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ORIGINAL RESEARCH

Evaluation of growth, survival and body composition of larval white shrimp (*Litopenaeus vannamei)* fed the combination of three types of algae

Hadi Jamali · Nasrollah Ahmadifard · Daruosh Abdollahi

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Abstract The larvae of the Pacific white shrimp *Litopenaeus vannamei* are fed, from the stage of protozoea I until to protozoea III (PZ III), with microalgae. The survival rate, duration of the terms of Z_I–Z_{III} and total length of protozoea larvae (PZ) of L. *vannamei* were survived. The shrimp larvae fed with the six treatments of microalgae (*Chaetoceros muelleri*, *Isochrysis galbana*, *Tetraselmis tetrathele*, *C. muelleri* : *I. galbana*, *C. muelleri: T. tetrathele* and *I. galbana: T. tetratheie*). The biochemical composition (protein, carbohydrate, lipid and energy) of the algae and the larvae fed those algae were also measured. The largest sizes were recorded for larvae fed with the mixture *C. muelleri and I. galbana* (4.35 mm) and smaller sizes were observed on larvae fed with *I. galbana* (3.04 mm) ($P < 0.05$). The larvae fed with mixture *T. tetrathele and C. muelleri* (88.42 %) and *I. galbana and T. tetrathele* (84.50 %) had the highest survival in experimental treatments. The lowest time for development was observed in larvae fed with mixture of *T. tetrathele and C. muelleri* (92.6 h). The larvae fed with mixture *I. galbana and T. tetrathele* had the highest protein and carbohydrate levels as compared with other treatments. Also highest lipid level and gross energy were shown in larvae fed with mixture *C. muelleri and I. galbana* ($P < 0.05$). The results showed that *L. vannamei* larvae fed with *C. muelleri* and the mixed diets containing this species (*C. muelleri* + *Isochrysis* sp., *C. muelleri* + *Tetraselmis* sp.) had the highest growth and survival among treatments.

Keywords Shrimp · Algae · Nutrition · Biochemical composition

Introduction

In production of shrimp post larvae, feeding is one of the most important aspects (Lopez-Elias et al. 2008). Cultivation of larval stages of various aquaculture species is still highly dependent on live food which is for herbivorous larvae, like molluscs and crustaceans (Boeing 2005). The variability of the nutritional value of the main live food organisms currently used in aquaculture (i.e., microalgae, rotifers and brine shrimp) is well documented, especially with respect to fatty acids (Merchie et al. 1997). Microalgae are utilized as live feed for all growth stages of bivalve molluscs (e.g., oysters, scallops, clams and mussels), early juvenile stages of abalone, crustaceans and some fish species, also for zooplankton culture (Catarina Guedes and Xavier Malcata

H. Jamali (✉) · N. Ahmadifard
Department of Fisheries, Faculty of Natural Resources, Urmia University, P.O. Box: 46414-356, Urmia, Iran
e-mail: saeed.jamali11@gmail.com

D. Abdollahi
Department of Fisheries, Gonbad Kavous University, Gonbad Kavous, Iran

2012). The most algae used in aquaculture are: *Chaetoceros*, *Thalassiosira*, *Tetraselmis*, *Isochrysis*, *Nannochloropsis*, *Pavlova* and *Skeletonema* (Brown et al. 1997; Enright et al. 1986; Thompson et al. 1993). These organisms are fed directly or indirectly through artemia, rotifers and Daphnia for feeding grown organism. It is widely accepted that microalgae play an important role in larval nutrition of shrimp; however, it is uncertain whether juveniles and adults feed on microalgae. Some reports suggest that microalgae are found in their gut because shrimp accidentally ingests them together with debris (Marinez-Cordova and Pena-Messina 2005; Kent et al. 2011). The microalgae that are usually used as feed of peneids larvae are among different genus, including *Chaetoceros* spp. and *Tetraselmis* spp. (Apt and Behrens 1999; Voltolina and Lopez-Elias 2002). This genus of algae is considered as a beneficial species, because they contain all the requirements of peneids larvae (Rodriguez et al. 1994; D'Souza and Kelly 2000). Some species of algae such as *Isochrysis* sp. (Okauchi et al. 1997), *Tetraselmis tetrathele* (Okauchi and Hirano 1986), and *T. chuii* (Tobias-Quinitio and Villegas 1982) have high nutritional value and potential growth. *Chaetoceros muelleri* has a high growth rate (Trujillo-Valle and Voltolina 1994) and it can grow in outdoor conditions with a wide range of temperature and illumination (Nelson et al. 1992). *Isochrysis galbana* is a free living marine unicellular phytoflagellate of the order Chrysomonadales. This species is rich in polyunsaturated fatty acids (Wood 1974) and has a high nutritional value for marine fish larvae (Scott and Middleton 1979) and juvenile stages of molluscs (Rhodes and Landers 1973).

Microalgae have an important role in aquaculture and also in the enrichment of zooplankton for feeding fish larvae. In addition to providing proteins (that contain essential amino acids) and energy, they carry nutrients such as vitamins, essential PUFAs, pigments and sterols—which are transferred to a high level through the food chain. For instance, PUFA-rich microalgae, such as *Pavlova* sp. and *Isochrysis* sp. to enrich the DHA, have been successfully fed to zooplankton (Nichols et al. 1989). Brown et al. (1998) described that rotifers fed with microalgae (e.g., *Isochrysis* sp. and *N. oculata*) become rapidly enriched with ascorbic acid (AsA), whereas rotifers fed on baker's yeast (which itself is deficient in AsA) contained only residual amounts of AsA.; after 16 h of starving, rotifers lost ca. 10 % of their AsA, while retaining ca. 50 % of the total AsA ingested. Similarly, the concentration of AsA in *Artemia* sp. may be increased by feeding with microalgae (Merchie et al. 1995). However, little information is available on the transfer of other vitamins from microalgae to fish larvae.

The feeding protocols of larvae in commercial shrimp hatcheries include a wide range of balanced feed and nutritional supplements, particularly in the early stages. However, live feed continues to be the principal nutritional basis for culture of larvae (Aguirre-Hinojosa et al. 1999; Voltolina and Lopez-Elias 2002; Richmond 2004). For Zoea larvae, and to a lesser degree for Mysis, phytoplankton is the main source of proteins, carbohydrates, lipids and other nutritional compounds. It has been proven that the proximal composition and growth rate of shrimp larvae are associated with the biochemical composition of microalgae used as feed (D'Souza and Loneragan 1999). The biochemical composition of microalgae varies depending on culture conditions, and is affected by factors such as light, pH, temperature and nutrients (Lopez-Elias et al. 1999). Variable culture conditions alter the biochemical composition of the microalgae and affect their quality as live feed (Lopez-Elias et al. 2003). The objective of the present study was to carry out the efficacy of micro algal species, such as *Chaetoceros* sp., *Tetraselmis* sp., *Isochrysis* sp, on shrimp growth, survival and body composition.

Materials and methods

The study was conducted in Gomishan shrimps research center (Gomishan, Golestan province, Iran). *I. galbana* was obtained from the Center of Urmia Lake Research Institute (Urmia-Iran). *C. muelleri* and *T. tetrathele* were obtained from the Gomishan shrimp production hatchery. Each algal species was grown under greenhouse conditions in 500 L opaque conical cylinders. The culture media used for the maintenance of inoculum and accomplishment of the experiments were: Guillard f/2 (Guillard 1975) for *C. muelleri* and *T. tetrathele* and Conway (Walne 1966) for *I. galbana*. Temperatures were 26 ± 1 °C, Salinity 32 ± 2.5 ppt, pH 7.2–8 and light 5,000 lux. Every day the algal cell densities were estimated using a hemocytometer in the algal culture medium and larval culture flasks so that the desired experimental algal cell densities could be maintained.

The feeding of bioassays was conducted in a completely randomized design with six treatments and four replicates per treatment for a total of 24 fiberglass tanks (each with a capacity of 10 L), at a stocking density of

100 nauplii L^{-1}, with a daily water exchange of between 20 and 50 %. A concentration of 100,000 cells mL^{-1} for monoalgal diets, and 50,000 cells mL^{-1} of each of the species for the mixed diet was used. Six treatments were fed to *Litopenaeus vannamei* larvae in the Zoea I to Zoea III stages that include three monoalgal diets (*C. muelleri*, *I. galbana, T. tetrathele*), and three mixture of species (*C. muelleri*:*I. galbana, I. galbana*:*T. tetrathele, T. tetrathele*:*C. muelleri*).

The protein, carbohydrate and lipid contents of the microalgae were quantified from samples collected from the conical cultivation cylinders. The dry weight was quantified gravimetrically by filtering 100–300 mL of the cultivated microalgae through a 47 mm diameter Whatman GFC glass fiber filters. For evaluation of proteins and carbohydrates, 10–30 mL and for lipids 30–50 mL of the cultivated microalgae were filtered. All samples were evaluated in quadruplicate. Microalgae cells were broken by homogenizing them for 10 min by ultrason (Branson sonifier B-12, energy of 80 W) (Molkenboer 1964). Proteins were extracted with NaOH 0.1 N (Lopez-Elias et al. 1999), according to the Lowry method (1951). The Dubois et al. method (1956) was used for carbohydrate extraction. Lipids were extracted with a mixture of methanol chloroform and water (Bligh and Dye 1959). Gross energy (GE) content was measured by combustion in a bomb calorimeter (Parr Instrument Company, Moline, IL, USA) using benzoic acid as a standard.

Larval development was evaluated by daily microscopic observation. The total length of the larvae at each developmental stage was measured under a binocular microscope from the tip of the rostrum to the end of the tail (total length = TL), and at the end of the experiment, percentage survival was quantified. The biochemical composition of the shrimp larvae was also determined at the end of each experiment by the same methods used for microalgae. To evaluate the effect of the diet treatments on proteins, carbohydrates and lipids, as well as on the growth survival and biochemical composition of the larvae, a one-way ANOVA and Tukey test for post comparison were performed (Zar 1984).

Result

The size of Zoea I was equal among experimental treatments ($P < 0.05$) with an average value of 1.05 mm. For Zoea II, the largest sizes were recorded for larvae fed with the mixture *C. muelleri*:*I. galbana* (2.32 mm) and *T. tetrathele*:*C. muelleri* (2.30 mm) ($P < 0/05$). For Zoea III, the largest sizes were recorded for larvae fed with the mixture *C. muelleri*:*I. galbana* (4.35 mm) and smallest sizes were observed on larvae fed with *I. galbana* (3.04 mm).

The proximal composition of the algae supplied in experimental treatments was different. Protein level was highest in *T. tetrathele* (42.6 %) and *C. muelleri* (39.8 %) as compared with *I. galbana* (30. 3 %). The carbohydrate and lipid levels and GE were higher in *T. tetrathele* treatment as compared with *I. galbana* and *C. muelleri* treatment.

The larvae fed with mixture *C. muelleri*:*T. tetrathele* (88.42 %) and *I. galbana*:*T. tetrathele* (84.50 %) had the highest survival vice versa larvae fed with *I. galbana* (72.33 %) had the lowest survival. The larvae fed with *I. galbana* (109.7 h) and *T. tetrathele* (107.5 h) had the highest duration of the terms of Z_I–Z_{III} and larvae fed with mixture *T. tetrathele* : *C. muelleri* (92.6 h) had the lowest duration of the terms of Z_I–Z_{III}.

The proximal composition of Zoea III was significantly different among the diets ($P < 0.05$). The larvae fed with mixture *I. galbana*:*T. tetrathele* (45.16 %) had the highest protein level as compared with other experimental treatments. The larvae fed with mixture *I. galbana* : *T. tetrathele* (31.81 %) and *T. tetrathele* (31.42 %) had the highest carbohydrate levels. Also highest lipid level and GE were in larvae fed with mixture *C. muelleri*:*I. galbana* (24.28 %), (4.14 kcal g^{-1}) and *T. tetrathele* (24.71 %) and (4.35 kcal g^{-1}), respectively.

Discussion

In our case, the higher growth was shown in larvae fed with mixture *C. muelleri*:*I. galbana* treatment. However, highest survival and lowest time for development were obtained in larvae fed with mixture *T. tetrathele*:*C. muelleri*. Pina et al. (2006) reported that *L. vannamei* larvae fed with *C. muelleri* and the mixed diets containing this species (*C. muelleri* + *Isochrysis* sp., *C. muelleri* + *Tetraselmis* sp.) had the highest

Table 1 Larval length (mm) in Zoea I, II and III stages, survival and duration of the terms of ZI to ZIII (h) of *Litopenaeus vannamei* fed with monospecific and mixture monoalgae

Diet	Survival (%)	Duration of the terms of Z_I–Z_{III} (h)	Total length(mm)		
			Z_I	Z_{II}	Z_{III}
Chaetoceros muelleri	79.21 ± 9.5^{b}	102.4 ± 2.7^{b}	1.05 ± 0.03	2.21 ± 0.07^{b}	3.12 ± 0.11^{d}
Isochrysis galbana	72.33 ± 7.6^{c}	109.7 ± 3.1^{a}	1.06 ± 0.05	2.25 ± 0.08^{ab}	3.04 ± 0.12^{e}
Tetraselmis tetrathele	76.14 ± 10.3^{bc}	107.5 ± 2.4^{a}	1.05 ± 0.05	2.20 ± 0.10^{b}	3.08 ± 0.10^{de}
C. muelleri and *I. galbana*	80.64 ± 7.5^{b}	100.2 ± 2.8^{b}	1.05 ± 0.04	2.32 ± 0.08^{a}	4.35 ± 0.11^{a}
I. galbana and *T. tetrathele*	84.50 ± 8.2^{ab}	102.5 ± 3.2^{b}	1.04 ± 0.04	2.27 ± 0.06^{ab}	3.65 ± 0.10^{c}
T. tetrathele and *C. muelleri*	88.42 ± 7.1^{a}	92.6 ± 1.5^{c}	1.05 ± 0.05	2.30 ± 0.09^{a}	4.11 ± 0.12^{b}

Different superscript letters indicate significant differences between the treatments

survival in Z_{II} (77 %), (68 %), (67 %) and Z_{III} (66 %), (61 %), (58 %), while the highest mortality was shown in *Tetraselmis* sp. treatment. D'Souza and Loneragan (1999) reported that survival of larvae fed *C. muelleri* or the mixed diet was always higher than that of larvae fed *Tetraselmis suecica*, *Isochrysis* sp. or *Dunaliella tertiolecta* alone. Furthermore, the development of larvae fed *C. muelleri* or the mixed diet was always at least as fast as those fed *T. suecica*, and always faster than that of larvae fed the other diets. In this study, the average growth of *L. vannamei* larvae from Zoea I to III was different in size range described by Treece and Yates (1990) and Rodriguez et al. (2012). Rodriguez et al. (2012) reported that the survival was similar between the monospecific treatments (*Chaetoceros* and *Isochrysis*) and the mixed treatment (Table 1).

Several factors contribute to the nutritional value of a microalga—including its size and shape, and digestibility as related to cell wall structure and composition (as mentioned above), as well as biochemical composition (e.g., accumulation compounds, enzymes and toxins) and specific requirements of the target animal (Catarina Guedes and Xavier Malcata 2012). D'Souza and Loneragan (1999) reported that differences in rates of development and dry weight of larvae were most likely related to the biochemical composition of the diets, while differences in survival may have been related to the spawning from which the larvae came. Crocos and Coman (1997) have shown that when the diet of *Penaeus semisulcatus* broodstock is constant, the survival of the PZI stage varies with such factors as age of the broodstock and season of spawning. The nutritional condition of the broodstock also appears to influence reproductive performance: the fatty acids, 20:5(n − 3) and 22:6(n − 3), in the eggs of *P. chinensis* were correlated with fecundity and the hatch rate of eggs (Xu et al. 1994).

Some authors have attributed the poor performance of prawn larvae to the large size of the algal cells in the diet (Tobias-Quinitio and Villegas 1982; Sanchez 1986). *C. muelleri* and *Isochrysis* are small and very similar in size (3–5 μm in length), while *Tetraselmis* sp. are larger in size (5–12 μm in length). In the current study, the larvae fed both the small algae (*C. muelleri*) and the large algae (*Tetraselmis* sp.) displayed high survival and development. Furthermore, the natural diet of *L. vannamei* PZI larvae includes algae as large as 1 mm length and algae >20 μm long are often ingested (Preston et al. 1992). It is, therefore, unlikely that the differences in larval growth performance between algal diets were due to the differences in size of the algal cells alone. *Tetraselmis* is considered a good food source for several species of penaeid shrimp (Liao et al. 1993; Loya-Javellana 1989). In particular, *Tetraselmis* has been shown to rank higher than *Isochrysis* for the protozoea stages of *P. monodon*, *P. semisulcatus* and *M. japonicas* (D'Souza and Loneragan 1999), but it is clearly inadequate as food for the zoea stages of *L. vannamei* (Pina et al. 2006). This was confirmed in a later experiment by Robles-Barraza (2003), who fed a different batch of larvae with *C. muelleri* until the stages of PZ II and PZ III, and with *Tetraselmis* sp. during the successive 5 and 4 days, respectively. Most literature points out that mixtures of microalgae perform better than monospecific diets for filter feeders (Treece 1984; Brown et al. 1989; Smith et al. 1992) (Table 2).

Dietary protein is a source of essential amino acids and energy for prawn larvae. D'Souza and Kelly (2000) reported that it is unlikely that the amino acid content of the diets was responsible for the large differences in development and dry weight of the larvae. Lipids are energy source and a component of the membrane structures of prawn larvae; in the form of steroids, they are precursors to vitamins, bile acids and hormones, including ecdysone, the vital molting hormone of crustaceans (D'Souza and Kelly 2000). Carbohydrates are not a dietary requirement for crustaceans, but they can be a cheaper source of energy than protein and lipid in

Table 2 Average percentage and standard deviation (s.d.) of the energy, protein, carbohydrate and lipid composition of monoalgae

Algal species	Protein (%)	Lipid (%)	Carbohydrate (%)	Energy (Kcal g^{-1})
Chaetoceros muelleri	39.8 ± 2.4^{b}	16.3 ± 1.8^{b}	7.5 ± 0.86^{c}	2.21 ± 0.36^{b}
Isochrysis galbana	30.3 ± 3.7^{c}	17.4 ± 2.3^{b}	14.2 ± 1.2^{b}	2.18 ± 0.71^{b}
Tetraselmis tetrathele	42.6 ± 2.5^{a}	21.3 ± 1.5^{a}	17.4 ± 2.3^{a}	3.07 ± 0.24^{a}

Different superscript letters indicate significant differences between the treatments

Table 3 Average percentage and standard deviation (s.d.) of the energy, protein, carbohydrate and lipid composition of *L. vannamei* larvae fed with monospecific diets (*Chaetoceros muelleri*, *Isochrysis galbana* and *Tetraselmis tetratheie*) and the mixture

Algal species	Proteins (%)	Carbohydrates (%)	Lipids (%)	Energy (Kcal g^{-1})
Chaetoceros muelleri	41.82 ± 3.19^{c}	28.77 ± 1.31^{c}	15.87 ± 1.12^{e}	2.20 ± 0.17^{c}
Isochrysis galbana	42.61 ± 2.71^{bc}	30.37 ± 1.54^{b}	18.54 ± 1.74^{d}	2.73 ± 0.24^{c}
Tetraselmis tetrathele	43.22 ± 3.46^{b}	31.42 ± 2.41^{a}	24.71 ± 1.62^{a}	4.35 ± 0.57^{a}
C. muelleri and *I. galbana*	43.34 ± 2.35^{b}	30.18 ± 1.20^{b}	24.28 ± 0.85^{a}	4.14 ± 0.31^{a}
I. galbana and *T. tetrathele*	45.16 ± 2.43^{a}	31.81 ± 1.63^{a}	20.15 ± 1.25^{c}	3.75 ± 0.40^{b}
T. tetrathele and *C. muelleri*	43.54 ± 2.50^{b}	28.50 ± 2.19^{c}	21.35 ± 2.05^{b}	3.84 ± 0.38^{b}

Different superscript letters indicate significant differences between the treatments

artificial diets (D'Abramo and Conklin 1995). Several studies have indicated that, in the late-logarithmic growth phase, microalgae contain typically 30–40 % (w/w) protein, 10–20 % (w/w) lipids and 5–15 % (w/w) carbohydrates (Brown et al. 1997; Renaud et al. 1999). When cultured through the stationary phase, the proximate composition of microalgae may significantly change; nitrate limitation leads carbohydrate levels to double at the expense of protein (Brown et al. 1993; Harrison et al. 1990). Hence, a strong correlation exists between composition of microalgae and their measurable nutritional value—even though diets containing high levels of carbohydrates have been reported to produce the best growth of juvenile oysters (Enright et al. 1986) and larval scallops (Whyte et al. 1989). Conversely, high dietary protein provides maximum growth for juvenile mussels (Kreeger and Langdon 1993) and oysters (Knuckey et al. 2002).

In this study, *T. tetrathele* had the highest percentage protein and lipid. Similar values were found by Lopez-Elias et al. (1999) for the same two species: percentage of protein was 39.3 % for Chaetoceros and 30.4 % for Isochrysis; carbohydrates and lipids' recorded values were 9.1 and 12.8 % for Isochrysis and 5.4 and 8.3 % for *Chaetoceros*. Pina et al. (2006) reported that *C. muelleri* had the highest percentage lipid content (25.81 %), arachidonic (5.27 Ag mg^{-1}) and eicosapentaenoic acids (9.03 Ag mg^{-1}), while *Isochrysis* sp. had the highest percentage protein (60.93 %) and docosahexaenoic acids (0.014 Ag mg^{-1}), also *Tetraselmis* sp. was richer in carbohydrates (24.75 %). Rodriguez et al. (2012) reported that although *Isochrysis* sp. had the lowest ratio of proteins, its carbohydrate and lipid ratio was high, and it also had the highest percentage of total polyunsaturated fatty acids. The larvae fed with mixture *I. galbana*:*T. tetratheie* had the highest protein and carbohydrate levels as compared with other experimental treatments (Table 3).

D'Souza and Loneragan (1999) reported that *C. muelleri* had the highest lipid level when compared with other treatments, while mixture of *C. muelleri* + *T. suecica* had the highest Polysaccharide level when compared with other treatments. Rodriguez et al. (2012) reported that protein and lipid levels of Zoea III were not significantly different among the diets (*Chaetoceros*, *Isochrysis* and mixture), in spite of the lipid level being significantly higher in Diet III (*Chaetoceros* + *Isochrysis*). The mixed diet was more complete with regard to the major constituents and fatty acid profile, with survival and growth comparable to monospecific diets (Pina et al. 2006). A mixture of *C. muelleri* and *T. suecica* might also provide a diet encouraging high survival and fast development in penaeid prawn (Penaeus spp.) larvae because of the presence of 18:2($n-6$), 18:3($n-3$) and 22:6($n-3$) in the *T. suecica* cells (D'Souza and Loneragan 1999). Based on literature and review, the algae C. *muelleri* show considerable amount of 20:4n − 6 and the EPA (20:5$n-3$). Puello Cruz et al. (2009) stated that, monospecific diets may cause nutritional deficiencies, because of the inadequate

content of one or more essential nutrients. To reduce this risk, several authors have suggested the use of mixed diets, because their combined nutrient contents are more likely to meet the nutritional requirements of the target species (Vidhya et al. 2014). The results of this research indicate that mixtures of microalgae perform better than monospecific diets for *L. vannamei*. In future studies, we can analyze the profile of fatty acids in algae and also examine the effects of *C. muelleri*, *I. galbana* and *T. tetratheie* diets supplemented with microencapsulated PUFAs poly-unsaturated fatty acids, HUFAs highly unsaturated fatty acids on growth, survival and fatty acid composition of penaeid larvae.

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