4</sup>Provided by Shanghai Lianshi Chemical Reagent Co., Ltd (Shanghai, China).

<sup>5</sup>Provided by Da Bei Nong Group (Huaian, China).

<sup>6</sup>Provided by Wuxi Hanove Animal Health Products Co., Ltd. (Wuxi, China).

<sup>7</sup>Vitamins premix (per kg mixture): Vitamin A, 1.5 g; Vitamin D<sub>3</sub>, 0.5 g; Vitamin K<sub>3</sub>, 3.5 g; thiamin, 1.3 g; riboflavin, 2.0 g; pyridoxine HCl, 2.4 g; cyanocobalamin, 0.8 g; niacin, 3.6 g; D-calcium pantothenate, 3.3 g; folic acid 0.4 g; D-biotin, 0.8 g; phosphate vitamin C, 40.0 g; inositol, 12.5 g and cellulose was used as a carrier.

<sup>8</sup>Mineral premix (g kg<sup>-1</sup> mixture): cupric sulphate, 10 g; ferrous sulphate, 66.7 g; manganese sulphate, 9.4 g; zinc sulphate, 34.8 g; magnesium sulphate, 150 g; potassium chloride, 23.6 g; sodium selenate, 4.5 g; calcium iodate, 6.5 g; cobalt sulphate, 1.7 g and zeolite was used as a carrier.

### *Experimental system and animals*

Healthy prawn fry were obtained from South Taihu Freshwater Hatchery Company, Huzhou, China. Prior to the feeding trial, prawn were fed with control diet (D1) for 2 weeks to acclimate to the experimental diet and conditions. After fasting for 24 h, prawn fry (initial weight  $0.243 \pm 0.003$  g,  $n = 50$ ) were randomly distributed into 30 concrete tanks ( $2.0 \text{ m} \times 1.50 \text{ m} \times 0.70 \text{ m}$ ) with 50 prawn in each tanks. Each diet was randomly assigned to six tanks. Some meshes were put into the tank to help the prawn avoid the attack from others. Water from an underground source was used. Daily silt was siphoned before feeding with an exchange of one-third of water in each tank once every two weeks.

Prawns were hand-fed with the experimental diets three times daily at 7:00, 12:00 and 18:00. The amount of feeding diet was 5% to 10% of the prawn weight based on the feed intake every day. The residue of the feed was collected half hour after feeding. During the 9 week feeding trial, the number and weight of dead prawn and feed consumption were recorded every day. Photoperiod was set at natural conditions. Water temperature fluctuated from 25 to 30 °C, pH was ranged 7.8-8.5 and ammonia nitrogen was less than  $0.5 \text{ mg L}^{-1}$ . Dissolved oxygen was proved by aeration round the clock.

### *Sample collection and analysis*

At the end of the feeding trial, prawns were starved for 24 h to evacuate the alimentary tract contents prior to sampling period. All prawns in each tank were counted and weighed for calculating the relative indexes. 18 prawns per group (three prawns per tank) were taken, and then blood samples were collected from the cardiocoelom using syringes. Alsever's solution was used as the anticoagulant, and the proportion of haemolymph to the anticoagulant was 1:1 (Rodriguez *et al.*, 1995). The blood was centrifuged ( $3500 \times g$ , 10 min, 4°C) to obtain plasma. Plasma was separated and stored at -80°C until use. At last twelve prawns from the same tank were dissected and got the muscle to detect the meat quality.

### *Biochemical measurements related to protein metabolism*

Twelve prawns of each group (2 prawns/each tank, 6 tanks per group) were selected randomly for haemolymph biochemical parameters. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by the colorimetric method using a Mindray Auto Bio-chemical Analyzer (BS-400, Mindray, P.R. China) described as Zhou *et al.* (2018). The total protein (TP), albumin (ALB) and urea nitrogen (UN) contents were detected by the colorimetric method using a Mindray Auto Bio-chemical Analyzer (BS-400, Mindray, P.R.

China).

### *Haemolymph non-specific immunity parameters*

Serum superoxide dismutase (SOD) activity, malondialdehyde (MDA) content, glutathione peroxidase (GSH-PX) activity and lysozyme activity were determined using kits of Jiancheng Bioengineering Institute (Nanjing, PR China) according to Liu *et al.* (2010b).

### *Meat quality of prawn*

#### *Compression loss and cooking loss*

The water holding capacity of the meat was evaluated by the compression loss according to the method of Zhou *et al.* (2018) with some modification. 12 prawns of each group (2 prawns/each tank, 6 tanks per group) were prepared to detect compression loss. Before packaging with layers of filter paper, the samples were weighed in advance. Then, they were pressured with the persistent power of 35 kg for 3 min using confining pressure-free strain control instrument (Nanjing Soil Instrument Factory, Nanjing, P.R. China). 12 prawns of each group (2 prawns/each tank, 6 tanks per group) were used to determine cooking loss. Cooking loss was performed according to Qi *et al.* (2018), with some modifications.

#### *Texture profile analysis*

Twelve prawns of each group (2 prawns/each tank, 6 tanks per group) were selected randomly for texture profile analysis (TPA) with XT plus texture analyser (Stable Micro Systems Ltd., Godalming, UK). Hardness, springiness, cohesiveness, gumminess, chewiness and resilience were determined according to De Huidobro *et al.* (2005) and analyzed with software Texture Expert Exceed (Stable Micro Systems Ltd).

#### *Sensory evaluation*

Sensory evaluation was carried out according to Yang *et al.* (2015) with some modifications. A ten-member sensory panel was trained before evaluation. According to the Chinese standard GB/T 22210-2008, each panellist was experienced in assessing sensory evaluation, including odour, whiteness, brightness, color, flavor, delicacy, succulency, fishiness and tenderness. All attributes were scored on five-point scales with small modifications (Kang *et al.*, 2011). Each evaluation session was conducted at the same time of all samples. A total of ninety prawn samples were evaluated, and each sample was evaluated twice.

#### *Calculation and statistical analysis*

Data were the mean of the raw data from the same tank. Therefore, a total of six data were used to analyze every parameter. Then data were analyzed using SPSS version 13.0 for Windows (SPSS Inc., Chicago, USA).

A one-way analysis of variance (ANOVA) was used to compare the means of the main effects and was followed by Duncan's test to detect significant differences between the means. All data are presented as mean  $\pm$  standard error (SE). Significance was determined as a  $P$  value  $< 0.05$ .

## RESULTS

### Growth performance and feed utilization

Growth performance is shown in Table II. 75-84% survival rate was observed in for all treatments and no significant differences were found among the all groups ( $P > 0.05$ ). All the treatment group of *I. cicadae* could significantly improve the specific growth rate (SGR) and protein efficiency ratio (PER) compared to the control group ( $P < 0.05$ ). Meanwhile, the feed conversion rate of the prawn fed with 100 mg Kg<sup>-1</sup> and 800 mg Kg<sup>-1</sup> *I. cicadae* was significantly lower than those fed with control diet ( $P < 0.05$ ). The feed intake in all treatments was significantly lower than those in control group ( $P < 0.05$ ).

### Biochemical measurements related to protein metabolism

The blood biochemical parameters are shown in Table III. The ALT activities of those fed with diet containing 100 mg Kg<sup>-1</sup> and 800 mg Kg<sup>-1</sup> *I. cicadae* were significantly higher than those fed with control diet ( $P < 0.05$ ). While the AST activity of prawn fed with 400 mg Kg<sup>-1</sup> *I. cicadae* were significantly lower than those fed with control diet ( $P < 0.05$ ). The total protein, albumin and globulin contents in the plasma of all treatments were significantly higher than that of control group ( $P < 0.05$ ). While the urea nitrogen of the treatment groups were keep similar with that of the control group except those fed with 800 mg Kg<sup>-1</sup> *I. cicadae*, which was significantly higher than control group ( $P < 0.05$ ).

### Haemolymph non-specific immunity parameters

The haemolymph SOD, GSH-PX, MDA and lysozyme are shown in Table IV. The SOD activity of prawns in the group of 400 mg Kg<sup>-1</sup> *I. cicadae* were significantly higher than that of the control group. The MDA contents of prawns in the group of 400 mg Kg<sup>-1</sup> *I. cicadae* and 800 mg Kg<sup>-1</sup> *I. cicadae* were significantly lower than those in the group of control and 100 mg Kg<sup>-1</sup> *I. cicadae*. The GSH-PX activities of prawns in the group of 100 mg Kg<sup>-1</sup> *I. cicadae* and 800 mg Kg<sup>-1</sup> *I. cicadae* were significantly higher than those in the control group. There were no significant differences of the lysozyme activity among the groups.

### Meat quality of the prawn

Compression loss and cooking loss of prawn are

shown in Table V. The compression loss of the prawn fed with diets including *I. cicadae* was significantly higher than the control group except the group of 100 mg Kg<sup>-1</sup> *I. cicadae* ( $P < 0.05$ ). However, the cooking loss of the prawn in all treatment groups was similar with that of control group, while the cooking loss in the group of 800 mg Kg<sup>-1</sup> *I. cicadae* was significantly lower than that in the group of 400 mg Kg<sup>-1</sup> *I. cicadae* ( $P < 0.05$ ).

The texture profile of giant freshwater prawn are shown in Table VI. The gumminess of the prawn fed diet with *I. cicadae* was significantly stronger than that of the control group ( $P < 0.05$ ). The hardness and chewiness of the prawn of the treatment group were significantly stronger than those of the control group ( $P < 0.05$ ), except of those fed with 400 mg Kg<sup>-1</sup> *I. cicadae*. The springiness, cohesiveness and resilience of the prawn were similar in all groups ( $P > 0.05$ ).

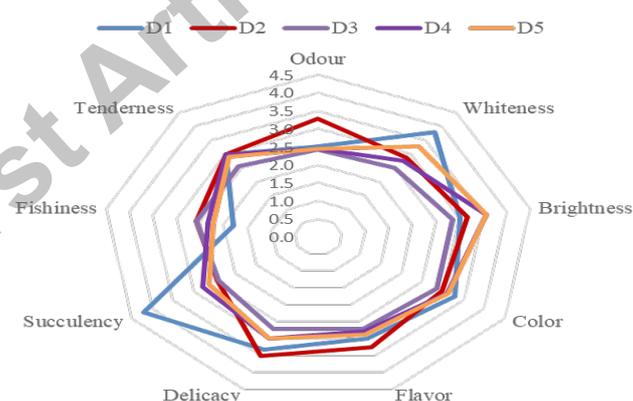


Fig. 1. Sensory evaluation of meat of giant freshwater prawn.

Note: D1(control diet), D2(100 mg Kg<sup>-1</sup>), D3(200 mg Kg<sup>-1</sup>), D4(400 mg Kg<sup>-1</sup>) and D5(800 mg Kg<sup>-1</sup>). Five-point scales were used. Odour, 1: abnormal taste, 5: odourless; whiteness, 1: not white, 5: very white; brightness, 1: lacklustre, 5: bright; color, 1: light, 5: bright; flavor, 1: dislike, 5: like; delicacy, 1: tasteless, 5: delicious; succulency, 1: dry, 5: succulent; fishiness, 1: without fishy, 5: very fishy; and tenderness, 1: hard, 5: very tender.

Sensory evaluations of the muscle, including odour, whiteness, brightness, color, flavor, delicacy, succulency, fishiness, and tenderness, of giant freshwater prawn were evaluated (Fig. 1). *I. cicadae* in diet did not significantly affect the brightness, color, flavor, delicacy, fishiness and tenderness of the giant freshwater prawns ( $P > 0.05$ ), however it significantly changed the odour, whiteness and succulency of the muscle of the prawn ( $P < 0.05$ ). The odour of the muscle of the prawn fed diet containing 100 mg Kg<sup>-1</sup>

**Table II. Growth performance and feed utilization of prawn fed diets with fruiting bodies of *Isaria cicadae*.**

Group	Initial weight (g)	Final weight (g)	WGR (%)	SGR (% d <sup>-1</sup> )	FCR	PER	FI (g d <sup>-1</sup> )	Survival (%)
Control	0.21±0.01	12.42±0.29 <sup>a</sup>	5748.14 ± 150.00 <sup>a</sup>	6.78±0.04 <sup>a</sup>	1.64±0.04 <sup>c</sup>	1.64 ± 0.04 <sup>a</sup>	4.92±0.11 <sup>b</sup>	84.33±2.03
100 mg Kg <sup>-1</sup>	0.21±0.01	14.82±0.59 <sup>b</sup>	6941.45 ± 310.72 <sup>b</sup>	7.08±0.07 <sup>c</sup>	1.40±0.03 <sup>a</sup>	1.96 ± 0.08 <sup>b</sup>	4.15±0.16 <sup>a</sup>	82.67±2.62
200 mg Kg <sup>-1</sup>	0.21±0.01	13.76±0.22 <sup>b</sup>	6327.62 ± 104.47 <sup>ab</sup>	6.94±0.03 <sup>b</sup>	1.53±0.10 <sup>abc</sup>	1.82 ± 0.03 <sup>b</sup>	4.44±0.07 <sup>a</sup>	82.67±5.28
400 mg Kg <sup>-1</sup>	0.21±0.01	14.05±0.36 <sup>b</sup>	6487.33 ± 171.64 <sup>b</sup>	6.98±0.04 <sup>bc</sup>	1.65±0.12 <sup>bc</sup>	1.86 ± 0.05 <sup>b</sup>	4.36±0.11 <sup>a</sup>	75.33±4.06
800 mg Kg <sup>-1</sup>	0.21±0.01	14.66±0.23 <sup>b</sup>	6779.78 ± 95.84 <sup>b</sup>	7.05±0.02 <sup>bc</sup>	1.44±0.04 <sup>ab</sup>	1.94 ± 0.03 <sup>b</sup>	4.17±0.06 <sup>a</sup>	81.33±2.35

Values are means ± SEM of six samples. Means in the same column with different superscripts are significantly different ( $P < 0.05$ ). Survival rate (%) =  $100 \times (\text{final amount of prawn}) / (\text{initial amount of prawn})$ ; WGR (weight gain rate, %) =  $100 \times (\text{final prawn weight} - \text{initial prawn weight}) / \text{initial prawn weight}$ ; SGR (specific growth rate, % day<sup>-1</sup>) =  $100 \times (\text{Ln final prawn weight} - \text{Ln initial prawn weight}) / \text{the experimental duration in days}$ ; FCR (feed conversion ratio) =  $\text{dry diet fed} / \text{wet weight gain}$ ; PER (protein efficiency ratio) =  $\text{wet weight gain} / \text{dry protein intake}$ ; Feed intake (FI, g d<sup>-1</sup>) =  $\text{dry diet fed} / (\text{amount of prawn} \times \text{the experimental duration in days})$ .

**Table III. Protein metabolism of prawn fed diets with the fruiting bodies of *Isaria cicadae*.**

Group	ALT (U/L)	AST (U/L)	TP (g/L)	ALB (g/L)	GLB (g/L)	UN (mmol/L)
Control	149.12 ± 8.05 <sup>a</sup>	80.22 ± 7.29 <sup>b</sup>	44.72 ± 2.34 <sup>a</sup>	5.60 ± 0.43 <sup>a</sup>	38.66±2.19 <sup>a</sup>	1.10 ± 0.11 <sup>a</sup>
100 mg Kg <sup>-1</sup>	195.59 ± 8.95 <sup>b</sup>	78.52 ± 5.72 <sup>ab</sup>	56.61 ± 1.75 <sup>b</sup>	7.28 ± 0.27 <sup>b</sup>	50.31±2.31 <sup>b</sup>	1.17 ± 0.09 <sup>ab</sup>
200 mg Kg <sup>-1</sup>	177.41 ± 11.48 <sup>ab</sup>	67.13 ± 8.01 <sup>ab</sup>	56.03 ± 2.67 <sup>b</sup>	7.35 ± 0.47 <sup>b</sup>	50.98±2.16 <sup>b</sup>	1.29 ± 0.10 <sup>ab</sup>
400 mg Kg <sup>-1</sup>	174.18 ± 7.98 <sup>ab</sup>	60.27 ± 4.81 <sup>a</sup>	55.62 ± 3.09 <sup>b</sup>	7.24 ± 0.34 <sup>b</sup>	48.45±2.85 <sup>b</sup>	1.03 ± 0.11 <sup>a</sup>
800 mg Kg <sup>-1</sup>	190.83 ± 10.47 <sup>b</sup>	76.73 ± 3.54 <sup>ab</sup>	57.50 ± 4.30 <sup>b</sup>	7.56 ± 0.74 <sup>b</sup>	49.26±3.61 <sup>b</sup>	1.45 ± 0.10 <sup>b</sup>

Values are means ± SEM of twelve samples. Means in the same column with different superscripts are significantly different ( $P < 0.05$ ). ALT, alanine aminotransferase activity; AST, aspartate aminotransferase activity; TP, total protein content; ALB, albumin content; GLB, globulin content; UN, urea nitrogen content.

**Table IV. Non-specific immunity of prawn fed diets with the fruiting bodies of *Isaria cicadae*.**

Group	SOD	MDA	GSH-PX	Lysozyme
Control	383.94 ± 6.31 <sup>a</sup>	52.28 ± 1.14 <sup>b</sup>	737.10 ± 14.66 <sup>b</sup>	30.75±1.33
100 mg Kg <sup>-1</sup>	392.49 ± 6.68 <sup>ab</sup>	51.47 ± 1.11 <sup>b</sup>	796.45 ± 10.27 <sup>c</sup>	21.46±3.70
200 mg Kg <sup>-1</sup>	368.24 ± 8.15 <sup>a</sup>	51.03 ± 1.28 <sup>ab</sup>	693.55 ± 20.13 <sup>a</sup>	23.29±2.31
400 mg Kg <sup>-1</sup>	416.23 ± 11.88 <sup>b</sup>	47.91 ± 1.06 <sup>a</sup>	742.26 ± 10.52 <sup>b</sup>	21.23±4.64
800 mg Kg <sup>-1</sup>	394.30 ± 7.82 <sup>ab</sup>	48.35 ± 1.09 <sup>a</sup>	771.29 ± 16.06 <sup>c</sup>	29.83±9.38

Values are means ± SEM of twelve samples. Means in the same column with different superscripts are significantly different ( $P < 0.05$ ).

**Table V. Compression loss and cooking loss of the prawn fed diets with the fruiting bodies of *Isaria cicadae*.**

Group	Compression loss (%)	Cooking loss (%)
Control	24.59 ± 1.06 <sup>a</sup>	21.63 ± 2.11 <sup>ab</sup>
100 mg Kg <sup>-1</sup>	26.56 ± 1.87 <sup>ab</sup>	16.50 ± 2.70 <sup>ab</sup>
200 mg Kg <sup>-1</sup>	35.87 ± 3.38 <sup>c</sup>	20.58 ± 1.90 <sup>ab</sup>
400 mg Kg <sup>-1</sup>	32.37 ± 2.52 <sup>bc</sup>	22.99 ± 4.50 <sup>b</sup>
800 mg Kg <sup>-1</sup>	32.67 ± 1.78 <sup>bc</sup>	13.91 ± 1.12 <sup>a</sup>

Values are means ± SEM of twelve samples. Means in the same column with different superscripts are significantly different ( $P < 0.05$ ).

*I. cicadae* was significantly stronger than that of the control group ( $P < 0.05$ ). The whiteness of the muscle of the prawn from the treatment group was significantly declined than that from the control group except the group of 800 mg Kg<sup>-1</sup> *I. cicadae* ( $P < 0.05$ ). The *I. cicadae* in the diet of giant freshwater prawn significantly decreased the succulency of the muscle compared with the control group ( $P < 0.05$ ).

## DISCUSSION

It was found that *I. cicadae* could improve the growth of giant freshwater prawn, increase the protein utilization in

**Table VI. Texture profile of prawn fed diets with the fruiting bodies of *Isaria cicadae*.**

Group	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Control	109.95±21.51 <sup>a</sup>	0.59±0.05	0.34±0.01	34.16±5.17 <sup>a</sup>	19.25±3.41 <sup>a</sup>	0.13±0.02
100 mg Kg <sup>-1</sup>	223.44±20.55 <sup>b</sup>	0.67±0.03	0.34±0.02	80.37±6.12 <sup>b</sup>	57.97±3.10 <sup>c</sup>	0.15±0.01
200 mg Kg <sup>-1</sup>	188.29±16.77 <sup>b</sup>	0.62±0.04	0.32±0.02	59.93±7.98 <sup>b</sup>	41.39±6.15 <sup>bc</sup>	0.14±0.02
400 mg Kg <sup>-1</sup>	159.51±19.28 <sup>ab</sup>	0.6±0.03	0.34±0.01	57.48±7.69 <sup>b</sup>	35.38±5.11 <sup>ab</sup>	0.14±0.01
800 mg Kg <sup>-1</sup>	199.23±26.57 <sup>b</sup>	0.62±0.02	0.36±0.03	70.17±11.39 <sup>b</sup>	44.67±8.37 <sup>bc</sup>	0.17±0.02

Values are means ± SEM of twelve samples. Means in the same column with different superscripts are significantly different ( $P < 0.05$ ).

the diet and reduce feed conversion ratio in this study. It was similar result of anthraquinones extracted from *Rheum officinale* Bail (Liu *et al.*, 2010a, 2010b) and *Ganoderma lucidum* polysaccharides (Mohan *et al.*, 2016), which suggested the suitable level of Chinese herbs in the diet could increase the growth performance of giant freshwater prawn. This may be related with the active components in the herb extraction (Liu *et al.*, 2010a, 2010b; Sriket *et al.*, 2011; Chang *et al.*, 2013; Rattanavichai and Cheng, 2015). Based on our detection, the concentration of polysaccharide in *I. cicadae* reaches 71.8 g Kg<sup>-1</sup>, and the concentration of adenosine is 0.85 g Kg<sup>-1</sup>. Dietary polysaccharides and adenosine can be digested and used as potential immune matters and energy sources in fish and prawn. It was reported that polysaccharides, such as  $\beta$ -glucan, chitin and fucoidan in the diet could significantly increase the survival, growth performance, and protein efficiency ratio on *M. rosenbergii*, *Litopenaeus vannamei* and *Penaeus monodon* (Sung *et al.*, 1994; Shiao and Yu, 1998; Chang *et al.*, 2000; Murthy *et al.*, 2009; Rodregeuz *et al.*, 2007; Meshram *et al.*, 2014; Sivagnanavelmurugan *et al.*, 2014; Kumar *et al.*, 2006). It was proved that dietary fructooligosaccharide at 0.4% significantly improved growth of *M. rosenbergii*, while the higher concentration (above 1%) induced oxidative stress (Chen *et al.*, 2017). In this study, the results showed that all the treatment groups of 100-800 g Kg<sup>-1</sup> *I. cicadae* could significantly improve the specific growth rate and protein efficiency ratio. The group pf 100 mg Kg<sup>-1</sup> and 800 mg Kg<sup>-1</sup> *I. cicadae* reduced feed conversion rate of the prawn compared with the control diet. Based on the feed conversion ratio and specific growth rates, the concentration of 100 mg Kg<sup>-1</sup> *I. cicadae* was suit for the growth performance of *M. rosenbergii*.

The influence of *I. cicadae* on the growth of *M. rosenbergii* was further confirmed with some blood parameters. It could be found that 400 mg Kg<sup>-1</sup> *I. cicadae* significantly reduced the AST activity compared to the control. Similarly, Liu *et al.* (2010b) found that the doses of 0.1% -0.2% anthraquinones could reduce ALT and AST activities compared to the control prawn (Liu *et al.*, 2010b). Nakano *et al.* (1999) who reported that AST

and ALT activities in rainbow trout fed a diet containing *astaxanthin* under the oxidative stress were significantly lower than those of the control fish (Nakano *et al.*, 1999). Rao *et al.* (2006) also found the AST and ALT activities in *Labeo rohita* significantly decreased in the dose of 0.05% *Achyranthes aspera* compared with the control fish (Rao *et al.*, 2006). AST is important indices for diagnosis of hepatopancreas function and damage for aquatic animals (Ozaki, 1978). So, 400 mg Kg<sup>-1</sup> *I. Miquel* in diet fed to *M. rosenbergii* may contribute to protect the hepatopancreas function.

The haemolymph protein content is used as an immune parameter indicating whether the prawn is healthy or not (Bachère *et al.*, 1997). Rainbow trout treated with some fructoses improved haemolymph total protein content (Jeney *et al.*, 1997). *M. rosenbergii* fed with 0.1%-0.2% anthraquinone extract also improved haemolymph total protein content. This experiment showed that the haemolymph total protein content significantly increased in fish fed with 0.4% anthraquinone extract before the stress, in fish treated with 0.2% anthraquinone extract at 6h and 12h after stress, and in fish treated with 0.4% anthraquinone extract at 12h, 24h after the stress as well. So, it indicated that the supplement of 0.1%~0.2% anthraquinone extract may elevate the haemolymph protein concentration, prevent the harm of the stress, improve the health of freshwater prawns and keep them in good condition to some degree. In this experiment, the total protein, albumin and globulin contents in the plasma of all treatments were significantly higher than that of control group. So, it indicated that the supplement of 100-800 mg Kg<sup>-1</sup> *I. cicadae* elevated the haemolymph protein concentration, improved the health of freshwater prawns and kept them in good condition.

Aquatic animals have a complex system of multiple types of antioxidants, such as glutathione, catalase, superoxide dismutase and various peroxidases to prevent the oxidation of non-self antigens, to protect the organism from oxidative damage (Holmblad *et al.*, 1999; Munoz *et al.*, 2000). Activation of non-specific defense mechanisms in prawn is evident by increasing SOD, GSH-PX, which

can lead to the decrease of MDA content (Liu *et al.*, 2010a, 2010b). In fish, Chinese herbal extracts in the diet can improve the antioxidant ability and reduce the mortality of fish (Xie *et al.*, 2008; Christyapita *et al.*, 2007). In prawns, it has been proved the Chinese herb such as anthraquinones extracted from *Rheum officinale* Bail could increasing the non-specific immunity of giant freshwater prawn even under the high temperature stress (Liu *et al.*, 2010a, 2010b). Consistent with these studies, in the present study the haemolymph SOD activity was enhanced significantly after feeding with 400 mg Kg<sup>-1</sup> *I. cicadae*, the haemolymph GSH-PX activity was increased significantly after feeding with 100, 800 mg Kg<sup>-1</sup> *I. cicadae* compared to the control group, which led to the significantly decrease of MDA content in the group of 400, 800 mg Kg<sup>-1</sup> *I. cicadae*. Thus, the fruit of *I. cicadae* could reduce the damage of oxidative stress.

Giant freshwater prawn is famous on its delicious meat quality (Mohan *et al.*, 2016), while no report concern on its meat quality. We evaluated the meat quality of giant freshwater prawn in this study for the first time based on the water holding capacity, texture profile and sensory evaluation. Cooking loss and compression loss are the primary indicators of water holding capacity of meat (Chen *et al.*, 2015). At present study, we found that 100 mg Kg<sup>-1</sup> *I. cicadae* did not affect the cooking loss and compression loss increase the water holding capacity of muscle with the decrease trend of cooking loss in the treatment group.

Water mainly exists between the myofibrils and most immobilized water is within the myofibrils (Ali *et al.*, 2015). Thus *I. cicadae* may lead to the increase of the water between the myofibrils, which may relate to the texture profile of the muscle. It was found that 100, 200, 800 mg Kg<sup>-1</sup> *I. cicadae* could increase the gumminess, hardness and chewiness of the muscle of *M. rosenbergii* compared with the control, which belong to the texture profile of muscle. The gumminess, hardness and chewiness of prawn are usually significantly lower than those of beef, pork and chicken. Thus, the increase of gumminess, hardness and chewiness may result in the harder of muscle than the normal one, which may lead to more delicious of prawn. According the data of the sensory evaluation, the odour of prawn fed diet with 100 mg Kg<sup>-1</sup> was increased and the whiteness and the succulency were reduced. It was a direct sensory that *I. cicadae* could make the prawn more delicious.

Therefore, it can be concluded that diet with 100 mg Kg<sup>-1</sup> *I. cicadae* could improve the growth performance by increasing the protein efficient ratio and by increasing protein metabolism, enhance haemolymph GSH-PX, TP, ALB, GLB and reduce feed conversion ratio and haemolymph AST, MDA. Meanwhile 100 mg Kg<sup>-1</sup> *I.*

*cicadae* in diet could improve the meat quality of giant freshwater prawn, especially the odour compression loss, gumminess, hardness and chewiness of the muscle. So, the supplement of 100 mg Kg<sup>-1</sup> *I. cicadae* in the diet can increase immune and anti-oxidation capability of prawns, enhance the meat quality and promote the growth of prawns. This work provides the theoretical basis for *I. cicadae* of the prawns feed.

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### Statement of conflict of interest

Authors have declared no conflict of interest.

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