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Substitution of *Artemia* nauplii by an artificial diet for larvae *Litopenaeus schmitti* (Pérez-Farfante and Kensley, 1997)

Sustitución de nauplios de *Artemia* por una dieta artificial para larvas de *Litopenaeus schmitti* (Pérez-Farfante y Kensley, 1997)

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Abstract

The crustaceans larviculture has been based on live food although the production of this food is expensive, it requires of handling specialized and it is not always possible to obtain a uniform quality. The current tendency is toward the search of live foods substitutes, artificial diets, microparticulate or microencapsulated are used. The present study was designed to examine the substitution, at least partial, of *Artemia* nauplii for artificial food in the *Litopenaeus schmitti* larviculture. An artificial food was elaborated using as additive *Spirulina* meal, and the zoothecnical indexes were evaluated: Growth, development index and survival. The chemical score to evaluate the protein quality of the foods is used. In the shrimp Hatchery YAGUACAM, Cienfuegos, Cuba, a randomized design was developed, with five treatments and three replicates, where they were substituted total and partially the rates of *Artemia* nauplii for the dry food. When concluding this study applied different statistical tests that allowed to end up concluding that: The survival was not affected when substituting 100 % of *Artemia* nauplii, the final length behaved in a similar way in all the treatments ($p > 0,05$); when adding microparticulate diet only diminished significant the development index, being only possible to replace until 75 % of the doses of *Artemia* nauplii in the feeding routing ($p < 0,05$). On the other hand, the chemical scores evidenced that the *Artemia* nauplii cover the aminoacids requirements of the larvae in bigger quantity that the microparticulate diet, although the combination of both are in better results.

Keywords: Litopenaeus schmitti, larvae, feeding, *Artemia*, microparticulate diet.

Resumen

El cultivo de las fases larvarias de los crustáceos se ha basado en alimento vivo, aunque la producción de dicho alimento es costosa, requiere de manejo especializado y no siempre es posible obtener una calidad uniforme. La tendencia actual es hacia la búsqueda de sustitutos de alimentos vivos, por dietas artificiales, microparticuladas o microencapsuladas. Por lo que con este estudio se propone la sustitución, al menos parcial, de nauplios de *Artemia*, por alimento microparticulado en la cría larval del camarón blanco del Caribe *Litopenaeus schmitti* (Pérez-Farfante y Kensley, 1997). Se elaboró un alimento artificial recomendado para esta especie, y se determinaron los índices zootécnicos: crecimiento, desarrollo y supervivencia, además se utilizó el cómputo químico para evaluar la calidad proteica de los alimentos utilizados. En el laboratorio del Centro de Producción de Postlarvas de Camarón YAGUACAM, Cienfuegos, Cuba, se desarrolló un diseño experimental completamente aleatorizado, con cinco tratamientos y tres réplicas, donde se sustituyeron total y parcialmente las raciones de *Artemia* por el alimento seco. Al finalizar este estudio se aplicaron diferentes pruebas estadísticas, que permitieron llegar a concluir que: no se afectó la supervivencia al sustituir el 100 % de las raciones de *Artemia*. El largo final de las larvas se comportó de forma similar en todos los tratamientos ($p > 0,05$). Al adicionar solo microparticulado disminuyó de forma significativa el índice de desarrollo, lográndose solamente reemplazar hasta el 75 % de las dosis de nauplios de *Artemia* establecidas en la rutina de alimentación de la especie ($p < 0,05$). Por otro lado, el cómputo químico evidenció que los nauplios de *Artemia* cubren los requerimientos aminoacídicos de las larvas en mayor cuantía que el alimento microparticulado utilizado, aunque la combinación de ambos resulta en mejores resultados.

Palabras clave: *Litopenaeus schmitti*, larvas, alimentación, *Artemia,* dieta microparticulada.

Introduction

Artemia sp. and microalgae species are conventional feed used for the production of shrimp postlarvae, yet important efforts are carried-out to substitute these feeds with microparticulate and microcapsulate artificial diets (Pedroza *et al.*, 1996; Jaime *et al.,* 2006). However, artificial diets are still costly and do not always result in uniform quality of postlarvae (Robinson *et al*., 2005).

There have been studies dealing with the biology and culture of the white shrimp *Litopenaeus schmitti*, as well as the development of efficient feeds for the larval stages of the species (Gelabert *et al*., 1988; Jaime *et al.,* 1996; Jaime y Galindo, 2006; Jaime, 2007). In Cuba, there is currently a need for an artificial diet that could be easily produced using local ingredients and that could be offered either, alone or combined with conventional feeds (Gelabert *et al*., 1988; Jaime *et al.,* 1996; Márquez *et al.*, 1997; Artiles *et al.,* 1999).

Artemia is a highly-priced feed that is increasingly being required for aquaculture operations worldwide. Consequently, there is a need to find substitutes of *Artemia* for the aquaculture of shrimp species (Wouters *et al*., 2004). This study is aimed at evaluating the feasibility of substituting *Artemia* nauplii by artificial microparticulate feed for larvae of the Caribbean white shrimp *Litopenaeus schmitti*, so as preliminary estimating an optimal substitution level from the economic point of view.

Materials and methods

Preparation of feeds

The composition of the microparticulate diet is presented in Table 1. The premix of vitamins and minerals was according to Davis y Arnold (2000) and the proximal composition was analyzed according to AOAC (1995). The dietary ingredients were ground and passed through a 60 µm mesh sieve and homogenized using a domestic blender (Hobart M-600, Hobart Corp., Troy, OH, USA). Oil and water (250 mL/kg) were gradually added during mixing. A meat grinder (Javar 32, JAVAR LTDA, Bogotá, Columbia) was used to pelletize the wet mixture. The pellets were dried in a forced-air oven at 60 °C for 10 h. Pellets were ground and passed through sieves to obtain particulates within 100-200 µm. The microparticulate was packed in plastic bags and refrigerated at 10 °C until use.

Table 1. Ingredients and proximate composition (dry weight percentage) of the experimental diet

Ingredient (%)	
Fish meal[1]	30,0
Soybean meal[1]	13,0
Shrimp meal[2]	15,0
Squid meal[1]	16,0
Spirulina meal[3]	5,0
Sunflower oil[1]	5,5
Fish oil[1]	5,6
Vitamin – mineral premix[4]	2,5
C vitamin	0,5
Cholesterol	0,5
Soy lecithin	1,0
Cholide chlorine	0,2
Grenetine (binder)	5,0
Proximate composition (%)	
Protein	57,00 ± 0,30
Ether extract	13,53 ± 0,04
Ash	9,83 ± 0,06
Crude fiber	2,55 ± 0,02
NFE	17,09 ± 0,43
Moisture	5,28 ± 0,05
Energy (cal g^{-1})	4 935,65 ± 5,23

[1]PIASA, La Paz, BCS. México.
[2] Shrimp whole meal (*Litopenaeus vannamei*) prepared in laboratory.
[3] *Spirulina platensis*. GENIX, La Habana, Cuba.
[4] Formula of the Union of Companies feeds MINAGRI. Cuba, for commercial feeds for shrimp culture. One metric ton of premix. Retinol 12.500 UI; Thiamine 10.000 mg; Riboflavin 20.000 mg; Pyridoxine 10.000 mg; Cianocobalamin 40,0 mg; Ascorbic acid 500.000 mg; Cholecalciferol 2.400.000 UI; DL-α tocoferol 100.000 mg; Menadione 4.000 mg; Pantothenic acid 40.000 mg; Choline chloride 1.600 mg; Folic acid 2.000 mg; Nicotinic acid, 140.000 mg; Biotin 1.000 mg; Inositol 300.000 mg; Paraminobenzoic acid 35.000 mg; Cobalt 200 mg; Copper 2.000 mg; Iron 20.000 mg; Iodine 1.500 mg; Manganese 40.000 mg; Zinc 20.000 mg; Selenium 100,0 mg.

Cysts of *Artemia franciscana* (INVE, Utah, USA) were incubated for 24 h using continuous illumination and vigorous aeration. Temperature was controlled at 27 ± 1°C and salinity was 35 ups. The proximal composition and the amino acidic profile of *Artemia* nauplii are presented in Table 2.

TABLE 2. Chemical and essential amino acid composition of newly hatched *Artemia* nauplii

Chemical composition (%)											
Crude protein		Ether extract		Crude fiber		Nitrogen-free extract				Ash	
52,2		18,9		14,8		14,8				9,7	
Essential amino acid composition*											
Arg	Cys	Met	Thr	Ile	Leu	Lys	Val	Trp	Tyr	Phe	His
7,3	0,6	1,3	2,5	3,8	8,9	8,9	4,7	1,5	5,4	4,7	1,9

* As percentage of feed org./100 g of total recovered amino acids: Arginine-Arg; Cysteine-Cys; Metionine-Met; Threonine-Thr; Isoleucine-Ile; Leucine-Leu; Lysine-Lys; Valine-Val; Tyrosine-Tyr; Tryptophan-Trp; Phenylalanine-Phe; Histidine-His. (Tacon, 1989)

Chemical scores were also determined according to García (1993), where amino acid composition of *L. schmitti* postlarvae served as reference (Gallardo *et al.*, 1989).

Larvae (protozoea III) from a commercial hatchery were cultivated to the postlarval stage in 15 L plastic tanks at an initial stocking rate of 100 individuals L^{-1}. Five levels of substitution of *Artemia* with microparticulate diet were used following the protocol in TABLE 3. Triplicate sets of plastic tanks were used for each substitution level.

TABLE 3. Feeding protocol used for the experimental trial

Substitution (%)	Feeding schedule (h)											
	AM						PM					
	2	4	6	8	10	12	2	4	6	8	10	12
0	A	A	A	A	A	A	A	A	A	A	A	A
25	A	M	A	A	A	M	A	A	A	M	A	A
50	A	M	A	M	A	M	A	M	A	M	A	M
75	M	A	M	M	M	A	M	M	M	A	M	M
100	M	M	M	M	M	M	M	M	M	M	M	M

A: *Artemia* nauplii (2 nauplii/mL) M: Microparticulate feed (1g/m^3)

Seawater (35,0 ± 0,00 ups salinity) was passed through sand (10 μm) and cartridge (5 μm) filters and a UV light unit before entering the tanks. Seawater was replaced daily at 30 % of volume. Temperature was maintained at 28,1 ± 0,25 °C, pH 8,05 ± 0,15, and constant aeration with air stone diffusers to maintain dissolved oxygen at 6,6 ± 0,65 mgL^{-1}. Ammonium reached values of 0,015 ± 0,008 mgL^{-1}. Water temperature and dissolved oxygen were monitored twice a day using an oxygen meter (YSI-58). Salinity was recorded with a refractmeter (Atago 2401) and pH was measured with a pH meter (UC-12) on a weekly basis. Ammonium concentration was determined using a photometer SQ 118 (MERCK®).

Data collection and analysis

Growth of larvae was determined by measuring length (n = 300 for each tank) and the development index

(n = 100 for each tank). (Villegas and Kanazawa, 1979) Growth, survival and development index data were tested for normality and homogeneity of variance using the Kolmogorov-Smirnov and Bartlett tests. One-way ANOVA was used to determine the effect of substitution level on the response parameters and the Duncan's multiple rank test was used to separate mean values. Significance was set at $p < 0,05$.

A cost-benefit analysis was conducted to preliminary estimate an optimal substitution level. For this an initial population of 2 million *L. schmitti* nauplii was assumed for every case. For projection of costs, income and net benefit, 5-6 additional days of culture period were assumed to reach postlarval stage 5. Costs were 45 USD per kilogram of *Artemia* cysts and 27 USD per kilogram of microparticulate feed. One gram of cysts was assumed to yield 230,000 cysts at a hatching rate of 85 %.

Results

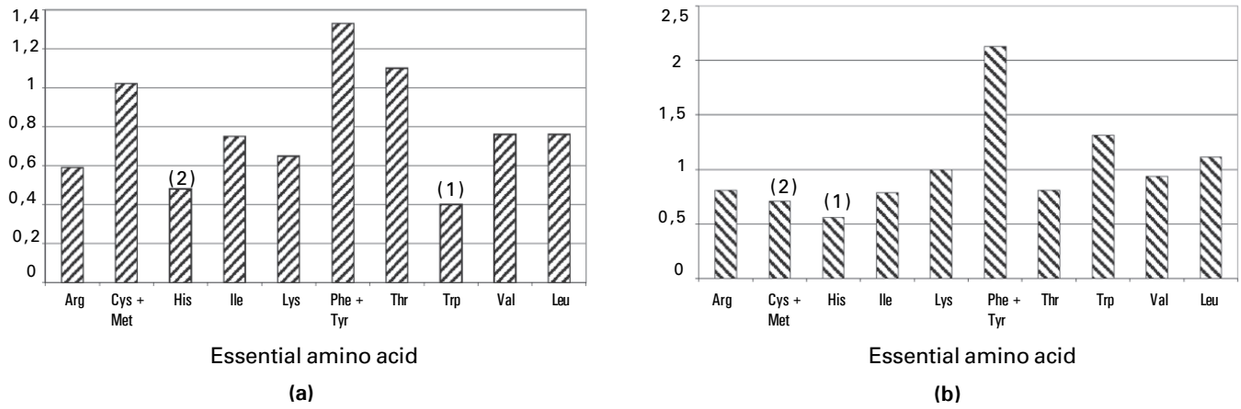
There were no significant differences between the levels of substitution of *Artemia* by microparticulate feed in terms of final larvae length and survival. Length ranged from 3,67-3,75 mm and survival averaged 70 % (Table 4). The development index varied from 6,69-6,83 among 0, 25, 50, and 75 % substitution levels, yet no significant differences were determined. Using the 100 % substitution level resulted in a significant lower development index (6.15). Net benefit ranged from 4 765-5 116 USD and the substitution level that yielded the maximum economic net benefit was estimated to occur at 80 %.

Table 4. Mean (± SE) final length, survival and development index of *Litopenaeus schmitti* larvae fed different substitution levels of *Artemia* nauplii by microparticulate feed

	Substitution (%)				
	0	**25**	**50**	**75**	**100**
Length (mm)	3,67 ± 0,08[a]	3,74 ± 0,08[a]	3,68 0,09[a]	3,71 ± 0,08[a]	3,75 ± 0,11[a]
Survival (%)	71,7 ± 15,27[a]	68 ± 7,64[a]	70 ± 8,66[a]	70 ± 10,00[a]	68 ± 2,89[a]
Development index	6,81 ± 0,06[a]	6,79 ± 0,11[a]	6,86 0,06[a]	6,69 ± 0,14[a]	6,15 ± 0,13[b]
Cost	497,3	380,0	263,2	146,2	34,2
Income	5 262,0	5 262,0	5 262,0	5 262,0	4 900,0
Net benefit	4 765,011	4 882,311	4 999,111	5 116,111	4 865,8

Note: Means with different superscripts indicate significant differences ($p < 0,05$) for the same line. Cost, income and net benefit are in USD and are calculated assuming a common survival rate for treatments where there were no significant differences in survival.

Chemical score results show that all amino acids are represented in the composition of *L. schmitti* postlarvae, although it is important to indicate that tryptophan and histidine appear as the first and second restrictive EAA respectively (Figure 1a). In the same Figure 1b are shown that the amino acid profile of *Artemia* nauplii, approaches to the nutritional necessities of *L. schmitt*i, however histidine and cysteine+metionine were restrictive.



(1) First limiting amino acid

(2) Second limiting amino acid

Figure 1 Chemical score of the micropartculate diet (a) and newly hatched *Artemia* nauplii (b).

Discussion

The results in this study indicate that it is feasible to substitute *Artemia* nauplii by artificial microparticulate feed for *L. schmitti* larvae. There was no evidence of final length and survival being negatively affected despite *Artemia* was totally substituted and, up to 80 % substitution, there was no evidence that using the microparticulate feed resulted in diminished rate of development.

Sizes similar to those observed in this study have been reported for postlavae when were fed with *Artemia* nauplii (3,71 mm, Jaime *et al*., 2005). These sizes are larger that the 3,4-3,44 mm reported by Márquez (1997) for the species when using microparticulate diets in conjunction with microalgae and *Artemia*. Other authors working with *L. schmitti* have neither found significant differences in postlarvae size when using artificial diets, even when these were enriched with *Chlorella vulgaris* (Artiles *et al*., 1999). However, studies in other shrimp species have shown that better larval growth can be obtained when live feeds (particularly *Artemia*) are offered, either alone or in combination with microparticulate diets (e.g. Arellano *et al*. 1993 with *Litopenaeus vannamei*, Gaxiola *et al*., 2002 with *Litopenaeus setiferus* and Robinson *et al*., 2005 with *Farfantepenaeus aztecus*).

Artificial diets, when have been used in combination with live feed; generally promote higher survival of shrimp larvae. (Subasinghe, 1995) Survival rates as high as 90 % have been reported for larval stages (Zoea to postlarvae) of *Penaeus monodon* when used flakes as substitute of live feed (Wouters *et al*., 2004). Gaxiola *et al*. (2002) found that 40 % substitution of microalgae by microparticulate feed results in significantly increased survival of *L. setiferus* larvae. However, it has been observed that total replacement of live feed produces a significant reduction in survival of *F. aztecus* larvae (Robinson *et al*., 2005). In our investigation, no significant evidence was found that total replacement of *Artemia* provoked lower survival, probably as a consequence of the nutritional value of the artificial diets used. Survival rates to the postlarval stage higher than 80 % have been observed for *L. schmitti* using the diet composition employed in this investigation (Jaime *et al*., 2005).

Artemia promotes larval growth of shrimp species (Gelabert, 1994). Ingestion, digestion and assimilation of

artificial feeds by marine species are enhanced when *Artemia* is incorporated as part of the diet (Kolkovski and Tandler, 1995). *Artemia* possibly stimulates the production of digestive enzymes, thus making protein in artificial diets more digestible (Jones 1998). In this study, the benefit of offering *Artemia* was evidenced in terms of larval development. When *Artemia* was totally excluded from the feeding regime, there was a significant reduction in the development index and only 15 % of the larvae reached the postlarval stage. At least three rations per day of *Artemia* were necessary to observe no significant differences in the development index. Delays of 1,5-2 days in larval development of *Fenneropenaeus indicus* have been observed when fed microcapsulate diets, as compared to the development obtained when using *Artemia* nauplii (Kumlu and Jones, 1995).

In this study, total exclusion of *Artemia* from the feeding regime resulted in a delay in larval development. A longer culture period was necessary to reach a marketable postlarval stage which, in turn, resulted in increased costs, lower survival and monetary income. Consequently, it was estimated that 80 % substitution was optimal from the economic point of view. However, it must be noted that natural feeds contribute to improve larvae and postlarvae quality (Subasinghe, 1995). Future studies must be conducted to validate the economic projection in this investigation and to evaluate the performance of postlarvae during the nursery and grow out stages, in terms of stress and diseases resistance, growth, survival and others.

We found that tryptophan and histidine were the limiting amino acids in the microparticulate diet. Thryptophan and arginine have been determined, respectively, as the first and second limiting amino acids in purified microparticulate diets for protozoea and mysis of *L. setiferus* and *L. vannamei* (Gaxiola *et al*., 2002; Cuzon *et al.*, 2004). The authors consider deficiencies in thryptophan as the main cause of lower growth and survival of the larvae when compared to the results obtained using live feed. In this study, the protein content of *Artemia* and the artificial diet was similar (52,8 and 57,0 %, respectively). Yet the amino acidic profile of *Artemia* more closely approximated the amino acid requirements of *L. schmitti*. Consequently, thryptophan can be considered the main limiting factor causing the significant reduction in the development index observed when *Artemia* was totally excluded from the feeding regime.

Environmental variables are inside the ranges recommended for *L. schmitti* larviculture according to Vega and De la Cruz (1988), although other authors recommend salinity values of 20 ups, during *L. schmitti* larviculture (Yakov *et al*. 2004).

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