
Effects of *Schizochytrium* sp. on growth performance and survival rate of Giant Freshwater Prawn , *Macrobrachium rosenbergii* (De Man)

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To study the effect of fatty acid from *Schizochytrium* sp. as a feed supplement for juvenile giant freshwater prawns, (*Macrobrachium rosenbergii*) was conducted by using 4 formulated diets to contain 0 (fish oil 2%), 2, 4 and 8 % *Schizochytrium*. The prawns were raised in the hatchery at Department of Fisheries, Faculty of Agriculture and Natural Resources for 60 days, compare to the prawns fed on commercial diet. It was found that the prawns fed on 0 % (fish oil 2%) showed percentage weight gain, average daily weight gain, specific growth rate and feed conversion ratios were not difference from the prawns fed on 2 and 4 % *Schizochytrium* ($p > 0.05$) but significantly different from that fed on 8 % and commercial diet ($p < 0.05$). But survival rates showed the best results at 2 % *Schizochytrium* which were not difference from the prawns fed on commercial diet ($p > 0.05$) but significantly different from the prawn fed on fish oil (2%), *Schizochytrium* 4 and 8 % ($p < 0.05$). This study showed that *Schizochytrium* sp. can be used to replace fish oil and indicated that supplement at 2 % was appropriate for growth performance and survival rate of *M. rosenbergii* juvenile.

Keywords: giant freshwater prawns (*Macrobrachium rosenbergii*), Fish oil replacement, *Schizochytrium* sp., essential fatty acids

Introduction

Lipid is the one of the important nutrients for crustaceans because it is the source of energy. (Sheen and Wu, 1999). Lipid also supply essential fatty acids (EFA) which need for maintenance, integrity of cellular membranes, and serve as precursors of steroid and moulting hormones (Harrison, 1990). Additionally, EFA required for growth, survival and the normal metabolic function of crustaceans.

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Polyunsaturated fatty acids (PUFA) such as linoleic (18:2n-6,LOA) and linolenic (18:3n-3, LNA) acids and highly unsaturated fatty acid (HUFA) such as eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) are EFA. They have been importance nutrients for the growth and survival of crustaceans. However the crustaceans have a limited ability for de novo synthesis of PUFA and HUFA, such as *Macrobrachium rosenbergii* (Reigh and Stickney, 1989), *Penaeus duorarum* (Sick and Andrews, 1973), *Penaeus indicus* (Colvin,1976), *Penaeus japonicas* (Guary et al, 1976) *Penaeus monodon* (Catacutan, 1991; Deering et al,1997; Vasagam et al,2005) *Penaeus vannamei* (Dominy &Lim,1989; Lim et al, 1997; Gonzales-Felix et al, 2002) *Procambarus clarikii* (Wen et al,2003) have been demonstrated. According to most shrimp are not able to synthesize all EFA mentioned above, but take from natural feed or completed feed.

The most important source of EFA use in shrimp feed formulation comes from fish oil, such as menhaden, cod, pollack, tuna and sardine oil, which limited by natural supply. Fish oil is a finite resource and by 2010 it is more than 90 % of global fish oil supplies will be used for aquafeeds (Barlow,2000). Moreover, global fish oil production has reached a plateau because of overfishing, high production cost, due to inadequate amount of fishes and limitation of amount of DHA 7-14%, concomitant with the possible accumulation of dioxins and dioxins like polychlorinated biphenyls (Zhou, Li, Liu, Chi &Yang, 2007). Due to increasing demand and limited supplies of fish oil if shrimp culture is to remain a viable industry, feed manufacturers must reduce dependence on fish oil. Therefore, it has become to find alternative sources of high quality oils are available to be used in aquafeeds.

Recently *Schizochytrium* sp. is the fast growing marine microalgae with 30-40 % of total fatty acid content (Bajpai et al, 1991). It has enriched biomass and oil, and produce as high as 500 g DHA Kg-1 lipid (Behrens & Kyle, 1996) and has high potential as fish oil substitute in aquaculture feed. Therefore, it is one alternative sources that has been developed as a commercial source of DHA (Barclay et al, 2005). It can be grown heterotrophically and are commercially available as dried products have been reported to be a good source of nutrients and EFA for laval live food enrichment and for formulated broodstock diets of marine teleostes (Harel, Koven, Lin, Bar, Behrens, Stubblefield, Zohar & Place 2002). Oil extracted from marine microalgae has been shown to successfully replace marine fish oil in the diet for Atlantic salmon *Salmo salar* parr without detriment to growth (Mill et al., 2007). Dried algae *Schizochytrium* sp. appears to be a good source of DHA for seabream *Sparus aurata* larvae (Ganuza et al., 2008). Patnaik, Samocha, Davis, Bullis and Browdy (2006) showed that fish oil can be successfully replaced in the

diets for *Litopenaeus vannamei* using spray-dried cells of *Schizochytrium* sp. obtained by a proprietary commercial fermentation process.

In recent years, the development of modern culture techniques for giant freshwater prawn, *Macrobrachium rosenbergii* has considerably expanded. Global production has been increased from 17,129 to 180,221 tonnes between 1993 and 2003 (FAO,2005). . In 2012, the worldwide production of the giant freshwater prawn, *Macrobrachium rosenbergii* was 220,254 tons/year, making it the 7th largest crustacean aquaculture industry. World leading producers of this species included China, Bangladesh and Thailand (124,713, 45,162 and 23,913 tons) in 2012 (FAO FishStatJ 2013). Consequently, the objective of this study is to evaluate the effectiveness of fish oil replacement strategies using fatty acids, particularly DHA from marine microalgae with different amount in practical diets and the subsequent influence on growth performance and survival rates of *M. rosenbergii*.

Objectives: To determine the effect of supplemented *Schizochytrium* sp. levels in base diets on growth performance and survival rate of Giant Freshwater Prawn , *Macrobrachium rosenbergii* (De Man)

Materials and methods

Experimental design and allocation of the test animals

Approximately one month old *M. rosenbergii* post larvae were purchased from a commercial farm in Chachengchao Province, Thailand and they were acclimatized in a 2.7 m³ cement pond for one month until the prawns are two months within the experimental hatchery unit at Rajamangala University of Technology Tawan-ok . A 5 × 3 completely randomized design (CRD) was used in the present study. The two month old juveniles, which should average weight 4.89±0.02 g; average total length = 8.00 ±0.02 cm, were randomly allocated between the 15 experimental tanks (160 L) at stocking density of 40 individuals per m² (15/tank).. Each tank was provided with two air-stones having similar air flows of 10 lmin-1. Tanks were filled with freshwater and 30% of the total volume was renewed daily. The experimental prawns were then fed twice daily the relevant experimental diet at 10 % of the wet body weight/day (at 07.00 and 18.00). The feed trial experiment was conducted over a 60-day period.

Experimental diets

Four isonitrogenous and isoenergetic experimental diets were formulated to meet the nutritional requirements of juvenile *M. rosenbergii* (Table 1). Each diet contained the same basal composition but differed in their *Schizochytrium* and fish oil level. Diet 1 contained fish oil 2% of total ingredients. Diet 2 to 4 contained *Schizochytrium* 2, 4 and 8% of total ingredients (Table 1).

The dry ingredients were weighed and mix in horizontal mixer prior to the addition of fish oil and *Schizochytrium*. The ingredients were pelleted in shrimp feed pellet machine. The final product was baked in the oven at 60 ° C for 12 hours. To verify the composition of the experimental diets the proximate composition was carried out in laboratory (Central Laboratory (Thailand) Co., Ltd.).

Experimental sampling protocol

At the start and the end of the trial, the weight and length of each *M. rosenbergii* individual was measured. After the experiment had concluded and the individuals had been measured, 10 *M. rosenbergii* individuals from each tank were shelled, minced and then stored at -20°C until the fatty acid content of each sample could be determined. Due to the amount of tissue required for proximate analysis.

Chemical composition of the experimental diets

The formulation of each experimental diet is presented in Table 1. The moisture, crude protein, crude lipid, ash content and nitrogen free extract (NFE) of the diets were determined according to standard procedures (AOAC 2005). The digestible energy of the experimental diets were calculated from standard physiological fuel values of 4, 4 and 9 Kcal/g for protein, carbohydrate and lipid respectively (Garling and Wilson, 1976).

Table 1.Ingredients and proximate composition (%) of the four experimental diets prepared for the current study

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal ¹	38.0	38.0	38.0	38.0

Soybean meal	28.0	28.0	28.0	28.0
Shrimp shell meal	14.0	14.0	14.0	14.0
Corn grain	3.0	3.0	3.0	3.0
Wheat meal	8.0	8.0	6.0	2.0
Rice bran	5.0	5.0	5.0	5.0
Tuna oil	2.0	-	-	-
<i>Schizochytrium</i> sp. ²	-	2.0	4.0	8.0
Binder ³	1.0	1.0	1.0	1.0
Vitamin premix ⁴	1.0	1.0	1.0	1.0
<i>Proximate composition</i>				
Moisture %	6.12	6.86	6.51	5.42
Protein%	40.88	40.88	40.68	40.28
Lipid%	10.33	8.73	9.27	11.76
Ash%	15.05	15.15	15.51	15.46
Fiber%	4.00	3.79	4.06	3.78
NFE	23.62	24.59	23.97	23.30
Digestible energy	328.73	316.88	319.79	336.91
(Kcal 100g ⁻¹)				

¹Mix of marine fish 55 % protein from Siam fish meal Lp.

²Drum dried algal meal DHA Gold™ made from *Schizochytrium* sp. from

Marine Leader Co., Ltd.

³ α-starch from Mario bio products., Co. Ltd.

⁴ premix prawn from Planet Aquatic Chemical Co., Ltd.

Water quality analysis

During the experiment period, temperature, pH (YSI, Model 63) and dissolved oxygen were daily monitored. Alkalinity (titration method detailed in APHA et al. 1980), hardness, ammonia (indophenol blue method detailed in Grasshoff 1976) and nitrate (the diazotization method provided in Grasshoff 1976) were determined on a weekly basis.

Growth parameter calculations

Feed conversion ratio (FCR), specific growth rate (SGR), survival rate (SR), weight gain (WG), percentage weight gain (%WG) and absolute daily weight gain (ADG) for each experimental tank were calculated according to the following equations:

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

Weight gain = final weight (g) - initial weight (g)

Length gain = final length (cm) - initial length (cm)

% weight gain = final weight (g) – initial weight (g) × 100/initial weight

Absolute daily weight gain = final weight (g) – initial weight/number of days

Specific growth rate = [ln final weight (g) - ln initial weight (g)]/days × 100

Feed conversion ratio = feed intake (g)/weight gain (g)

Statistical analysis

The effects of dietary treatment were determined by a one-way analysis of variance (ANOVA) using the SPSS programme (ver. 11, SPSS, Inc.). Statistical significances were set at $p < 0.05$ and the mean differences among treatments were determined using Duncan's new multiple range test.

Results

The proximate compositions of the experimental diets were similar (Table 1). Each experimental diet contained approximately 40 % protein and 10 % lipid whilst the ash and NFE content of each diet was approximately 15% and 23%, respectively. The digestible energy content of each diet was between 316 and 336 Kcal 100g⁻¹.

Table 2. Growth performance of *Macrobrachium rosenbergii* fed different experimental diets for 60 days.

Growth performance	Experimental diets				
	Diet1	Diet2	Diet3	Diet4	control
Initial weight (g)	4.96±0.08	4.85±0.05	4.97±0.07	4.83±0.05	4.84±0.11
Initial length (cm)	7.99±0.03	7.98±0.07	8.01±0.13	8.00±0.02	8.04±0.14
Weight gain (%)	106.36±5.08 ^a	106.21±2.49 ^a	97.48±2.99 ^{ab}	89.03±6.53 ^b	83.44±5.42 ^b
ADG(g)	0.09±0.01 ^a	0.09±0.01 ^a	0.08±0.01 ^{ab}	0.07±0.01 ^{bc}	0.07±0.01 ^c
SGR(%/day)	1.21±0.04 ^a	1.21±0.02 ^a	1.13±0.10 ^{ab}	1.06±0.06 ^b	1.01±0.09 ^b
FCR	1.46±0.04 ^a	1.45±0.02 ^a	1.52±0.10 ^{ab}	1.59±0.06 ^b	1.64±0.09 ^b
Survival rate (%)	86.67±2.88 ^{bc}	93.33±2.89 ^a	81.67±2.88 ^c	81.67±2.88 ^c	88.33±2.89 ^{ab}

ADG = absolute daily weight gain; SGR = specific growth rate.

Values are given as the mean ± standard deviation of three groups of juvenile *M. rosenbergii* per treatment. Values with differing superscript letters highlight significant differences ($p \leq 0.05$).

Growth performance of freshwater prawns

At the start of the trial, there were no statistical differences ($p > 0.05$) in the weight or length of the experimental juvenile prawns (Table 2). After eight weeks, however, there were significant differences ($p < 0.05$) in growth performances and survival rates, e.g. percentage weight gain (WG), absolute daily weight gain (ADG), the specific growth rate (SGR) and in the feed conversion ratio (FCR).

The prawns fed on diet 1 (fish oil 2%) diet 2 (2 % *Schizochytrium*) had the highest percentage weight gain (WG), absolute daily weight gain (ADG), the

specific growth rate (SGR) and in the feed conversion ratio (FCR) significantly different ($p < 0.05$) from those fed on diet 4 (8 % *Schizochytrium*) and control diet. However, they were not significantly different ($p > 0.05$) from those fed on diet 3 (4 % *Schizochytrium*). Furthermore, prawns fed on diet 2 showed the best survival rates, significantly different ($p < 0.05$) from those fed on other diet (1, 3, and 4) but were not significantly different ($p > 0.05$) from those fed on control diet. Prawns fed on diet 3 and 4 had the lowest survival rate (Table 2).

Proximate composition of the flesh

The flesh of prawn compositions (Table 3) typically corresponded to dietary fatty acid compositions (Table 1). Prawn flesh moisture content did not differ significantly among treatments. There were significant differences ($p < 0.05$) in protein, lipid, ash and fiber composition of prawn fed all experimental and natural prawns.

Protein in flesh of natural prawn higher than other groups but not significantly different ($p > 0.05$) from prawn fed on diet 2. Lipid in flesh of prawn fed diet 4 higher than other groups but not significantly different ($p > 0.05$) from prawn fed on diet 3. Ash in flesh of prawn fed control diet higher than other groups but not significantly different ($p > 0.05$) from prawn fed on diet 1, 2, 3 and natural prawn. The flesh fiber content of prawn fed on diet 1 higher than other groups ($p < 0.05$).

Table 3. Muscle proximate composition of *Macrobrachium rosenbergii* fed experimental diets for 60 days

proximate composition%	Natural prawn	Experimental diets				
		Diet1	Diet2	Diet3	Diet4	Control diet
Moisture	0.83±10.70	0.30±9.63	0.84±11.85	0.86±10.80	0.45±9.52	0.63±10.07
Protein	0.38±25.38 ^a	0.13±24.26 ^b	0.36±24.52 ^{ab}	0.23±24.26 ^b	0.17±24.36 ^b	0.38±24.09 ^b
Lipid	0.26±3.15 ^{cd}	0.27±4.61 ^{bc}	0.23±4.08 ^{bc}	0.54±4.69 ^{ab}	0.31±5.26 ^a	0.3±2.576 ^d
Ash	0.23±17.37 ^{ab}	15.0.99±93 ^{ab}	0.12±15.76 ^{ab}	0.12±17.27 ^{ab}	0.14±15.24 ^b	1.44±18.01 ^a
Fiber	0.05±7.65 ^{bc}	0.40±10.70 ^a	0.02±8.11 ^b	0.01±8.04 ^b	0.01±6.89 ^d	0.16±7.29 ^{cd}

Water quality of the experimental trial

Water quality was consistent through the experimental period and across experimental tanks. The temperature of the water during the experimental trial

ranged from $28.38 \pm 0.24 - 29.25 \pm 0.32$ °C, the dissolved oxygen from $3.37 \pm 0.73 - 7.70 \pm 0.42$ mg L⁻¹, pH from $7.62 \pm 0.27 - 7.94 \pm 0.19$, alkalinity from $64.04 \pm 10.48 - 84.50 \pm 0.86$ mg L⁻¹, hardness from $75.33 \pm 2.21 - 83.33 \pm 6.54$ mg L⁻¹ and, the concentration of ammonia from $0.09 \pm 0.02 - 0.22 \pm 0.04$ mg L⁻¹.

Discussions

In the present study, five experimental diets were tested, among diet contained the same basal composition but differed in their *Schizochytrium* and fish oil level and commercial diet fed the juvenile prawn for 8 weeks. The results showed no statistically significant differences in %WG, ADG, SGR and FCR of the juvenile *M. rosenbergii* fed diet with fish oil 2 % and *Schizochytrium* 2 and 4 %. Furthermore, prawn fed diet with *Schizochytrium* 2 % showed the best results of survival rates and significant different from prawn fed diet with fish oil 2%. These results agree with several reports that partially or entirely replacing fish oil in aquafeeds with *Schizochytrium* did not negatively effect their production (Boeing 1996; Langdon and Onal 1999; Carter et al. 2003; Miller 2007; Ganuza et al. 2008; Li et al. 2009; Van Hoestenbergh et al. 2014). Although fish oils are best known and highly regarded for their abundance of LC-PUFA of the n-3 series-EPA, and DHA but increasing demand and limited supplies of fish oil. Therefore this study suggested that replacing fish oil by varying amount of *Schizochytrium* in the diets of the juvenile prawn because *Schizochytrium* is the fast growing marine microalgae with 30-40 % of total fatty acid content (Bajpai et al, 1991). It has enriched biomass and oil, and produce as high as 500 g DHA Kg⁻¹ lipid (Behrens & Kyle, 1996). Similarly, *Schizochytrium* spp. oil has been successfully tested on Atlantic salmon (Miller *et al.*, 2007). Patnaik et al. (2006) demonstrated that fish oil can be successfully replaced in diets for *Litopenaeus vannamei* using cells of *Schizochytrium* sp. and *Mortierella* sp. obtained from commercial microbial fermentation.

In a nutritional point of view, the proximate biochemical composition of any edible organism is vary crucial. The nutritive values of crustaceans depend upon their body biochemical constituents (Vifayavel and Balasubramanian, 2006). Body biochemical composition is a good indicator for physiological condition and easy to assess in cultivable organisms. In this study, biochemical constituents such as protein, lipid, ash and fiber contents were significantly ($p < 0.05$) but there were no significant differences in moisture. Lipid composition of the juvenile *M. rosenbergii* increased when fed the diets with more *Schizochytrium* level. The juvenile *M. rosenbergii* fed diet with

Schizochytrium 2 % no significant differences in protein contents compared with natural prawns and prawns fed diet with fish oil 2%. Ash contents of prawns fed diet with fish oil 2%, *Schizochytrium* 2, 4 % and company diet were no significant differences ($p>0.05$). However, use of Schizochytrium ingredients contribute to optimal biochemical composition contents in prawns tissue, comparable to that of fish oil. These results indicated that fish oil can be successfully replaced with *Schizochytrium* in diets for the juvenile *M. rosenbergii*.

Conclusion

The results of this study reaffirm that can replace dietary fish oil with *Schizochytrium* sp. for juvenile *M. rosenbergii* without negatively affecting their growth performance. The optimal of *Schizochytrium* for juvenile growth performance and survival rate were 2%.

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