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# Evaluation of *Schizochytrium* meal in microdiets of Pacific white shrimp (*Litopenaeus vannamei*) larvae

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## Abstract

A feeding trial was conducted to assess the effects of dietary *Schizochytrium* meal supplementation on survival, growth performance, activities of digestive enzymes and fatty acid composition in Pacific white shrimp (*Litopenaeus vannamei*) larvae (initial body weight 4.21 ± 0.10 mg). Four isonitrogenous and isolipidic diets were formulated to contain graded levels of *Schizochytrium* meal: 0% (S0, the control diet), 2% (S2), 4% (S4) and 6% dry matter (S6). Results showed that there was no significant difference in survival of shrimps among dietary treatments ($P > 0.05$). Shrimps fed diets with 2% and 4% microalgae meal had significantly higher specific growth rate (SGR) than that of shrimps fed diets with 0% and 6% microalgae meal, and no significant differences were observed between shrimps fed diets with 2% and 4% microalgae meal ($P > 0.05$). Activity of trypsin in the pancreatic and intestinal segments, and activity of amylase in the pancreatic segments were not significantly affected by dietary microalgae meal levels ($P > 0.05$). Specific activities of both alkaline phosphatase and leucine-aminopeptidase in intestine and purified brush border membrane of intestine were significantly higher in shrimps fed diet with 2% microalgae meal ($P < 0.05$). There were no significant differences in C18:2n-6, n-3 fatty acids, n-6fatty acids, PUFA and n-3/n-6 in muscle samples among dietary treatments. C16:1n-7, C18:1n-9, MUFA, C18:3n-3 and C20:5n-3 decreased, however, C20:4n-6 increased in the muscle as dietary microalgae meal level increased. In conclusion, 4% *Schizochytrium* meal in microdiets of shrimps can improve growth performance and may be a valuable additive in the microdiets of shrimps.

**Keywords:** digestive enzymes, fatty acid, growth, *Litopenaeus vannamei*, *Schizochytrium* meal

## Introduction

The products derived from wild-caught marine fisheries are ingredients commonly used in balanced feed formulations for many aquaculture animals because of their excellent quality and palatability (Tacon & Akiyama 1997). However, due to overexploitation of fishery resources, increasing demand and high price of fish meal and fish oil with the rapid expansion of global aquaculture industry, hence, considerable research is occurring worldwide in an effort to find alternative, sustainable feed ingredients to fish meal and fish oil in aquafeeds (Lewis, Nichols & McMeekin 1999; Li, Robinson, Tucker, Manning & Khoo 2009; Vizcaíno, López, Sáez, Jiménez, Barros, Hidalgo, Camacho-Rodríguez, Martínez, Cerón-García & Alarcón 2014).

Microalgae contain many valuable nutrients for aquafeeds, such as proteins, essential amino acids, carbohydrates, minerals, water-soluble vitamins, sterols and bioactive compounds (Ju, Deng & Dominy 2012; Atkinson 2013). Microalgae are also rich in antioxidant pigments such as carotenoids, chlorophylls and phycobiliproteins, and



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have commonly been added in diets to be tested as pigmentation sources for shrimps, salmon and trout (Chien & Shiau 2005; Güroy, Şahin, Mantoğlu & Kayal 2012). Moreover, most microalgae are rich in n-3 long-chain polyunsaturated fatty acids (LC-PUFAs), particularly eicosapentaenoic (EPA), docosahexaenoic (DHA) and arachidonic acid (AA), which improve and stabilize the nutrition, promotes growth and appetites, high survival rate and normal development of larval and juvenile fish, crustacean and mollusc (Ronquillo, Fraser & McConkey 2012; Shan & Lin 2014).

According to some studies, the microalgal-derived products could be a promising alternative for fish meal and fish oil or as a valuable additive in aquafeed formulations (Patnaik, Samocha, Davis, Bullis & Browdy 2006; Macias-Sancho, Poersch, Bauer, Romano, Wasielesky & Tesser 2014). The application of algal meal in aquafeeds can induce positive effects on growth, feed utilization, protein digestibility, physiological activity, carcass quality, starvation tolerance, disease resistance and immune responses, reproductive performance and induced early maturation (Güroy *et al.* 2012; Ju *et al.* 2012; Atkinson 2013; Patterson & Gatlin 2013; Macias-Sancho *et al.* 2014; Vizcaíno *et al.* 2014). However, the adverse effects on growth, fish metabolism and physiology caused by high inclusion level or long-term utilization of microalgae have been reported for goldfish *Carassius auratus* (Coutinho, Rema, Otero, Pereira & Fabregas 2006), Atlantic cod *Gadus morhua* (Walker & Berlinsky 2011), red drum *Sciaenops ocellatus* (Patterson & Gatlin 2013) and white shrimps (Macias-Sancho *et al.* 2014). The disparate responses to the dietary inclusion of microalgae may be related to aquatic species, mouth size and growth stage, microalgae species and nutritional value, inclusion level, type of algal product and method of processing (Reitan, Rainuzzo, Øie & Olsen 1993; Vizcaíno *et al.* 2014). Unfortunately, the major constraint to the wide use of microalgae meal to replace fish-based ingredients in aquaculture is their high production cost and culture inefficiency.

*Schizochytrium* is a unicellular, heterotrophic thraustochytrid, with a relatively high lipid levels (~10–50%), and high level of total lipids as DHA (30–70%) (Lewis *et al.* 1999; Arney, Liu, Forster, McKinley & Pearce 2015). Previous studies have suggested that *Schizochytrium* can be used as a feed to effectively enrich both n-3 and n-6 LC-PUFAs contents of rotifers and *Artemia nauplii* prior to feeding these to fish and shrimp larvae (Lewis *et al.* 1999; Li *et al.* 2009). Thraustochytrid-derived products, such as *Schizochytrium* meal, are recognized as prominent sustainable sources of n-3 LC-PUFAs for farmed fish species, such as Atlantic salmon *Salmo salar* (Miller, Nichols & Carter 2007), white shrimps (Patnaik *et al.* 2006) and sea bream (Ganuza, Benítez-Santana, Atalah, Vega-Orellana, Ganga & Izquierdo 2008). *Schizochytrium* meal has never been investigated as an alternative protein or lipid ingredient in shrimp microdiets. Therefore, the objective of the current study was to evaluate the effects of dried *Schizochytrium* meal (*ALLTECH* SP1, Alltech, Nicholasville, KY, USA) supplementation on survival, growth, activities of digestive enzymes and fatty acid composition in Pacific white shrimps (*Litopenaeus vannamei*) larvae.

## Materials and methods

### Experimental diets

The *Schizochytrium* meal was provided by Alltech. The *Schizochytrium* meal contained 12.06% crude protein and 40.80% crude lipid. Four isonitrogenous and isolipidic diets were formulated to contain graded levels (0%, 2%, 4% and 6% dry matter) of *Schizochytrium* meal. These four test diets were named: S0, S2, S4 and S6. Feed ingredients and proximate composition of the experimental diets are shown in Table 1. Fatty acid composition of dried *Schizochytrium* meal and diets are shown in Table 2.

Microdiets were manufactured by micro-bonding technology. Diets were prepared by thoroughly mixing dry ingredients with the oil, lecithin and water to produce stiff dough. Diets were pelleted by passing the dough through an experimental feed mill and dried for about 12 h in a ventilated oven at 60°C. After drying, the diets were broken up, sieved into proper pellet sizes (150~250 μm and 250~380 μm), and sealed in plastic bags and stored at −20°C until used.

### Shrimps and growth trial

Larvae used in this study were obtained and reared at the hatchery of Guangdong Hisenor Group for shrimp in Wenchang, Hainan, China. The 16-day-old shrimp larvae (initial body weight

**Table 1** Ingredients and nutrients of the experimental diets

| | Diet *No* (*Schizochytrium* level, % dry matter) | | | |
|---|---|---|---|---|
| **Ingredient** | **S0** | **S2** | **S4** | **S6** |
| Fish meal* | 35.00 | 35.00 | 35.00 | 35.00 |
| Shrimp meal* | 7.00 | 7.00 | 7.00 | 7.00 |
| Squid meal* | 5.00 | 5.00 | 5.00 | 5.00 |
| Mussel powder* | 6.00 | 5.50 | 5.00 | 4.50 |
| Soybean meal* | 7.00 | 7.00 | 7.00 | 7.00 |
| Corn gluten meal* | 8.00 | 8.00 | 8.00 | 8.00 |
| *Schizochytrium* meal | 0.00 | 2.00 | 4.00 | 6.00 |
| Fish oil* | 2.54 | 1.80 | 1.00 | 0.20 |
| Wheat flour* | 23.07 | 22.31 | 21.61 | 20.91 |
| Sodium alginate | 2.00 | 2.00 | 2.00 | 2.00 |
| Sodium benzoate | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin premix† | 0.20 | 0.20 | 0.20 | 0.20 |
| Mineral premix† | 0.50 | 0.50 | 0.50 | 0.50 |
| $Ca(H_2PO_4)_2$ | 0.50 | 0.50 | 0.50 | 0.50 |
| Choline chloride | 0.30 | 0.30 | 0.30 | 0.30 |
| Astaxanthin | 0.10 | 0.10 | 0.10 | 0.10 |
| Glycine betaine | 1.50 | 1.50 | 1.50 | 1.50 |
| Ethoxyquin | 0.05 | 0.05 | 0.05 | 0.05 |
| Lecithin | 1.00 | 1.00 | 1.00 | 1.00 |
| Exuviate hormone | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin C | 0.04 | 0.04 | 0.04 | 0.04 |
| Proximate composition (% dry matter) | | | | |
| Moisture | 3.08 | 3.21 | 3.52 | 3.50 |
| Crude protein | 49.84 | 50.16 | 50.25 | 50.09 |
| Crude lipid | 9.90 | 9.98 | 10.28 | 10.39 |

*Those ingredients were supplied by Qingdao Great Seven Bio-Tech, (Qingdao, China).

†Vitamin premix and Mineral premix were supplied by Qing Dao Master Bio-Tech, (Qingdao, China).

**Table 2** Fatty acid composition of dried *Schizochytrium* meal and experimental diets

| Fatty acids (% of total fatty acids) | | Diet *No* | | | |
|---|---|---|---|---|---|
| | ***Schizochytrium*** | **S0** | **S2** | **S4** | **S6** |
| C14:0 | 6.43 | 6.03 | 6.24 | 5.55 | 5.48 |
| C16:0 | 35.43 | 28.17 | 33.23 | 34.31 | 37.59 |
| C18:0 | 1.43 | 5.10 | 4.83 | 4.41 | 4.08 |
| C20:0 | 0.19 | 1.41 | 1.43 | 1.55 | 1.78 |
| Total SFA | 43.48 | 40.71 | 45.73 | 45.82 | 48.93 |
| C16:1n-7 | 0.18 | 4.96 | 4.46 | 3.78 | 3.28 |
| C18:1n-9 | – | 13.04 | 13.14 | 10.46 | 9.55 |
| Total MUFA | 0.18 | 18.00 | 17.60 | 14.24 | 12.83 |
| C18:3n-3 | 0.66 | 1.80 | 1.89 | 1.45 | 1.44 |
| C20:5n-3 | 0.38 | 6.95 | 5.64 | 5.43 | 5.18 |
| C22:6n-3 | 40.64 | 12.48 | 12.70 | 15.06 | 16.21 |
| Total n-3 | 41.68 | 21.23 | 20.23 | 21.94 | 22.83 |
| C18:2n-6 | 0.53 | 13.45 | 12.93 | 11.54 | 11.66 |
| C20:4n-6 | 1.17 | 0.58 | 0.62 | 0.67 | 0.75 |
| Total n-6 | 1.7 | 14.03 | 13.55 | 12.21 | 12.41 |
| Total PUFA | 43.38 | 35.26 | 33.78 | 34.15 | 35.24 |
| n-3/n-6 | 24.52 | 1.51 | 1.49 | 1.80 | 1.84 |
| DHA/EPA | 106.95 | 1.80 | 2.25 | 2.77 | 3.13 |

4.21 ± 0.10 mg) were stocked randomly and distributed into 12 white plastic tanks (100 L capacity, water volume 80 L), and each tank was stocked with 2000 individuals. Each diet was randomly assigned to triplicate tanks. All shrimps were hand-fed to apparent satiation five times daily (07:00, 11:00, 15:00, 19:00 and 23:00). All tanks were supplied with seawater that had been filtered through two-grade sand filter, and freshwater was added to establish a water salinity level of 16‰. During the experimental period, the flow rate of water was maintained at 2.5 L $min^{-1}$, each tank was provided with continuous aeration to maintain the dissolved oxygen level above 6 mg $L^{-1}$, temperature was 26~27°C, pH was 7.8~8.2, ammonia-N was less than 0.2 mg $L^{-1}$, the photoperiod was set at 12-h light and 12-h dark. The water quality parameters were measured by using a multiparameter water survey instrument (YSI 556; YSI Inc., Yellow Springs, OH, USA). Mortality and feeding behaviour was monitored every day. The experiment lasted for 24 days.

### Sample collection and analysis

At the end of the feeding trial, all shrimps were fasted for 24 h before harvest. Total number of shrimps in each tank was measured to determine the survival (S). Twenty shrimps were randomly collected from each tank to monitor final body weight (FBW) and final body length (FBL). Fifty shrimps per tank were randomly collected in centrifuge tubes, and then immediately stored at −80°C for enzyme activity assays.

The shrimps were dissected to separate pancreatic and intestinal segments. Dissection was conducted on a glass plate maintained at 0°C. For enzymatic assays, the dissected samples were homogenized in 2 mL cold ultrapure water (0°C). The homogenates were centrifuged at 4°C at 3300 *g* for 3 min, and then the supernatant was gently collected and frozen at −70°C until analysis of enzyme activity. Purified brush border membranes (BBM) from homogenate of intestinal segment were obtained according to a method developed for intestinal scraping (Crane, Boge & Rigal 1979). Before $CaCl_2$ solution addition, 1 mL of homogenate was diverted for intestinal enzyme

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assays. After addition of 0.1 M $CaCl_2$, the homogenate was centrifuged at 3300 *g* for 3 min in a centrifuge at 4°C. The supernatants were kept in new vials and stored frozen (−80°C) until assayed for the digestive enzymes or protein content.

Trypsin activity was assayed according to Holm, Hanssen, Krogdahl and Florholmen (1988). Alkaline phosphatase (AP) and Leucine-aminopeptidase (LA) were assayed both in intestinal segment and BBM according to Bessey, Lowry and Brock (1946) and Maroux, Louvard and Baratti (1973) respectively. Protein concentration was determined by the Bradford procedure (Bradford 1976) using bovine serum albumin (BSA, A-2153; Sigma, Saint Louis, MO, USA) as a standard. All the enzyme activity assays were carried out in triplicate.

The fatty acid profiles were analysed using the detail procedures described by Zuo, Ai and Mai (2012). About 100 mg of freeze-dried muscle samples were added into a 10 mL volumetric screwed glass tube with plastic cover. Then 3 mL of potassium hydroxide methanol (1 N) was added and heated on 75°C water bath for 25 min. After that, 3 mL HCL-methanol (2 N) was added and the mixture was heated on 75°C water bath for another 25 min. Previous test has been conducted to make sure that all fatty acids can be esterified following the procedures above. At last, 1 mL hexane was added into the mixture above, shaken vigorously for 1 min, and then allowed to separate into two layers. Fatty acid methyl esters in the upper layer were separated, and quantified by HP6890 gas chromatograph (Agilents Technologies, Santa Clara, CA, USA) with a fused silica capillary column (007-CW; Hewlett Packard, Palo Alto, CA, USA) and a flame ionization detector. The column temperature was programmed to rise from 150°C up to 200°C at a rate of 15°C $min^{-1}$, and from 200°C to 250°C at a rate of 2°C $min^{-1}$. Injector and detector temperature were 250°C respectively.

### Calculations and statistical analysis

The following parameters were calculated:

$$\text{Survival}\,(S,\%) = \text{final shrimp number}/\text{initial shrimp number}$$

$$\text{Specific growth rate}\,(\text{SGR},\%\,\text{day}^{-1}) = (\text{Ln}\,W_f - \text{Ln}\,W_i) \times 100/t$$

Where $W_f$ is final body weight, $W_i$ is initial body weight, $t$ is experimental duration in day.

All data from each treatment were subjected to one-way analysis of variance (ANOVA) to determine if there was a significant difference related to experimental diets. If a significant difference was found ($P < 0.05$), Duncan's multiple range test was used to compare the mean values between individual treatment. All statistical analyses were carried out by using SAS 9.12 (Statistical Analysis System Institute, Cary, NC, USA) for Windows. The data are presented as means ± SD ($n = 3$).

## Results

### Growth performance

In this study, survival of shrimps fed diets was 40.3~44.5% and showed no significant difference ($P > 0.05$). Shrimps fed the diet with 2% *Schizochytrium* meal showed a significantly higher specific growth rate (SGR) value than that of the shrimps fed the diets with 0% and 6% microalgae meal ($P < 0.05$), and shrimps fed the diet with 2% microalgae meal had similar SGR value to shrimps fed diet with 4% microalgae meal ($P > 0.05$). Growth of shrimps fed diet with 6% microalgae meal was significantly lower than that of shrimps fed diets with 2% and 4% microalgae meal ($P < 0.05$). No significant differences were observed for the final body length value among all dietary treatments ($P > 0.05$) (Table 3).

### Specific activities of digestive enzyme

In this study, no significant differences in activities of amylase in the pancreatic segments were observed among all dietary treatments ($P > 0.05$). Activities of amylase in the intestinal segments of shrimps fed the control diet was significantly higher than those of shrimps fed diet with 2% microalgae meal, and no significant difference was observed among shrimps feed diets with 2%, 4% and 6% microalgae meal. No significant differences in activities of trypsin in the pancreatic and intestinal segments were observed among all dietary treatments ($P > 0.05$). Specific activities of alkaline phosphatase (AP) in purified brush border membrane (BBM) of intestine was significantly higher in the diet with 2% microalgae meal than in the diets with 0% and 6% microalgae meal ($P < 0.05$), and no significant differences were

**Table 3** Effect of different level of *Schizochytrium* meal on growth and survival of *Litopenaeus vannamei* larvae

| Diet *No* | IBW (mg) | FBW (mg) | SGR (% day$^{-1}$) | FBL (cm) | SR (%) |
|---|---|---|---|---|---|
| S0 | 4.21 ± 0.10 | 125 ± 8$^{b}$ | 14.1 ± 0.3$^{b}$ | 2.22 ± 0.24 | 42.7 ± 3.2 |
| S2 | 4.21 ± 0.10 | 149 ± 4$^{a}$ | 14.9 ± 0.1$^{a}$ | 2.54 ± 0.36 | 43.0 ± 1.1 |
| S4 | 4.21 ± 0.10 | 139 ± 7$^{ab}$ | 14.6 ± 0.2$^{ab}$ | 2.42 ± 0.48 | 42.9 ± 3.6 |
| S6 | 4.21 ± 0.10 | 130 ± 8$^{b}$ | 14.3 ± 0.3$^{b}$ | 2.30 ± 0.18 | 40.3 ± 2.8 |

IBW, initial body weight; FBW, final body weight; FBL, final body length; SR, survival rate.
Data represent as means ± SD, $n = 3$; values in the same column with different superscripts are significantly different ($P < 0.05$).

observed between diets with 2% and 4% microalgae meal ($P > 0.05$). Specific activities of AP in intestine was significantly higher in the diet with 2% microalgae meal than in the control diet ($P < 0.05$), and no significant differences were observed among diets with 0%, 4% and 6% microalgae meal ($P > 0.05$). Specific activity of leucine-aminopeptidase (LA) in BBM of intestine was not different between shrimps fed the diets containing 2% and 4% microalgae meal ($P > 0.05$), and shrimps fed the 2% microalgae meal diet had significantly higher specific activities of LA than shrimps fed the diets containing 0% and 6% microalgae meal ($P < 0.05$). Specific activity of LA in intestine was elevated in shrimps fed all microalgae meal-supplemented diets than that in control group ($P > 0.05$), and shrimps fed the 2% microalgae meal diet had significantly higher specific activities of LA than shrimps fed the control diet S0 ($P < 0.05$) (Table 4).

### Fatty acid composition

The percentages of all the identified fatty acids in the muscle of shrimps fed graded levels of microalgae meal are shown in Table 5. There were no significant differences in C16:0, C18:0, total saturated fatty acids, C18:2n-6, C22:6n-3, total n-3 fatty acids, total n-6 fatty acids, total PUFA and n-3/n-6 in muscle samples among dietary groups ($P > 0.05$). However, C16:1n-7, C18:1n-9, total MUFA, C18:3n-3 and C20:5n-3 in the muscle decreased as dietary microalgae meal level increased, 20:4n-6 in the muscle increased as dietary microalgae meal level increased ($P < 0.05$).

## Discussion

This study was performed to test the feasibility of *Schizochytrium* meal supplementation in microdiets for *L. vannamei* larvae. The low survival rates (40.3~44.5%) of shrimp larvae in this study could be attributed to two aspects. During early life stages, shrimp larvae feed mainly on artemia nauplius and copepod, which have more active substances, while these substances were not found in formula feeds. In addition, it may be a little bit hard for the shrimp larvae to adapt the test diets. The inclusion of microalgae meal in diets did not affect the survival rate. Ganuza *et al.* (2008) and Li *et al.* (2009) also found no significant differences in survival of fish fed diets containing various levels of dried microalgae meal.

Shrimps fed diets with 2% and 4% microalgae meal had good growth than that of shrimps fed the diets with 0% and 6% microalgae meal. These results indicate that 4% microalgae meal can enhance growth performance and used as a promising additive in the microdiets of shrimps. Li *et al.* (2009) also indicated that the supplementation of 1.0–1.5% dried *Schizochytrium* meal in all-plant diets resulted in increased weight gain of channel catfish *Ictalurus punctatus*. Similarly, other studies indicated that microalgae meal can be used as a feed additive to stimulate shrimps and fish growth and improve feed utilization (Ju, Forster & Dominy 2009; Güroy *et al.* 2012; Ju *et al.* 2012). The improved growth effects may be due to some unknown growth factors and biologically active substances, such as growth hormones or insulin-like growth factors, and compounds inducing gene expression, contained in the microalgae or from some of the health benefits of microalgae (Ju *et al.* 2009, 2012). The improved growth might also be associated with physiological conditions such as increasing protein assimilation, lipid metabolism and liver function (Nakagawa 2011).

In this study, shrimps fed diet with 6% microalgae meal had poor growth than that of shrimps fed diets with 2% and 4% microalgae meal. Similarly, Macias-Sancho *et al.* (2014) observed that 100% replacement of fish meal with *Spirulina*

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**Table 4** Effects of different level of *Schizochytrium* meal on activities of digestive enzymes of *Litopenaeus vannamei* larvae

| Digestive enzymes | | S0 | S2 | S4 | S6 |
|---|---|---|---|---|---|
| Trypsin (mU $mg^{-1}$·protein) | PS* | 40.26 ± 2.12 | 44.85 ± 1.89 | 45.59 ± 1.62 | 43.70 ± 1.93 |
| | IS* | 31.20 ± 0.54 | 37.62 ± 3.40 | 35.93 ± 0.33 | 33.45 ± 2.12 |
| Amylase (mU $mg^{-1}$·protein) | PS* | 2.02 ± 0.16 | 1.95 ± 0.09 | 1.98 ± 0.13 | 1.97 ± 0.11 |
| | IS* | 2.31 ± 0.06$^{a}$ | 2.11 ± 0.07$^{b}$ | 2.21 ± 0.05$^{ab}$ | 2.20 ± 0.15$^{ab}$ |
| Trypsin (I)/trypsin (P)† | | 0.78 ± 0.06 | 0.84 ± 0.05 | 0.79 ± 0.03 | 0.77 ± 0.05 |
| Specific activities of digestive enzymes in purified brush border membrane of intestine | | | | | |
| Leucine-aminopeptidase (mU $mg^{-1}$·protein) | | 60.62 ± 10.91$^{c}$ | 86.65 ± 6.32$^{a}$ | 82.89 ± 9.31$^{ab}$ | 68.44 ± 3.76$^{bc}$ |
| Alkaline phosphatase (U $mg^{-1}$·protein) | | 75.21 ± 12.41$^{c}$ | 103.57 ± 6.32$^{a}$ | 99.81 ± 9.31$^{ab}$ | 85.37 ± 3.76$^{bc}$ |
| Specific activities of digestive enzymes in intestine | | | | | |
| Leucine-aminopeptidase (mU $mg^{-1}$·protein) | | 23.77 ± 1.93$^{b}$ | 29.49 ± 2.54$^{a}$ | 28.99 ± 3.33$^{a}$ | 25.00 ± 1.20$^{ab}$ |
| Alkaline phosphatase (U $mg^{-1}$·protein) | | 9.57 ± 1.06$^{b}$ | 13.96 ± 2.54$^{a}$ | 13.12 ± 1.80$^{ab}$ | 12.80 ± 2.87$^{ab}$ |

Data represent as means ± SD, $n = 3$; values in the same row with different superscripts are significantly different ($P < 0.05$).
*PS, pancreatic segments; IS, intestinal segments.
†Trypsin (I), trypsin of intestinal segment; trypsin (P), trypsin of pancreatic segment.

**Table 5** Fillet fatty acid composition of *Litopenaeus vannamei* larvae fed microdiets containing various levels of *Schizochytrium* meal

| Fatty acids (% of total fatty acids) | Diet *No* | | | |
|---|---|---|---|---|
| | S0 | S2 | S4 | S6 |
| C16:0 | 24.66 ± 0.88 | 24.88 ± 1.98 | 24.59 ± 2.14 | 26.27 ± 0.43 |
| C18:0 | 11.84 ± 0.28 | 11.58 ± 1.01 | 11.61 ± 0.55 | 11.18 ± 0.16 |
| Total SFA | 36.50 ± 1.16 | 36.45 ± 2.99 | 36.21 ± 2.69 | 37.45 ± 0.27 |
| C16:1n-7 | 5.14 ± 0.01$^{a}$ | 4.74 ± 0.12$^{b}$ | 4.41 ± 0.07$^{c}$ | 4.22 ± 0.08$^{c}$ |
| C18:1n-9 | 15.07 ± 0.52$^{ab}$ | 15.42 ± 0.45$^{a}$ | 13.09 ± 0.87$^{b}$ | 12.91 ± 0.20$^{b}$ |
| Total MUFA | 20.22 ± 0.51$^{a}$ | 20.16 ± 0.33$^{a}$ | 17.50 ± 0.94$^{b}$ | 17.13 ± 0.28$^{b}$ |
| C18:3n-3 | 2.01 ± 0.03$^{a}$ | 2.18 ± 0.08$^{a}$ | 1.66 ± 0.11$^{b}$ | 1.59 ± 0.05$^{b}$ |
| C20:5n-3 | 17.01 ± 0.53$^{a}$ | 15.47 ± 1.24$^{ab}$ | 14.32 ± 0.33$^{ab}$ | 13.28 ± 0.16$^{b}$ |
| C22:6n-3 | 17.64 ± 0.72 | 17.38 ± 1.24 | 20.13 ± 1.10 | 19.83 ± 0.08 |
| Total n-3 | 36.67 ± 0.16 | 35.03 ± 2.56 | 36.11 ± 1.54 | 34.71 ± 0.12 |
| C18:2n-6 | 11.34 ± 0.56 | 11.59 ± 0.73 | 10.48 ± 0.79 | 10.93 ± 0.05 |
| C20:4n-6 | 3.05 ± 0.08$^{b}$ | 3.12 ± 0.04$^{b}$ | 3.32 ± 0.02$^{ab}$ | 3.53 ± 0.10$^{a}$ |
| Total n-6 | 14.39 ± 0.48 | 14.71 ± 0.69 | 13.80 ± 0.77 | 14.46 ± 0.15 |
| Total PUFA | 51.06 ± 0.64 | 49.74 ± 1.87 | 49.91 ± 2.30 | 49.16 ± 0.27 |
| n-3/n-6 | 2.55 ± 0.07 | 2.39 ± 0.29 | 2.62 ± 0.03 | 2.40 ± 0.02 |

Data represent as means ± SD, $n = 3$; values in the same row with different superscripts are significantly different ($P < 0.05$).

*platensis* reduced the growth of shrimps, and Walker and Berlinsky (2011) reported that replacement of 15% or 30% fish meal protein with a mixture of dried microalgae meal resulted in significant and proportional reductions in feed intake and growth of Atlantic cod. Depressed palatability was likely a major cause of poor growth in fish or shrimps fed diets containing higher level of microalgae meal (Coutinho *et al.* 2006; Walker & Berlinsky 2011; Ju *et al.* 2012). However, for the current study, it is difficult to test whether the inclusion of dried microalgae meal affected the palatability of shrimp larvae because of the size of the larvae. Additionally, the lack of amino acids and a lower digestibility may impair growth and development of fish or shrimp larvae (Coutinho *et al.* 2006; Jaime-Ceballos, Hernández-Llamas, Garcia-Galano & Villarreal 2006).

As with most crustacean species, the requirement of white shrimps for HUFA is determined not

only by its minimum content in the diet but also that a balanced dietary ratio of n-3/n-6 as well as EPA/DHA, which are particularly critical during the early life stages (Patnaik *et al.* 2006; Benítez-Santana, Masuda, Juárez Carrillo, Ganuza, Valencia, Hernández-Cruz & Izquierdo 2007). It has been reported that fatty acids deficiency or excessive in diet resulted in low larval growth, survival and stress resistance, impaired predator behaviour, skeletal deformities and immune-suppression of aquaculture species (Glencross & Smith 2001; Ganuza *et al.* 2008; Carboni, Vignier, Chiantore, Tocher & Migaud 2012). In the present study, fatty acids analysis revealed that dietary EPA content decreased from 6.95% to 5.18%, while dietary DHA content increased from 12.48% to 16.21%, DHA/EPA increased from 1.80 to 3.13, with increasing microalgae meal inclusion level from 0% to 6%. Changes in the DHA/EPA ratio destroy the balance and structure of the cell membrane of fish larvae, consequently affecting the growth, quality and pigmentation of the larvae (Reitan *et al.* 1993; Rainuzzo, Reitan & Olsen 1994). Therefore, the imbalance DHA/EPA ratio may be one of the reasons responsible for poor growth of fish and shrimps fed diets that contain high level of microalgae meal.

Changes in enzymatic activities had been used as indicators for studying the effects of the dietary additives that might modulate the maturation process of the digestive tract (Gisbert, Giménez, Fernández, Kotzamanis & Estévez 2009). The ratio of trypsin (I)/trypsin (P) reflects the secretion level of pancreatic enzymes (Zambonino-Infante, Cahu, Peres, Quazuguel & Le Gall 1996). In the present study, no significant difference was found in this ratio among all dietary treatments. This indicated that the addition of microalgae meal to the diets of shrimp larvae may not significantly affect enzyme secretion. No significant differences in activity of trypsin in the pancreatic and intestinal segments were observed among all diets groups in this study. Vizcaíno *et al.* (2014) found that trypsin activities tended to increase quadratically in fish fed on microalgae meals supplemented diets, and fish fed 12% microalgae meals showed higher trypsin activity than fish fed on microalgae-free diet. Microalgae growth regulators, such as polyamides, have been shown to stimulate cholecystokinin release in rats, which mediates the release of pancreatic enzymes (Fioramonti, Fargeas, Bertrand, Pradayrol & Bueno 1994).

Brush border membrane (BBM) enzymes assays have been successfully used to determine the degree of the maturation process of the digestive function in intestine in fish larvae (Cahu & Zambonino-Infante 1995; Ma, Cahu, Zambonino, Yu, Duan, Le Gall & Mai 2005). Leucine-aminopeptidase (LA) and alkaline phosphatase (AP) are regarded as indicators for a well-differentiated intestinal BBM and have previously been found to exhibit high activities in fish larvae fed appropriate diets (Cahu, Zambonino-Infante, Quazuguel & Le Gall 1999; Zambonino-Infante & Cahu 2001; Ma *et al.* 2005). In this study, shrimps fed diets with 2% and 4% microalgae meal presented comparable specific activities of AP and LA in BBM of intestine, but higher than those fed diets with 0% and 6% microalgae meal. Specific activity of AP and LA in the intestine showed the same trend. Results obtained confirmed that microalgae meal had not caused negative effects on both enzymatic activities at lower inclusion levels tested. This is in agreement with Vizcaíno *et al.* (2014) who found that AP and LA activities tended to increase quadratically in fish fed on *Scenedesmus almeriensis*-supplemented diets. An increase in specific activity of aminopeptidase has been previously related to maturation of the intestinal membrane and enhanced survival in fish (Cahu & Zambonino-Infante 1995).

In this study, the level of DHA in shrimp muscle increased from 17.38% to 20.13% of total fatty acids with increasing levels of dried microalgae meal in the diet, denoting the high nutritional value of the microalgae meal as an alternative source of this fatty acid. This result is similar to observations reported on channel catfish (Li *et al.* 2009) and olive flounder *Paralichthys olivaceus* (Qiao, Wang, Song, Ma, Li, Liu, Zhang, Wang & Zhang 2014) fed the dried microalgae meal. The EPA levels in muscle decreased as dietary dried microalgae levels increased.

Both n-3 and n-6 fatty acids are essential fatty acids for human health. However, high levels of n-6 fatty acids are not appropriate from a human nutrition standpoint, which promotes the pathogenesis of many diseases, especially cardiovascular health, therefore, it is critical to ensure that fish and shrimp retain high levels of n-3 HUFA and high n-3/n-6 ratios for human health. In this study, there were no significant differences in muscle total n-3 fatty acids, total n-6 fatty acids levels and n-3/n-6 ratio among shrimps fed diets with

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various levels of microalgae meal. Similar results were observed in Atlantic cod (Walker & Berlinsky 2011) and olive flounder (Qiao *et al.* 2014). In contrast, researches had shown that feeding the dried microalgae increases n-3 LC-PUFAs in fillet of Atlantic salmon (Miller *et al.* 2007) and channel catfish (Li *et al.* 2009), without adverse effects on flavour quality of fish product, which would improve health status of humans.

## Conclusion

In summary, results of this study indicated that 4% *Schizochytrium* meal in microdiets improved growth performance of shrimp larvae. The use of this microalgae meal in shrimp microdiets did not significantly affect the n-3 and n-6 fatty acids levels in the muscle, and therefore *Schizochytrium* meal could be used as a valuable additive in the microdiets of shrimps.

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