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Original Article

Effects of different levels of live food replacement with microdiet on growth factors, survival and resistance to salinity stress of Indian white shrimp post-larvae (*Fenneropenaeus indicus*)

Seyed Hossein Hoseinifar*, Parviz Zare

Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Abstract: In this study, partial and complete replacement of live food (*Artemia* nauplii) with a microdiet was investigated in post-larval Indian white shrimp, *Fenneropenaeus indicus*. Post-larvae (PL_1) were stocked into nine 20-L vessels (5-L water volume) at a density of 50 L^{-1}. Shrimp were fed six times a day over 10 days. At the end of the feeding period, there was no significant difference in quality index (stress-test survival) among treatments. Statistical analysis of results showed that partial or complete replacement of live food with microdiet significantly decreased survival, total length, carapace length, weight, growth rate, performance index, and the number of spines in upper rostrum of the post-larvae. However, substitution of live food with microdiet had no negative effects on resistance to salinity stress. These results showed that the commercial microdiet used in this study is not a good replacement of live food; further studies are required to determine the nutritional requirements of Indian white shrimp larvae and post-larvae before microdiets can be utilized effectively.

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Introduction

Crustacean larval stage is considered as a critical stage in their life history. Success of larval rearing depends mainly on the availability of suitable diets that are readily consumed, efficiently digested and that provide the required nutrients to support good growth and health (Giri et al., 2002). Larval diets are typically composed of different species of microalgae and newly hatched *Artemia* nauplii (Robinson, 2005). While supplying live food is difficult and requires considerable space and expense, microdiets are easier to maintain and usually have lower production costs (Jones et al., 1993; Person-Le Ruyet et al., 1993; Wang et al., 2005). Sorgeloos and Leger (1992) described finfish and crustacean larviculture diets for the first feeding stages as being the major bottleneck for complete replacement of live feeds. The development of cost-effective and nutritionally complete formulated larval diets would be of tremendous benefit to commercial hatchery operations (Robinson et al., 2005). The possibility of replacing live food with formulated diets has been investigated in several studies (Kanazawa, 1982; Kanazawa, 1985; Jones et al., 1989; Jones et al., 1997; Samocha et al., 1999; Gallardo et al., 2002; Wouters et al., 2003; Robinson et al., 2005; Tang et al., 2010).

Microparticulate foods are in various forms (e.g. microencapsulated, microbound and microcoated) have yielded some degree of success in commercial hatcheries as partial replacements for live foods (Langdon et al., 1985) but have deleterious effects deterioration on water quality (Muir et al., 1991). Nutrients leaching from microparticulated foods can

* Corresponding author: Seyed Hossein Hoseinifar
E-mail address: hoseinifar@gau.ac.ir
Tel: +98 1716622251

affect growth and survival of shrimp post-larvae and depredate the water quality via proliferation of bacteria (Jones et al., 1987; Muir et al., 1991).

Growth and survival data are powerful tools for understanding the effects of both live and formulated diets on first-feeding larvae (Cannavate and Fernandez-Diaz, 1999). Therefore, in the present study, growth, survival and resistance to salinity stress were used to evaluate the effects of partial and complete replacement of *Artemia* nauplii with commercial microdiet on Indian white shrimp (*Fenneropenaeus indicus*) post-larvae.

Materials and methods

Source of shrimp and stocking: This study was conducted at the Abziparavar Chabahar hatchery, Chabahar. Larvae were obtained from three eyestalk-ablated spawners (47.6, 37.16, 45.2 g). Shrimp nauplii were kept in spawning tanks (1000 l) containing natural sea water (37 g l^{-1} salinity, 30.5 ^{o}C, 8.1-8.2 pH) supplemented with a mixture of the microalgae *Chaetoceros* and *Tetraselmis*, which was added daily at a rate of 2×10^{6} cells ml^{-1} (Ziaei Nejad et al., 2006). Beginning at post larval stage 1 (PL_1), shrimp post-larvae were harvested and stocked into nine 20-L vessels (5-L water volume) at a density of 50 individual L^{-1}.

Feeding trial: Three treatments, each with varying levels of live food substitution [0% (Treatment *a*), 50% (Treatment *b*) and 100% (Treatment *c*)] with commercial Microdiet (Royal™ (BernAqua BVBA, Belgium; with particle size of 100-200 μ; Table 1) were used with three replicates. Indian white shrimp post-larvae were fed with experimental treatment from PL_1 to PL_{11} stage. Post-larvae in Treatment *a* were fed with newly hatched *Artemia franciscana* nauplii at a rate of 15 nauplii for each post larvae, 6 times a day. Along with post-larvae growth the number of nauplii was increased to 25 nauplii per post-larvae (Ziaei Nejad et al., 2006). To evaluate the effects of complete replacement of live food post-larvae fed with microdiet according to feeding schedule suggested by Shakoori (2005) (Table 2). In Treatment *c* this amount divided by half and live food was used in combination with microdiet.

Table 1. Nutritional profile of the commercial Microdiet Royal™ (BernAqua bvba, Belgium) according to manufactures.

Ingredient	Approximate amount	
Crude protein	50%	
Crude fat	15%	
Crude fiber	2%	
Ash	20%	
Calcium	2%	
Phosphorous	1.5%	
Vitamin A	20000	IU/KG
Vitamin D_3	4000	IU/KG
Vitamin E	400	IU/KG
Vitamin C	1100	ppm
HUFA	25	mg/gr
DHA	12	mg/gr
EPA	8	mg/gr

Indices: At the beginning and the end of trial 20 specimens were sampled randomly from each vessel to determine the following growth factors: carapace length, total length, and number of spines over the rostrum. Survival was calculated on the basis of the final number of post-larvae (PL_{11}) in relation to the initial number of post-larvae (PL_1). The final number of post-larvae was obtained by counting all individuals in each treatment. Specific growth rate (SGR) was determined using the following:

$$SGR = \frac{(\ln W_t - \ln W_0)}{t} \times 100$$

Where t is the culture period in days, $\ln W_0$ is the natural logarithm of the weight (g) of the shrimp at beginning of the experiment, and $\ln W_t$ is the natural logarithm of the weight (g) of the shrimp at day t.

Quality index (QI) was calculated from surviving post-larvae (%) after an acute salinity challenge (from 37 to 15 g L^{-1}) (Gallardo et al., 2002):

$$QI = \frac{(PLa)}{(PLb)} \times 100$$

Where *PL*a is the number of surviving post-larvae (%) after the sudden change of salinity and *PLb* is the total number of post-larvae before the sudden change of salinity.

Table 2. Feeding schedule of Indian white shrimp during the experiment (Shakoori, 2005).

Stage	Pl_1	Pl_2	Pl_3	Pl_4	Pl_5	Pl_6	Pl_7	Pl_8	Pl_9	Pl_{10}	Pl_{11}
Feeding amount (mg MD l^{-1} Day^{-1})	3	6	9	12	15	18	21	24	27	30	33

MD= microdiet

Table 3. Growth factors of *Fenneropenaeus indicus* post-larvae fed different level of live food replacement with microdiet.

	Total Length (mm)	Carapace Length (mm)	Weight (mgr)	W/CL ratio	SGR (mm day^{-1})
Live feed	12.76 ± 0.82^a	2.58 ± 0.18^a	11.7 ± 1.3^a	0.40 ± 0.09^a	0.88 ± 0.09^a
100% replacement	10.35 ± 1.00^c	2.29 ± 0.20^c	6.1 ± 1.0^b	0.26 ± 0.04^b	0.54 ± 0.06^b
50% replacement	11.04 ± 0.73^b	2.46 ± 0.14^b	7.8 ± 0.2^b	0.31 ± 0.02^b	0.61 ± 0.03^b

Values (mean ± SE) in the same column not sharing a common superscript are significantly different (P<0.05).

The performance index (PI) was calculated according to Gallardo et al. (1995):

PI = [Growth ×Survival ×Ql]

Statistical analysis: All statistical analyses were conducted using SPSS statistical package version 10.0 (SPSS Inc., Chicago, IL, USA). Data were examined for normality and homogeneity of variance, and were subjected to a one-way analysis of variance (ANOVA). When significant differences were observed, Duncan's multiple range tests were performed (Zar, 1994). Mean values were considered significantly different at P<0.05. Data are expressed as mean values ± SD.

Results

Growth and development: Measurement of growth factors showed that post-larvae fed live food had significantly higher weight, carapace length, SGR and total length than both other treatments (P<0.05) (Table 3). There was no significant difference between growth factor of post-larvae in treatment *b* or *c* (P>0.05)

Survival, quality index and performance index: Survival data analysis showed that feeding with microdiet significantly decreased survival but the survival of shrimp fed 50% or 100% microdiet were not significantly different compared to the other (Fig. 1).

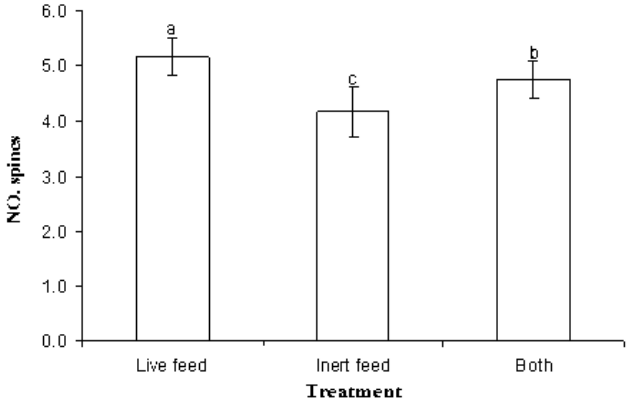

Figure 1. The number of spines in upper rostrum of the post-larvae of *Fenneropenaeus indicus* fed with different treatment (P<0.05).

Quality index calculation demonstrated that post-larvae fed with different dietary treatments had same resistance to salinity stress (Fig. 2). Shrimp fed on live food had significantly higher performance index but no differences were observed between the 50% and 100% groups (Table 1).

Discussion

Brine shrimp nauplii are widely used in hatcheries throughout the world. The annual available brine shrimp cysts were 800 metric tons, or 40%, of the total demand from aquaculture (Li, 2003).

Fluctuations in the supply of cysts have resulted in unstable prices and higher production costs for the hatchery (Sorgeloos et al., 2001). In response to the limitations of supplying live food for early stages of shrimp culture several attempts have been made to reduce dependency of hatchery to live food and varying levels of success achieved in these trials (Kanazawa, 1982; Kanazawa, 1985; Jones et al., 1989; Jones et al., 1997; Samocha et al., 1999; Gallardo et al., 2002; Wouters et al., 2003; Robinson et al., 2005; Tang et al., 2010).

The result of our study showed that complete (100%) or partial (50%) replacement of *Artemia* nauplii with a commercial microdiet decreased growth and performance index of Indian white shrimp post-larvae. Similar result was reported by Robinson (2005) who replaced live food with Zeigier^{TM} Microbound diets in *Farfantepenaeus aztecus* post-larvae culture. Moreover replacement of live food with crumbled experimental microbound diets in Zoea-Mysis stages of *Litopenaeus setiferus* (Gallardo et al., 2002) or with microencapsulated diet FRIPPAK® in *P. monodon* Zoea-PL culture (Wouters et al., 2003) was not successful and resulted in reduction weight gain. One possible reason of growth reduction is due to nitrogenous products including ammonium, nitrite and nitrate which are toxic to several marine organisms at extremely low concentrations (Spotte, 1979). Robinson et al. (2005) found that sub-lethal ammonia–nitrogen concentrations were detected within a short time after feeding (24 h).

In contrast, feeding of *L. vannamei* post-larvae with low level crumbled microbound diet FRIPPAK® had no negative effect on growth (Wouters et al., 2003). They assessed varying replacement levels of live food with crumbled microbound diet for *Litopenaeus vannamei* mysis and post-larvae and observed that replacement levels below 25% did not impact growth performance.

The result of the present study showed that 50% or 100% replacement of *Artemia* nauplii with microdiet resulted in lower survival. Similar result was reported in several studies (Gallardo et al., 2002;

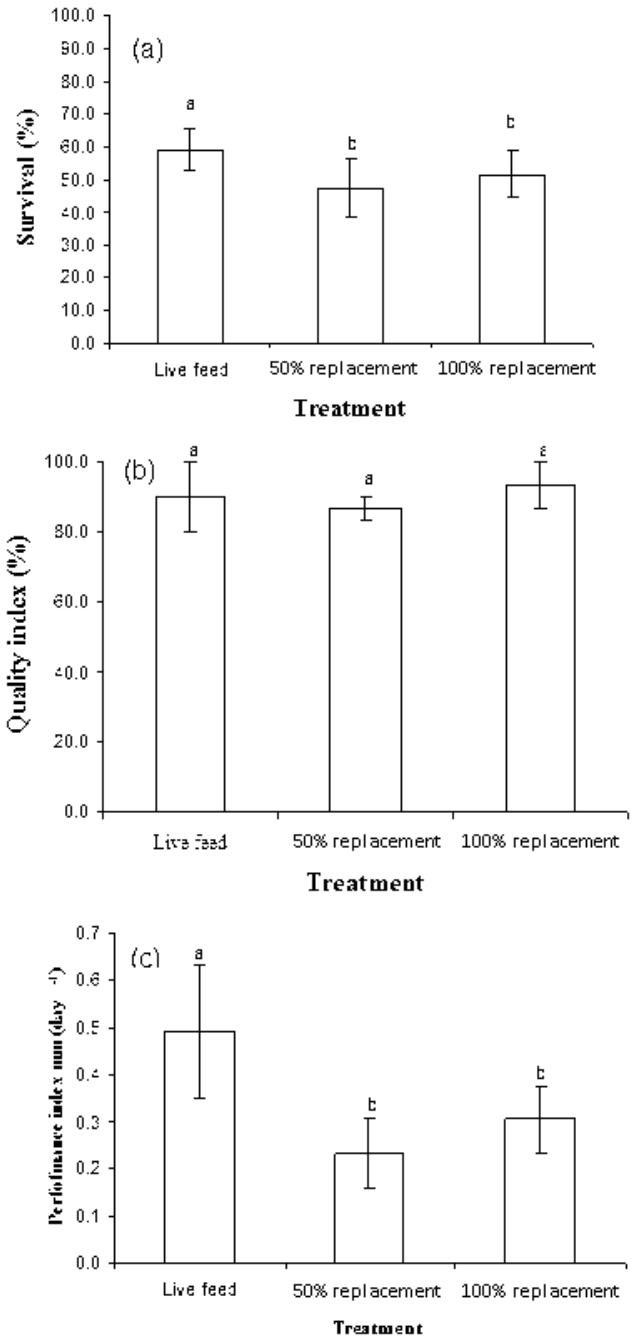

Figure 2. Survival (*a*, S, %), quality index (*b*, QI, %) and performance index (*c*, PI, mmday^{-1}) of the post-larvae of *Fenneropenaeus indicus* fed with live food and different level of live food substitution. Different letters indicate significant differences (P<0.05).

Wouters et al., 2003; Robinson, 2005). However, Samocha (1999) indicated that only complete replacement resulted in a reduction of survival and that 25% and 50% replacement had no negative effects on *Litopenaeus vannamei* post-larvae survival. In contrast, substitution of Artemia with microencapsulated diet FRIPPAK® had no negative

effects on *P. monodon* post-larvae survival (Wouters et al., 2003). The contradictory nature of the obtained results is possibly attributed to the type of microdiet, route of administration or species.

Resistance to salinity stress test shows the quality of shrimp post-larvae (Palacious et al., 2007) and our results revealed that substitution of live food with a microdiet in post-larval rearing did not affect their quality in this respect. In addition there was no significant difference in resistance of post-larvae fed with different level of live food replacement (P>0.05). However, previous studies on *F. azetcus* and *L. setiferus* showed that post-larvae feeding with microdiet diet significantly decreased quality index (P<0.05) (Gallardo et al., 2002; Robinson et al., 2005).

It is concluded that replacement of live food (50 or 100%) the present microdiet negatively affects growth performance and survival. Future studies are necessary to evaluate the potential of lower level replacements.

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