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EVALUATION OF THE NUTRITIONAL QUALITY OF *CHAETOCEROS MUELLERI* SCHÜTT (CHAETOCEROTALES: CHAETOCEROTACEAE) AND *ISOCHRYSIS* SP. (ISOCHRYSIDALES: ISOCHRYSIDACEAE) GROWN OUTDOORS FOR THE LARVAL DEVELOPMENT OF *LITOPENAEUS VANNAMEI* (BOONE, 1931) (DECAPODA: PENAEIDAE).

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Abstract – The biomass, proximal composition and fatty acid profile of *Isochrysis* sp., *Chaetoceros muelleri* and their mixture, grown under greenhouse conditions, were evaluated. The nutritional value of both species supplied as the monoalgal (*Chaetoceros muelleri*: Diet I, and *Isochrysis* sp. Diet II) and mixed diet (Diet III) for larval *Litopenaeus vannamei* was also assessed on the basis of the development and biochemical composition of the larvae. The highest protein levels were obtained in Diets I and II (40% and 35%, respectively). No significant differences in larval survival were found among the diets; however, larvae fed on Diet II had the lowest mean larval length.

*Key words***:** Microalgae, nutrition, shrimp larvae, outdoor cultures, chemical composition

INTRODUCTION

The production and distribution of farmed, high quality, disease-free shrimp postlarvae is one of the most important issues for sustainable shrimp aquaculture. In commercial hatcheries, biosecurity for the disease control of broodstock and larvae, as well as quality diets for farmed organisms, are the two most important aspects to consider for this purpose.

It is extremely important to account for the nutritional requirements of larvae, especially during larval development. Larvae have specific energy requirements, particularly to progress through certain stages of development, such as metamorphosis (Müller-Fegua et al. 2003). 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 López-Elías 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 (López-Elías et al. 1999).

For the commercial production of postlarvae in northwest Mexico, the species of microalgae most commonly fed to shrimp larvae are: *Chaetoceros muelleri* Shütt; *Isochrysis sp.*; *Tetraselmis suecica* (Kylin) Butch; and *Dunaliella tertiolecta* Butcher. All of these species can be produced in open environment and in different types of containers where culture conditions vary widely. Variable culture conditions alter the biochemical composition of the microalgae and affect their quality as live feed (López-Elías et al. 2003). It is very important to regularly evaluate the effect of the composition of microalgae production on the composition of the farmed larvae and overall. This was an objective of the present study.

MATERIALS AND METHODS

The study was conducted using the facilities of the Peñasco Experimental Unit, University of Sonora, Puerto Peñasco, Sonora, Mexico.

Three treatments were fed to *Litopenaeus vannamei* (Boone, 1931) larvae in the Zoea I to Zoea III stages. There were two monoalgal diets (Diet I, *Chaetoceros muelleri,* Diet II*, Isochrysis* sp), and a mixture of both species (Diet III).

Microalgae were obtained from the AREMAR S.A. DE C.V. shrimp production hatchery. Each algal species was grown under greenhouse conditions in 800 L opaque conical cylinders, using a batch system with f/2 medium (Guillard and Ryther 1962) in exponential phase of growth, at temperatures between 20 and 34°C, and light from 450 to 516 $\mu mol \cdot m^{-1} \cdot s^{-1}$.

The feeding of bioassays was done twice during the study, in quadruplicate each time, in 8 L experimental units, at a stocking density of 100 $nauplii \cdot L^{-1}$, with a daily water exchange of between 20 to 50%. A concentration of 100,000 $cells \cdot mL^{-1}$ for monoalgal diets, and 50,000 $cells \cdot mL^{-1}$ of each of the species for the mixed diet were used.

The dry weight and the protein, carbohydrate, lipid, and fatty acid content of the microalgae were quantified from samples collected from the conical cultivation cylinders. The dry weight was quantified gravimetrically by filtering 100 to 300 mL of the cultivated microalgae through a 47 mm diameter Whatman GFC glass fiber filters. For evaluation of proteins and carbohydrates, 10 to 30 mL, for lipids 30 to 50 mL, and for fatty acids 100 to 500 mL of the cultivated microalgae were filtered. All samples were evaluated in quadruplicate.

Proteins were extracted with NaOH 0.1 N (López-Elías et al. 1999), according to the Lowry method (1951) and modified by Malara and Charra (1972a). The Dubois et al. method (1956) modified by Malara and Charra (1972b) was used for carbohydrate extraction. Lipids were extracted with a mixture of methanol chloroform and water (Bligh and Dyer 1959), for colorimetric determination pursuant to the Pande et al. method (1963).

Lipid extraction to quantify fatty acids was performed according to Bligh and Dyer (1959); thereafter, the sample was evaporated in a R-Buchi CH 9230 vacuum rotovapor, followed by methyl esterification (AOAC 1993). The sample was analyzed in a Model Varian gas chromatograph in an Omegawax 250 silica column (0.25 mm inside diameter x 30 cm long). The standard used was PUFA-1 (marine origin) No. 4-7033 Supleco Inc.

Larval development was evaluated by daily microscopic observation. The total length of the larvae at each developmental stage was measured, 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 organic matter, 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).

RESULTS

The dry organic biomass ($g \cdot L^{-1}$) supplied to the organisms was equal among the diets in both experiments ($F = 0.18$, $p > 0.83$; $F = 0.01$, $p > 0.98$, respectively), with a mean of 0.0414 $g \cdot L^{-1}$. The microalgae biomass decreased over time, despite efforts to maintain a constant number of cells. The decrease was not significant for *Chaetoceros muelleri* ($F = 0.18$, $p > 0.84$), but it was for *Isochrysis* sp. and the mixed diet (on average, 43 and 31%, respectively, less than the initial value) during the feeding of Zoea I to III ($F = 15.79$, $p < 0.0001$; $F = 5.56$, $p < 0.01$, respectively) (Table I).

The proximal composition of the diets supplied in both experiments was different. Protein level was highest in Diet I (*Chaetoceros*) (40.39 %) and Diet III (mixture) (34.93 %) compared with Diet II (*Isochrysis*) (28.83 %). The carbohydrate level was higher in Diets II (*Isochrysis*) and III (mixed) in the first experiment, whereas in the second, it was equal among the diets. The percentage of lipids was variable in all treatments, although high values were always recorded in Diet II (Table II).

The profile of fatty acids in Diet I (*Chaetoceros muelleri*), Diet II (*Isochrysis* sp.) and Diet 3 (mixture) was similar in both experiments, with high ratios of saturated fatty acids (between 62.7% and 76.9% on average), followed by monounsaturated (18.9% and 26.6%) and polyunsaturated (4.3% and 10.8%) (Table III). The most abundant polyunsaturated fatty acids in Diet I were 20:5w3 and 22:5w3. In Diet II, the highest fatty acid was 22:6w3; Diet III (mixed) provided a more complete fatty acid composition, according to the established ratios for both microalgae in the diet.

The survival of shrimp larvae was equal among treatments in both experiments, but survival was significantly higher in the second experiment compared with the first (Table IV).

The size of Zoea I was equal among treatments in both experiments ($F_{exp.\ 1} = 0.31$, $p > 0.7$; $F_{exp.\ 2} = 0.07$, $p > 0.9$), with an average value of 0.93 mm. For Zoea II, in both experiments the largest sizes were recorded for larvae fed with the mixture ($F_{exp.\ 1} = 4.15$, $p < 0.05$; $F_{exp.\ 2} = 5.13$, $p < 0.05$). For Zoea III, in the second experiment, smaller sizes were observed on larvae fed with *Isochrysis* sp. ($F = 11.7$, $p < 0.001$) (Table V).

The proximal composition of nauplii was relatively similar in both experiments, with a large ratio of proteins, followed by carbohydrates and lipids. For the zoeas, the pattern was the same. At the end of the experiments, zoea larvae had a proximal composition similar to nauplii with the three diets. The protein and lipid levels of Zoea III were not significantly different among the diets in any of the experiments, in spite of the lipid level being significantly higher in Diet III in the first experiment (Table VI).

The profile of fatty acids of larvae fed with the different diets was different to that recorded for the corresponding microalgae (Table VII). The high content of linoleic acid was evident in larvae fed with Diet II. As regards polyunsaturated fatty acids, larvae fed Diet I had acids 20:5w3 and 22:5w3; larvae fed Diet II, had mostly 20:5w3, 22:5w3 and 22:6w3; while larvae fed the mixed diet had acids 20:5w3 and 22:5w3.

DISCUSSION

The amount of organic matter provided to shrimp larvae with the same cell concentration was similar among the diets. In all cases, it was enough for the survival of shrimp larvae, despite the slight decrease in organic matter over the course of the experiment.

Overall, the chemical composition of the diets was similar in both experiments. The protein level of microalgae used in the two experiments varied from 23.9 to 43.9%. However, all values within this range promoted adequate larval growth. The mean protein level from the two experiments was lower for *Isochrysis* (Diet II), although this diet contributed the largest percentage of carbohydrates and lipids. Similar values were found by López Elías et al. (1999) for the same two species: percentage of protein was 39.3

Table 1. Average amount of organic matter provided as food to *Litopenaeus vannamei* larvae with Diet I (*Chaetoceros muelleri*), Diet II (*Isochrysis* sp.) and Diet III (mixture) in two experimental runs. Letters different indicate significant differences, a<b<c.

Algal Diet	Organic matter ($g \cdot L^{-1}$)	
	1st experiment	2nd experiment
Chaetoceros muelleri		
Nauplius to Zoea I	0.0492 ± 0.0104^{bc}	0.0486 ± 0.0131^{c}
Zoea I to Zoea II	0.0402 ± 0.0043^{abc}	0.0364 ± 0.0106^{abc}
Zoea II to Zoea III	0.0372 ± 0.0058^{abc}	0.0401 ± 0.0081^{abc}
Isochrysis sp.		
Nauplius to Zoea I	0.0504 ± 0.0145^{c}	0.0481 ± 0.0193^{bc}
Zoea I to Zoea II	0.0425 ± 0.0076^{abc}	0.0472 ± 0.0099^{bc}
Zoea II to Zoea III	0.0281 ± 0.0171^{a}	0.0280 ± 0.0185^{a}
Mixture		
Nauplius to Zoea I	0.0498 ± 0.0120^{c}	0.0491 ± 0.0162^{c}
Zoea I to Zoea II	0.0416 ± 0.0064^{abc}	0.0418 ± 0.0114^{abc}
Zoea II to Zoea III	0.0335 ± 0.0120^{ab}	0.0341 ± 0.0150^{ab}

Table 2. Average percentage and standard deviation (s.d.) of the protein, carbohydrate and lipid composition of Diet I (*Chaetoceros muelleri*), Diet II (*Isochrysis* sp.) and Diet III (mixture) during their use as *L. vannamei* larvae feed in two experimental runs. Letters different indicate significant differences, a<b<c.

Algal Diet	1st experiment			2nd experiment		
	Prot	Carboh	Lip	Prot	Carboh	Lip
Chaetoceros muelleri						
Nauplius to Zoea I	43.17^{d} (3.98)	7.18^{a} (1.58)	12.49^{abc} (1.94)	34.55^{cd} (4.06)	6.57^{a} (3.95)	9.81^{a} (2.07)
Zoea I to Zoea II	38.67^{bcd} (8.23)	5.03^{a} (0.27)	8.70^{a} (0.47)	40.95^{d} (2.32)	8.69^{a} (1.10)	8.87^{a} (1.21)
Zoea II to Zoea III	43.94^{d} (1.62)	8.20^{a} (1.22)	16.42^{bc} (2.04)	41.07^{d} (5.72)	8.16^{a} (1.96)	13.19^{ab} (3.73)
Isochrysis sp.						
Nauplius to Zoea I	41.08^{cd} (7.50)	8.21^{a} (0.70)	16.40^{bc} (2.44)	24.96^{ab} (5.30)	12.55^{a} (6.17)	16.81^{ab} (7.67)
Zoea I to Zoea II	23.93^{a} (1.00)	15.08^{bc} (0.70)	13.18^{abc} (2.53)	33.80^{bcd} (4.08)	10.41^{a} (1.84)	25.71^{b} (9.48)
Zoea II to Zoea III	29.10^{ab} (1.14)	19.77^{c} (2.74)	18.51^{c} (8.43)	20.16^{a} (3.91)	16.03^{a} (8.74)	17.60^{ab} (5.98)
Mixture						
Nauplius to Zoea I	42.13^{d} (5.49)	7.70^{a} (1.23)	14.45^{ab} (2.91)	28.16^{abc} (6.68)	10.56^{a} (6.05)	14.48^{a} (7.08)
Zoea I to Zoea II	31.30^{abc} (9.63)	10.05^{ab} (5.52)	10.94^{ab} (2.95)	37.38^{d} (4.91)	9.55^{a} (1.65)	17.29^{ab} (11.03)
Zoea II to Zoea III	36.52^{bcd} (8.22)	13.98^{b} (6.61)	17.46^{c} (5.61)	34.10^{cd} (11.56)	10.78^{a} (6.08)	14.66^{a} (4.75)

Table 3. Fatty acid profiles (%) of Diet I (*Chaetoceros muelleri*), Diet II (*Isochrysis* sp.) and Diet III (mixture) during experimental runs.

Fatty Acids	Diet I		Diet II		Diet III	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Saturated	66.55	62.68	76.95	73.22	71.74	67.94
Monounsaturated	23.79	26.56	18.92	19.31	21.35	22.92
Polyunsaturated	9.66	10.76	4.13	7.47	6.89	9.1

Table 4. Average percentage of survival of *Litopenaeus vannamei* larvae fed with Diet I (*Chaetoceros muelleri*), Diet II (*Isochrysis* sp.) and Diet III (mixture). Letters different indicate significant differences, a<b<c.

Experiment	Diet I *Chaetoceros*	Diet II *Isochrysis*	Diet III Mixture
1	57.21 %[a] (20.94%)	56.75 %[a] (34.26%)	61.60 %[a] (18.49%)
2	82.52 %[a] (11.51%)	81.98 %[a] (9.10%)	76.47 %[a] (9.55%)

Table 5. Larval length (mm) in Zoea I, II and III stages of *Litopenaeus vannamei* fed with Diet I (*Chaetoceros muelleri*), Diet II (*Isochrysis* sp.) and Diet III (mixture). Letters different indicate significant differences, a<b<c.

Diet	Experiment 1			Experiment 2		
	Zoea I	Zoea II	Zoea III	Zoea I	Zoea II	Zoea III
Chaetoceros	0.94±0.05[a]	1.61±0.15[a]	2.50±0.13[a]	0.92±0.04[a]	1.64±0.06[a]	2.54±0.08[b]
Isochrysis	0.95±0.05[a]	1.69±0.10[ab]	2.43±0.14[a]	0.91±0.04[a]	1.65±0.06[a]	2.47±0.10[a]
Mixture	0.93±0.06[a]	1.74±0.15[b]	2.47±0.14[a]	0.91±0.04[a]	1.68±0.06[b]	2.55±0.05[b]

Table 6. Average percentage and standard deviation (s.d.) of the protein, carbohydrate and lipid composition of *L. vannamei* larvae fed with monospecific diets (*Chaetoceros muelleri* and *Isochrysis* sp.) and the mixture of both in two experimental runs. Letters different indicate significant differences, a<b<c.

Composition of shrimp larvae	Proteins (%)		Carbohydrates (%)		Lipids (%)	
	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II
Nauplii	40.58 (0.00)	37.06 (9.74)	31.64 (0.00)	32.13 (1.07)	12.64 (0.00)	10.16 (0.08)
Chaetoceros Zoea III	39.94[a] (6.53)	45.94[a] (6.73)	30.77[a] (0.09)	30.84[a] (0.49)	15.87[a] (4.75)	15.12[a] (9.80)
Isochrysis Zoea III	43.68[a] (7.69)	42.34[a] (4.73)	31.37[a] (0.39)	30.82[a] (0.94)	18.54[a] (3.18)	12.05[a] (5.78)
Mixture Zoea III	38.94[a] (2.36)	48.63[a] (6.95)	31.20[a] (0.11)	30.57[a] (0.11)	26.28[b] (3.11)	13.75[a] (6.72)

Table 7. Fatty acid profile (%) of *L. vannamei* larvae fed with Diet I (*Chaetoceros muelleri*), Diet II (*Isochrysis* sp.) and Diet III (mixture) in two experimental runs.

Fatty Acids	Diet I		Diet II		Diet III	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Saturated	72.07	73.79	33.15	41.76	64.11	73.19
Monounsaturated	6.83	4.76	20.52	24.32	20.07	19.32
Polyunsaturated	21.10	21.45	46.33	33.92	15.82	7.49

% for *Chaetoceros* and 30.4 % for *Isochrysis*; carbohydrates and lipids recorded values of 9.1 and 12.8 % for *Isochrysis* and 5.4 and 8.3 % for *Chaetoceros* under similar conditions during the same season.

The profile of fatty acids was different among the species. For polyunsaturated fatty acids, a larger ratio of EPA was found for *Chaetoceros* and a greater ratio of DHA was found for *Isochrysis*. The mixture represented the composition of both species with all the polyunsaturated fatty acids present. It has been reported that DHA is the most abundant polyunsaturated fatty acid in *Isochrysis*, whereas for *Chaetoceros muelleri,* it is EPA (Brown et al. 1997, D´Souza and Loneragan 1999). In this research, EPA was not detected in *Isochrysis* sp. The high variability of lipid composition in this microalgae has been documented for both laboratory and greenhouse conditions (Pernet et al. 2003, Piña, et al. 2006), and coincidently, Liu and Lin's (2001) research on the lipid formation of *Isochrysis* sp. found DHA present but did not detect EPA.

López-Elías et al. (2003) reported values of DHA for *Chaetoceros* between 0.03 to 5.23 %, but this acid was not detected in our study. It is possible that under greenhouse culture the production of DHA was very low and therefore undetectable.

In general, the proximal composition of nauplii and larvae fed with monoalgal diets and a mixed diet were similar. The major constituents in the larvae were proteins, which is consistent with research by Rodríguez et al. (1994), followed by carbohydrates and lipids.

Saturated fatty acids were the main lipid components of larvae fed with the monoalgal diets, followed by polyunsaturated and monounsaturated fatty acids. These results are similar to those previously reported by D´Souza and Loneragan (1999) with *Penaeus* spp. larvae fed with *Isochrysis* sp. and *Chaetoceros muelleri*.

Cultivated larvae present a larger proportion of monounsaturated and highly unsaturated fatty acids as compared to the diet they were fed. This implies that larvae bio-convert fatty acids as reported by Teshima et al. (1992) and Lim et al. (1997).

The content of essential fatty acids in shrimp larvae depends on their availability in the diet and the remnant from nauplii (Jones et al. 1997).

The average growth of *Litopenaeus vannamei* larvae from Zoea I to III recorded in this study, was similar to the size range described by Treece and Yates (1990). The survival obtained was similar between the monospecific treatments (*Chaetoceros* and *Isochrysis*) and the mixed treatment. This result differs from the results reported by D´Souza and Loneragan (1999), who found that a monoalgal diet based on *Isochrysis* was unsatisfactory for shrimp larvae nutrition. In addition, Piña et al. (2006) found that a monoalgal diet with Isochrysis sp. did not improve the survival rate and rate of development in *L. vannamei* protozoa larvae.

In this study however, *Isochrysis* was cultivated in a greenhouse and was able to synthesize a sufficient amount of DHA and other important cell constituents, which had a positive effect on the larval culture.

The use of *Chaetoceros* as a food for larval *L. vannamei* zoeas was more than adequate with respect to growth and survival. 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 size and survival of larvae fed with *Isochrysis* sp. was equal for both diets. The mixed diet was more complete, with regard to the major constituents and fatty acid profile, with survival and growth comparable to monospecific diets. In this research, the fatty acid profile was rich in the mixed diet, more than in the monospecific diets. Although some authors considered that mixed algal diets are better in order to improve the nutritional quality to sustain the growth and development of shrimp larvae, most of the commercial laboratories for *L. vannamei* larvae production used monospecific diets (Piña et al. 2006). The results of

this research indicate that *Isochrysis* sp. could be included in the diets used in commercial hatcheries.

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