African Journal of Biotechnology Vol. 10(55), pp. 11594-11599, 21 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.710
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Efficacy of micro algae and cyanobacteria as a live feed for juveniles of shrimp *Penaeus monodon*

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Accepted 11 July, 2011

Growth performance and survival of giant tiger shrimp *Penaeus monodon* fed with five different micro algal diets as a live feed, was evaluated under laboratory condition. The experimental design consisted of feeding the juvenile with micro algal diets and cyanobacterial diets at the same concentrations. Fresh biomass of *Chlorella* sp., *Tetraselmis* sp., *Isochrysis* sp., *Synechococcus* sp. and *Phormidium* sp. were used as feed for shrimp *P. monodon*. Mean total length of shrimp was higher when fed with *Chlorella* sp. (4.8 cm) followed by *Phormidium* sp. (4.4 cm) and mean total weight was also higher in shrimp fed with *Chlorella* sp. (0.59 g) followed by *Phormidium* sp. (0.569 g). The survival rate of the shrimp was improved in shrimp fed *Phormidium* sp. (83.33%) and it was decreased in shrimp fed with *Isochrysis sp.* (36.67%). The shrimp that performed best had significantly more edible flesh (59.35%) (with *Chlorella* sp.) protein and lipid content (with *Phormidium* sp.) carbohydrate (with *Tetraselmis* sp.) Further more, water quality of the tank was better in shrimp fed with *Chlorella sp*. In general, the research of this study revealed that *Chlorella* sp. and *Phormidium* sp. could be used as a live feed for better growth of *P. monodon*.

Key words: *Penaeus monodon*, micro algae, *Synechococcus, Phormidium*, Shrimp growth, *Chlorella*.

INTRODUCTION

Penaeus monodon is the most valuable and profitable commercial species of the shrimp industry in India and other South Asian countries. Due to the decrease supply of fishery byproducts and concerns over its quality, the aquaculture industry is now actively investigating alternative nutrient source (Naylor et al., 2000). Studies have shown that diets containing fish-based ingredients have generally performed better in terms of growth and feed efficiency than diets containing alternative plant based sources (Moyan et al., 1992; Webster et al., 1992; Kikuchi, 1999). Phytoplankton is the main food of larval stages of some crustaceans (Preston et al., 1992) and of the early growth stages of some fishes (Ritan et al., 1994). However, little work has been done on the nutritional requirements of shrimp larvae. There are large differences between the survival and growth of prawn larvae fed different species of algae (Naranjo et al., 1995). Some mixed-algae diets have resulted in higher survival and faster development of larvae than the component species alone (Kurmaly et al., 1989). Hence it is clear that successful prawn culture still depends to a large extent on live feeds (Liao et al., 1990).

The following species of algae, *Nannochliris aculata*, *Nannochloropsis oculata*, *Chlorella* sp., *Chlamydomonas* sp., *Tetraselmis tetrathels* and *Tetraselmis chuii* are used as live feed for cultured shrimp larvae (Duerr et al., 1998). Fresh algal culture is a major bottleneck in the aquaculture industry. Algal concentrates made by centrifugation was fed to bivalves and prawn larvae which have promising results (D'Souza et al., 2000; Heasman et al., 2000; Robert et al., 2001). Algae such as *Chaetoceros calcitrans* and *T. chuii* were fed to *P. monodon* larvae and produced the same rate of survival (Millamena et al., 1990). A number of studies have documented the nutritional excellence of dried algal products in shrimp and fish diet (Wood et al., 1991). The

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objectives of the present study was to carry out the efficacy of micro algal species such as, *Chlorella* sp., *Tetraselmis* sp., *Isochrysis* sp, and cyanobacteria such as *Synechococcus* sp. and *Phormidium* sp. on shrimp growth and their survival.

MATERIALS AND METHODS

Source of feed

Three micro algal strains and two cyanobacterial strains were selected for the present study. Microalgae such as *Chlorella* sp. (LF-1), *Tetraselmis* sp. (LF-2), and *Isochrysis* sp. (LF-3) were collected from the Algal Division, Central Marine Fisheries Research Institute (CMFRI), Mandabam Regional Centre, Ramanad. Stock cultures of these micro algae were maintained in 100 ml of f_2 medium with vitamin and sodium metasilicate (Guillard and Ryther, 1962; Guillard, 1975). Filtered seawater (35 ppt) was used for the preparation of the media (25 µm sand filter). Cyanobacterial cultures [*Synechococcus* sp. (LF-4) and *Phormidium* sp. (LF-5)] were collected from Cuddalore coastal area (East Coast of India). Cultures were incubated at 25°C and illuminated from one side with artificial white light (2000 Lux) under a 12/12 h light/dark cycle. The stock cultures were sub cultured once in 15 days to maintain actively growing cells. Working cultures were prepared by inoculating 1L Erlenmeyer flasks with 25 ml of stock culture in 700 ml of f_2 medium.

Experimental setup

The feeding trials were conducted in plastic troughs of 50 L capacity. Plastic troughs of uniform size and uniform colour for each experiment were selected and filled with 25 L saline water (adjusted to 25 ppt). Each trough stored ten juveniles of shrimp and was provided aeration continuously. The acclimatized shrimps were stored for 48 h (periods of experimentation). They were weighed accurately after wiping with blotting paper and stocked in each experimental trough. The whole experiment was conducted in the temperature-controlled laboratory. The growth of the shrimp was analyzed by, the size, total body weight, mortality ratio and its proximate composition. Water quality parameters such as salinity, water temperature, pH, dissolved oxygen, nitrates, ammonia and sulphates were also analyzed (AOAC, 2005).

Feeding trial

A total of 375 juvenile shrimps were equally distributed into 15 tanks (n = 25). Three replicates were maintained for each feeding study. Feed quantity was adjusted according to the shrimp density (Lavens and Sorgeloos 1996). Concentrated centrifuged live feed biomass was used as a diet. Equal weight of the live feed biomass was fed to all the experimental shrimps. The experiment was conducted for 30 days and the shrimp growth was recorded. The experiment was replicated (n = 3 simultaneously) and the mean values were calculated. Shrimp juveniles were counted every day of the experiment. Percentage of survival (%) was calculated based on the number of surviving shrimp at a particular day of the experiment. Total length (mm) and the body weight (g) of the shrimp were analyzed once in ten days up to 30 days of the experiment. Shrimp carcasses were dry at 105°C for 12 h in an oven and were weighed by using electronic balance (Shimadzu, UniBloc, 0.001 g accuracy). Moisture content, crude protein, ash, total lipids and nitrogen free extract (NFE) were also calculated by FAO methods (FAO, 1994).

Water quality study

The physico-chemical parameters of the saline water used in various culture tanks were recorded twice in a week during the whole experimental duration. Water temperature was measured by mercury thermometer having 0.1°C accuracy, salinity was measured by refractometer, dissolved oxygen (DO) was estimated by Winkler's method, pH was measured by digital pH meter and other parameters such as nitrates, ammonia and sulphates were estimated by standard estimation methods (AOAC, 2000).

RESULTS

The trial on microalgal and cyanobacterial live feed had a significant effect on the size [length (cm)] of *P. monodon*. The highest increase in mean length was generally observed after 20 days of shrimp growth, and the highest mean length of 4.8 cm was achieved on the 30th day of feeding by *Chlorella* sp. (LF-1) (Figure 1). The results show that addition of live algal food supported the growth of the juvenile of shrimp *P. monodon*. The lowest mean length of 4.1 cm was observed with LF-2 (*Tetraselmi*s sp.). Shrimp juvenile fed with LF-1 had significantly greater length ($p < 0.05$) than that of shrimp fed with other diets. Similarly, weight of the shrimp was also higher with diet LF-1 (0.592 g fresh weight) and lower with diet LF-2 (0.44 g fresh weight) after 30 days. The mean weight of shrimp fed with diet LF-1 was significantly greater ($P < 0.05$) than that of shrimp fed with the other diets (Figure 2). *Tetraselmis* sp. (LF-2) did not support the growth of shrimp (Figure 2). *Phormidium* sp. when used as the sole microalgal diet gave the highest survival rate (Figure 3) (diet LF-5). Maximum survival of 83.33% was observed in feed LF-5 at the 30th day, followed by LF-1 (*Chlorella* sp.) (77%). All the other microalgal feeds such as *Tetraselmis* sp. (LF-2), *Isochrysis* sp. (LF-3) and *Synechococcus* sp. (LF-4) showed poor survival (Figure 3).

The range in water quality parameters during the study is given in Table 1. The fluctuation of temperature, pH and salinity was very low in all the experimental tanks. Variations in DO, nitrate, ammonia and sulphates were also recorded. Low level of DO was recorded in LF-2 (*Tetraselmis* sp.) tank and maximum DO was observed in LF-3 (*Isochrysis* sp) tank. Nitrate concentration was low in LF-1 (*Chlorella* sp.) tank and higher in *Phormidium* sp. (LF-5) tank. Ammonia level was high in LF-3 (*Isochrysis* sp.) and was low in LF-4 (*Synechococcus).* Sulphate was high in LF-3 *(Isochrysis* sp.) tank and low in *Chlorella* (LF-1) tank (Table 1).

Observation of the proximate composition of juvenile shrimp carcass after the experimental period is shown in Table 2. There was a significant variation in percentage of edible flesh of juvenile shrimp fed with all type of microalgal diets, but there was no significant variation in total body protein. Crude lipid in the juvenile shrimp fed with diet LF-1 showed significantly higher value. Ash content of the shrimp was lowest in shrimp fed with diet

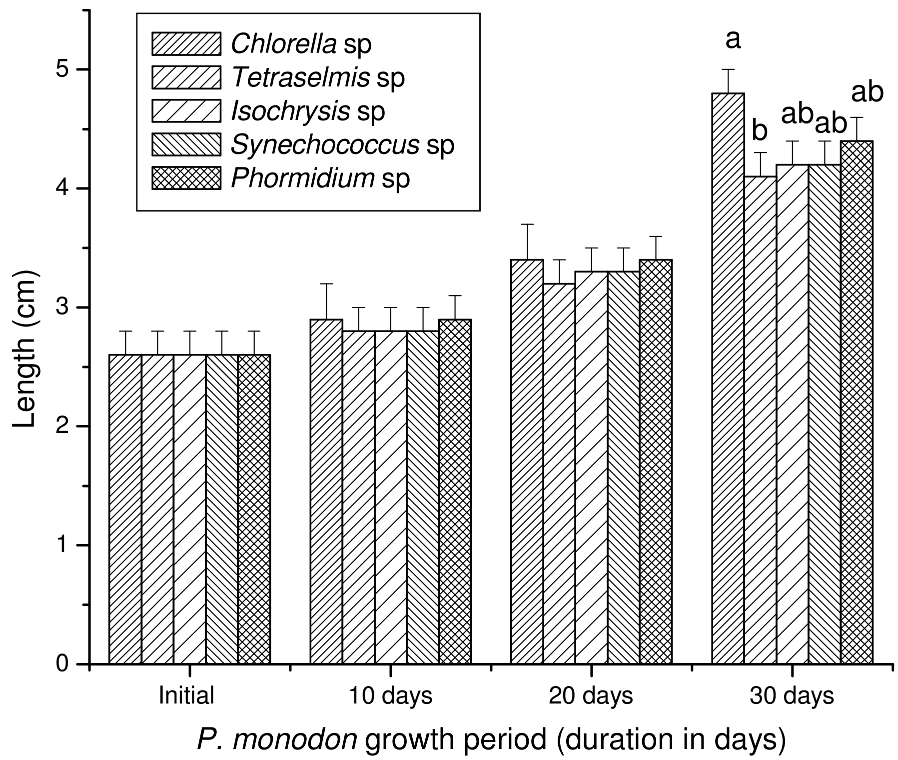

Figure 1. Mean total length of *P. monodon* fed with different live feeds. All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P > 0.01).

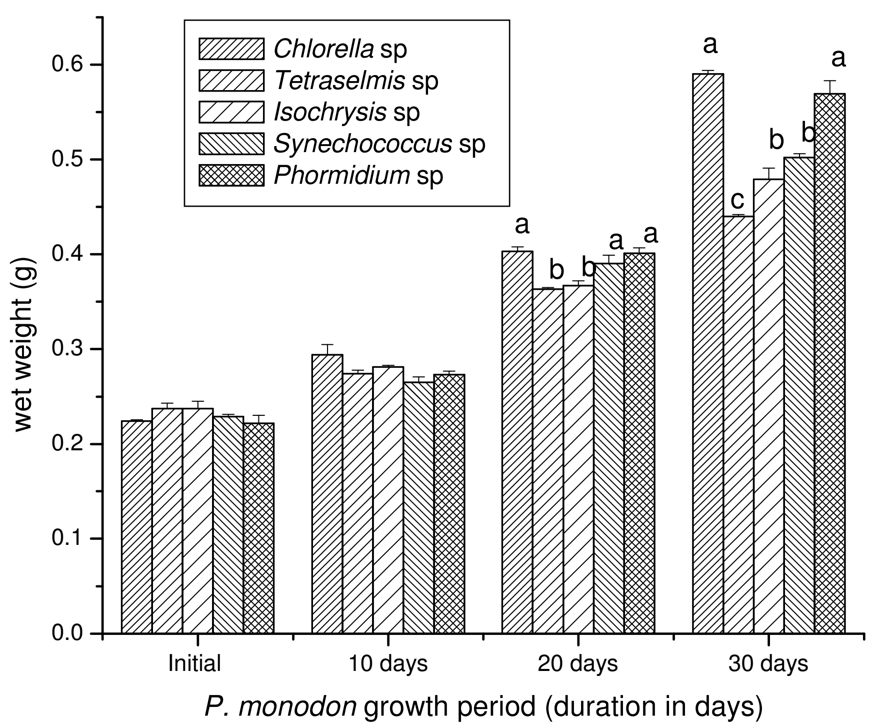

Figure 2. Mean weight gain of *P. monodon* fed with different live feeds. All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P > 0.01).

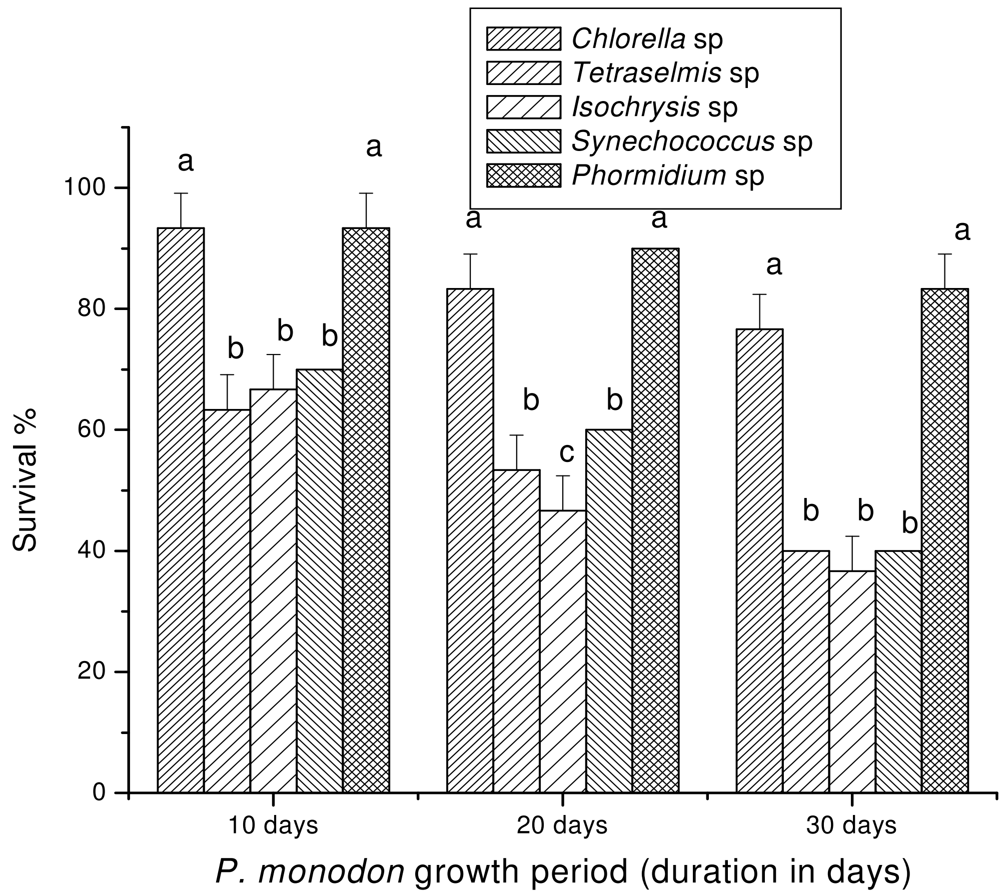

Figure 3. Mean survival rate of *P. monodon* fed with different live feeds. All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P > 0.01).

Table 1. Ranges of water quality parameters in *P. monodon* growth tanks during feed experimental studies.

Parameter	Quality of water in experimental tank				
	LF-1	**LF-2**	**LF-3**	**LF-4**	**LF-5**
Temperature (℃)	25.4 – 26.3	25.4 – 26.3	25.3 – 26.4	25.3 – 26.4	25.4 – 26.4
pH	7.87 – 8.25	8.04 – 8.27	7.93 – 8.18	7.83 – 8.26	7.91 – 8.12
Dissolved oxygen (mg/l)	6.4 – 7.1	5.7 – 6.2	6.9 – 7.4	6.7 – 7.4	6.7 – 7.3
Salinity (ppt)	24.3 – 25.6	24.0 – 25.3	24.6 – 25.2	24.2 – 25.3	24.2 – 25.4
Nitrate (µg/l)	3.7 – 4.7	5.3 – 6.3	5.9 – 6.4	5.4 – 6.1	5.6 – 7.1
Ammonia (µg/l)	2.1 – 4.0	2.0 – 3.0	2.0 – 4.8	0.8 – 2.1	2.0 – 3.4
Sulphate (µg/l)	4.8 – 6.9	5.2 – 8.3	6.1 – 9.2	5.2 – 7.8	5.8 – 8.6

LF-1; *Chlorella* sp.; LF-2, *Tetraselmis* sp.; LF-3, *Isochrysis* sp.; LF-4, *Synechococcus* sp.; LF-5, *Phormidium* sp.

LF-1. Higher total lipid content was found in shrimp fed with LF-5 diet and lower lipid was found in LF-3.

DISCUSSION

Result of the study showed that the fresh *Chlorella* sp. and *Phormidium* sp. had significant effect on the growth of shrimp *P. monodon*. The physicochemical characters of all the experimental tank waters were well within the normal limit even after the addition of marine algal diets. This showed that the microalgae live feed did not affect the water quality of the aquaculture systems. There were no significant differences in the mean total length of the

Table 2. Proximate composition of shrimp fed with different micro algal diet.

Content	Proximate contents of shrimp fed with different diet				
	LF-1	LF-2	LF-3	LF-4	LF-5
Moist weight basis (%) g					
Edible flesh ((%)	59.35[a] ± 2.45	54.35 [c] ± 3.15	57.66[b] ± 1.24	53.31 [c] ± 0.91	54.0[c] ± 0.5
Waste	40.65 ± 2.45	45.65 ± 3.15	42.34 ± 1.24	46.69 ± 0.91	46.0 ± 0.5
Moisture content	70.02 ± 4.25	68.44 ± 3.14	70.76 ± 2.0	69.9 ± 2.3	70.65 ± 0.45
Dry weight basis (%) g					
Dry weight	29.98 ± 4.55	31.56 ±3.14	29.24 ± 2.01	30.1 ± 2.1	29.35 ± 0.4
Protein	55.85 [b] ± 1.65	56.4 [b] ± 2.5	56.35 [b] ± 6.15	55.5[b] ± 4.5	58.4 [a] ± 8.6
Lipid	5.3[b] ± 0.1	6.15 [a] ± 0.05	5.1 [b] ± 0.2	6.4 [a] ± 0.1	6.65 [a] ± 0.05
Ash	11.1 ± 0.2	11.25 ± 0.45	10.7 ± 0.2	11.3 ± 0.1	11.5 ± 0.1
NFE	15.7[b] ± 2.3	26.5[a] ± 2.4	15.75 [b] ± 2.1	14.25 [b] ± 1.9	16.25 [b] ± 2.4

LF-1, *Chlorella* sp; LF-2, *Tetraselmis* sp; LF-3, *Isochrysis* sp; LF-4, *Synechococcus* sp; LF-5, *Phormidium* sp.
All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P> 0.01).
NFE = Nitrogen free extracts.

juveniles fed with the experimental diets, *Chlorella* sp and *Phormidium* sp. Generally, survival decreased with time in both algal and cyanobacterial feeds, whereas growth was increased with time in both types of feeds. Previous studies showed that larvae fed with a mixed diet of *Chaetoceros muelleri* and *Thalassiosira suecica* performed well (D'Souza and Loneragan, 1999). Survival and development of *P. monodon* may be better on a mixed diet of *C. muelleri* and *T. suecica* (Kurmaly et al., 1989). Some authors have reviewed the poor performance of prawn larvae to the large size of the algal cells in the diet (Tobias-Quinitio and Villegas, 1982; Sanchez, 1986). Shrimp fed with micro algal diet showed better weight gain. In this study, shrimp fed with *Chlorella* sp. showed considerable weight gain. However, *Tetraselmis* sp. fed shrimp showed lower body weight.

Micro algae such as *Chlorella* sp., *T. tetratheca* and *T. chuii* were used as feed for shrimp and fish. Survival of *P. monodon* fed with the five diets showed considerable variation. Survival rates were observed to be higher in diet with *Phormidium* sp. (83.33%) followed by *Chlorella* sp. (76.67%). Unlike micro algae, the *Phormidium* sp. cells is easily ingested and assimilated by shrimps. Larvae of *P. monodon* fed fresh micro algae had high survival and development (D'Souza et al., 2000, 2002). The survival and development of prawn larvae varied according to the species of algal food. Survival and development of spawning feed with algal diet have been reported for *Metapenaeus ensis* larvae (Chu and Lui, 1990). Crocos and Coman (1997) showed that the diet of *Penaeus semisulcatus* brood stock PZ1 stage varies with such factors as age of the brood stock and season of spawning. Fresh micro algae *Tetraselmis chuii* was a suitable microalgal diet for *P. monodon* (Heasman et al., 2000). *T. suecica* grown in the higher nitrogen medium was the better diet for the growth of shrimp (Jackson et al., 1992; D'Souza and Kelly, 2000). Larval survival declined linearly with time particularly in the case of *Tetraselmis* sp., *Isochrysis* sp. a*nd Synechococcus* sp. and was less in the case of *Chlorella* and *Phormidium* sp. The survival rate of the prawn larvae fed on both algae was high compared with that of sole feeding trials (Okauchi and Tokuda, 2004). In this study, the shrimp mortality was observed to be lower in shrimp fed with *Phormidium* sp. and *Chlorella* compared with the other microalgal and cyanobacterial feed.

Survival alone is not a suitable measure of the nutritional value of diets for prawn larvae. Some species of algae have high nutritive value and growth capacity, such as *Isochrysis* sp. (Okauchi et al., 1997), *Tetraselmis tetrathele* (Okauchi and Hirano, 1986), and *T. chuii* (Tobias-Quinitio and Villegas, 1982). The proximate compositions of the microalgae fed shrimp are shown in Table 2. The shrimp that performed best had significantly more lipid and carbohydrate than others. Similar findings were reported when *P. monodon* larvae were fed microalgal feed (D'Souza and Loneragan, 1999; Tobias-Quinitio and Villegas, 1982). D'Souza and Kelly (2000) measured the biochemical composition of the larvae fed by those algae, and found that; carbohydrate increased three fold in the lower nitrogen algae, while protein and lipid were reduced slightly compared to the control. In this study, maximum protein was accumulated in shrimp fed with *Phormidium* sp. and lowered in shrimp fed *Synechococcus* sp. larvae and juvenile oyster grew faster when fed algal diets with a higher content of carbohydrate (Wiktora et al., 1984; Thompson and Harrison, 1992). Juvenile oyster fed a high content of carbohydrate in the algal diet, showed improved growth (Enright et al., 1986).

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