Agriculture and Natural Resources 51 (2017) 103–108

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Effects of alternative oil sources in feed on growth and fatty acid composition of juvenile giant river prawn (*Macrobrachium rosenbergii*)

Chanpim Kangpanich,[a, c, *] Jarunan Pratoomyot,[b] Wansuk Senanan[c]

[a] *Department of Fisheries, Faculty of Agriculture and Natural Resources, Rajamangala University of Technology Tawan-ok, Chonburi 20110, Thailand*
[b] *Institute of Marine Science, Burapha University, Chonburi 20131, Thailand*
[c] *Department of Aquatic Science, Faculty of Science, Burapha University, Chonburi 20131, Thailand*

ARTICLE INFO

Article history:
Received 3 January 2016
Accepted 1 December 2016
Available online 24 May 2017

Keywords:
Essential fatty acids
Fish oil replacement
Giant river prawn
Macrobrachium rosenbergii
Schizochytrium sp.

ABSTRACT

To relieve the pressure on the future use of fish oil (FO), alternative oil sources need to be explored. Alternative oil sources were evaluated—*Schizochytrium* sp. (SZ) and soybean oil (SO)—on the growth performance and flesh quality of juvenile river prawn, *Macrobrachium rosenbergii*. Five experimental diets differed in the types of oil used (oil comprised 3% of dietary ingredients): 3% FO, 1% SZ + 2% SO, 1.5% SZ + 1.5% SO, 2% SZ +1% SO and 3% SZ. After 60 d of the experiment, the survival rates of prawns fed non-FO diets did not significantly ($p > 0.05$) differ from those fed the FO diet (77.82 ± 4.45–93.38 ± 0.00%). Moreover, prawns fed diets containing both SZ and SO had significantly ($p < 0.05$) better growth performance than those fed a single oil source. Prawns fed 2% SZ+1% SO showed the best final weight, percentage weight gain, absolute daily weight gain, specific growth rate and feed conversion ratio ($p < 0.05$) while those fed 1.5% SZ +1.5% SO or 3% SZ had the highest survival. Tissues of prawns fed the non-FO diets contained higher amounts of n−6 polyunsaturated fatty acid (PUFA) but were lower in n−3 long-chain PUFAs (eicosapentaenoic acid and docosahexaenoic acid) than those fed the FO diet. Among the non-FO groups, prawns fed 3% SZ had the most similar flesh fatty acid profile to those fed 3% FO. Substitution of FO with combinations of SZ and SO significantly improved growth performance and feed utilization. The study recommended diets containing 2% SZ + 1% SO or 1.5% SZ + 1.5% SO for *M. rosenbergii* juveniles.

Introduction

Global crustacean aquaculture has continued to expand, while at the same time, the amount of fish meal (FM) and fish oil (FO) derived from fisheries available for the formulation of aquatic diets has steadily declined (Turchini et al., 2010). Food and Agriculture Organization (2012) projected that the total amount of FO to be used in aquaculture will increase by more than 16%, from 782,000 t in 2008 to 908,000 t by 2020. The giant river prawn, *Macrobrachium rosenbergii* is one of the most widely cultured freshwater prawns in the world; in 2012, the worldwide production of *M. rosenbergii* was 220,254 t/yr, making it the seventh largest crustacean aquaculture industry with the world's leading producers of this species being China (124,713 t, Bangladesh (45,162 t) and Thailand (23,913 t) in 2012 (Food and Agriculture Organization, 2013)..

FO has been used traditionally as a source of lipids to supply energy and essential fatty acids in the diets fed to *M. rosenbergii* (Hasan et al., 2007). Like other crustaceans, *M. rosenbergii* cannot *de novo* synthesize the n−3 and n−6 essential fatty acids (EFAs) from saturates or from monoenes and must obtain them through its diet (Xu et al., 1993). *M. rosenbergii* also lacks the ability to synthesize linolenic acid (LNA) and linoleic acid (LA) (D'Abramo and Sheen, 1993) and has limited ability to elongate and desaturate short-chain n−3 and n−6 polyunsaturated fatty acids (C18) to long-chain polyunsaturated fatty acid (C ≥ 20; Reigh and Stickney, 1989). The main species of fish that are harvested and processed for their fish oil are pelagic types (such as *Engrauhs ringens*, *Brevoorita* spp. and *Pollachius* spp.), which are known to have a lipid content of about 8% or more (Turchini et al., 2010). These fish species typically have a high n−3 polyunsaturated fatty acid (PUFA) content, particularly in long chain polyunsaturated fatty acids (LC-PUFA), namely, eicosapentaenoic acid (EPA) and docosahexaenoic

* Corresponding author. Department of Fisheries, Faculty of Agriculture and Natural Resources, Rajamangala University of Technology Tawan-ok, Chonburi 20110, Thailand.
E-mail address: chanpimk@hotmail.com (C. Kangpanich).

http://dx.doi.org/10.1016/j.anres.2016.12.004

acid (DHA) (FAO, 2012). Depending on the fish species, the EPA and DHA contents are in the range 4–22% and 2–13%, respectively (Turchini et al., 2010). Since the future manufacture and availability of FO is limited by the uncertain sustainability of natural supplies and has high costs of production, and because of the possible accumulation of dioxins and dioxin-like polychlorinated biphenyls in the FO (Zhou et al., 2007), the search for alternative oil sources to replace FO is necessary.

Marine algae are known to contain high levels of lipids and fatty acids (for example, De Swaaf et al., 2003; Wu et al., 2005). Among these, the marine algal species, *Schizochytrium* spp., are of interest as their fatty acid composition is suitable as a feed ingredient and provides for rapid growth rates under culture conditions (for example, Ren et al., 2010). *Schizochytrium* spp. contain lipids in amounts as high as 55% of the cell weight, of which the DHA, EPA and arachidonic acid (ARA) can represent 35%, 7% and 5%, respectively, of the total fatty acids fraction (Barclay, 1997), which is close to the composition in FO (Nakahara et al., 1996). Studies have suggested that *Schizochytrium* spp. could serve as good substitutes for fish oil or as PUFA supplements in the diets for crustaceans, including the penaeid shrimp, *Litopenaeus vannamei* (Poungchor et al., 2009; Wang et al., 2016) and freshwater prawn, *M. rosenbergii* (Kangpanich and Senanan, 2015; Sisouvong et al., 2013). In juvenile *M. rosenbergii*, a diet containing *Schizochytrium* sp. (SZ) at 2% of ingredient dry weight yielded growth and survival comparable to the FO control diet (Kangpanich and Senanan, 2015). Also, juveniles fed a commercial diet (32% protein) supplemented with 7.5% and 10% SZ (75 mL/kg feed and 100 mL/kg feed, respectively) improved growth and survival compared to the commercial diet without SZ (Sisouvong et al., 2013).

Unlike marine fish and shrimp, freshwater animals require a high content of both n−3 and n−6 PUFAs (Takeuchi and Watanabe, 1977), resulting in a ratio of n−3 to n−6 close to or less than 1 (Teshima et al., 1994). Essential sources of n−6 PUFAs used in commercial prawn diets are typically derived from plant oils (Tacon, 1990). Several vegetable oils are used within the shrimp feed industries, including oils derived from soybean (Goda et al., 2011), corn (Kamarudin and Roustaian, 2002; Goda et al., 2011), palm, canola, sunflower and linseed (Kim et al., 2013). However, the traditional use of these vegetable oils as a substitute of FO in aquafeeds only provides a partial replacement of the overall requirements of n−3 and n−6 fatty acids, especially LC-PUFA. A diet containing an optimal n−3 to n−6 ratio may be necessary to fulfill the growth requirement of *M. rosenbergii*.

Therefore, the present study investigated the total replacement of fish oil either through the incorporation of a mixture of oils derived from *Schizochytrium* sp. and soy or through the sole use of *Schizochytrium* sp. oil added to experimental diets fed to juvenile *M. rosenbergii* over 2 mth. The study determined the survival and growth performance of the prawns throughout the trial and the fatty acid content of the flesh on termination of the trial. The effect of a range of dietary ratios of n−3 to n−6 on the growth performance and flesh fatty acid compositions was also tested.

Materials and methods

Experimental design and animals

Approximately *M. rosenbergii* post larvae age 1 mth, purchased from a commercial farm in Chachoengsao province, Thailand, were acclimatized in a 2.7 m^3 cement pond at Rajamangala University of Technology Tawan-ok, Chonburi, Thailand for 1 mth prior to the start of the trial.

Fifteen juvenile *M. rosenbergii* (average weight = 3.33 ± 0.20 g; average total length = 6.4 ± 0.03 cm) were randomly allocated to each of the 15 experimental plastic tanks each with a capacity of 160 L containing fresh water. Each tank was supplied with two airstones having similar air flows of 10 L/min. The daily water replacement rate was 30% of the total volume. Each of five experimental diets was assigned at random to three replicates of the tanks containing the juvenile prawns. The experimental prawns were fed twice daily (0700 and 1800 h) with the assigned experimental diet at 10% of the wet body weight/day. The feeding trial was conducted for 60 d.

Experimental diets

Five isonitrogenous and isoenergetic experimental diets were tested (Table 1). The diets were modified from the feed formulation developed by the Thai Department of Fisheries (Somsueb, 2009) and derived from the best diet treatment (2% SZ diet) suggested by Kangpanich and Senanan (2015). Each diet contained the same basal composition but differed in n−3 PUFA and n−6 PUFA oil compositions (ingredient source) and ratios. The fish oil/alternative oil types constituted 3% of the total ingredients of the experimental diets. The FO component in all but the control diet was completely substituted by oil derived from *Schizochytrium* sp. (SZ) and from soybean (SO) in the following ratios: Diet 1 contained 3% FO; Diet 2 contained 1% SZ and 2% SO; Diet 3 contained 1.5% SZ and 1.5% SO; Diet 4 contained 2% SZ and 1% SO; and Diet 5 contained 3% SZ.

Data collection

Measurements were recorded for the growth performance (initial and final weight and length of each individual prawn) and the survival rate, and the fatty acid compositions in the muscle tissue of prawns in each treatment were analyzed. The total amount of feed intake (in grams) was recorded for each tank for the calculation of the feed conversion ratio. After the experiment had concluded, 10 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 analysing the fatty acid composition, individuals were pooled from

Table 1
Composition of the five experimental diets (%) prepared for the current study.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal[a]	35.0	35.0	35.0	35.0	35.0
Soybean meal	25.0	25.0	25.0	25.0	25.0
Shrimp shell meal	12.0	12.0	12.0	12.0	12.0
Corn grain	6.0	6.0	6.0	6.0	6.0
Wheat flour	9.0	9.0	9.0	9.0	9.0
Rice bran	8.0	8.0	8.0	8.0	8.0
Tuna oil	3.0	–	–	–	–
Soy oil	–	2.0	1.5	1.0	–
Schizochytrium sp.[b]	–	1.0	1.5	2.0	3.0
Binder[c]	1.0	1.0	1.0	1.0	1.0
Vitamin and mineral premix[d]	1.0	1.0	1.0	1.0	1.0
Proximate composition					
Moisture	5.24	5.52	6.13	6.54	6.16
Protein	38.97	39.91	39.68	39.81	39.28
Lipid	11.40	10.42	10.48	10.15	9.48
Ash	12.68	12.61	12.67	12.70	12.58
NFE[e]	31.71	31.54	31.04	30.80	32.50
DE[f]	337.72	333.35	331.77	329.09	324.64

[a] Mix of marine fish 55% protein from Siam fish meal Lp.
[b] Drum-dried algal meal DHA Gold™ made from *Schizochytrium* sp. from Marine Leader Co., Ltd.
[c] α-starch from Mario Bio Products., Co. Ltd.
[d] premix prawn from Planet Aquatic Chemical Co., Ltd.
[e] NFE = nitrogen free extract + fiber.
[f] DE = Digestible energy (Kcal/100 g).

all three replications for each treatment (30 individuals per treatment) and a duplicate analysis was performed on each pooled sample.

Chemical composition of the experimental diets

The moisture, crude protein, crude lipid and ash contents and the nitrogen free extract (NFE) of the diets were determined according to standard procedures (Association of Official Analytical Chemists, 2005). The digestible energy of the experimental diets was calculated from the standard physiological energy values of 4 KcaL/g for crude protein and nitrogen-free extract and 9 KcaL/g for lipid (Cavalli et al., 1999).

Fatty acid composition of the experimental diets and flesh samples

The experimental diets and flesh samples were sent to Central Laboratory (Thailand) Co., Ltd. for fatty acid compositions analysis. The total lipid fraction in each diet and the flesh samples were subjected to extraction by ether and then the fats were methylated to fatty acid methyl esters (FAMEs) using 14% BF_3 in methanol. According to the standard method of Association of Official Analytical Chemists (2005), the FAMEs were quantified using capillary gas chromatography (GC). GC analysis was performed using an Agilent 6890 (Santa Clara, CA, USA); column type: capillary, equipped with DB-MS and HP-INNOWAX columns (30 m × 0.25 mm; film thickness, 0.25 μm). The column features, in combination with the temperature program applied, allowed the separation of the FAMEs, which were eventually detected using a flame ionization detector (FID) at various temperatures. The FID detector used a temperature of 210 °C, and a pressure setting range of 0–100 psi.

Water quality analysis

On a daily basis, the temperature, pH (Model 63; YSI; Yellow Springs, OH, USA) and dissolved oxygen were monitored during the experiment. On a weekly basis, alkalinity (using the titration method detailed in American Public Health Association et al., 1980), hardness, ammonia (using the indophenol blue method detailed in Grasshoff, 1976) and nitrate (using the diazotization method provided in Grasshoff, 1976) were determined.

Growth parameter calculations

Individuals in each experimental tank were measured for their specific survival rate (SR), weight gain (WG), percentage weight gain (%WG), absolute daily weight gain (ADG), growth rate (SGR, % per day) and feed conversion ratio (FCR). These growth parameters were calculated using the following calculations.

$$SR(\%) = (\text{final number of prawns}/\text{initial number of prawns}) \times 100$$

$$WG = \text{final body weight} - \text{initial body weight}$$

$$\%WG = (WG/\text{initial body weight}) \times 100$$

$$ADG = WG/\text{number of days}$$

$$SGR = ((\ln \text{final weight} - \ln \text{initial weight}) \times 100)/\text{number of days}$$

$$FCR = \text{total feed intake}/WG$$

where all weights were measured in grams and *ln* represents the natural logarithm function.

Statistical analysis

The effects of dietary treatments were determined by one-way analysis of variance using the SPSS software package (version 11; SPSS, Inc., Chicago IL, USA). Statistical significance was set at $p < 0.05$ and the mean differences among treatments were determined using Duncan's new multiple range test.

Results

Proximate compositions and fatty acid contents of the experimental diets

All experimental diets contained similar proximate compositions (Table1), with approximately 39% protein, 10% lipid, 13% ash and 32% NFE content. The digestible energy content of each diet was in the range 331.77–337.72 Kcal/100 g.

The major fatty acid classes of each diet generally reflected its oil sources (Table 2). In all diets, the amounts of total monoenes and saturates were higher than those of total n−3 PUFA, n−3 LC-PUFA (EPA + DHA) and n−6 PUFA. The amounts of total saturates, monoenes, n−3 PUFA, n−3 LC-PUFA and n−6 PUFA in Diets 1 and 5 were almost identical. In contrast to Diets 1 and 5, Diets 2, 3 and 4 contained lower levels of total saturates, n−3 PUFA and n−3 LC-PUFA but higher levels of total monoenes and n−6 PUFA. In Diets 2, 3 and 4, the level of total saturates and total n−3 PUFA increased slightly with an increasing SZ content. However, the percentage of total monoenes and n−6 PUFA in these diets gradually decreased with the increasing SZ content. The percentages of total n−3 PUFA and n−6 PUFA contents in Diets 1 and 5 were both approximately 19%, resulting in a ratio of total n−3 PUFA to total n−6 PUFA close to 1 (0.96 for both diets). By comparison, the levels of total n−3 PUFA in Diets 2, 3 and 4 were much lower than those of total n−6 PUFA in the same diets, resulting in ratios of total n−3 PUFA to total n−6 PUFA of 0.45–0.65.

All diets contained LA as a principal component of total n−6 PUFA but Diets 2, 3 and 4 contained higher levels of total n−6 PUFA compared to those determined in Diets 1 and 5. For n−3 PUFAs, DHA and LNA were the main components of all diets, however, the DHA content of Diets 2, 3 and 4 was lower than that of both Diets 1 and 5. The LNA content also steadily declined from Diet 2 through

Table 2
Major fatty acid classes (% of total fatty acids) of the experimental diets.

Fatty acid[a]	Experimental diet				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
18:2n6 (LA)	17.8	27.4	25.1	23.1	18.8
20:4n6 (ARA)	1.02	0.54	0.58	0.62	0.70
18:3n3 (LNA)	4.36	4.91	4.79	4.71	4.57
20:5n3 (EPA)	3.89	2.03	2.12	2.26	2.54
22:6n3 (DHA)	9.90	5.42	6.68	8.16	11.40
∑saturates	33.80	28.20	29.60	30.70	32.50
∑monoenes	28.28	30.84	30.38	29.56	28.14
∑n−6 PUFA	19.10	28.19	25.91	23.99	19.84
∑n−3 PUFA	18.40	12.60	13.90	15.50	19.00
∑n−3 LC-PUFA (EPA + DHA)	13.79	7.45	8.8	10.42	13.94
∑n−3/∑n−6	0.96	0.45	0.54	0.65	0.96

[a] LA = linoleic acid, ARA = arachidonic acid, LNA = linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, PUFA = poly unsaturated fatty acid.

Table 3
Growth performance (mean ± standard deviation) of *Macrobrachium rosenbergii* fed different experimental diets for 60 d[b].

Growth performance[a]	Experimental diet				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Initial weight (g)	3.11 ± 0.02a	3.13 ± 0.01a	3.17 ± 0.02a	3.12 ± 0.02a	3.14 ± 0.02a
Final weight (g)	7.65 ± 0.08c	8.12 ± 0.03b	8.25 ± 0.07b	8.76 ± 0.08a	7.59 ± 0.06c
Final length (cm)	7.87 ± 0.09a	8.11 ± 0.16a	7.78 ± 0.26a	7.98 ± 0.19a	7.65 ± 0.27a
Survival rate (%)	80.04 ± 3.85ab	80.04 ± 7.70ab	93.36 ± 3.84a	77.82 ± 4.45b	93.38 ± 0.00a
Weight gain (g)	4.54 ± 0.05bc	4.99 ± 0.03b	5.08 ± 0.04ab	5.64 ± 0.09a	3.97 ± 0.41c
Length gain (cm)	1.53 ± 0.11a	1.62 ± 0.0a	1.30 ± 0.21a	1.55 ± 0.12a	1.27 ± 0.27a
Weight gain (%)	145.97 ± 0.45c	159.36 ± 0.38b	160.13 ± 0.72b	181.20 ± 4.07a	141.38 ± 2.45c
ADG (g)	0.08 ± 0.00b	0.08 ± 0.00b	0.08 ± 0.00b	0.10 ± 0.00a	0.07 ± 0.00b
SGR (%/day)	1.50 ± 0.00c	1.59 ± 0.00b	1.59 ± 0.00b	1.72 ± 0.03a	1.47 ± 0.02c
FCR	2.05 ± 0.01a	1.88 ± 0.01b	1.87 ± 0.01b	1.65 ± 0.04c	2.12 ± 0.04a

[a] ADG = absolute daily weight gain, SGR = specific growth rate FCR = feed conversion ratio.
[b] Values with different lowercase superscript letters are significantly different ($p < 0.05$).

Diet 4. The control diet (Diet 1) and Diet 5 contained twice as much DHA as LA. In contrast, DHA and LA in Diets 2, 3 and 4 were present in similar amounts, although the DHA level in Diet 4 was higher than in the other two diets (Table 2).

Growth performance of giant river prawn

The initial weight and length of the experimental juvenile prawns did not differ among treatments ($p > 0.05$). After 8 wk, there were significant differences in the survival rates and some growth performance parameters among experimental groups (Table 3). The prawns fed Diets 3 and 5 had the highest survival rates, which were significantly different from those fed Diet 4. The survival rates of the juveniles fed Diets 3 and 5 were not significantly different from those fed on Diets 1 and 2. Although prawns fed Diet 4 had the lowest survival rate, this rate was not significantly different from that of the juveniles fed the control diet.

The prawns fed Diet 4 showed the best final weight, which was significantly different from those fed the other four experimental diets. The final weights of juveniles fed Diets 2 and 3 did not significantly differ, but were significantly higher than those fed Diets 1 and 5. Furthermore, the prawns fed Diet 4 had the highest percentage weight gain, ADG and SGR values and the lowest FCR compared to juveniles fed the other four experimental feeds ($p < 0.05$). In addition, the prawns fed Diet 4 also had the lowest feed conversion ratio. Apart from this, no other marked differences in growth performance were observed between the prawns fed either Diets 2 and 3 or between those fed on Diets 1 and 5, although those fed Diets 2 and 3 showed significantly better growth than those juveniles fed Diets 1 and 5 (Table 3).

Fatty acid composition of the flesh

The flesh fatty acid compositions (Table 4) typically corresponded to the dietary fatty acid compositions (Table 2) with substantially increased proportions of total saturates and decreased proportions of total EFAs in the flesh. The major fatty acid classes found in the flesh across all experimental groups were total saturates and total monoenes, with the quantity of total saturates being higher than that of the total monoenes in all samples. Prawns fed Diet 5 had the highest total saturates in the flesh ($p < 0.05$). For EFAs, the percentages of both LA and LNA were reduced in the flesh across all experimental groups. Although the total n−3 LC-PUFA in the flesh was similar to the dietary levels in most experimental groups, the flesh EPA levels increased compared to the dietary levels across all experimental groups.

Regarding the flesh EFA composition, only the prawns fed the FO diet (Diet 1) presented similar values of total n−3 PUFA and n−6 PUFA in their flesh, resulting in the ratio of n−3 to n−6 being close to 1. Prawns fed non-FO diets showed lower ratios of n−3 to n−6 (0.43–0.82; Table 4) with the flesh ratios of n−3 to n−6 corresponding to the oil combinations. Individuals fed the FO diet had the highest levels of flesh total n−3 PUFA and n−3 LC-PUFA, and lowest n−6 PUFA. The flesh LNA content was approximately 2% of total lipid across all experimental groups, but the EPA and DHA contents of individuals fed the FO diet were significantly higher

Table 4
Major fatty acid classes (%) of *Macrobrachium rosenbergii* muscle (whole body) fed different experimental diets.

Experimental diets[b]					
Fatty acid[a]	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
18:2n6(LA)	13.02 ± 0.03e[c]	20.84 ± 0.06a	18.05 ± 0.01c	19.85 ± 0.06b	14.02 ± 0.03d
20:4n6(ARA)	2.68 ± 0.01b	1.79 ± 0.01d	2.42 ± 0.01c	2.39 ± 0.01c	2.81 ± 0.02a
18:3n3(LNA)	2.12 ± 0.01d	2.23 ± 0.01c	2.29 ± 0.01b	2.45 ± 0.01a	2.09 ± 0.01e
20:5n3(EPA)	7.38 ± 0.02a	4.59 ± 0.01e	5.42 ± 0.01d	6.09 ± 0.03b	5.72 ± 0.06c
22:6n3(DHA)	6.42 ± 0.08a	2.67 ± 0.01d	4.93 ± 0.06c	5.09 ± 0.01c	6.13 ± 0.20b
∑saturates	38.39 ± 0.05b	35.63 ± 0.02d	36.91 ± 0.08c	34.75 ± 0.07e	40.40 ± 0.32a
∑monoenes	28.91 ± 0.05b	31.04 ± 0.05a	28.76 ± 0.03c	28.16 ± 0.08d	27.95 ± 0.06e
∑n−6 PUFA	15.80 ± 0.04e	22.72 ± 0.05a	20.59 ± 0.01c	22.35 ± 0.06b	17.00 ± 0.01d
∑n−3 PUFA	16.16 ± 0.11a	9.71 ± 0.01d	12.93 ± 0.07c	13.92 ± 0.04b	13.93 ± 0.25b
∑n−3LC-PUFA (EPA + DHA)	13.80 ± 0.11a	7.26 ± 0.01e	10.35 ± 0.07d	11.18 ± 0.03c	11.85 ± 0.26b
∑n−3/∑n−6	1.03 ± 0.01a	0.43 ± 0.01d	0.63 ± 0.01c	0.62 ± 0.01c	0.82 ± 0.01b

[a] LA = linoleic acid, ARA = arachidonic acid, LNA = linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, PUFA = poly unsaturated fatty acid.
[b] Values are given as the mean ± standard deviation of a duplicate analysis of a pooled flesh samples per treatment.
[c] Values with different lowercase superscript letters are significantly different ($p < 0.05$).

than other groups. Compared to the other non-FO groups, prawns fed the diet with 3% SZ had higher flesh DHA but comparable EPA levels. Individuals that consumed Diet 2 had the highest level of flesh n–6 PUFA ($p < 0.05$).

Water quality during the experimental trial

Water quality was consistent through the experimental period and across experimental tanks. The temperature of the water during the experimental trial was in the range 28.4 ± 0.2–29.2 ± 0.3 °C, dissolved oxygen was in the range 7.04 ± 0.48–7.70 ± 0.42 mg/L, pH was in the range 7.86 ± 0.10–7.90 ± 0.08, alkalinity was in the range 105.88 ± 8.13–110.42 ± 7.90 mg/L, hardness was in the range 97.04 ± 12.89–110.08 ± 5.77 mg/L and the concentration of ammonia was in the range 0.05 ± 0.02–0.13 ± 0.04 mg/L.

Discussion

LC-PUFA-rich *Schizochytrium* sp. was shown to be a viable alternative to FO in the diets for juvenile *M. rosenbergii*. The results confirmed that replacing FO with varying amounts of SZ had no negative influence on the survival of the juvenile *M. rosenbergii*. All prawns fed non-FO diets had similar survival rates to those fed the FO diet ($p > 0.05$), although the prawns fed diets containing SZ at 1.5% or 3% showed slightly better survival rates than those fed the FO diet. The juvenile prawn survival rates in this study were satisfactory and comparable to other similar diet studies (Kangpanich and Senanan, 2015; Sisouvong et al., 2013; Kim et al., 2013). Increasing the oil percentage in the diet from 2% in Kangpanich and Senanan (2015) to 3% in the current study did not compromise juvenile growth and slightly improved the weight gain and SGR. In addition, most of the non-FO diets yielded better growth performance for the prawns than those fed the FO diet. The current results concurred with several studies reporting that partially or entirely replacing FO in aquafeeds with SZ did not negatively affect fish or shellfish production (Langdon and Onal, 1999; Miller et al., 2007; Li et al., 2009).

However, for improved growth performance, juvenile *M. rosenbergii* appeared to respond better to diets containing a combination of SZ and SO that corresponds to a dietary ratio of n–3 to n–6 of approximately 0.54–0.65. Juvenile *M. rosenbergii* fed the diets containing a combination of SZ and SO (Diets 3 and 4; dietary n–3:n–6 between 0.54 and 0.65) had better final weight and weight gains, as well as better feed conversion ratios than those fed the diet containing only FO or SZ (Diets 1 and 5, respectively; n–3:n–6 of approximately 0.96). The best growth performance of *M. rosenbergii* occurred for prawns fed Diet 4, which may have been due in part to their slightly lower survival rates that led to less competition among individual prawns within the tank. These oil combinations may have better satisfied the juvenile's needs for fatty acids compared to a single oil source. Kamarudin and Roustaian (2002) and Leela et al. (2005) observed similar growth benefits in larval *M. rosenbergii* fed diets containing combinations of cod liver oil and a vegetable oil source.

The lipid levels in the experimental diets provided comparable contents of monoenes and saturates across all experimental diets (28–33% of total fatty acids) and appeared adequate for the juveniles' growth. Increased proportions of saturated fatty acid in flesh compared to the dietary levels across all experimental groups indicated juvenile *M. rosenbergii*'s ability to synthesize and elongate this fatty acid class from shorter chain fatty acids (C2:0) (Tacon, 1987; Kim et al., 2013). They may also be able to synthesize monoenes from saturates through elongation and desaturation reactions.

Juvenile *M. rosenbergii* seemed to require both n–3 and n–6 fatty acid series for growth. The results also suggested the need for higher dietary n–6 than n–3 fatty acids for juvenile *M. rosenbergii* (dietary n–3:n–6 = 0.54 to 0.67; n–6 PUFA = 25.91 to 23.99% of total fatty acids) given an adequate dietary LC-PUFA level (8.8% and 10.42% of total fatty acids in Diets 3 and 4, respectively). D'Abramo and Sheen (1993) reported growth benefits from LC-PUFA (DHA) when added at 0.075% of diet ingredients while adding LA or LNA alone did not stimulate growth. In the current study, negative impacts were not detected from high levels of n–6 fatty acids (23.99–28.19% of total fatty acids). These results were consistent with current knowledge on the EFA requirements of this species, which are different from marine shrimp species (reviewed by D'Abramo, 1998; Mukhopadhyay et al., 2003; National Research Council, 2011). The ratios of n–3 to n–6 observed in Teshima et al. (1994) (dietary n–3:n–6 = 0.083) and in the current study were much lower than those required by marine *Penaeus* species (for example, *Penaeus sylisostris* n–3:LA = 1.18, Fenucci et al., 1981; *Penaeus monodon* n–3:n–6 = 2.5, Glencross et al., 2002). The amphidromous behavior of *M. rosenbergii* may explain the importance of both fatty acid classes, especially n–6, for juvenile growth. The juvenile stage requires a brackish water environment for the larval development and relies on n–3 rich, marine-based diets (Alam et al., 1995). As the juveniles migrate upstream to freshwater systems, they would rely on a freshwater food web, which typically is rich in n–6 fatty acids (Desvilettes et al., 1997).

In addition, the optimal dietary ratios discovered in the current study seemed well reflected in the flesh ratios of n–3 to n–6. The ratios were also similar to those detected in adult *M. rosenbergii* collected from natural and semi-natural environments (0.41–0.8, Chanmugam et al., 1983; Bhavan et al., 2010; Li et al., 2011). The current results and Kim et al. (2013) suggest that it is possible to entirely replace FO with vegetable oils with appropriate fatty acid contents and this replacement can enhance the growth of juvenile *M. rosenbergii*. Kim et al. (2013) tested various vegetable oils as alternatives to FO. Compared to juvenile *M. rosenbergii* fed a diet containing FO (5.5% of diet ingredients), they found significantly better growth for groups fed diets containing canola oil (dietary n–3:n–6 = 0.4; flesh n–3:n–6 = 0.8; dietary LC-PUFA = 5.9% of total fatty acids). The outcomes of the current study and Kim et al. (2013) may be attributed to adequate quantities of both classes of essential fatty acids, LC-PUFA as well as the appropriate ratios of n–3 to n–6 in the diets.

Seafood is recommended in human diets to increase the ratio of n3 to n6 and to promote human health by preventing cardiovascular disease, cancer and inflammatory autoimmune diseases and by supporting brain development and function (Ruxton et al., 2007). Replacing traditional LC-PUFA rich FO with vegetable oils in aquafeed may lower the concentrations of n–3 fatty acids (Gunstone, 2010), thereby affecting the final quality of farmed aquaculture species. In the current study, the percentage of n–3 PUFA in tissue of the *M. rosenbergii* fed FO and SZ diets was similar, although the n–3 LC-PUFA level of the latter group was slightly reduced and the n–6 PUFA content was increased. The n–3 PUFA contents were much reduced in the prawn groups that consumed Diet 2. This result suggested that it is possible to utilize SZ as a sole oil source in the juvenile *M. rosenbergii* diet without negatively affecting tissue n–3 PUFA accumulation. However, to achieve both good growth performance and desirable flesh fatty acid profiles, the diet of *M. rosenbergii* juveniles may need to contain a combination of SZ and a vegetable oil with high LC-PUFA and relatively low n–6 content. Additionally, feeding a diet rich in LC-PUFA to sub-adult shrimp before harvest may also improve flesh fatty acid profiles although the feeding duration needs further investigation.

Conflict of interest

There is no conflict of interest.

Acknowledgements

The authors would like to acknowledge the National Research Council of Thailand for funding. Special thanks are recorded to the technical staff of the Rajamagala University of Technology Tawan-Ok and Burapha University for their valuable practical assistance.

References

Alam, M.J., Ang, K.J., Begum, M., 1995. Use of egg custard augmented with cod liver oil and *Moina micrura* on production of freshwater prawn postlarvae. Aquac. Int. 3, 249–259.

Association of Official Analytical Chemists, 2005. Official Methods of Analysis of the Association of Official Analytical Chemists, eighteenth ed. Gaithersburg, MD, USA.

American Public Health Association, American water works association, water environment federation, 1980. In: Standard Methods for the Examination of Water and Wastewater, fifteenth ed. American Public Health Association, Washington, DC, USA.

Barclay, W.R., 1997. Method of aquaculture feeding microflora having a small cell aggregate size. United States Patent 5,688,500.

Bhavan, P.S., Ruby, S.A., Poongodi, R., Seenivasan, C., Radhakrishnan, C.S., 2010. Efficacy of cereals and pulses as feeds for the post-larvae of the freshwater prawn *Macrobrachium rosenbergii*. J. Ecobiotechnol 5, 9–19.

Cavalli, R.O., Lavens, P., Sorgeloos, P., 1999. Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid composition. Aquaculture 179, 387–402.

Chanmugam, P., Donovan, J., Wheeler, C.J., Hwang, D.H., 1983. Differences in the lipid composition of freshwater prawn (*Macrobrachium rosenbergii*) and marine shrimp. J. Food Sci. 48, 1440–1443.

D'Abramo, L.R., Sheen, S.S., 1993. Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquaculture 115, 63–86.

D'Abramo, L.R., 1998. Nutritional requirements of the freshwater prawn *Macrobrachium rosenbergii*: comparisons with species of penaeid shrimp. Rev. Fish. Sci. 6, 153–163.

Desvilettes, C.H., Bourdier, G., Amblard, C.H., Barth, B., 1997. Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. Freshw. Biol. 38, 629–637.

De Swaaf, M.E., Sijtsma, L., Pronk, J.T., 2003. High-cell-density fed-batch cultivation of the docosahexaenoic acid producing marine alga *Crypthecodinium cohnii*. Biotechnol. Bioeng. 81, 666–672.

Food and Agriculture Organization, 2012. The State of World Fisheries and Aquaculture, 2012. Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations, Rome, Italy.

Food and Agriculture Organization, 2013. Fisheries and Aquaculture Department, Statistics and Information Service FishStatJ: Universal Software for Fishery Statistical Time Series. Copyright 2011. Version 2.1.0. (March, 2013). Rome, Italy.

Fenucci, J.L., Lawrence, A.L., Zein-Eldin, Z.P., 1981. The effects of fatty acid and shrimp meal composition of prepared diets on growth of juvenile shrimp, *Penaeus stylirostris*. J. World Maricul. Soc. 12, 315–342.

Glencross, B.D., Smith, D.M., Thomas, M.R., Williams, K.C., 2002. Optimising the essential fatty acids in the diet for weight gain of the prawn, *Penaeus monodon*. Aquaculture 204, 85–99.

Goda, A.M.A.S., Habashy, M.M., Hanafy, M.A., Mohamed, R.A., Mabrouk, H.A., El-Afifi, T.M., Nada, S.A., Mohamed, K.A., 2011. Effect of altering linoleic acid and linolenic acid dietary ratios on the performance and tissue fatty acid profiles of prawn, Macrobrachium rosenbergii (de Man 1879) post larvae. J. Agri. Sci. Tech. B (1), 1032–1044.

Grasshoff, K., 1976. Methods of Seawater Analysis. Verlag Chemie, Weinheim, Germany.

Gunstone, F.D., 2010. The world's oils and fats. In: Turchini, G.M., Wing-Keong, N.G., Toucher, D.R. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. Taylor Francis Group, Boca Raton, FL, USA.

Hasan, M.R., Hecht, T., De Silva, S.S., Tacon, A.G.J. (Eds.), 2007. Study and Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development. FAO Fisheries Technical Paper. No. 497. Rome, Italy.

Kamarudin, M., Roustaian, P., 2002. Growth and fatty acid composition of freshwater prawn, *Macrobrachium rosenbergii* larvae fed diets containing various ratios of cod-liver oil-corn oil mixture. J. Appl. Ichthyol. 18, 148–153.

Kangpanich, C., Senanan, W., 2015. Effects of *Schizochytrium* sp. on growth performance and survival rate of giant freshwater prawn, *Macrobrachium rosenbergii* (de man). J. Agri. Tech. 11, 1337–1348.

Kim, Y.C., Romano, N., Lee, K.S., Teoh, C.Y., Ng, W.K., 2013. Effects of replacing dietary fish oil and squid liver oil with vegetable oils on the growth, tissue fatty acid profile and total carotenoids of the giant freshwater prawn, *Macrobrachium rosenbergii*. Aquac. Res. 44, 1731–1740.

Langdon, C., Onal, E., 1999. Replacement of living microalgae with spray-dried diets for the marine mussel, *Mytilus galloprouincialis*. Aquaculture 180, 283–294.

Leela, G., Ramanathan, N., Francis, T., 2005. Growth performance of *Macrobrachium rosenbergii* in nursery phase with varying levels of n3:n6 fatty acids. Indian J. Fish. 52, 433–439.

Li, M.H., Robinson, E.H., Tucker, C.S., Manning, B.B., Khoo, L., 2009. Effects of dried algae *Schizochytrium* sp. a rich source of docosahexaenoic acid, on growth, fatty acid composition and sensory quality of channel catfish, *Ictalurus punctatus*. Aquaculture 292, 232–236.

Li, G., Sinclair, A.J., Li, D., 2011. Comparison of lipid content and fatty acid composition in the edible meat of wild and cultured freshwater and marine fish and shrimps from China. J. Agr. Food Chem. 59, 1871–1881.

Miller, M.R., Nichols, P.D., Carter, C.G., 2007. Replacement of fish oil with thraustochytrid *Schizochytrium* sp. L oil in Atlantic salmon parr (*Salmo salar* L) diets. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 148, 382–392.

Mukhopadhyay, P.K., Rangacharyulu, P.V., Mitra, G., Jana, B.B., 2003. Applied nutrition in freshwater prawn, *Macrobrachium rosenbergii* culture. J. Appl. Aquacult 13, 317–340.

Nakahara, T., Yokochi, T., Higashihara, T., Tanaka, S., Yaguchi, T., Honda, D., 1996. Production of docosahexaenoic and decosapentaenoic acids by *Schizochytrium* sp. isolated from Yap islands. J. Am. Oil Chem. Soc. 73, 1421–1426.

National Research Council, 2011. Nutrient Requirements of Fish and Shrimp. National Academy Press, Washington, DC, USA.

Poungchor P., Ratanaarporn P., Limsuwan C., Chuchird N., 2009. Effects of Schizochytrium sp. on growth and survival rate of Pacific white shrimp (Litopenaeus vannamei, Boone) in low and normal salinity conditions, pp. 588–597. In: Proceedings of the 47th Kasetsart University Annual Conference. Bangkok, Thailand.

Reigh, R.C., Stickney, R.R., 1989. Effects of purified dietary fatty acids on the fatty acid composition of freshwater shrimp, *Macrobrachium rosenbergii*. Aquaculture 77, 157–174.

Ren, L.J., Ji, X.J., Huang, H., Qu, L., Feng, Y., Tong, Q.Q., Ouyang, P.K., 2010. Development of a stepwise aeration control strategy for efficient docosahexaenoic acid production by *Schizochytrium* sp. Appl. Microbiol. Biotechnol. 87, 1649–1656.

Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A., Millington, K.J., 2007. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J. Hum. Nutr. Diet. 20, 275–285.

Sisouvong, A., Limsuwan, C., Chuchird, N., Wongmaneeprateep, S., 2013. Effects of dietary *Schizochytrium* sp. supplementation on growth and survival rate of giant freshwater prawn (*Macrobrachium rosenbergii*). Khon Kaen Agric. J. 41, 123–128.

Somsueb, P., 2009. Diet Formulation for Macrobrachium rosenbergii. Inland Fisheries Research and Development Bureau. Inland Feed Research Institute. http://webcache.googleusercontent.com/search?q=cache:mSWGLbt2XRQJ:%3ca%20href=>http://webcache.googleusercontent.com/search?q=cache:mSWGLbt2XRQJ:www.fisheries.go.th/if-inland_feed/distribute/sample/macrob.htm+&cd=1&hl=th&ct=clnk&gl=th 4 November, 2016.

Tacon, A.G.J., 1987. The Nutrition and Feeding of Farmed Fish and Shrimp, a Training Manual. The Essential Nutrients. GCP/RLA/075 ITA Field Document. Food and Agriculture Organization of United Nations, Brasilia, Brazil.

Tacon, A.G.J., 1990. Standard Methods for the Nutrition and Feeding of Farmed Fish and Shrimp. Argent Laboratories Press, Washington, DC, USA.

Takeuchi, T., Watanabe, T., 1977. Effect of eicosapentaenoic acid and docosahexaenoic acid in pollock liver oil on growth and fatty acid composition of rainbow trout. Bull. Jpn. Soc. Sci. Fish. 43, 947–953.

Teshima, S., Koshio, S., Kanazawa, A., Oshida, K., 1994. Essential fatty acid of prawn Macrobrachium rosenbergii. In: Chou, L.M., Munro, A.D., Lam, T.J., Chen, T.W., Cheong, L.K.K., Ding, J.K., Hooi, K.K., Khoo, H.W., et al. (Eds.), Proceedings of the 3rd Asian Fisheries Society Forum. Asian Fisheries Society. Manila, the Philippines, pp. 705–708.

Turchini, G.M., Ng, W.K., Tocher, D.R., 2010. Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, Boca Raton, FL, USA.

Wang, Y., Li, M., Filer, K., Xue, Y., Ai, Q., Mai, K., 2016. Evaluation of *Schizochytrium* meal in microdiets of Pacific white shrimp (*Litopenaeus vannamei*) larvae. Aquac. Res. http://dx.doi.org/10.1111/are.13068.

Wu, S.T., Yu, S.T., Lin, L.P., 2005. Effect of culture conditions on docosahexaenoic acid production by *Schizochytrium* sp. S31. J. Process Biochem. 40, 3103–3108.

Xu, X., Ji, W., Castell, J.D., O'Dor, R., 1993. The nutritional value of dietary n–3 and n–6 fatty acids for the Chinese prawn (*Penaeus chinensis*). Aquaculture 118 (3–4), 277–285.

Zhou, Q.C., Li, C.C., Liu, C.W., Chi, S.Y., Yang, Q.H., 2007. Effects of dietary lipid sources on growth and fatty acid composition of juvenile shrimp, *Litopenaeus vannamei*. Aquac. Nutr. 13, 222–229.