Effect of *Artemia* nauplii replacement by an artificial feed containing krill hydrolysate on ingestion rate, oxygen consumption, and energy budget in the mysis of *Litopenaeus vannamei* (Boone, 1931).

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Abstract

The effects of complete replacement of *Artemia* nauplii in a live food regimen (LFR) by a microencapsulated diet containing krill hydrolysate (MDKH) on microalgae ingestion, oxygen consumption, and energetic budget was evaluated in Mysis of *Litopenaeus vannamei*. *Chaetoceros gracilis* ingestion rate was affected significantly only in Mysis III (M III). *Tetraselmis chuii* ingestion rate was significantly lower in larvae fed MDKH, between MI and M III ($p < 0.05$). Oxygen consumption of larvae fed MDKH was similar to that obtained with the LFR diet, decreasing along the Mysis sub-stages. A significant higher oxygen consumption was obtained in PL1 fed LFR ($p < 0.05$). Energy expended in metabolism (R) during all the mysis sub-stages was lower in larvae fed MDKH than in those fed LFR. The same trend was shown for growth (production, P), which was 14% lower with MDKH than with LFR. Losses by exuvia (E_u) presented lower values as compared to P and R. The assimilation efficiency was higher with MDKH (3.5%) than with LFR (3%). The net efficiency production (K_2) was lower with MDKH (18.3%) than with LFR (19.2%). and that related to respiration was 81.7% for MDKH and 82.3% for LFR.

Key words: shrimp larvae, energetic budget, oxygen consumption, ingestion rate

Introduction

Replacement of live food by artificial microdiets in shrimp larviculture has been used for a long time (Jones *et al.*, 1997; Jones *et al.*, 1998; Kovalenko, *et al.*, 2002). According to Jones *et al.* (1998) total replacement of live feeds results in poorer growth than that achieved with live feeds. Jones *et al.* (1987) reported *Artemia* nauplii replacement by microcapsules (X-linked protein) for *Litopenaeus vannamei* larvae, but included yeast, and a mix of *Chaetoceros spp* (as initial inoculate), and *Skeletonema* (added daily), in the feeding regimen. Although live food (generally *Artemia* nauplii and rotifers) replacement has been proven successful for raising larvae of many shrimp species, its effects on the energetic demands and benefits for the different larval sub-stages have been poorly studied (Lemos and Phan, 2001).

When *Artemia* nauplii are replaced by inert food, changes in the alimentary behavior of mysis, which already present well-defined raptorial habits, may take place (Lovett and Felder, 1989; Jones *et al.*, 1997). These changes can affect metabolism (through oxygen consumption, Anger, 2001), and the ingestion rate of microalgae and *Artemia* nauplii by the larvae, as compared

to larvae fed a combination of live and artificial feed, needs to be evaluated. The conversion of ingestion values (I), respiration (R), metabolism, and growth (P, production) into their equivalents of work energy, through the integration of an energetic budget model, allows to visualize the proportions of energy that are channeled into growth and metabolism; therefore, allowing to evaluate the efficiency of energy utilization from different kinds of feeding regimens

The present work was designed to determine if the *Artemia* nauplii replacement by microencapsulated diet containing krill hydrolysate diet (MDKH) produced variations on microalgae ingestion rate, oxygen consumption, body carcass composition, and energetic budget of the mysis of *Litopenaeus vannamei.*

Materials and methods

Origin of larvae

L. vannamei larvae in nauplii II-III sub-stages were obtained from the shrimp farm of Industrias Pecis, S.A. de C.V., located in Sisal, Yucatán, Mexico. Animals were transported to the laboratory, where they were acclimated to seawater temperature. Nauplii were reared to protozoea III (P III) using the feeding regimen of Trece & Yates (1990).

Experimental design and diet composition

A completely randomized design with two treatments (microencapsulated diet and microalgae, as MDKH regimen, and microalgae and *Artemia* nauplii, as LFR, live food regimen) with three replicates per treatment was employed. Diet composition and proximate analysis are presented in Table I. The microencapsulated diet was obtained by encapsulating a shrimp larvae diet previously tested as microparticles (Gallardo, 2002) (Table I) using a selected polysaccharide blend as encapsulating mix (Pedroza-Islas *et al.*, 1999, 2000, and in press). To produce the microcapsules, the shrimp larvae formulation was added to the polysaccharide blend aqueous solution (composed by a mix of mesquite and arabic gum) obtaining dispersions with 25% solids content before spray drying. The proportion of polysaccharide blend and ingredients was 2:1, to maintain the encapsulation power of the diet. The aqueous dispersions were dried in a Mobile Minor Niro atomizer (Copenhagen, Denmark) spray dryer, equipped with a rotary centrifugal atomizer. The dispersions were fed to the spray dryer at a rate of 20 ml/min, a 2 bar air pressure, an inlet air temperature of $170 \pm 5°C$, and an outlet temperature of $110 \pm 5°C$. The mean volumetric $(D_{4,3})$ particle size (Pedroza-Islas *et al.*, 1999) of the MDKH diet was determined with a Malvern droplet and particle size analyzer series 2600 (Malvern Instruments, Malvern, Worcs, UK). The result of this measurement indicated that the mean microcapsule size was of 8.4 μm, within a range of 2 and 27 μm. The proximal analysis of the microcapsules (Table II) was made by the Centro de Control Total de Calidades, S.A. de C.V., using the following methods: humidity, gravimetric method (NOM-116-SSA-1994); ash, gravimetric method (NMX-F-607-NORMEX-2002); lipids, gravimetric method (NOM-86-SSA-1994, Ap. C.1.1.3.1); proteins, Kjedahl method (NMX-F-608-NORMEX-2002); and crude fiber, gravimetric method (NMX-F-90-S-1978).

Live food

The microalgae and *Artemia* nauplii concentrations used in the experiment are presented in Table III, and were adjusted daily every 12 hours (8:00 and 20:00 hours). The feeding frequency of the microencapsulated diet was every 4 hours (0:00, 4:00, 8:00, 12:00, 16:00 and 20:00 hours).

Experimental condition

Protozoea III (P III) were placed in a battery of conic bottom white tanks made of fiberglass with a 10 L capacity, constant aeration, adding 400 larvae to determine ingestion rate and oxygen consumption. The seawater was treated with ultraviolet light and filtered through a

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sand filter, cartridge filters of 20, 5, and 1μm, and re-circulated for 3 hours in a biologic filter (FLUVAL model 403) to eliminate suspended organic material. The physicochemical conditions during the course of the experiment were: average temperature, 28.5 ±1°C; salinity, 35 ±1 g‡ L^{-1}; dissolved oxygen, 5.7 ± 0.7 mg‡ L^{-1}; pH, 8.1 ± 0.2. The experiment covered from P III to PL1 stages.

Carcass energy content of larvae

To obtain the energy content of the larvae of *Litopenaeus vannamei,* the rearing conditions of Industrias PECIS farm were used, which consisted of 14 000 L tanks following the commercial practice. From each tank, three pools of larvae (0.5 g each pool) were obtained considering the development index in each tank. The feeding regimen included two microalgae (*Chaetoceros gracilis* and *Chlorella spp)*, *Artemia* nauplii. The samples were placed in Eppendorff tubes and then in liquid nitrogen until the caloric measurements were made. The samples were dried at 60°C until constant dry weight and caloric content had been obtained, using an Oxygen Bomp calorimeter Parr (2901EB, Parr Instrument Co. Moline, IL, USA), previously calibrated with benzoic acid at the start and end of each experiment, according to Pascual *et al.* 2004.

Table I: Diet composition (%) of the microcapsules used in the experiment.

Ingredients	Inclusion level (%)
Fish muscle (*Scomberomourus sierra*)	27
Krill hydrolysate*	17
Shrimp muscle (*Litopenaeus setiferus*)	17
Saccaromices cereviseae	15
Soybean meal	14
Wheat starch	1.05
Cod liver oil	2
Sunflower oil	2
Cholesterol	0.5
Vitamin and mineral premix**	2.5
Robimix Stay C***	2.0
Crude protein (%)	53
Total carbohydrates (%)	14
Total lipids	11
Diet energy (kJ/g)	15

* Krill hydrolysate liquid (Specialty Marine Products).
** Agribrands Purina de México.
*** Roche.

Table II: Proximate composition (%) of microencapsulated diet (MDKH)*

Humidity	5.29
Gross protein	17.15
Lipids	3.09
Ash	6.72
Crude fiber	0.10
Carbohydrates	67.65
Digestible energy (kJ/g)	17.33

* The proximal composition was made by Centro de Control Total de Calidades, S.A de C.V. using the following methods: humidity, gravimetric method (NOM-116-SSA-1994); ash, gravimetric method (NMX-F-607-NORMEX-2002); lipids, gravimetric method (NOM-86-SSA-1994, Ap. C.1.1.3.1); proteins, Kjedahl method (NMX-F-608-NORMEX-2002); and crude fiber, gravimetric method (NMX-F-90-S-1978).

Table III: Live feeding regimen used for *L. vannamei* mysis for microcapsule (mg^{-1} larvae^{-1} day^{-1}), microalgae (Cell mL^{-1}) and *Artemia franciscana* (Nauplii mL^{-1}).

Stage	Sub-stage	MDKH (mg larvae^{-1}day^{-1})	*Chaetoceros gracilis* (Cell mL^{-1})	*Tetraselmis chui* (Cell mL^{-1})	*Artemia franciscana* (Nauplii mL^{-1})
Protozoea	I		40 000	10 000	
	II		75 000	10 000	
	III	0.16	85 000	25 000	0.2
Mysis	I	0.32	60 000	25 000	1.0
	II	0.32	50 000	20 000	1.5
	III	0.32	50 000	20 000	2.0
Postlarvae	1	0.32	50 000	15 000	3.0

Indices

Physiological indices

Microalgae and Artemia ingestion rate (cell h^{-1} larvae^{-1})

Ingestion rate was determined according to Rosas *et al.* (1995). Three 1-L round bottom sterile glass flasks per treatment with 50 larvae per flask, was used. The flasks were maintained in a 28°C thermostatically controlled water bath. The residual microalgae concentration of each experimental flask was counted using a Neubauer haematocytometer (Fox, 1983); 2 mL of water were taken and the microalgae were counted in the Neubauer chamber. The ingestion rate was calculated only from the difference between the night algae concentration and the morning algae concentration, adjusted according to the established design to exclude the possible effects of algae growth during daylight hours under semidarkness conditions, according to Gallardo *et al.* (1995). The ingestion rate (IR) was calculated with the equation (Paffenhoffer, 1971):

$$IR = \frac{V(Co - Ct)}{TN}$$

Where: V is the water volume in the experimental tank, C_O and are the algae concentrations at 20:00 and 8:00 h, respectively. T is the experimental period (hours) and N is the number of larvae in each tank.

Regarding *Artemia*, the number of ingested nauplii by the shrimp larvae was calculated by measuring the nauplii decrease in the experimental flasks. A minimum of 10 samples of 10 ml was taken to determine the concentration of nauplii every 12 hours. The ingestion rate was calculated using the same equation used to measure the ingestion values of microalgae, but substituting concentration by nauplii mL^{-1} (Rosas *et al*, 1995).

Ingestion of the microencapsulated diet was assumed constant and estimated by the individual daily ration, which was of 0.32 mg^{-1} larvae^{-1} day^{-1} (16 mg L^{-1}) according to Gallardo *et al.* (2002) and Pedroza-Islas *et al.* (in press)

Oxygen consumption (μg O$_2$ h^{-1} μg of larvae^{-1})

Oxygen consumption determinations were made in a close system of oxygen consumption. Two organisms of each sub-stage were placed in the respirometric chamber RC 300 of a micro-respirometer (Strathkelvin Instruments, Glasgow, UK) with 0.5 mL water from the experimental

tank. Twelve respirometric chambers were connected to a thermostatically re-circulation bath at 28°C (Fisher Scientific Isotherm Refrigerated Circulator, Model 900). Readings were taken after 30 minutes of larvae acclimation to the chamber, during 5 min. Readings from 10 chambers with organisms were made for each larval sub-stage and treatment. Two chambers without larvae were used to correct the values. Once the oxygen consumption measurements were finished, larvae were washed with distilled water and dried at 60°C for 24 hours until constant dry weight. Later, they were placed in a dryer and weighed using a microbalance (0.1 µg, CAHN-33).

Evaluation method of energetic budget parameters

Ingestion (I)

The ingestion rates of microalgae and *Artemia* nauplii were converted into joules according to Lemos and Phan (2001), using the following values: *Chaetoceros gracilis*, 30 X 10^{-6} µg dw cell^{-1} and 9.66 J dw mg^{-1}; *Tetraselmis chuii*, 269 pg dw cell^{-1} and 47.88 J dw mg^{-1}; *Artemia*, 2.42 J µg dw nauplii^{-1} and 24.21 J mg^{-1}dw. Oxygen consumed during a single developmental stage was converted to energy, using 14.06 J mg^{-1} 0_2 as conversion factor (Gnaiger, 1983). The energetic value of the microcapsule (13.94 J‡mg^{-1}) was estimated using the apparent digestibility coefficients (ADC) reported by Cuzon and Guillaume (1997).

Growth (P)

The individual dry weight of the larvae from oxygen consumption measurements was converted into joules by using the model reported for *Farfantepenaus paulensis* by Lemos and Phan (2001). The equation is:

$$Log C = a + b * \log dw$$

Where: C is expressed in joules and dw is dry weight in milligrams and the constants are: a = 37.15 J mg dw^{-1} and b = 0.145 (r= 0.9), obtained from the calorimetric measurements of the *Litopenaeus vannamei* larvae.

Exuvia (E_v)

The energy content of exuvia was calculated according to Lemos and Phan (2001), considered as 5% of the total carcass energy content.

Other energetic parameters

With the conversion into joules of the parameters previously shown, we estimated an energetic budget model, including the following parameters, according to Lucas (1993) and Lemos and Phan (2001):

Assimilation (As) = Respiration (R) + Production (P) + Exuvia (E_v)

Assimilation efficiency (As Ef) = (Assimilation/Ingestion) X 100

Respiration efficiency (R Ef) = (Respiration/assimilation) X 100

Net growth efficiency (K_2) = [(P + E_v) / (P + R + E_v)] X100

Statistical analysis

Significant differences for all evaluated indices were determined through Student's t test, after analysis of the normality of results for each parameter. Ontogenetic changes were analyzed using one-way ANOVA and Tuckey's multiple comparisons test to determine the existence of

significant differences among sub-stages in each treatment. In all cases, a probability level of 0.05 was considered (Zar, 1996).

Results

Growth

The individual dry weight (dw) of larvae fed the MDKH regimen was not significantly different among sub-stages, between M II and PL1 (mean value 59.12 µg dw). A significantly lower value was obtained in M I (47.8 ± 2.27 µg dw), and also for P III (34.8 ± 2.1µg dw) (p>0.05) (Fig 1). Larvae fed LFR showed a significant increment in dry weight between M I (36.9 ± 1.3 µg dw) and M II (55.2 ± 3 µg dw) (p<0.05). Between M II and PL1 (mean value 61.5 µg dw) no significant differences were found (p>0.05). Regarding analysis per treatment in each sub-stage, only M I revealed significant differences (p<0.05), with a higher value for MDKH regimen (47.8 ± 2.3 µg dw) as compared with MI fed LFR (36.9 ± 1.3 µg dw) (Fig 1).

Carcass energy content

The carcass energy content of each sub-stage of *L. vannamei* obtained by combustion did not show significant differences (p>0.05) (Fig 2).

Ingestion rate (IR) of microalgae and Artemia

The IR of *C. gracilis* was higher for all mysis sub-stages fed on LFR than for those fed on MDKH, being significant (p<0.05) only in M III (76 210 ± 712 and 40,277.8 ± 3674.6 cell^{-1} h^{-1} larvae^{-1}, respectively, Fig 3a).

Regarding the IR of *T. chuii*, maximum microalgae consumption was presented by larvae fed with LFR in all sub-stages and the IR was significantly different from that observed in larvae fed on MDKH (p<0.05) except at PL1. A maximum peak in M III was obtained with LFR treatment (24 928 + 712 cell h^{-1} larvae^{-1}) (Fig 3b)

IR of *Artemia* nauplii was affected during the mysis sub-stages (p<0.05). Values obtained showed a significant increase as the mysis sub-stages changed to reach the highest rate in PL1 (4 ± 0.1 nauplii larvae• h^{-1}) (Fig 3c).

Estimation of the IR in joules per day, for each food in both feeding regimens, is presented in Table IV. Larvae fed LFR had the possibility to increase total ingestion per sub-stage by increasing the ingestion of *Artemia* nauplii (1.96 to 5.7 J larvae^{-1} day^{-1}) which increased the total energy input from M I –PL1 (38.63 Joules), whereas larvae fed MDKH, in which the joules per day of the microencapsulated diet were maintained constant (4.46 J larvae^{-1} day^{-1}), the total energy input (28.58 Joules) was 26% lower than that of larvae fed LFR.

Oxygen consumption

Oxygen consumption decreased gradually during mysis sub-stages (Fig 4). No significant differences between the dietary regimens tested were found, except in PL1, in which larvae fed with LFR presented a higher value (0.06 ± 0.009 mg O$_2$ h^{-1} mg dw^{-1}) than those fed MDKH (0.03 ± 0.005 mg O$_2$ h^{-1} mg dw^{-1}) (p<0.05). Converted into energy (R): 0.17 and 0.31 joules day^{-1} larvae^{-1} respectively.

Energetic budget

An estimation of an incomplete energetic budget model in joules per larvae per day showed that intake of gross energy (IGE), as a whole, was higher for larvae fed with LFR (38.63 J• larvae^{-1} from M I to PL1) than for those fed with MDKH (average of 28.58 J larvae^{-1}) (Table V). A difference of 0.10 J invested in respiration was obtained between larvae fed with MDKH (0.82 J larvae^{-1}) and those fed LFR (0.92 J larvae^{-1}) (Table V), whereas a difference favored the assimilation of larvae fed with LFR (1.26 J larvae^{-1}), as compared to those fed with MDKH (1.12 J larvae^{-1}, Table V).

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Energy partition strategy is shown in Table VI. Respiratory efficiency (R/As), which is the assimilated quantity of energy invested in respiration, was lower for larvae fed with LFR (mean value 76.3%) than for those fed MDKH (mean value 79.7%). Assimilation efficiency (As Ef) maintained a difference between MDKH and LFR regimens (3.5 for MDKH, and 3% for LFR) (Table VI). The net production efficiency (K_2) yielded an average higher value with the LFR treatment (23.7%) than that calculated for larvae fed MDKH (20.3%).
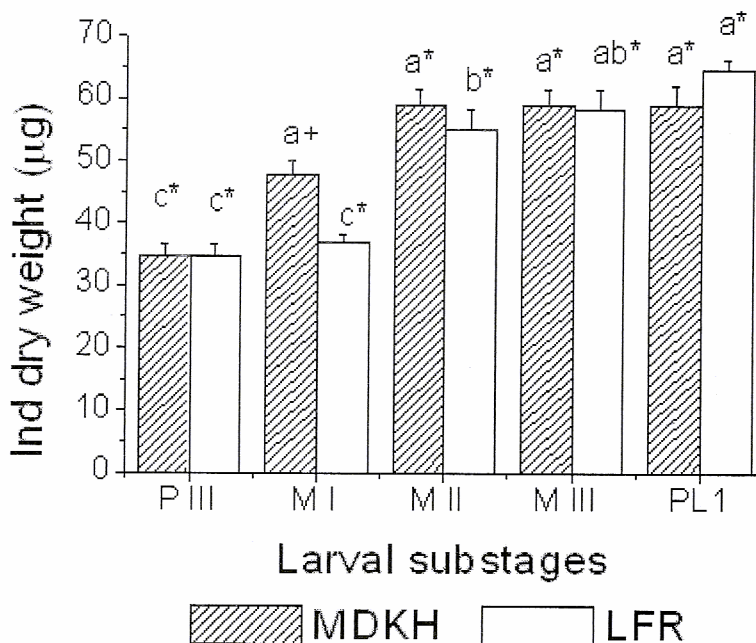

Figure 1: Individual dry weight (µg) of the Mysis of *Litopenaeus vannamei* larvae for each different sub-stage fed microencapsulated diet containing krill hydrolysate and microalgae (MDKH) or on a live food schedule (LFR). Different letters indicate significant differences among sub-stages in each treatment, different symbols (*, +) indicate significant differences between treatments in each sub-stage (Mean± SE.) N=10.
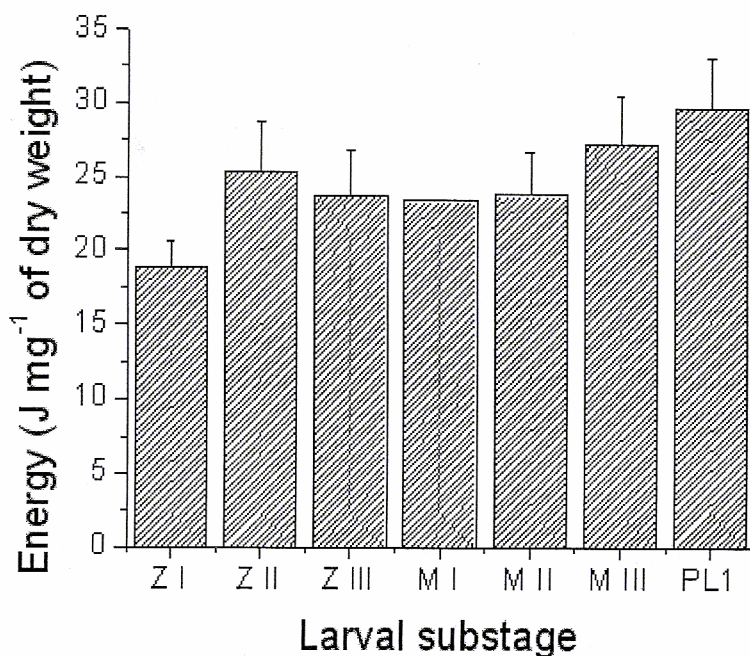
Figure 2: Carcass energy content measured by combustion of *Litopenaeus vannamei* larvae from P I until PL1. No significant differences were observed (p>0.05).
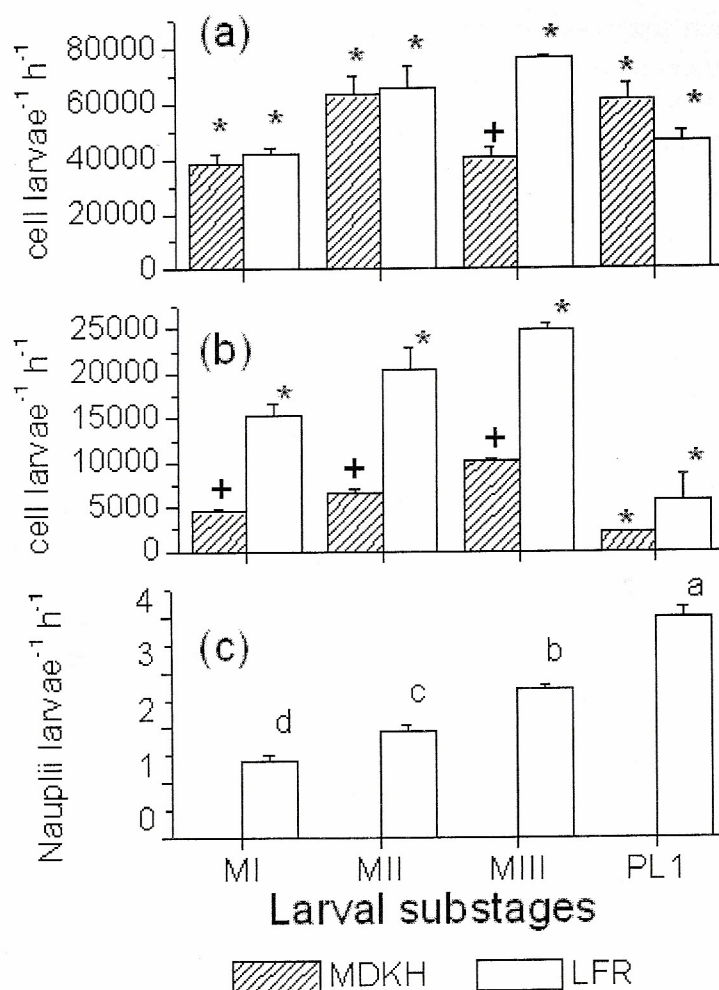
Figure 3: Ingestion rate (IR)(cell h^{-1} larvae^{-1}) of *Litopenaeus vannamei* larvae for each different sub-stage fed microencapsulated diet containing krill hydrolysate and microalgae (MDKH) or on a live food schedule (LFR). **(a)** IR of *Chaetoceros ceratosporum*, **(b)** IR of *Tetraselmis chuii* and **(c)** IR of *Artemia* nauplii (Mean $\pm$ SE). Different letters indicate significant differences among sub-stages in each treatment. Different symbols (*, +) indicate significant differences between treatment in each sub-stage (p<0.05).
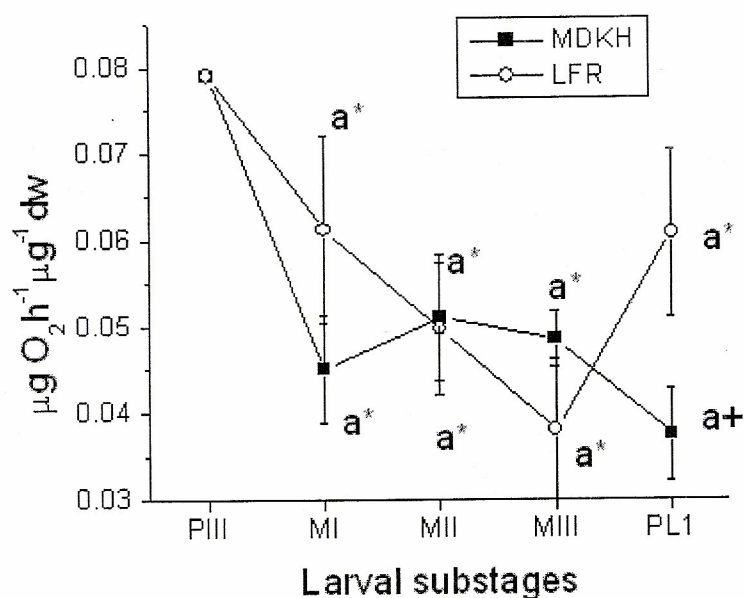
Figure 4: Oxygen consumption (mg O$_2$ h^{-1} mg dw^{-1}) for routine metabolism (HiE+Hem) of *Litopenaeus vannamei* larvae fed microencapsulated diet with krill hydrolysate and microalgae (MDKH) or on a live food schedule (LFR). (Mean$\pm$ SE.) N=10.

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Table IV: Energy intake (J larvae^{-1} day^{-1}) of *Litopeaneus vannamei* mysis fed MDKH and LFR regimens. (Mean± SE). Different letters in the same column indicate significant differences among sub-stages in each treatment. Different symbols (* and †) in each sub-stage indicate significant differences between treatments Feed ration with µcaps is constant.

Treatment	Sub-stage	C gracilis	T chuii	Artemia	Microcapsule	total energy intake
MDKH	M I	0.66±0.06	1.38±0.1*		4.46	6.51
	M II	1.1±0.12	2.04±0.09*		4.46	7.6
	M III	0.7±0.06*	3.15±0.07*		4.46	8.31
	PL1	1.06±0.1	0.64±0.00		4.46	6.16
LFR	M I	0.7±0.04	4.7±0.4+	1.96±0.2^d		7.36
	M II	1.15±0.13	6.2±0.8+	2.8±0.2^c		10.15
	M III	1.32±0.01†	7.6±0.22†	3.9±0.14^b		12.82
	PL1	0.8±0.07	1.8±0.9	5.7±0.23^a		8.3

Table V: Energy (joules larvae^{-1} day^{-1}) channeled into growth: Production (P), routiine metabolism (R), exuvia (E$_v$) during larval sub-stages of *L. vannamei* fed MDKH or LFR. A = P + R + E$_v$.

	Sub-stage	Ingestion	Respiration	E$_v$	Production	Assimilation
MDKH	M I	6.5	0.18	0.009	0.17	0.36
	M II	7.6	0.25	0.005	0.11	0.37
	M III	8.3	0.22	0.00003	Neg	0.22
	PL1	6.2	0.17	0.00002	0.0004	0.17
Total		**28.6**	**0.82**	**0.014**	**0.29**	**1.12**
LFR	M I	7.36	0.19	0,002	0.042	0.23
	M II	10.15	0.23	0.01	0.20	0.44
	M III	12.82	0.19	0.001	0.03	0.22
	PL1	8.24	0.31	0.002	0.05	0.36
Total		**38.61**	**0.92**	**0.016**	**0.32**	**1.26**

Table VI: Energy efficiencies estimated for the Mysis of *Litopenaeus vannamei* fed MDKH or LFR regimens (%).

Treatment	Sub-stage	Respiration %	Assimilation %	K$_2$ %
MDKH	M I	49.8	5.5	50.2
	M II	69.5	4.8	30.5
	M III	100	2.6	0
	PL1	99.7	2.8	0.3
	Mean	**79.7**	**3.5**	**20.3**
LFR	M I	81.01	3.2	19
	M II	52.2	4.3	48
	M III	86.7	1.7	13
	PL1	85.3	4.4	14.7
	Mean	**76.3**	**3**	**23.7**

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Discussion

Although the *Artemia* nauplii replacement by microencapsulated diet containing krill hydrolysate diet (MDKH) produced variations on microalgae ingestion rate, oxygen consumption, energy carcass content, and energetic budget of the mysis, the postlarvae raised from the MKDH regimen presented mean dry weight comparable to that providing from LFR, which indicate the possibility of *Artemia* nauplii replacement by the artificial diet for larval rearing of *Litopenaeus vannamei*.

Intake is difficult to assess in larvae and it always lessens the impact of ration and energy expenditure. Intake is dependent on many factors, such as composition of feed, energy content, nutritional status (fasted or fed), size of feed, texture, color, live food versus inert particles, i.e., it remains the weakest point for any assessment whether on feed conversion, nitrogen, or energy retention (Papand *et al.*, 1999). Basically, food composition will exert one of the main influences on intake and the present study provides a comparison between inert feed particles and the regular live food sequence.

Concerning ingestion rate, a high consumption of algae by larvae maintained with LFR was observed, as compared to values of larvae from the MDKH regimen. In this sense, less usage of algae by larvae fed with formulated diet reflects that this last diet met the nutritional needs of *L. vannamei* larvae, allowing an optimal development, but ingestion of microencapsulated diet remained below the live diet. These results coincide with those obtained for the mysis of *Marsupenaeus japonicus* by Marin-Magán and Cañavate (1995). These authors concluded after fluorometric determination of selectivity of live and inert foods, that the mysis fed on algae far below to the control, when fed a mix of both live and inert particles. When intake is controlled with certain accuracy, it is possible to consider the energy budget and determine the benefit of both regimens. Although microcapsules provided enough energy for survival and development as compared to those fed LFR, without significant increase in time (Gallardo *et al.*, 2002), growth (P) diminished during M II to PL1 (Table V) for larvae fed MDKH regimen, coinciding with the fixed daily ratio of microencapsulated diet (0.32 mg larvae^{-1}), which, transformed to its energetic equivalent, was of 4.46 J larvae^{-1} day^{-1} (Table IV). Otherwise in larvae fed on LFR regimen, growth increased (P) as the energetic contribution of *Artemia* nauplii increased (Table IV). However to the end of the experiment, no significant differences were found in dry weight of the postlarvae from the two treatments (Fig 1). This evidences the relevance of the actual contribution of artificial diets in larvae rearing. In the literature, the daily ratio of artificial diet is frequently given in fixed values. For example, Gallardo *et al.* (2002) reported that, in the presence of algae, the microbound diet allowed to replace *Artemia nauplii*, between 40 and 60%, with rations of 0.11-0.13 mg larvae^{-1} day^{-1} for *L. setiferus* mysis (Gallardo *et al.*, 2002); Galgani and AQUACOP (1988) recommended levels of 0.025 mg larvae^{-1} day^{-1} for *F. indicus*, *L. vannamei,* and *P. monodon* larvae in which they observed up to 85% survival. Kumlu and Jones (1995) showed that the best feeding level for the culture of *F. indicus* protozoeal stages seems to be 0.06-0.08 mg larvae^{-1} day^{-1} of microencapsulated diets with the necessary day per day adjustment of the ration according to leftovers and the degree of feed and feces accumulation in the larval tank.

Anger (2001) pointed out that development and growth imply material and potential chemical energy conversions, originated from ingested food or internally stored reserves. This conversion process is synthesized in metabolic terms and, because of the intermediate metabolism in crustaceans, depends on oxygen consumption (commonly referred as respiration) and is, therefore, highly correlated with the metabolic global rate. Larval respiration is continuously affected by the quality and quantity of the available food; in general terms, it is proposed that feeding increases the respiration rate, this effect has been termed specific dynamic

action (Lehninger et al., 1993) or apparent heat increase (Rosas et al., 1996) and represents the energetic costs of feeding and digestion. In the present study, in which only the feeding metabolism was measured, the two feeding regimens required the same energetic level, expressed in larvae oxygen consumption decreasing as mysis sub-stages elapsed (Fig 3). The high oxygen consumption by PIII might be associated to a metamorphic change (Lemos and Phan, 2001) as reported for other species, like F. paulensis (Lemos and Phan, 2001) and M. japonicus (Laubier-Bonichon et al., 1977; Kulkarmi and Joshi, 1984). According to Lemos and Phan (2001) this phenomenon might be attributed mainly to the decrease of the surface:volume ratio that limits the supply and removal process (for example, gas exchange, digestion) and to the change from typical nauplii and protozoean plankton, through the transition phase to the benthonic habits at mysis and first postlarval stages. The significant difference found in the respiratory rate of postlarvae, feeding from either dietary regimen, could be related to the major effort involved in the capture of Artemia nauplii. In L. setiferus mysis larvae, it has been observed that optimal concentrations of microalgae and Artemia nauplii supply (increased protein) produce higher oxygen consumption than low concentrations of the same food, which was reflected in a higher amount of energy for larval development of this species (Rosas et al, 1995). The only sub-stage in which significant differences were displayed between the two dietary regimens was in PL1. Integration of all indices at organism level, through the energetic budget model, allowed to estimate the global yield of both feeding regimens evaluated for L. vannamei mysis and to decide whether, in fact, microencapsulated food can replace Artemia nauplii. Average respiration results (R) calculated for the two nutritional regimens (mean value of 0.2 J larvae^{-1} day^{-1} for MDKH and 0.23 J larvae^{-1} day^{-1} for LFR) were higher than those reported by Kumarly et al. (1989), who obtained an average value of 0.11 J larvae^{-1} day^{-1} but were lower than those reported by Lemos and Phan (2001) for F. paulensis (average value of 0.48 J larvae^{-1} day^{-1}). Likewise, assimilation values between M I and PL1 were almost 50% lower in larvae fed either MDKH (1.34 J larvae^{-1}) or LFR (1.43 J larvae^{-1}) than the reported values for F. paulensis (2.5 J ind^{-1}) by Lemos and Phan (2001).

On the other hand, production values (P) between M I and PL1 of larvae fed either MDKH (0.034 J larvae^{-1}) or LFR (0.039 J larvae^{-1}) were similar to those reported by Kumarly et al., (1989) for P. monodon (0.55 J ind^{-1}), but lower than those reported by Lemos and Phan (2001) for F. paulensis larvae (1.51 J ind^{-1})

Finally, K$_2$ (average net production efficiency) per sub-stage in larvae fed MDKH (20.3%) and those fed on LFR (23.7%) maintained very low values as compared to F. paulensis larvae (62%, Lemos and Phan, 2001) and P. monodon larvae (48%, Kumarly et al, 1989), which could indicate that L. vannamei mysis are less efficient in transforming assimilated energy (A) into growth (P).

The results of the present work showed that with 26% less energy input during mysis sub-stages fed MKDH (Table V), postlavae with similar dry weights to those fed live food (Fig 1), can be successfully reached. The artificial diet containing krill hydrolysate can replace Artemia nauplii as animal protein source for the larval rearing of Litopenaeus vannamei.

Acknowledgement

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