Journal of the World Aquaculture Society
April 2013, Volume 44, Issue 2, pages 187–197
http://dx.doi.org/10.1111/jwas.12031

Replacement of *Artemia franciscana* Nauplii by Microencapsulated Diets: Effect on Development, Digestive Enzymes, and Body Composition of White Shrimp, *Litopenaeus vannamei*, Larvae

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Abstract :

The effects of two microencapsulated feeds were evaluated on development, growth, survival, proteolytic activity, and biochemical composition of white shrimp, Litopenaeus vannamei, larvae. The treatments were: (1) basal microcapsules (BM), (2) microcapsules containing krill hydrolysate (BMK), and (3) live food control (LFC: Artemia franciscana nauplii) with all treatments receiving algae (Chaetoceros ceratosporum and Tetraselmis chuii). No significant differences were found in development index and survival among larvae. Growth rate was significantly higher in larvae fed LFC (15 ± 0.06%/d) as compared with those offered the BM diet (7.5 ± 0.5%/d) with the BMK (11 ± 0.04%/d) treatment producing intermediate results. The activity of total proteases and chymotrypsin decreased significantly after Mysis I (MI) in larvae fed LFC or BMK. Protein content of larvae increased significantly toward PL1. The acylglycerides content in MIII fed on LFC (2.3 ± 0.2%/dw) was higher than that MI fed BM (1 ± 0.01%/dw). No difference was observed in the cholesterol (CH) content of the larvae. The acylglycerides/protein and cholesterol/protein ratios showed a decreasing pattern between MI and PL1, indicating that these two ratios were related to ontogenetic shifts. These results demonstrate improvements in microparticulate diets when krill hydrolysates are included in the formulation.

The substitution of live food with artificial diets in crustacean larvae has been associated with reduced digestibility, hindering nutritional assimilation (Kumlu and Jones 1997). Recent research has demonstrated the efficiency of microparticulate and microencapsulated diets when used as partial substitute of live feed, obtaining survival and development rates similar to those obtained with live food, albeit, sometimes with lower growth or survival (Gallardo et al., 2002; Pedroza et al., 2004; Sarvi et al, 2010)., More recent trials have indicated that when high quality inter feeds are utilized some live foods may be replaced with artificial feeds (Cuzon, pers. comm.).

Protein hydrolysates have been considered as stimulators of enzymatic activity in fish and crustacean larvae (Cahu and Zambonino 1994, 1997; Carvahlo et al. 1998; Cahu et al. 1999; Ravallec-Plé, 2000). Reduction in developmental time and increases in survival have been reported in larvae of carp (Carvahlo et al. 1998) and seabass (Cahu et al. 1999) when protein hydrolysates were included in the diet. The presence of protein hydrolysates in larval shrimp feeds has resulted in shifts in digestive enzymes. (Ravallec-Plé, 2000) which may result in improved of digestive performance and in growth and larvae survival.

Endogenous secretory peptides, such as gastrin and cholecystokinin (CKK), have been identified in the gastric tract of many crustacean species (Reifel et al., 1983; Aldman et al. 1989; Reinecke et al. 1992; Van Wormhoudt and Dircksen 1990). Gastrin and CKK depict a large spectrum of activities, including stimulation of protein synthesis (Johnson et al., 1978), control of intestinal peristalsis, and secretion of digestive enzymes (Ravallec-Plé 2000). Ravallec-Plé (2000) reported the presence of these peptides in fish and shrimp hydrolysates produced by enzyme controlled hydrolysis using the alkalase (enzyme of bacterial origin) as control, and purified enzymes obtained from the hepatopancreas of L. vannamei juveniles. An enzymatic activity inductor at different larval stages was determined in larvae of the shrimp L. vannamei, and it was concluded that it could be used as an indicator to test the secretory effect of peptides included in protein hydrolysates of marine origin (Ravallec-Plé, 2000). Also krill hydrolysate can contain secretory peptides that might induce digestive enzyme activity in mysis of L. vannamei when they are included in the artificial feed. The krill hydrolysate is made by partial enzymatic hydrolysis under controlled digestive conditions; it contains high levels of short chain polypeptides (in two populations: one corresponding to peptides of less than 14.4 kDa and the other to peptides bigger than 66 kDa) (Córdova-Murueta and García-Carreño 2002). Its use as an ingredient in aquaculture feed has been investigated (Kolkowski et al., 2000; Storebakken, 1988; Nicol and Endo, 1997). This research was designed to analyze the influence of krill hydrolysate (Euphausia superba) included in a microencapsulated diet (Gallardo et al. 2002) as a substitute of A. franciscana nauplii in the feeding of L. vannamei larvae. Special focus was placed on characterizing the enzymatic activity of proteinases, variations of the body chemical composition (total soluble protein, acylglycerides and cholesterol) at different larval stages, as well as effects on growth, development index and survival rate.

1. Materials and Methods

Litopenaeus vannamei larvae in the nauplii III-IV sub-stage was 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 (28 $\pm$ 0.5 ^{o}C).

1.1. Experimental Design and Diet Composition

A complete randomized design with 3 feeding treatments and 3 replicates per treatment was used. The krill hydrolysate was included replacing squid meal in a previous formulation tested in microparticles reported by Gallardo et al (2002). Both diets (BM and BMK) were produced according to the procedures reported by Pedroza et al (1999, 2000). The shrimp larvae formulation was added to the polysaccharide blend in aqueous solution obtaining dispersion with 25% solids content before spray drying. The proportion of agent's encapsulants: diet was 2: 1 to maintain power encapsulant of diet and according to the procedure established by Pedroza *et al* (2000). Table 1 presents the formulation and chemical composition of diets microencapsulated.

The aqueous dispersion was dried in a mobile minor niro atomizer (Niro, Copenhagen, Denmark) spray dryer, equipped with a rotary centrifugal atomizer (Niro, Copenhagen, Denmark). The dispersion was fed to the spray dryer at a rate of 20 ml/min, a 2-bar air pressure, an inlet air temperature of 170 $\pm$ 5º C, and an outlet temperature of 110 $\pm$ 5º C.

The mean volumetric particle size of the microcapsules was determined according to Pedroza et al. (2000) with a Malvern droplet and particle size analyzer series 2600 (Malvern Instruments, Malvern, Worcs., UK). The mean particle size of basal microcapsules was 12 µm (range of 1-42 µm) and that of microcapsules with krill hydrolysate was 9 µm (range of 1-36 µm). The feeding frequency of the formulated diet was every 4 hours (0:00, 4:00, 8:00, 12:00, 16:00, and 20:00 hours) considering the degree of hydration maxima and leaching of microcapsules for their stay in seawater according to Pedroza et al. (2000).

Live food control (LFC) consisted of microalgae (C. ceratosporum and T. chuii) and A. franciscana nauplii according feeding schedule for Treece and Yates (1990) and were adjusted daily every 12 hours (8:00 and 20:00 hours) according to Gallardo et al. (2002). The microalgae were used in the same density and frequency in treatments with BM and BMK.

1.2. Experimental Condition

To evaluate development and growth groups of 400 larvae (protozoea III; PZ_{III}) per tank were placed in white conical-bottom tanks made of fiberglass in 8 L of seawater, with constant aeration. To evaluate survival rates groups of larvae (50 PZ_{III}) per flask were placed in round bottom 1-L glass flasks. Each treatment was replicated three times for both culture systems. Additionally, 4000 larvae were placed in 100-L tanks and were sampled in three groups (50 organisms each one) by larval sub-stage to evaluate digestive enzymatic activity, total protein, acylglycerides, and cholesterol content.

Seawater was filtered through sand filter, cartridges of 20, 5, and 1µm and then passed through ultraviolet light and re-circulated for 3 hours in a biological filter (FLUVAL, Mod. 403, HAGEN, U.S.A) to eliminate suspended organic material and to control bacteria populations. The physicochemical conditions during the experiment were: average temperature 28.5 $\pm$ 1º C, salinity of 35 $\pm$ 1 g/l, dissolved oxygen of 5.7 $\pm$ 0.7 mgO_2/L and pH of 8.1 $\pm$ 0.2. The experiment began from PZ_{III} up to PL_1.

1.3. Specific Growth Rate (SGR), Development Index (DI) and Survival

Growth and DI were evaluated in 15 larvae from each treatment (5 larvae by tank) each day. For the wet weight, the organisms were placed in a microbalance, CAHN-33 (Orion Inc., Beverly, MA, USA), with a 0.0001 mg accuracy. From weight data given in micrograms (µg), a specific growth rate (SGR = [((lnWf-lnWi) / t) * 100]) was calculated, considering PL_1 (final) and PZ_{III} (initial) according to Gallardo et al. (1995, 2002) and Pedroza et al. (2004). The DI was assessed every 24 hours with the same organisms used for growth determination along the larval sub-stage identification. Larval sub-stages were defined according to García (1972). To determine the ratio, we used the Villegas and Kanazawa's (1979) equation:

$$DI. = \frac{\sum A}{N}$$

where A is the number of organisms at each sub-stage multiplied by the number assigned to each sub-stage, and N is the total number of sampled larvae. The absolute number value assigned to each larval sub-stage was: PZ_{III}=3, M_{I}=4, M_{II}=5, M_{III}=6 and PL_1=7.

Percent survival was obtained at the end of the experiment by counting produced postlarvae in the 1-L flasks as, compared to the number of PZ_{III} placed at the beginning of the experiment.

Extract Preparation. From each sub-stage, 150 larvae were sampled (one hour after being fed) and distributed in three pools each containing 50 organisms. Larvae were washed with distilled water, dried on a 10-µm mesh provided with an absorbent paper to extract all the water, and immediately frozen in liquid nitrogen in Ependorff tubes until processing (Palacios 1999). To prepare the extracts, 500 µL deionized cold water were added to each tube and the larvae were homogenized over ground ice in a tissue homogenizer for approximately 30 sec. Homogenates were centrifuged for 6 min at 16000 *g* at 4 C. The supernatant was used to analyze and determine soluble protein, acylglycerides, cholesterol, and digestive enzyme concentrations.

1.4. Specific Activity of Total Proteases,Ttrypsin and Chymotripsin

Total proteases activity was measured according to Walter (1984) using azocoll hydrolysis (Sigma A 4341, Sigma Chemical St, Louis, MO, U.S.A.) at 1%, in a 10 mM phosphate buffer, pH 7, at 37ºC for 1 h. The reaction was blocked with 5% trichloroacetic acid (TCA) to precipitate the soluble proteins. After centrifugation, the supernatant was recovered and mixed in equal volumes with a solution of 0.5 M NaOH, and read at 420 nm in a spectrophotometer (SPECTRONIC model 21 D, Spectronic Instruments, U.S.A).

Trypsin activity was measured through hydrolysis of the substrate L-benzoil-arginine-p-nitroanilide (BAPNA, 1 mM), in a 0.1 M TRIS buffer, pH 8, and read at 405 nm (Geiger and Fritz, 1988). A unit of trypsin specific activity corresponds to 1 µM p-nitroanilide liberated per minute.

Chymotrypsin activity was estimated by the hydrolysis of succinil-alanine-2-proline-phenylalanine p-nitro anilide (SAPPNA, 1mM) in a 0.1 M TRIS buffer, pH 8, and read at 405 nm (Geiger, 1988). A unit of chymotrypsin activity corresponds to 1 µM p-nitroanilide liberated per minute.

For all the evaluated enzymes the specific activity was calculated using IU per milligram of protein per dry weight. For this reason, it was considered dry weights obtained from the larvae used for growth determination.

1.5. Total Soluble Protein

Total protein was measured by the Bradford method (1976), using the microprotein determination of the Bio-Rad kit (Procedure no. 610) for Elisa microplates. Samples were read after 10 minutes in a microplates Bio-Rad reader (Mod. 550, Bio- Rad, U.S.A) with a 595 nm filter.

1.6. Acylglycerides (AG)

The extract (10 μL) was placed on a microplate, adding 200 μL of the reactive solution from the Sera-Pak commercial kit Cat. 6684 (50 mM Buffer pH 7; lipoproteinlipase ≥ 50 U/mL; glycerokinase 0.055 U/mL; glycerol-phosphate-oxidase ≥ 2.0 U/ mL; peroxidase ≥ 3.0 U/mL; 0.7 mM adenosine-5´-triphosphate (ATP); 1.0 mM 4- aminophenazone; 7. 0 μM potassium ferrocyanide, 0.6 mM magnesium salts; 1.2 mM N-ethyl-N- (3-sulfopropyl)-m-anisine; surfactant 2.0 g/ L). The reaction was incubated at room temperature for 10 min and the absorbance was read at 540 nm. Acylglycerides concentration (mg mL^{-1}) was calculated from the commercial kit standard.

Cholesterol (CH)

The extract (10 μL) was placed in to the well of the microplates, adding 200 μL of reactive solution from the commercial kit Sera-Pak, Cat 6670 (100 mM phosphate buffer, pH 7.2, cholesterol oxidase ≥ 170 U/L; cholesterol ester hydrolase ≥ 400 U/L; peroxidase ≥ 400 U/L; 9 mM 2 hydroxyphenylacetic acid; 0.5 mM 4 amino-phenasone; 7μM potassium ferrocyanide; tensoactives 6 g/L). Samples were incubated at room temperature for 15 min and the absorbance was read at 540 nm. Cholesterol concentration (mg/mL) was calculated based on the commercial kit standard.

1.8. Statistical Analysis

To determine whether growth rates, survival, development index and ontogenetic changes in digestive enzymes, lipid and soluble protein reserves were significantly different between the dietary treatments and larval substages, a bifactorial ANOVA and Tukey's multiple comparisons test were used (Zar, 1996). All variables expressed as percent or proportion, were transformed to their arcsine prior to analysis. Value of P was set at the confidence level of 0.05.

2. Results

2.1. Specific Growth Rate (SGR), Development Index (DI) and Survival

SGR (PZ_{III} – PL1) obtained by the larvae maintained on treatment BM (7.49 ± 0.01%/day) was less than that shown by the larvae fed with LFC (15.3 ± 0,039%/ day) (Fig. 1a) ($P<0.05$). A value of 10.8 ± 0.02%/day was observed for larvae maintained

with BMK treatment which was not statistically different to larvae maintained on the other treatments (Fig. 1a).

Dietary treatments did not result in differences in survival with ranged from 53 ± 5% (BM) to 78 ± 0.02% (BMK) (Fig. 1b). Development index (DI) was not significantly different ($p<0.05$) among the three treatments evaluated, between the 72 and 144 hours (Fig 1c). Only at the beginning (24-48 hours) a significant delay was observed in larvae fed BM or BMK as compared to those fed LFC.

2.2. Digestive enzymes

A significant peak of total proteases specific activity was observed in the M_{II} sub-stage, for the larvae fed on BM (355 ± 53 mU/mg protein/µg dw) as compared to the values obtained with BMK (66 ± 36 mU/mg protein/ µg dw) and LFC (57 ± 2 mU/mg protein/µg dw) treatment (Fig. 2a).

Concerning the ontogenetic changes of total proteases activity, different patterns were observed according to dietary treatment. In larvae fed LFC or BMK, a significant reduction ($P<0.05$) was observed (Fig. 2a) from M_I to PL_1, whereas for those fed BM a significant reduction ($P<0.05$) in proteolytic activity, after the peak obtained at M_{II}, was recorded (Fig. 2a).

In regard to trypsin activity, no significant differences were observed among treatments in each sub-stage. For this enzyme, a significant reduction was recorded during development from M_I to PL_1 only in larvae fed on the LFC and BMK treatment (Fig. 2b).

A significant reduction in chymotrypsin activity (85%) from ontogenetic origin was obtained in larvae fed LFC from M_I to PL_1 ($P<0.05$). In relation to larvae fed BMK treatment no significant reduction of the activity was observed. Statistical comparison of chymotrypsin specific activity in each larval sub-stage showed that, only during M_{II} was significantly higher level recorded in larvae fed BM (Fig. 2c).

2.3. Biochemical composition of larval stages

The highest soluble protein content (P) values were obtained for larvae fed on LFC with significant differences ($P<0.05$) observed in PL1 (32 ± 0.4%/dw) as compared to shrimp fed on artificial diets (Fig. 3a). Acylglycerides content (AG), did not show any differences whatever dietary treatments. However, ontogeny development indicated a significant increment at M_{III} fed LFC compared to M_I fed BM (Fig. 3b). The cholesterol content (CH) evaluated in L. vannamei mysis did not show significant fluctuations during all sub-stages with any of the dietary treatments, except a peak to M_{III} with LFC treatment (Fig. 3c).

The acylglycerides:protein ratio (AG/P) diminished along the larval development with each dietary treatment; with a significant decreased observed only during PL_1 with LFC (Fig. 3d). The acylglycerides:cholesterol ratio (AG/CH) revealed a stable evolution along the mysis sub-stages and at PL_1. However, in larvae fed on the BMK diet, a significantly increased in M_{III} was observed (Fig. 3e).

The cholesterol:protein ratio (CH/P) tended to decrease during larval development similarly to the AG:P ratio. The effect of dietary treatment on this ratio was significant in M_I and M_{II}, reaching significantly higher values with the LFC and BM treatments respectively (Fig. 3f). A low CH/P ratio was observed in PL1 for all treatments ($P<0.05$)

3. Discussion

The use of krill hydrolysate in the feed has been reported to affect a number of digestive enzymes. The secretagogue effect of krill hydrolysate included in the microencapsulated (BMK) diet was not evident through the total proteinases, trypsin, and chymotrypsin specific activities when compared to the BM and LFC. Furthermore, the BM diet, which did not contain krill hydrolysate, induced a peak of activity for total proteinases and chymotrypsin enzymes during M_{II} as compared to both BMK and LFC treatments. BM diet contained native squid, which had been substituted with krill hydrolysate in BMK. Le Moullac et al. (1994) reported that squid produced the highest chymotrypsin activity in L. vannamei larvae. However, growth and survival were not positively affected by BM as with the BMK treatment compared to control (LFC). The ontogenetic reduction of enzymatic activities was confirmed, derived from the ontogenetic changes of the digestive tube of shrimp larvae, as reported for other penaeid shrimp species by Lovett and Felder (1990a), Le Vay et al. (2001), Anger (2001) and Lemos and Phan (2001). This ontogenetic change is related to the disappearance of the anterior caeca observed in mysis sub-stages of L. setiferus by Lovett and Felder (1989) and by Abubakr and Jones (1992) in Penaeus monodon. These changes are associated with a variation in feeding habits of omnivorous crustacean larvae. An increase in trypsin content during herbivorous sub-stages of protozoea, reached a peak in the transition to mysis, and declined afterwards, during the carnivorous phase, in the stages of mysis and first postlarvae as shown before (Laubier-Bonichón et al., 1977; Lovet and Felder, 1990b; Le Vay et al., 1993; Jones et al., 1993; and Le Vay et al. 2001).

In other studies, the use of probiotics in the rearing water of larval L. vannamei, promoted survival to PL1 and increased digestive activity but only in the early postlarvae (PL1-PL8) stages (Zhou et al., 2009). As stated by Le Vay et al. (2001), in herbivorous and omnivorous shrimp larvae and under laboratory conditions with high feed available, ingestion rates tended to be high and are accompanied by a high exchange rate in the digestive tract (Kurmaly et al. 1989; Lovett and Felder 1990b); therefore, as a strategy, a high enzymatic activity allowed larvae to extract most of the digestible compounds, retrieve nutritional entities with a relatively low assimilation efficiency and gain more net energy (Le Vay et al., 2001). In the present study proteinases activity, as an indicator of digestive activity of shrimp larvae showed that the secretagogue effect of krill hydrolysate only was evident during Mysis I stage, indicating that MBK and LFC diets had an important role into the digestive stimulation, just when shrimp larvae requires a change in digestive capacity due to raptorial behavior. Total proteinases and chymotrypsin activities showed no differences between mysis sub-stages and PL1 stage fed all diets showing a stable behavior in activity during this 3-4 days period. The digestive enzymes activity is very similar in L. vannamei mysis larvae fed with either BMK or LFC, and indicate the wide flexibility of the digestive system of larvae at this stage, where the digestive enzymes to be active received equivalent substrates (A. franciscana nauplii or microcapsules).

Weight gain is a direct response of the diet effect and summarizes the physiological, biochemical, and nutritional adaptations (in terms of requirements) of organisms. In this study, the high percentage of survival, specific growth rate, and development obtained with BMK treatment was close to those obtained with LFC, suggesting that the artificial diet was well consumed and covered the nutritional requirements of shrimp larvae. Specific growth rate was significantly lower when fed BM indicating that in this diet there are some missing nutrients that affected larval performance. This could be related to the diet's composition with the squid that in spite of its stimulant role in digestive activity (analyzed though differences found in each substage) could produce

a poor assimilation of the diet, with consequences into biomass accumulation and survival.

The decapod larvae are composed of proteins (> 30%), lipids (> 20%), chitin (> 15%) and free carbohydrates (< 5%). One aspects of crustacean larval nutrition is the effect of diet on larval biochemical composition, leading to modification in the pattern of developmental growth (Anger, 2001). In larvae of decapod crustacean, triacylglycerides, phospholipids, and free sterols compose the predominant lipid fractions (Anger, 2001). Among these, triacylglycerides (TAG) constitute the main source of energy reserve (Sasaki et al., 1986; Galois, 1987; Chandumpai et al., 1991; Dall et al., 1992, Mourente et al., 1995). Teshima and Kanazawa (1982) also reported the presence of mono- and di-acylglycerides in the different larval stages of Marsupenaeus japonicus. The technique to evaluate the total quantity of acylglycerides in L. vannamei included all acylglycerides representing lipid reserves. Nutrient intake provided by BM, BMK, and LFC maintained similar acylglycerides reserves during the mysis stage, indicating that both microcapsules and A. franciscana nauplii presented a similar lipid quality, which later on could be assessed through the absence of significant differences in larval development (Fig 1c). Quality lipids will contribute to success in efficient development of shrimp larvae as well as adequate use of energy reserves (Mourente et al., 1995). A lower acylglycerides content in larvae fed with BM as compared to that of larvae fed on BMK and LFC treatments was observed at PL_1 stage only suggesting that some lipids could be missing into the BM diet affecting larval growth and survival.

Cholesterol is an ecdysteroid hormone precursor (Lehninger, 1993), which can not be synthesized by crustaceans (Teshima and Kanazawa, 1982). It is a stable structural compound due to its participation in cell membranes structure, independently of feeding conditions (Fraser, 1989; Anger, 2001). This pattern of stability of the values from the three dietary treatments though all the larval substages was confirmed (Fig. 1i).

In terms of lipid reserves the ratio of tricylglycerides:cholesterol has been proposed as a reference for larvae condition. This ratio was modified to a acylglycerides:cholesterol (AG/CH) ratio in the present study. Although acylglyceride reserves did not show significant differences during mysis stage and PL_1 among dietary treatments (except a lower value in larvae fed BM in PL_1), a decrease of lipid reserves in animals fed with LFC during M_I was observed, whereas BM and BMK treatment maintained a more constant lipid reserves supply (Fig 3a). However, these variations did not effects larval survival and development (Fig. 1b and 1c). This does not mean that variation does not exist. Simply, at the level of sampling it was not possible to detect a variation. Such variation in cholesterol is known in relation with molting in crabs and the lipocytic coefficient increases as a physiological response for water absorption at tissue level to trigger exuviation. On the other hand and with regard to the AG/protein ratio, which can be used as a parameter for energetic condition of crustaceans larvae (Anger, 2001), the significant decrease observed at mysis sub-stages according to dietary treatments, indicates that lipid reserves were maintained constant (Fig 3d). The exponential increase in protein content produced an exponential decrease of AG/protein ratio values, evidencing that, at mysis stage, an exponential growth rate is common. The ratio cholesterol:protein (CH/P) can be used as an indicator of an increase in biomass structure (Anger, 2001). Accordingly, the pattern observed in this research contrasts with those remaining constant in Homarus americanus during the larval phase (Sasaki, 1986).

Larval growth, survival and development are often used in diet evaluation for shrimp (Kanazawa, 1985; Jones et al., 1993; Cahu and Zambonino, 1997) and integrate

directly the effects of food type supplied on shrimp larvae (Jones et al., 1997). Similar responses evidenced that A. franciscana nauplii commonly used in feeding mysis larvae can be substituted with the microencapsulated diet containing krill hydrolysate.

4. Conclusion

Replacement of Artemia franciscana by inert dry particles is going to be more and more efficient with progress in formulation, increase in palatability, number of meals per day and optimum rearing conditions (T ^{o}C, aeration, light) and a constant survey of the larvae during critical phases before metamorphosis.

Acknowledgments

We thank UNAM IN 234596 and IN- 220505 and CONACYT 38193 for financial support. To CONACyT for the graduate (Ph.D) studies grant to MS. Pedro Pablo Gallardo. Also to Ingrid Mascher for language assistance.

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Tables

TABLE 1.- Ingredient composition (g/kg) of the microparticulate diets for the microcapsules used in the experiment. The proportion of agent's encapsulants: microparticulates diets were 2: 1.

Ingredients	BM	BMK
Fish muscle (*Scomberomorus sierra*)	270	270
Squid mantle muscle	170	
Krill hydrolysate*		170
Shrimp muscle (*Litopenaeus setiferus*)	170	170
Saccaromices cereviseae	150	150
Soybean meal	140	140
Wheat starch	15	15
Cod liver oil	20	20
Sunflower oil	20	20
Soybean lecithin	10	10
Cholesterol	5	5
Vitamin and mineral premix**	25	25
Stay C***	5	5
Proximate composition of microcapsules (g/kg) [1]		
Moisture	47	53
Crude protein	177	172
Total lipids	33	31
Ash	61	67
Nitrogen free extract	681	687
Digestible energy (MJ/kg) [2]	17.3	17.3

* Krill hydrolysate under liquid form (Specialty Marine Products).

** Agribrands Purina de México property information only vitamin types are given; A UI/kg; D; E; K; Beta-caroten; thiamin; pyridoxine; riboflavin; cyanocobalamin; ac.folique; nicotinamid; panthotenic; biotine; C; carophyll red; choline; inositol.
***Roche.

[1] Moisture, protein, lipids, and ash contents were determined following methods established by A.O.A.C. (1990) (protocols 934.01, 976.05, 920.39 and 942.05, respectively).
[2] Estimated from Cuzon & Guillaume (1997) on the basis of energy content estimates of 21.3, 17.6, and 39.5 kJ g^{-1} for proteins, carbohydrates, and lipids, respectively.

Figures

FIG.1. (a) Specific growth rate (%/day), (b) survival (%) and (c) development index (ID) of Litopenaeus vannamei larvae fed microencapsulated diets (BM and BMK) and live food (microalgae and artemia nauplii). Means ± S.E. N= 3 repetitions. Different letters indicate significant differences between sub-stages; different symbols (*, +) indicate significant differences among treatments in each sub-stage. T = treatments, S = Substage and N.S. = Not significant.

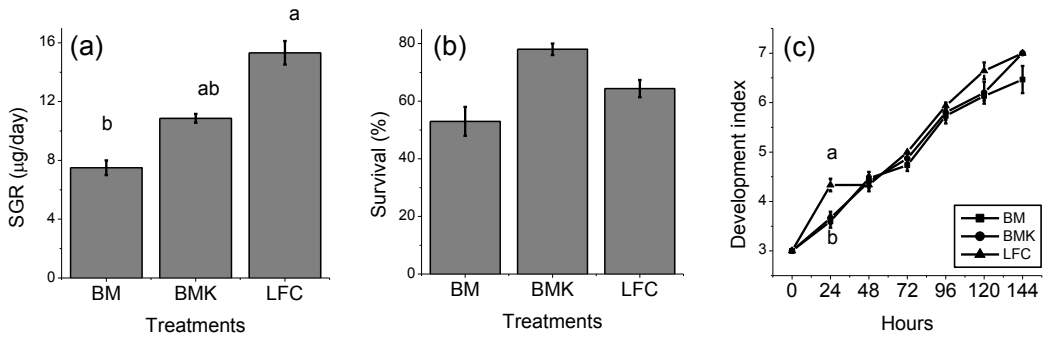

Fig. 2. Specific activity of (a) total proteases, (b) trypsin and (d) chymotrypsin (UI mg protein/g dw) of Litopenaeus vannamei larvae fed microencapsulated diets (BM and BMK) and live food (microalgae and artemia nauplii). Means ± S.E. N= 3 repetitions. Different letters indicate significant differences between sub-stages; different symbols (*, +) indicate significant differences among treatments in each sub-stage. T = treatments, S = Substage and N.S. = Not significant.

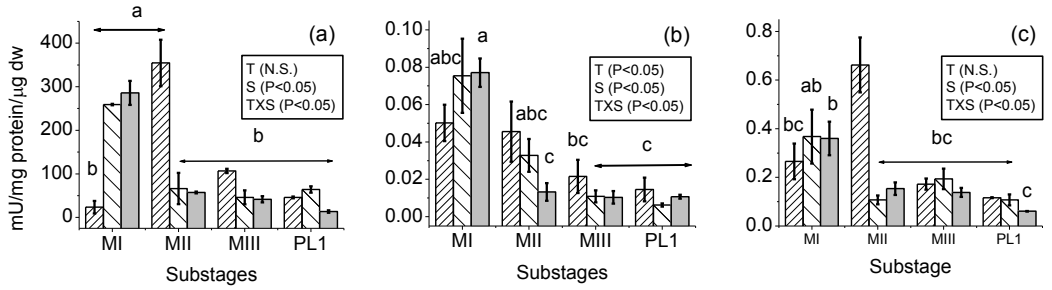

Fig. 3. (a) Total protein, (b) acylglycerides (AG), (c) cholesterol content (%/dw) and (d) AG/cholesterol, (e) AG/protein and (f) cholesterol/protein ratios of Litopenaeus vannamei larvae fed microencapsulated diets (BM and BMK) and live food (microalgae and artemia nauplii). Means ± S.E. N= 3 repetitions. Different letters indicate significant differences between sub-stages; different symbols (*, +) indicate significant differences among treatments in each sub-stage. T = treatments, S = Substage and N.S. = Not significant.