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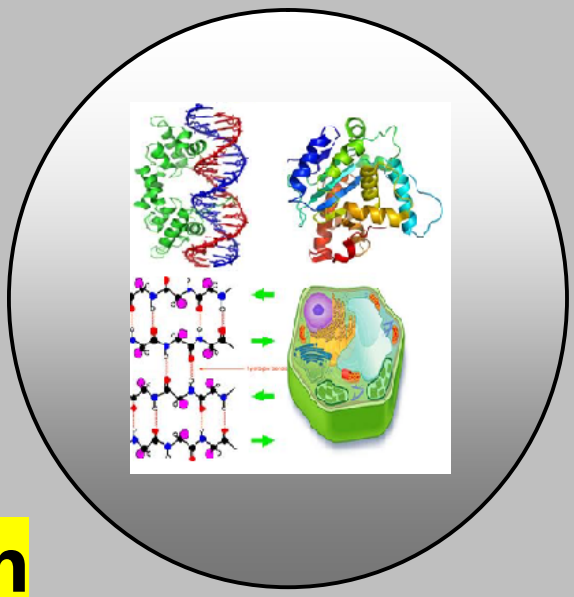

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RESEARCH PAPER

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Effect of *Spirulina platensis* Meal as Feed Additive on Growth, Survival and Development in Tropical Penaeid Prawn *Penaeus indicus* Larvae

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ABSTRACT

To evaluate the effect of Spirulina platensis inclusion in micro diets for white shrimp Penaeus indicus larvae, a completely randomized experimental design was developed with 4 treatments and three replicates. Diets with 3 inclusion levels (0, 2.5 and 5%) were prepared. A control was fed Artemia nauplii. Larvae were fed for 120 hours from mysis I to post larvae 1 every 4 h, and water quality parameters were measured daily. At the end of the experiment survival was determined, and the development index of the larvae was calculated. The chemical score of the diet was also determined, using the P. indicus post larvae amino-acid pattern as reference. Tryptophan and hystidine were the first and second limiting amino acids in the diets. Survival was around 80% for all treatments. When compared to the control, final post larval size was significantly smaller. However, the development index (DI) showed that larvae fed diets containing 5% Spirulina platensis meal was superior to the rest of the diets and similar to the Artemia nauplii (p<0.05).

Key words: Shrimp culture, larvae, feed additive, Penaeus indicu , and Spirulina platensis.

INTRODUCTION

Changes in abundance and distribution of wild penaeid postlarvae and the increasing world market demand for prawns has led to rapid development of commercial larval breeding systems for penaeids (Hudinaga and Miyamura, 1962).

Penaeid shrimp hatcheries are highly dependent on live phytoplankton and zooplankton cultures, which are costly and labor intensive (Robinson *et al*., 2005). However, recent advances in formulation and microparticulate diet technology will soon permit the completion of the larval cycle exclusively on inert diets (Le Moullac *et al*., 1987; Kanazawa 1990; Wouters *et al*., 2004). The search of efficient feeds for the white shrimp larvae is necessary in order to increase postlarvae quality and culture profitability. The dependence on *Artemia* nauplii for production is a concern for commercial hatcheries due to the possibility of a global shortage and the rising cost of *Artemia* cyst, as well as the possible variations in nutritional value and the risk of disease transfer. Over 80% of hatcheries reported decreasing *Artemia* cyst utilization in the last few years as a result of these concerns (Wouters *et al*., 2003). One solution to this problem is to develop a practical and economical formulation capable of to satisfying the nutritional requirement of the different larval stages. In India, commercial postlarvae production of *Penaeus indicus* still depends on live food such as *Artemia* cysts and microalgae (*Chaetoceros* spp. and *Tetraselmis* spp.), as well as on imported microparticulated feeds in order to guarantee the post larvae demands from the shrimp farms. As feed additives, dried algae improve growth, feed efficiency, carcass quality, and physiological response to stress and disease in several species of fish (Mustafa and Nakagawa, 1995). Experimental formulations for white prawn larvae have also been successful (Jaime *et al*., 1996; Márquez 1997; Artiles *et al*., 1999). *Spirulina* is considered a rich source of protein, vitamins, minerals, essential amino acids, and fatty acids (gamma - linolenic acid (GLA), and antioxidant pigments, such as carotenoids (Belay *et al*., 1996). In addition, it is effective as an immunomodulator (Takeuchi *et al*., 2002). Several studies have been conducted using dried *Spirulina* as a feed supplement (Chow and Woo, 1990; Watanabe *et al*., 1990), and *Spirulina platensis,* which is commercially produced, has been considered for partial substitution of microalgae in the feeding of tropical penaeid prawn *Penaeus indicus* protozoeas (Jaime, *et al*., 2004). The present study evaluates the effect of *Spirulina platensis* meal, as feed additive, on body length, survival and development index in *P.indicus larvae*.

MATERIAL AND METHODS

Three artificial diets (0, 2.5 and 5% of *Spirulina* meal) and a control (*Artemia* fed at 5 nauplii. ml-1) were tested in a totally randomized design during 120 hours, using *Penaeus indicus* (Pérez-Farfante and Kensley, 1997) larvae obtained from a commercial rearing tank of the laboratory of postlarvae production. Mysis I larvae were stocked at a density of 120 organisms per liter, in 12 fiber-glass tanks, using three replicates per diet, with 100 liters of filtered (5 µm) and uv sterilized seawater at 35 ups. Constant aeration was provided via air stones using a 5 hp turbo blower. Na_2 EDTA was added to the water at 10 mg.l-1 (Lawrence *et al*., 1981). To eliminate faecal residues and food remains, a 50% water exchange was done daily. Constant illumination was provided with fluorescent lights, temperature (ºC), oxygen (mg.l- 1), salinity (psu), pH and ammonium (µg.l-1) were registered daily before the water exchange.

Three microparticulated diets were elaborated (Table 1), using the MIP-CIP 2 formulation evaluated by Artiles *et al*., (1999) as reference. Particle size ranged from 100 to 250 µm. Feeding was carried out every 4 hours (06.00, 10.00, 14.00, 18.00, 22. 00 and 02.00 hours) at a rate of 0.3 mg/larvae/day of food. Proximal composition of diets was determined according to the procedures of AOAC (1995). Total pigments were determined using the trichromatic method (Strickland and Parson, 1972). The chemical score of the experimental diets was carried out using the amino acid composition of *P.indicus* postlarvae (Gallardo *et al*., 1989) as reference. Survival was determined using the volumetric method, taking five 100 ml samples per tank daily. The number of mysis and postlavae in each sample was determined in order to calculate the development index (DI) following Villegas and Kanazawa (1979). Larval growth was determined at the end of the study. Using an optical microscope, 40 animals were measured per tank from the tip of the rostrum the end of the tail, excluding the thorns. ANOVA and Duncan's multiple range tests were used to analyze the final data after using Kolmogorov-Smirnov and Bartlett tests to confirm homoscedasticity (Sigarroa, 1985).

RESULTS AND DISCUSSION

Water quality parameters were maintained within the range recommended by Vega and de la Cruz (1988) for the species. Dissolved oxygen was 7.25 $\pm$ 0.25 mg.l-1, temperature was maintained at 27.6 $\pm$ 1°C, and pH was 8.05 $\pm$ 0.25. NH_4-N had a range between 328.1- 736.3 µg.l-1. Mean body length, survival and the DI are presented in Table 2. Larvae fed the *Artemia* nauplii control were significantly bigger ($p<0.05$). There were no significant differences between treatments for larval size when fed artificial diets. The results are similar those of Artiles *et al*., (1999). Jones (1998) indicated that only when a formulated artificial diet is accepted, ingested and assimilated at rates comparable to live feeds will it be possible to investigate specific nutritional requirements. Consequently, the size, shape, attractiveness and nutritional content of an artificial diet must be optimized so that rates of ingestion result in growths and survivals similar to those achieved with live feeds. In the present study, the results when feeding artificial diets resulted in similar rates of survival compared to the control. Kumlu and Jones (1995) reported similar survivals for *Farfantepenaeus indicus* when feeding microparticulated diets. In this case, mortality was probably associated to handling during the water exchange process. This is in agreement with Alfonso (1996), who stated that, when the quality of food is good, the survival is determined by manipulation. Arellano *et al*., (1993) feeding *Litopenaeus vannamei* mysis with microparticulated diets, achieved smaller growth rates and survival that when they combined the artificial diet with *Artemia* nauplii, suggesting that these constitute an important source of nourishment in this phase. However, Kurmaly *et al.,* (1989) achieved the metamorphosis from MII - PL1 of *Penaeus monodon*, feeding a microencapsulated diet rich in high quality proteins and lipids demonstrating the possibility to completely replace live food during the larval development with artificial rations, whenever these meet the nutritional requirements for the species. Márquez (1997) testing diets with different levels of torula yeast

(*Sacharomyces cereviseae*) with *L. schmitti* achieved similar final postlarvae sizes to the ones obtained in this work. The growth results indicate that supplementation of *S. platensis* meal did not favor an increment in size of the shrimp. Jaime *et al.,* (1996) evaluating different commercial microparticules for *L. schmitti* larvae found that better growth rates were obtained with diets containing 17% lipids, when compared with experimental diets containing 8.6% lipids. Artiles *et al.,* (1999), on the other hand, obtained the best growth results when combining *Artemia* nauplii with a microdiet containing 14% lipids. This formula was used in this work as control. The results show that, for the formulated diets, the inclusion of *S. platensis* did not affect survival or final size. Kumlu and Jones (1995) reported delays of up to 2 days in larval development, of *Fenneropenaeus indicus* when fed with microencapsulated diets. On the other hand, Artiles *et al.,* (1999) and Márquez (1997) report that *L. schmitti* reach the PL1 stage after 120 hours when fed with a combination of microparticulated diet and *Artemia* nauplii. The time of development was similar for this trial. However, the rate of metamorphosis, expressed through the development index (DI) was statistically different for the four treatments, indicating that the concentration of *Spirulina platensis* influenced development, and that an inclusion rate of 5%, DI was not statistically different to that of the *Artemia* control. Further studies should incorporate higher inclusion levels of *S. platensis* in the diet so as to assess the potential to increase final size, and the potential for partial substitutions of the *Artemia* nauplii. The determination of nutrient essentiality to larval penaeid shrimp is complicated by a life cycle consisting of a series of lanktotrophic stages resulting in substantial trophic level changes (Jones *et al*., 1993). Cuzon (2004) states that there is a relationship between whole body composition, in terms of essential amino acids for *Penaeus monodon* juveniles, and its protein requirement. So, we suggest that the chemical score can be considered an index to evaluate the protein quality of feeds for aquatic species. Fig. 1 shows that the formulations of the experimental diets are similar in terms of amino acid balance. However, the chemical score shows that the experimental diets are limiting for several amino acids, when compared with *L. schmitti* postlarvae. Hysitidine and Thriptophan are the first limiting amino acids. This is similar to the report of Gaxiola *et al*., (2002) for *Litopenaeus setiferus* and *Litopenaeus vannamei*. The reduction in final size could be related to this, and further research should evaluate microparticulated diets that supply all of the essential amino acids. Table 1 shows the level of total pigments in the diets. It appears that as the level of *S. platensis* increased in the diet and the pigment concentration increases, the physiological and metabolic responses of *L. schmitti* larvae improves. Several authors mention that this microalgae has an important role in metabolic, antioxidant, respiratory and immunological roles of aquatic organisms (Mustafa *et al*., 1994; Miyasaki *et al.,* 1995; Gabaudan 1998; Takeuchi *et al*., 2002). This is in agreement with the report by Meyer (2000) who suggests that diets for aquatic larvae must be formulated to provide suitable amounts of supplemental carotenoids, specifically astaxanthin, which cannot be synthesized *"the novo",* but depends on the assimilation of a precursor Ringelberg (1980).

It is important to indicate that the use of microparticles for shrimp larvae production would represent a significant decline in production costs, particularly due to the reduction or elimination of areas required for supporting cultures, such as microalgae and *Artemia*. This use could also provide advantages in terms of disease and water quality control, and the possibility of recycling the water, further improving the operational and practical efficiency of the culture (Robinson 2005).

CONCLUSION

The development index showed that *Litopenaeus schmitti* larvae fed diets containing 5% *S. platensis* meal produced results similar to those obtained by feeding *Artemia* nauplii. Final length was significantly smaller when feeding microparticles, which may be related to nutritional deficiencies, particularly associated to essential amino acids. Further improvement in formulation and management of rearing tanks should lead to the full replacement of live food by inert particles in commercial hatcheries for the species.

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