**Original Research Article**

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**Marine yeasts as feed supplement for Indian white prawn**

*Fenneropenaeus indicus*: Screening and Testing the Efficacy

Pathissery J. Sarlin

1Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, Fine Arts Avenue, Cochin-16, Kerala, India

2Department of Zoology, Fatima Mata National College, Kollam, India

*Corresponding author

**ABSTRACT**

25 marine yeasts were used for the study based on their performance in a feeding experiment in *Fenneropenaeus indicus*. In the present study, the yeast incorporated feeds were found to be giving better performance in terms of various growth parameters in shrimps compared to the control feed. Yeast wise variation could be noted in the performance, which may be due to the variation in the protein, lipid, and carbohydrate and vitamin profile of the yeast biomass. Production (weight gain) was found to be more than twice for some of the yeast diets when compared to the control (free of yeast) and it was found to be significantly different for all the animals fed on yeast diets. The study showed that *Candida sake* S165, *Debaryomyces hansenii* S8, *Candida utilis* S186 and *Debaryomyces hansenii* S100 supported better growth in prawns compared to other strains. The study showed that marine yeasts can serve as potential feed supplements in aquaculture.

**Keywords**

Marine yeast, *Fenneropenaeus indicus*, *Candida sake* S165, *Debaryomyces hansenii*

**Article Info**

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**Introduction**

Yeasts are a rich source of proteins and B-complex vitamins. They have been used as supplements in animal feeds to compensate for aminoacid and vitamin deficiencies of cereals, and are recommended as a substitute for soybean oil in diets for fowl (Gohl, 1991). Yeast products (primarily brewer’s yeast and baker’s yeast) are frequently used as feed ingredients in aquaculture because of the nutritional value of these products, which include proteins, lipids, B-complex vitamins etc (Mahnken, 1991; van derMeeren, 1991). Yeast based diets are rich in proteins, lipids, attractants and other nutrients. Some of the yeast species used as fishmeal substitutes are *Candida* sp., *Kluyveromyces* sp. and *Phaffia* sp. Among unconventional protein sources, Single Cell Protein (SCP) of microbial origin appears to be a promising candidate. Many workers...
have reported partial replacement of fishmeal with yeast, bacteria and soybean protein (Bergstrom, 1979; Spinelli et al., 1978).

The protein component ranged from 29 to 63%, carbohydrate 21-39% and fat 1 to 23%. Protein content was found to be comparatively high in Candida, Saccharomyces, Torula and Geotrichum. Phaffia was reported to contain 23% fat (Sanderson and Jolly, 1994) and comparatively less protein (22%). Carbohydrate expressed as NFE was maximum in Torulopsis and Candida (Kamel and Kawano, 1986). Another concern with SCP is their high concentration of nucleic acids, ranging from 5-12% in yeast and 8-16% in bacteria (Schulz and Oslage, 1976). In brewer’s yeast, nucleic acid nitrogen is present mostly in the form of RNA and represents about 20-25% of the nitrogen (Rumsey et al., 1991b).

Baker’s yeast, Saccharomyces cerevisiae, chemically treated with sulphhydryl compounds to improve its digestibility were tested on juvenile Mercenaria mercenaria (Lavens et al., 1989; Couteau et al., 1990). Such a yeast-based diet has proven to be a valuable algal substitution in the larval culture of marine shrimps (Naessens-Foucquaert et al., 1990). In juvenile Sydney rock oyster spat (Brown et al., 1996), substitution with 86% (dry weight basis) live yeasts produced a weight increase, 63-81% of those obtained on algal diet.

Rumsey et al. (1990) showed that the lower performance of fish fed diets containing high levels of brewer’s yeast may be caused by intact yeast cells, as probably not all intracellular ingredients become available to the fish. Rumsey et al. (1991b) found that digestibility of intact brewer’s yeast in rainbow trout is significantly lower than that of disrupted cells. In accordance to this finding, Rumsey et al. (1990) observed that brewer’s yeast could replace 50% of total nitrogen in the diet of lake trout when the yeast cell walls were disrupted but growth depression was observed when intact yeasts were used.

The efficacy of live yeasts as diet compliments in aquaculture diets have been tested by many workers (Couteau et al., 1991; Roques and Dussert, 1991; Couteau et al., 1993; Couteau et al., 1994) as substitutes for algae. Phaffiarhodozyma is a species of yeast, containing astaxanthin, the most abundant carotenoid in the marine environment (Johnson and Ann, 1991).

In contrast, it has been shown that common carps can utilize a high percentage of their dietary protein requirement from the yeasts Candida tropicalis, Candida utilis and Candida lipolytica with better results than those obtained with soybean or meat and bone meal. For carp larvae diets, it has been shown that from 62 to 88% of Candida utilis and Candida lipolytica can be used in combination with other materials such as fishmeal and other animal by-products (Atack et al., 1979; Hccht and Viljoen, 1982; Dabrowski et al., 1983; Alami-Durante et al., 1991).

Experiments conducted on diets for tilapia (Oreochromis mossambicus) to evaluate the effects of substituting animal protein with a mixture of plant feed stuffs including 25, 30, 35, 40 and 45% of the protein with torula yeast (Candida utilis), 20% with soybean meal and 15% with Alfaalfa Leaf protein Concentrate (ALC). Feeding efficiency was compared against a diet with fishmeal as the sole protein source and the results showed that 30% yeast diet showed the best growth performance.
The results suggested that it is possible to replace up to 65% of animal protein with a mixture of plant proteins, including 30% torula yeast, in tilapia fry diets without adverse effects on fish performance and culture profit (Novoa et al., 2002). Experiments conducted on Nile tilapia (Oreochromis niloticus) evaluated the effects of three types of probiotics, two bacteria and one yeast on growth performance. Three diets were formulated with the optimum protein level (40%) for tilapia fry 1) supplemented at 0.1% with a bacterial mixture containing Streptococcus faecium and Lactobacillus acidophilus 2) supplemented at 0.1% with the yeast Saccharomyces cerevisiae; and 3) control diet without supplements. Of the four treatments, the 40% protein diet supplemented with yeast produced the best growth performance and feed efficiency, suggesting that yeast is an appropriate growth stimulating additive in Tilapia cultivation (Lara-Flores et al., 2003). Growth appeared to increase with the amount of protein in the diet. The protein requirement for optimal growth of Penaeus monodon has been reported to be between 35-61% (Lee, 1970; Ting, 1970; Chen and Liu, 1971; Deshimaru and Kuroki, 1975; New, 1976; Lin et al., 1981; Alava and Lim, 1983; Shiau et al., 1991). This work is focused on screening and testing the efficacy of marine yeasts as a feed supplement in Fenneropenaeus indicus and selection of the potential yeasts for aquaculture applications.

**Materials and Methods**

**Selection of Strains**

Representative isolates (25 numbers) of various genera were selected for nutritional quality evaluation. Following were the strains subjected to proximate composition analysis \textit{i.e.} S3, S8, S13, S28, S30, S42, S48, S50, S56, S69, S70, S81, S87, S100, S165, S169, S170, S186, S297, S303, S382, S394, S425, S434, and S437.

**Preparation of Yeast Biomass**

The selected 25 yeast cultures were swab inoculated onto malt extract agar plates, incubated at 28±2°C for 72 hrs and harvested with sterile saline. The cell suspensions were centrifuged at 7000 rpm for 20 minutes in a refrigerated centrifuge (Remi C-30, Mumbai) and the yeast biomass stored at 4°C in a refrigerator.

**Proximate composition of the yeast biomass**

Biochemical composition of the yeast biomass was analyzed to assess their nutritional quality. Protein was estimated by microkjeldhal method (Barnes, 1959) and lipid by phosphovanillin method following chloroform methanol extraction of the sample (Folch et al., 1957) and carbohydrate by Roe (1955). Based on the nutritional quality analysis 14 yeast strains were selected for the feeding experiment (Fig.1).

**Feeding Experiment with \textit{F.indicus} Post Larvae**

**Experimental Animals**

Post larvae (PL-21) of Indian white prawn, \textit{(Fenneropenaeus indicus} H.Milne Edwards) of the size range 20-30 mg were brought to the laboratory from a commercial prawn hatchery in Kannamali, Kochi, India.

**Experimental Feed Preparation**

Powdered ingredients as given in Table 1 were mixed well into a dough with 100ml water. This was steamed for 10 minutes in an autoclave and pelleted using a
laboratory model pelletizer having 1mm die. Pellets were dried in an oven at 50°C for 18hrs. The pellets were broken into pieces of 4-5mm size. 14 different feeds were prepared incorporating the biomass of 14 yeast strains plus the control diet without the yeast biomass. Water stability of feed was checked by immersing pellets in seawater for 15 hrs and examining stability by visual observation. Feeds were stored in airtight polythene bags at -20°C in a freezer.

Proximate Composition of the Experimental Diets

Protein content of the experimental diets was determined by microkjeldhal method (Barnes, 1959) and lipid by chloroform-methanol extraction (Folch et al., 1957). Ash was determined by incineration at 550°C in a muffle furnace for 5 hrs and moisture content by drying in an oven at 80°C to constant weight. Fiber content was determined by acid and alkali treatment following AOAC (1990). The nitrogen free extract (NFE) was computed by difference (Crompton and Harris, 1969) (NFE=100 - (% protein + % lipid + % fiber + % ash).

Feeding Schedule

Prawns were fed twice daily at 10 a.m. and 5 pm with fourteen different feeds including control diet at the rate of 10-15% of the body weight per day. Pre-weighed experimental diets were placed in petridishes in the tank. Faecal matter was removed by siphoning twice daily.

Rearing Facility

Fiber reinforced rectangular plastic (FRP) tanks of 30L capacity were used for the study. Water quality was monitored daily and was maintained as per Table (2). On alternate days after removing the faeces and unconsumed feed, 50% of water was exchanged from all the experimental tanks. Aeration was provided from a 1HP compressor through air stones. Physico-chemical parameters like salinity, nitrogen and dissolved oxygen of the rearing water were estimated daily by following standard procedures (APHA, 1995).

Design of Experiment

The post larvae of F. indicus were maintained on control diet for a period of one week. The larvae were then stocked into 30L rectangular fiberglass tanks containing 20L seawater with 25 individuals per tank and reared on the experimental diets for 21 days. Feeding trials were conducted using triplicate tanks for each treatment.

Measurements

The initial body weight of the prawns in each rearing tank was recorded. For this they were weighed on a precision balance after being blotted free of water with tissue paper. The mean weight of all the prawns in a tank was calculated (mean±0.01g). After 21 days, final weights of all the prawns were measured and mean weight was found. Parameters including individual increase in weight (production), food conversion ratio (FCR), specific growth rate (SGR), relative growth rate (RGR), gross growth efficiency (GGE), and protein efficiency ratio (PER) were determined based on the data collected during the experimental period.

The formula used for calculating the growth parameters are given below:

Production = Final weight - Initial weight

FCR = Food consumed / Live weight gain

SGR = (In final weight - In initial weight) x
100 / days of feeding experiment

RGR = (W2 - W1) / Mean weight/No of days

GGE = [(W2 -- W1) / Food consumed] x 100

PER = Live weight gain/ protein consumed in dry weight

Challenge Experiment

After termination of the feeding experiment (21 days) all treatment groups including the control, were maintained under the same rearing conditions. Challenge with white spot virus (WSSV) was performed through oral administration. For this, prawns were fed with white spot virus infected prawn flesh (F. indicus adult) in the morning (after a starvation period of 12 hrs) and evening ad libitum for one day ensuring availability of infected meat to all the prawns in the tank and then maintained on the corresponding experimental diets for the following days. All the rearing conditions were also maintained as earlier. Survival rates were recorded everyday for a period of 7 days. Mortality by WSSV infection was confirmed by checking the characteristic circular white spots on the carapace and other exoskeletal parts of the infected animal.

Data Analysis

The data obtained in the feeding experiments were subjected to one-way analysis of variance (ANOVA). When a significant difference was found among the various treatments, Duncan's multiple range tests were done to bring out the difference between the treatments means. The statistical analysis was performed using the SPSS 11 package for windows.

Results and Discussion

Proximate Composition of Yeast Biomass (SCP) and Feed

Proximate Composition of Yeast Biomass

Protein content of the yeast biomass of various strains belonging to different genera was found to be in the range 22-30% and the maximum was found in S169 (30.45%) belonging to Debaryomyces (Fig 1). No significant difference could be observed between the genera in biochemical composition. Lipid content of yeast biomass varied between 2 to 8.25% the maximum being in S28 (Kluyveromyces sp.). There was no significant variation in the carbohydrate content in yeast biomass (22.36 to 29.68%) with a minimum in S48 (Lodderomyces sp.) and maximum in S70 (Homoascus sp.).

Proximate Composition of Feeds

Protein content of the feeds ranged from 40.2 to 55.4% with the maximum in F165 (55.4%) followed by F303 (53.9%). Lipid was maximum in F69 and F165 (11.2%) followed by F28, F87 and F434 (10.8%). Nitrogen Free Extract was maximum in the control feed C (36.8%). No significant variation could be obtained in the fiber content of various feeds and the value ranged from 1.9 to 2.2%. Ash content was higher in F434 (7.7%) followed by F186 and F303 (7.5%). Moisture content of the feeds ranged from 3.2 to 9.6% (Table 3).

Feeding Experiment

The data collected from the experiments were analyzed and the biogrowth parameters like production, food conversion ratio (FCR), specific growth rate (SGR), relative growth rate (RGR), gross growth efficiency (GGE), protein efficiency ratio (PER) were
determined. All the yeast biomass incorporated feeds supported better biogrowth parameters compared to the control feed. Performance of F8, F87, F165, F170, F186 and F303 was notable (Table 4). The highest production was recorded in prawns fed feed F 165 (115.93 mg) followed by F186 (103.48mg) and the lowest for control feed (32.70mg) (Fig.2).

Food conversion ratio (FCR) was found to be the best with feed F8 (0.61), followed by F186 (0.63) and F100 (0.68) (Fig.3). Specific growth rate (SGR) was found to be maximum for F186 (9.82) followed by F165 (8.59) and the lowest for control feed (3.24) (Fig.4). Gross growth efficiency (GGE) was found to be maximum for F8 (165.23) followed by F186 (158.52) and the lowest was recorded for F30 (68.74) (Fig.4). Relative growth rate (RGR) was highest for F186 (0.058) followed by F165 (0.057) and the lowest for F30 (0.022) (Fig.6). Protein efficiency ratio (PER) was found to be best with F8 (3.37) followed by F186 (3.29) (Fig.7).

**Post challenge Survival**

Post challenge survival is presented in Fig.3.3. No mortality was observed for prawns fed with feed F8, F165, F169, F186 and F434 (Fig.8). All the yeast incorporated feeds showed better survival compared to control feed. Death by (White Spot Virus) WSV infection was confirmed by the presence of white spots on the carapace of the infected prawns.

**Statistical Analysis**

Duncan’s multiple range analysis of the various growth parameters affected by the different feeds showed that the performance of the yeast incorporated experimental feeds varied significantly from that of the control feed. Within the various yeast incorporated feeds itself significant variation could be observed. Generally the performance of F8, F186, F87 and F100 was found to be best compared to other feeds.

Protein content of yeast biomass of various genera was found to be in the range of 22-30% and the maximum was encountered for S169 (30.45%) belonging to *Debaryomyces*. Brown *et al.* (1996) found about 21% protein in *Debaryomyces hansenii*, and 42% *Candida utilis*. Han *et al.* (1976) recorded 44.3% protein in *Candida utilis*. However, according to Kamel and Kawano (1986) *Candida* sp. contained only 34.9% protein. In this study *Candida* was found to contain lower protein content (25-28%) when compared to earlier studies. As per Brown *et al.* (1996) various yeast strains of *Dipodascus* sp. contained 25-30% protein which is in agreement with the report for *Dipodascus* (26.5%) isolated in the present study. In *Torula* yeast 46% protein was recorded by Olivera Novoa *et al.* (2002) whereas in the present study only 28% could be noticed. *Saccharomyces* sp. was found to contain larger quantity of protein (48-83%) by Kamel and Kawano (1986) whereas Brown *et al.* (1996) reported only 29% protein.

Lipid content of yeast biomass was found to be in the range 2 to 6.78% which is found to be almost similar with the earlier reports (Kamel and Kawano, 1986 and Brown *et al.* 1996) where a range of 1.05 to 7.7 was noted. However, Sanderson and Jolly (1994) have reported a very high content of fat (23%) in *Phaffia*. As per the literature, carbohydrate content in yeasts ranged from 21 to 39%. In the present study the amount of carbohydrate varied from 22.36 to 29.68%. This high carbohydrate content make yeast SCP a good carbon source for animal feeds.
Table 1 Rearing Conditions and Water Quality Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tr>
<td>Initial body weight (average)</td>
<td>20-30 mg</td>
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<tr>
<td>Stocking density</td>
<td>25 prawns/tank</td>
</tr>
<tr>
<td>Tank capacity</td>
<td>30 L</td>
</tr>
<tr>
<td>Feeding level</td>
<td>10-15% body weight</td>
</tr>
<tr>
<td>Feeding frequency</td>
<td>twice daily</td>
</tr>
<tr>
<td>Feeding period</td>
<td>21 days</td>
</tr>
<tr>
<td>Water temperature</td>
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<td>pH</td>
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<tr>
<td>Salinity</td>
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<td>NH₃</td>
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<tr>
<td>NO₃</td>
<td>Below detectable level</td>
</tr>
<tr>
<td>NO₂</td>
<td>0.01 mgL⁻¹</td>
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<tr>
<td>Dissolved O₂</td>
<td>7-8 mgL⁻¹</td>
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Table 2 Composition of Experimental Diets

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<th>Ingredients</th>
<th>Control diet g</th>
<th>Experimental diet g</th>
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<td>Prawn shell powder</td>
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<td>10</td>
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<td>Yeast¹</td>
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<td>Fish meal</td>
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<tr>
<td>Soybean meal²</td>
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<td>Maida³</td>
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<td>Rice bran⁴</td>
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<td>Vitamin and mineral mix⁵</td>
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<td>2</td>
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<tr>
<td>Agar</td>
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<td>2</td>
</tr>
<tr>
<td>Carboxy methyl cellulose</td>
<td>20</td>
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</tr>
<tr>
<td>Water</td>
<td>100 ml</td>
<td>100 ml</td>
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</table>

Table 3 Proximate Composition of the Experimental Feeds

<table>
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<tr>
<th>Feed</th>
<th>Protein</th>
<th>Lipid</th>
<th>Fiber</th>
<th>Ash</th>
<th>Moisture</th>
<th>NFE*</th>
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<td>7.2</td>
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<tr>
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<td>44.3</td>
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<td>6.7</td>
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<td>31.5</td>
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<td>F28</td>
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<td>F434</td>
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<td>7.7</td>
<td>5.9</td>
<td>23.7</td>
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</table>

NFE* - nitrogen free extract
### Table 4
Relative position of various feeds with respect to their performance in terms of bio-growth parameters and percentage survival in *F. indicus* post larvae maintained on experimental diets (Four best feeds are given in Bold)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PRO</th>
<th>FCR</th>
<th>SGR</th>
<th>GGE</th>
<th>RGR</th>
<th>PER</th>
<th>*Survival</th>
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<tbody>
<tr>
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<tr>
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<td>F 8</td>
<td>F 186</td>
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*Survival : 7th day post challenge survival when infected with WSV

### Fig. 1
Mean (±S.D) Proximate Composition of Yeast Biomass

- **a. Protein**
- **b. Lipid**
Fig. 2 Mean (±S.D) Weight Gain (Production) obtained in *F. indicus* post larvae when fed various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05)

Fig. 3 Mean (±S.D) phenoloxidase (PO) values of *F. indicus* fed on different experimental diets and challenged with WSSV (via diet). Data at the same exposure time with different letters are significantly different (p<0.05)
**Fig. 4** Mean (±S.D) Superoxide anion values of *F. indicus* fed on different experimental diets and challenged with WSSV (via diet). Data at the same exposure time with different letters are significantly different (p<0.05)

(0hr: Just before challenge, PC-1: Post challenge 1 day, PC-2: Post challenge 2 day, PC-3: Post challenge 3 day, PC-5: Post challenge 5 day, PC-7: Post challenge 7 day)

**Fig. 5** Mean (±S.D) Alkaline Phosphatase activity of *F. indicus* fed on different experimental diets and challenged with WSSV (via diet). Data at the same exposure time with different letters are significantly different (p<0.05)

(0hr: Just before challenge, PC-1: Post challenge 1 day, PC-2: Post challenge 2 day, PC-3: Post challenge 3 day, PC-5: Post challenge 5 day, PC-7: Post challenge 7 day)
**Fig. 6** Mean (±S.D) Acid Phosphatase activity of *F. indicus* fed on different experimental diets and challenged with WSSV (via diet). Data at the same exposure time with different letters are significantly different (p<0.05)

(0hr: Just before challenge, PC-1: Post challenge 1 day, PC-2: Post challenge 2 day, PC-3: Post challenge 3 day, PC-5: Post challenge 5 day, PC-7: Post challenge 7 day)

**Fig. 7** Mean (±S.D) Protein Efficiency Ratio (PER) obtained in *F. indicus* post larvae when fed various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05)
Fig.8 Mean (±S.D) Percentage survival of *F.indicus* post larvae when fed various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05)

The protein content of the feeds ranged from 44-55%. This range was found acceptable for optimum growth in penaeid prawns as shown by various earlier workers. Dietary protein has been reported as the most essential nutrient for the growth of prawns (Venkataramiah *et al.*, 1975; Alava and Lim, 1983). Penaeid shrimps require 35 to 40% protein, 8-10% fat rich in PUFA and 35% carbohydrate in their diet. Vitamins, minerals, fish oil, highly unsaturated fatty acids, phospholipids and cholesterol are essential additives to the basal diet (Ali, 1989) for optimal growth in shrimp. The protein quantity of a feed ingredient depends on several variables, digestibility and content of essential aminoacids, which are also crucial to biological value of the protein. Juveniles or adult penaeids have been shown to attain optimum growth on diets containing 22-60% protein (Hanson and Goodwin, 1977). In the present study, a part of protein in the feed is contributed by yeast protein. The nutritional value of the microorganisms used in aquaculture depends on their digestibility and assimilation characteristics and the target animal. In the present study most of the yeast incorporated feeds supported better growth.

The quantity of lipid in the diet was not found to have much effect on the growth parameters. However, the influence of the qualitative composition of the lipids in the various yeasts cannot be ruled out. Microorganisms contain a diverse range of fatty acid composition and are rich sources of useful unsaturated fatty acids like PUFA (Brown *et al.*, 1996). Recommended lipid levels for commercial shrimp feeds range from 6 to 7.5% and a maximum level of 10% was suggested by Akiyama and Dominy (1989). Lipid content in the feeds ranged from 7 to 10.8%. Qualitative composition was not estimated and therefore the role of lipids in the performance of the feeds cannot be explained. Among the lipid components in the diet of shrimps, polyunsaturated fatty acids, phospholipids and sterols have received the most attention in crustacean lipid nutrition. Sheen and Chen (1993) found that growth of *P. monodon* fed iso-nitrogenous diets
supplemented with 8, 10 and 12% lipid was significantly higher than those with lower lipid content. Fatty acids are reported to promote growth in penaeids (Guay et al., 1976). Millamena et al. (1988) noted greater growth in Penaeus monodon larvae that were fed lipid enriched Artemia nauplii. A qualitative analysis of the lipids in yeast biomass is essential to comment on its role as nutritional parameters.

Various studies with Penaeus japonicus have demonstrated that dietary phospholipids enhance growth and survival of larvae (Teshima et al., 1982; Kanazawa et al., 1985) and growth and stress resistance in post larval/juvenile stages (Sandifer and Joseph, 1976; Levin and Sulkin, 1984; Kanazawa et al., 1979a, b; Camara et al., 1997; Kontara et al., 1977). Watanabe et al. (1974) have reported that yellowtail fed diets with alternative protein sources replacing fish meal, had lower levels of plasma lipid components with increased susceptibility to infectious disease. This correlation between plasma lipid level to resistance and immunity has been further shown by Maita et al. (1998). Manomaitis (2001) determined that the crude protein requirement of newly released juvenile red claw to be at least 40%. He also concluded that a diet of 30% should be utilized for 9 to 19 week red claw. The nutritional value of brewer’s yeast S. cerevisiae has been studied in lake trout (Rumsey et al., 1990), rainbow trout (Rumsey et al., 1991a and b) and sea bass (Oliva-Teles and Goncalves, 2001) by comparing growth performance, feed efficiency, liver uricase and nitrogen retention.

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