

Lipid digestibility and performance index of *Litopenaeus vannamei* fed with *Chaetoceros muelleri* cultured in two different enriched media

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Abstract The in vitro digestibility of lipids from *Chaetoceros muelleri* that were cultured in Guillard medium (F/2) and agricultural fertilizer medium (AF/2) and performance index was examined in enzymatic extracts of *Litopenaeus vannamei* larvae. The highest enzymatic activity (EA) of lipases was observed in Zoea I (1.53 ± 0.14 EA/10 EU) and Mysis I (1.26 ± 0.01 EA/10 EU (EU are units of enzymatic activity)) when *C. muelleri* was grown in the F/2 medium. The highest concentration of the unsaturated fatty acids (UFAs) in *C. muelleri* was observed with F/2 medium ($9.96 \mu\text{g FA}/10^6$ cells), and the lowest was obtained with (AF/2) medium ($6.68 \mu\text{g FA}/10^6$ cells). The percentage of the total highly unsaturated fatty acids (HUFAs) in Zoea (18.23 %) and Mysis I (F/2=19.32 %, AF/2=20.25 %) larvae was similar between culture media. The performance indices (PI) of shrimp larvae (Zoea I to Mysis I) differed between culture media, with higher values of PI recorded with the F/2 medium-cultured algae (14.3 ± 1.1) with respect to the values with the AF/2 medium-cultured algae (8.0 ± 1.8). We concluded that due to the effect of the nitrogen source in AF/2 medium, *C. muelleri* cells have a lower HUFA content and *L. vannamei* larvae

experience decreased lipid digestibility and performance index.

Keywords Performance index · Digestibility · Enzymatic activity · *Litopenaeus vannamei* larvae · *Chaetoceros muelleri*

Introduction

The white shrimp *Litopenaeus vannamei* is a native species of the western Pacific Coast of Latin America, from Perú to México (Liao and Chien 2011). Penaeid shrimp progress through several larval stages, the first of which is a naupliar stage, when nutrients are obtained from the yolk sac. From the Zoea I to Mysis III stages, they feed on microalgae, and at the Mysis stage, they usually begin to feed on brine shrimp nauplii until metamorphosis (González-Félix et al. 2002).

More data on lipid digestibility in shrimp is needed to improve commercial larval production. To this end, many techniques have been developed, such as in vivo digestibility (apparent digestibility coefficients and markers), digestibility in vitro (Versaw et al. 1989), the *o*-phthaldialdehyde (OPA) method, and the pH-shift method. For shrimp, a method has been designed to evaluate in vitro digestibility by measuring lipids and lipases (Nolasco et al. 2006).

The biochemical composition of the culture medium can influence microalgal growth and quality. The main factors that alter the biochemical composition in microalgal cultures are temperature, pH, culture age, chemical forms, availability of nutrients, and quantity and spectral composition of light (Sánchez-Saavedra and Voltolina 2006).

Agricultural fertilizers have been used as nutrient sources (nitrates, ammonium, and urea) in substitution of nitrates of F/2 medium (Guillard 1975) for microalgal culture medium with excellent results. Additionally, agricultural fertilizers that are

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used as nutrient sources can modify the biochemical composition of *Chaetoceros muelleri* (Pacheco-Vega and Sánchez-Saavedra 2009). When various nitrogen sources are used for microalgae production, it is necessary to determine the biochemical composition of the cells that are supplied as food and evaluate lipid digestibility in vitro. Regarding the lipid content in microalgae used as feed, its effects on growth, metabolism, assimilation, and survival of the marine organisms that cannot synthesize them must be determined (Brown et al. 1989).

The aim of the present study was to examine the performance index and lipid digestibility at various larval stages of *L. vannamei* that were fed on *C. muelleri*, in two culture media.

Materials and methods

Diatom cultures

Non-axenic batch cultures of *Chaetoceros muelleri* (strain Ch m, UABCS culture collection, México) were maintained in F/2 medium (Guillard 1975) used as a control, and the experimental medium, agricultural fertilizer (AF/2), was prepared with liquid agricultural fertilizers. AF/2 (Table 1) was prepared with 72 % phosphoric acid as the phosphorus source and with a liquid fertilizer, with 32 % of the total nitrogen as urea (16.4 %) and ammonium nitrate (15.6 %), which were added to achieve the same P and N concentrations as in F/2 medium. The concentration of the nitrogen and phosphorus in the experimental media was measured spectrophotometrically on a DR/4000 Hach. Nitrates were measured by the cadmium reduction method, nitrite was measured by diazotization method, ammonia was measured by salicylate method, and orthophosphates were measured by acid method (Hach 1997).

Both media were prepared with 1- μm filtered seawater that was enriched with 0.107 mol L⁻¹ sodium silicate (Pacheco-Vega and Sánchez-Saavedra 2009), and a magnetic stirrer was used to mix the solution. The semi-continuous cultures were maintained in triplicate in 9-L polyethylene bags (28 × 66 cm) for each culture medium at 19 °C, with continuous light at 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 20 % daily dilutions to allow periodic harvest of cultured cells. To determine the content of lipids and fatty acids in microalgae, 600 mL of the microalgae culture was harvested and centrifuged at 1076 × g for 40 min at 10 °C. The cell pellet was washed with 5 % ammonium formate solution, frozen at -40 °C, and lyophilized.

Extraction and measurement of fatty acids

Triplicate samples from each experimental culture were dried with a Labconco freeze dry system/freezone 4.5. Fatty acids were extracted by direct transesterification (Carrapiso and

Table 1 Nutrient composition and concentration of culture medium-based agricultural fertilizers (AF/2) and medium of Guillard (F/2)

Component	AF/2	F/2
Macronutrients	mL L ⁻¹	mg L ⁻¹
Nitrogen mix	75.0	
Nitrate (7.8 %)		
Ammonium (7.8 %)		
Urea (16.4 %)		
Nitrate (100 %)		75.0
Phosphoric acid	5.0	–
Sodium phosphate	–	5.0
Silicate	30.0	30.0
Micronutrients	g L ⁻¹	g L ⁻¹
EDTA-Fe	4.3	4.3
Copper sulfate	9.8	9.8
Zinc sulfate	22.0	22.0
Cobalt chloride	10.0	10.0
Manganese chloride	180.0	180.0
Sodium molybdate	63.0	63.0
Vitamins	mg L ⁻¹	mg L ⁻¹
Biotin	1.0	1.0
Cyanocobalamin (B ₁₂)	1.0	1.0
Thiamine HCl (B ₁)	20.0	20.0

García 2000) and analyzed (1 μL) on a Hewlett Packard G1800B automatic injection gas chromatograph with an Omegawax 250 capillary column (30 m, 25 mm diameter) and 0.25 μM of liquid phase. High-purity (99 %) helium was used as the carrier gas at 1.2 mL min⁻¹. Fatty acids were identified by comparing retention times with individual fatty acid standards (SIGMA Fatty Acids kit). Each fatty acid was quantified by measuring the proportion of its peak area to the percentage of total fatty acid peak area. The concentration of fatty acids in microalgae was measured using the lipid content per Bligh and Dyer (1959).

Nauplii maintenance

Shrimp nauplii of *L. vannamei* (Boone 1931) were supplied by AQUALAB, Bahía de La Paz, Baja California Sur, México, and transported to the Pichilingue Aquaculture Laboratory, Baja California Sur State University (UABCS), La Paz, Baja California Sur, México. Seven hundred thousand nauplii were transported in an oxygen-saturated polyethylene bag and acclimated gradually for 2 h at 29.5 ± 0.5 °C. Each experimental unit contained 1500 larvae at the start of the experiment. The buckets contained 15 L of seawater at a daily exchange rate of 20 %. The nauplii were maintained on a 12:12 light/dark cycle that was provided by natural ambient conditions of the laboratory and with an air supply. The developmental

Table 2 Composition of reaction mixture used to measure the enzymatic activity

Volume (mL)	Reactive	Final concentration (mM)
7.00	Sodium taurocholate	20
3.00	Lipid emulsion	11
2.00	Sodium chloride	1
1.00	Calcium chloride	20
0.20	Enzyme	–

stages of the shrimp were analyzed by random sampling of five shrimp larvae per treatment every 2 h on ×100 Olympus II-CHK microscope.

Each treatment of shrimp nauplii that were fed *C. muelleri* cultivated in the two culture media (AF/2 and F/2) was administered in quadruplicate. At the beginning of the experiment, each experimental container was supplied with 7×10^4 cells mL⁻¹. The cell density was increased daily by adding new cells every day, to reach a concentration of 15×10^4 cell mL⁻¹ on the third day. The cell density of the microalgae was measured every 2 h by cell counting using hemocytometer, and when necessary, algal cells were added to attain the required concentration. The shrimp larvae were weighed in different live stages in each treatment. The survival of shrimp larvae was calculated by differences between the numbers of organisms at the initial time related with the evaluated at the end of the experiment.

Enzymatic extract preparations

Samples at the Zoea I stage (2×10^5 larvae) were homogenized in cold 50 mM Tris–HCl buffer, pH 7.5. Enzyme homogenates from Mysis I were also processed at the end of the experiment. The samples were frozen and kept at –30 °C for 1 day until analysis. Homogenates for lipase activity were prepared in 1.5-mL vials using glass rods to macerate the samples, which were centrifuged at 16,000×g for 15 min at 5 °C. The supernatants were recovered, adjusted to pH 8, aliquoted into 1-mL samples, and frozen at –20 °C until analysis.

Table 3 Mean values and standard deviations of protein and specific activity in Zoea I and Mysis I of *Litopenaeus vannamei* fed with *Chaetoceros muelleri* lipids cultured in medium with agricultural fertilizers (AF/2) and medium of Guillard (F/2)

Larval stage/composition	Protein (µg mL ⁻¹)	Specific activity (units µg ⁻¹ of protein)
Zoea I	6.84±0.42b	1.07±0.13c
Mysis I ^a	11.48±0.22a	1.41±0.19b
Mysis I ^b	3.52±0.18c	2.91±0.33a

Different letters indicate significant differences (ANOVA, α=0.05, a>b>c, n=3)

^a Mysis larvae fed with *C. muelleri* cultured in F/2 medium

^b Mysis larvae fed with *C. muelleri* cultured in AF/2 medium

In vitro digestibility

The in vitro digestibility of lipids (enzymatic activity) is a new method of correlating data from in vivo digestibility experiments (Nolasco et al. 2006). We used this technique to measure lipase activity in Zoea I and Mysis I larvae using emulsified lipids according to Nolasco et al. (2006), as described in Table 2. Lipids from *C. muelleri* were extracted according to Bligh and Dyer (1959) and dried with nitrogen gas. Samples were immediately stored at –20 °C for analysis. The emulsion was prepared by homogenizing the mixture of a solution distilled water/gum arabic/lipids (91.3:4.8:3.9). Lipase activity was measured by continuous monitoring pH-STAT (718 Stat Tiotrino, Metrohm) method. To calculate the enzymatic activity (EA), the reaction was run for 4800 s at 25 °C by mixing and NaOH consumption was measured every 300 s. The EA was calculated according to the following equation:

$$EA = \frac{(\Delta V / \Delta T) \times (NaOH)}{Vol\text{-enzyme}}$$

where ΔV=consumed volume of 0.1 N NaOH (mL) to keep the reaction at pH 8.0 and ΔT=slope of the equation for the linear function between NaOH consumed (Y-axis) versus elapsed time (X-axis). Distilled water was used as blank, NaOH=normality of NaOH solution, and Vol. enzyme=volume of enzymatic extract in microliter.

Determination of enzymatic activity

Lipase activity was calculated as 1 µg naphthol released per minute using a coefficient of molar extinction of 0.02 at 540 nm (Versaw et al. 1989) and was related to the soluble protein content in the homogenates. Each unit of lipase represented the amount of enzyme that was required to increase the optical density by 0.01 unit min⁻¹ at 540 nm. The soluble protein concentration in the homogenates was measured spectrophotometrically at 595 nm according to Bradford (1976) method using bovine serum albumin as the standard. For the pH-Sat test 10, enzymatic units were used.

Table 4 Mean values of different fatty acid fractions and lipid (% of total dry weight) content of *Chaetoceros muelleri* cultures in medium with agricultural fertilizers (AF/2) and medium of Guillard (F/2)

Fatty acid	Culture medium	
	F/2	AF/2
Sum saturated	29.92±3.23a	28.12±2.51a
Sum monounsaturated	19.60±2.51a	18.94±1.59a
Highly unsaturated fatty acids		
20:4 (n-6)	0.72±0.05a	0.14±0.01b
20:5 (n-3)	4.30±0.34a	2.41±0.21b
22:6 (n-3)	– ^a	0.49±0.03a
Sum polyunsaturated	9.96±1.01a	6.68±0.72b
Lipids	7.00±0.20a	7.30±0.20a

Data are expressed as microgram per 10^6 cells⁻¹. Values with the same letter showed an insignificant difference ($P>0.05$; $a>b$, $n=3$)

^a Not detected

The effect of urea on lipid digestibility was measured according to Versaw et al. (1989). The urea concentrations in the reaction were 6, 12.5, 25, 50, and 100 $\mu\text{mol L}^{-1}$. Digestive gland homogenates of shrimp larvae were prepared by removing the insoluble material by centrifugation at 70,970 $\times g$ for 10 min at 4 °C immediately. The digestibility assay was performed by mixing enzymatic extract with lipid substrate. This reaction was incubated at 25 °C for 90 min and stopped with trichloroacetic acid (TCA) and ethanol/ethyl acetate (1:1).

Performance index

Performance index (PI) was calculated using the following equation (Díaz-Iglesias et al. 1991):

$$PI = \frac{(P_{\text{fin}} \times n) - (P_{\text{ini}} \times n)}{(P_{\text{fin}} \times n)} \times 100 \times \frac{1}{T}$$

where T =time elapsed in days, n =number of survivors, P_{fin} =average weight at the end, and P_{ini} =average initial weight.

For the fatty acid analysis, samples of Zoea I *L. vannamei* (2×10^5 organisms) were collected and frozen at -40 °C. Fatty acids were extracted as described above.

Table 5 Mean values of enzymatic activity (EA), dry weight, survival, and performance index (PI) at various stages of *Litopenaeus vannamei* fed with *Chaetoceros muelleri* lipids cultured in medium with agricultural fertilizers (AF/2) and medium of Guillard (F/2)

Culture media	Developmental stage	EA/10 EU	Dry weight (mg)	Survival (%)	PI
F/2	Zoea I	1.53±0.14a	0.130±0.076a	–	–
	Mysis I	1.26±0.01b	0.268±0.152a	52.01±5.69 a	14.3±1.1a
AF/2	Zoea I	1.02±0.00bc	0.130±0.076a	–	–
	Mysis I	0.80±0.05c	0.152±0.054b	25.27±4.32 b	8.0±1.1b

The activity is for each ten enzymatic units (EU) added. Different columns indicate significant differences (two-way ANOVA and Tukey test, $\alpha=0.05$, $a>b>c$, $n=3$)

Statistical analysis

Before the statistical analysis, the data were tested for homoscedasticity and normality. Differences between enzymatic activities were analyzed by a two-way analysis of variance (ANOVA). The effects of urea concentration included in the media and lipid digestibility were analyzed by one-way ANOVA. Differences in performance index were analyzed by one-way ANOVA. The percentages that were obtained from the fatty acid profiles of shrimp larvae were arcsine square root-transformed before analysis and evaluated by one-way ANOVA. When significant differences were noted Tukey a posteriori test was used. Statistical analyses were performed using STATISTIC 7.0 for Windows (Statsoft, USA) and $\alpha=0.05$.

Results

The concentration of total soluble protein from *L. vannamei* enzymatic extracts was higher at stages Zoea I and Mysis I in larval that were fed *C. muelleri* that was raised in (F/2) versus (FA/2) (Table 3). However, lipase-specific activity was significantly higher (51 %) in larvae that were fed FA/2-cultured algae than in Zoea I to Mysis I larvae that were fed F/2-cultured algae (Table 3).

The amounts of saturated fatty acids (SFAs) and monounsaturated acids (MUFAs) in *C. muelleri* cultured in F/2 and AF/2 media showed insignificant difference did not differ ($P>0.05$). However, the concentrations of HUFAs, such as arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), differed between media (Table 4).

Mean in vitro enzymatic activity (EA) was significantly higher ($P<0.05$) in Zoea I (1.53 ± 0.14) and Mysis I (1.26 ± 0.01) larvae when the *C. muelleri* was grown in F/2 medium compared with the AF/2 medium (Table 5). Mean survival differed significantly for Mysis I larvae between treatments, with higher values in F/2 (52.01 ± 5.69) versus AF/2 (25.27 ± 4.32). The dry weight was higher from Zoea I to Mysis I in F/2 (0.268 ± 0.152 mg) compared with AF/2 (0.152 ± 0.054 mg).

Lipid digestibility by shrimp lipase was significantly affected by the culture medium of microalgae. This was confirmed with the result shown in Fig. 1.

The fatty acid composition of the shrimp larvae reflected the composition of the microalgal diets cultured in F/2 and AF/2 media (Table 6). Zoea I shrimp larvae had a higher percentage of saturated fatty acids (SFAs) than Mysis I larvae fed with *C. muelleri* that was cultured in F/2 and AF/2 media (Table 6). Zoea I larvae had a lower total monounsaturated fatty acid content (Σ MUFA) than Mysis larvae fed with *C. muelleri* cultured with AF/2 and F/2 media. No significant differences were observed with regard to polyunsaturated fatty acids (Σ PUFAs), highly unsaturated fatty acids (Σ HUFAs), and eicosapentaenoic acid (EPA) ($P>0.05$) between samples. Arachidonic acid (ARA) and docosahexaenoic acid (DHA) content differed between treatments (Table 6).

Discussion

The differences in polyunsaturated fatty acids are attributed to the differences in the chemical nitrogen sources in *C. muelleri* and other species as described by several groups (Fidalgo et al. 1995; Lourenço et al. 2002; Liang et al. 2006). Changes in highly polyunsaturated fatty acids in algal cells depend not only on the nitrogen source and the characteristics of a species inter alia. Our results show that *C. muelleri* grown with urea, nitrates, and ammonium (AF/2 medium) as nitrogen sources produced lower amounts of polyunsaturated fatty acids.

The differences detected in lipid digestibility by Zoea I and Mysis I *L. vannamei* were also observed by González et al. (1994) during the ontogenesis of *Litopenaeus schmitti*, noting

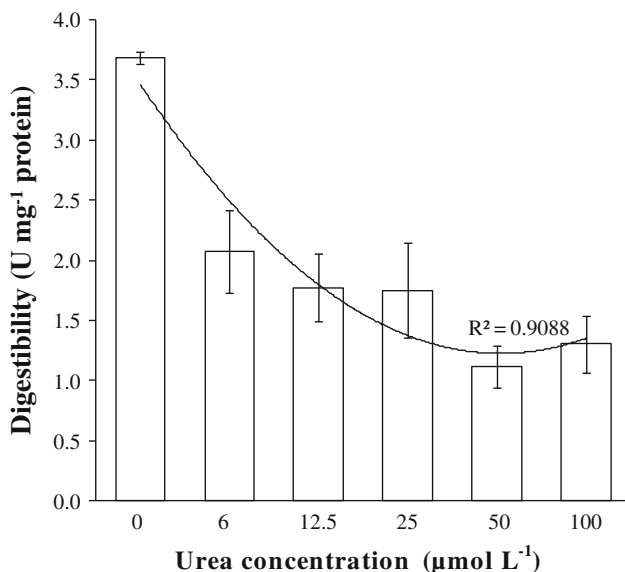


Fig. 1 Mean values and standard deviations of the effect of different concentrations of urea on the digestibility of lipids incubated for 90 min, $n=3$

Table 6 Mean values and standard deviation of fatty acids (% of total fatty acids) at various stages of *Litopenaeus vannamei* (Boone, 1931) fed with *Chaetoceros muelleri* (Lemmerman, 1898) cultured in medium with agricultural fertilizers (AF/2) and medium of Guillard (F/2)

Fatty acid	Stage/treatment		
	Zoea I	Mysis I (F/2)	Mysis I (AF/2)
Saturated			
12:00	0.05±0.02a	0.0±30.00a	0.03±0.00a
13:00	0.15±0.02a	0.04±0.01b	0.02±0.00b
14:00	4.85±0.77a	5.77±0.31a	5.54±0.12a
15:00	0.69±0.11a	0.65±0.02a	0.69±0.04a
16:00	35.10±4.40a	24.21±0.74b	24.84±1.34b
17:00	1.02±0.08a	0.75±0.04b	0.70±0.02b
18:00	11.91±0.76b	12.44±0.10ab	13.59±0.52a
20:00	1.03±0.04a	0.57±0.43a	0.95±0.09a
22:00	0.32±0.06a	0.47±0.07a	0.47±0.08a
Total	55.15±6.05a	44.96±1.32c	46.86±0.69b
Monounsaturated			
16:1 (n=7)	7.67±0.90b	18.37±0.34a	17.76±1.35a
18:1 (n=9)	8.78±3.97a	12.44±0.10a	1.67±0.06b
18:1 (n=6)	5.54±0.27b	1.38±0.03c	10.95±0.29a
18:1 (n=3)	0.24±0.01a	0.25±0.06a	0.26±0.04a
20:1 (n=11)	1.03±0.04	– ^a	– ^a
20:1 (n=9)	0.20±0.02a	0.08±0.01b	0.08±0.01b
20:1 (n=7)	1.50±0.03a	0.42±0.01b	0.42±0.01b
22:1 (n=9)	0.04±0.00b	0.15±0.02a	0.12±0.06ab
22:1 (n=7)	– ^a	– ^a	0.04±0.02
Total	25.04±1.90b	33.12±0.34a	31.31±1.11a
Polyunsaturated			
16:3 (n=3)	0.29±0.03a	0.29±0.03a	0.08±0.05b
18:2 (n=9)	1.54±0.04a	1.27±0.03b	1.36±0.10b
18:2 (n=6)	0.04±0.01c	0.40±0.01a	0.24±0.01b
18:3 (n=6)	0.09±0.01b	0.28±0.01a	0.25±0.05a
18:3 (n=3)	0.06±0.01	– ^a	– ^a
20:2 (n=6)	1.40±0.14a	0.11±0.00b	0.18±(0.06b
20:3 (n=6)	– ^a	– ^a	0.04±0.03
20:4 (n=6)	2.35±0.26b	4.41±0.30a	4.95±0.49a
20:5 (n=3)	10.69±1.15a	12.78±1.15a	11.35±1.16a
22:6 (n=3)	5.16±1.32a	2.13±0.24c	3.95±1.18b
Total HUFAs	18.23±0.95a	19.32±1.43a	20.25±0.74a
Total	21.62±0.83a	21.67±1.43a	22.40±0.40a

Letters indicate significant differences (ANOVA, $\alpha=0.05$, $a>b>c$, $n=3$)
^a Trace levels

increased enzymatic activity from Nauplii to Zoea I, as in *L. vannamei*. Our results in shrimp larvae demonstrate that the enzymatic activities were constant until Mysis I, following a similar pattern as in *L. vannamei* in Rivera-Pérez et al. (2010) in *L. vannamei*. The present results suggest that the digestive gland, which is the principal organ in the synthesis

and secretion of enzymes, is maturing and the midgut caeca is degenerating or experiencing a slowdown in enzyme production. Further, the activities of various enzymes are considered hallmarks of the mature hepatopancreas during early development (Lovett and Földes 1990). The resulting enzymatic activity followed a similar pattern as in Ziaei-Nejad et al. (2006) with an induced strong enzymatic inhibition in early larvae. The variations in digestibility that we observed might be attributed to ontogenetic changes in the digestive system that caused variations in enzymatic activity in *L. vannamei* larvae. In addition, the differences in enzymatic activity correlated with the changes in lipid composition in *C. muelleri*; higher concentrations of *n*-3 HUFA increased the efficiency and absorption of lipids (by approximately five to ten times) (Geurden et al. 1997).

The presence of urea in the experimental medium (AF/2) also affected digestibility. Urea denatures proteins, which affects an increase in the ionic strength of a solution (Madigan et al. 2003). Urea concentrations from 0.5 to 8 M affect lipase activity (Abuin et al. 2005) by the incorporation of urea to the micellar interface producing a decrease of the association of 2-NA with the micelles on lipid digestibility. The concentration of urea in the experimental medium (AF/2) was 35 μ M, which altered the lipase activity in shrimp larvae, seen in Fig. 1; the digestibility decreased if any quantity of urea was added.

In the feed of shrimp cultures, the proportion of saturated and unsaturated fatty acids can affect the digestibility of lipids with a diet that contained a higher proportion of SFAs is lower than one with a higher proportion of the HUFAs (Merican and Shim 1995). Glencross et al. (2002) reported high levels of digestibility in *Penaeus monodon* for all fatty acids with unsaturated bonds. Longer-chain saturated fatty acids were the least digestible; lower digestibility values were obtained as fatty acid chain length increases. The results of this study demonstrate that the amount of lipids in the diet influences their digestibility and that the fatty acid composition of the total lipids in a diet affects their digestibility in total and individually. Thus, differences in the fatty acid composition of *C. muelleri* were affected by the chemical composition of the culture medium (F/2 or AF/2).

The fatty acid composition at various stages of *L. vannamei* was altered by the nitrogen source that was used to grow *C. muelleri* in F/2 and AF/2 media; changes in the SFAs occurred at the Zoea I and Mysis I stages. At the Zoea I stage, the fatty acid composition depends primarily on the contribution of the yolk sac, not on exogenous feeding (Fraser 1989). Nauplii shrimp are physiologically prepared to metabolize endogenous yolk reserves, which are the chief metabolic substrate for energy production, allowing larvae to become independent of external feeding before the opening of the mouth (Rivera-Pérez et al. 2010).

However, low EPA content in shrimp eggs has been associated with a low survival to Zoea III (Palacios et al. 2002). Therefore, the difference in performance index from the Zoea I to the Mysis I might be attributed to high levels of SFAs, MUFAs, ARA, and DHA in the *C. muelleri* that was added as feed in the treatments. The various fatty acid contents in *C. muelleri* feed were used for the biosynthesis of new tissue and maintaining cell tissue during the early developmental stages of shrimp larvae. The results of this study showed equal HUFA percentages in Zoea I and Mysis I. An increase in the proportion of HUFAs from Zoea I to Mysis I was observed by D'Souza and Loneragan (1999) and González-Félix et al. (2002) in various crustacean species (*L. vannamei*, *L. japonicus*, *Penaeus semisulcatus*, and *P. monodon*).

Our results show that regardless of the HUFA content in *C. muelleri* and Mysis I of *L. vannamei*, a constant level of HUFAs is maintained. Glencross et al. (2002) concluded that the fatty acid composition in the diet influences the digestibility of lipids and the fatty acid composition in shrimp larvae. Furthermore, the differences in the PI in shrimp larvae show that lipolytic digestion has a direct effect on the development of Zoea *L. vannamei*. The differences in digestibility reflect the ability of Mysis I *L. vannamei* to maintain constant HUFA proportion. Moreover, the presence of urea in the culture medium decreases the lipolytic digestibility in *L. vannamei* shrimp, affecting PI in the AF/2 treatment.

Due the nutritional requirements during the development of *L. vannamei* larvae, we recommend feeding shrimp larvae with *C. muelleri* that is grown in F/2 medium to obtain better results with regard to performance index.

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