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RESEARCH ARTICLE

EFFICACY OF MARINE YEASTS AS FEED SUPPLEMENT FOR *FENNEROPENAEUS INDICUS* IN CULTURE SYSTEMS

***, [1]Sarlin, P.J. and [2]Rosamma Philip**

[1]Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, Fine Arts Avenue, Cochin -16, Kerala , India
[2]PG and Research Department of Zoology, Fatima Mata National College, Kollam, India

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ABSTRACT

Efficacy of three marine yeasts (*Debaryomyces hansenii* S8, *Debaryomyces hansenii* S100 and *Candida tropicalis* S186) as feed supplement for *Fenneropenaeus indicus* was estimated in comparison with *Saccharomyces cerevisiae* MTCC 36, a commercial feed and a control feed were used for the study. Biomass of yeast strains was prepared using Malt extract agar and incorporated into a standard diet to prepare yeast diets of varying concentrations. *F.indicus*were fed these diets for a period of 28 days and various growth parameters such as production, FCR, SGR, GGE, RGR, PER, CUD were performed. Among the three marine yeast diets *Debaryomyces hansenii* S8 supported the best biogrowth parameters. Commercial feed was found to be better in efficiency compared to the Bakers yeast diet and control diet. Present study showed that the three marine yeasts used in the study could very well be used as feed supplement in aquaculture.

Key words: *Fenneropenaeus indicus, Debaryomyces hansenii, Saccharomyces cerevisiae,* hepatopancreas, Culture systems.

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INTRODUCTION

Yeasts have high protein content and can be produced on the basis of various raw materials, independently of the climate and at relatively low production costs (Kihlberg, 1972). Yeast of the genus *Candida* is most commonly used for the production of microbial protein from cellulose hydrolysates. Feeding experiments on mirror carp with yeast, bacterial, algal and soybean meal proteins showed that the major portion of protein requirement could be met by bacterial and yeast proteins (Atack *et al.,* 1979). Rainbow trout fed with various novel proteins such as herring meal, soybean, petroleum yeast, brewer's yeast, bacteria, algae etc. exhibited the highest specific growth rate with the fishmeal and petroyeast diets. Maximum Protein Efficiency Ratio was obtained with petroyeast. The petroyeast also had good physical characteristics, producing a hard water-soluble pellet (Atack *et al.,* 1979). Bivalve molluscs grew as fast or faster than controls when fed diets containing 50% yeast (*Candida utilis*). Growth of soft tissue in *Crassostrea virginica*, decreased with the amount of yeast in the diet (Epifanio, 1979). The abundance and availability of proteins of vegetable origin has increased research emphasis on their potential utilization. As a protein source, SCP of yeast or bacterial origin appear especially attractive because the protein content and amino acid composition of these organisms compare well with those of fishmeal (Spinelli *et al.,* 1979). It was found that *Geotrichum candidum* single cell protein could replace 100, 75 or 50% of fishmeal in a pelleted diet when fed to rainbow trout (Dabrowski *et al.,* 1980). Yeasts were found to be a suitable substitute for fishmeal upto 40% level in rainbow trout. Up to 25% substitution in diet was judged to be acceptable for marine coho-salmon (Mahnken *et al.,* 1980). Feeding a diet consisting solely of fresh baker's yeast (*Saccharomyces cerevisiae*) under controlled conditions led to poor growth (Coutteau *et al.,* 1990). However, removing or permeabilizing the yeast cell wall by an enzymatic treatment could improve the growth performance. In this way Coutteau *et al*. (1990) could reveal that the ineffectiveness of untreated baker's yeast is mainly due to its low digestibility. The development of techniques to improve the digestibility (Coutteau *et al.,* 1990) and the nutritional composition of (Leger *et al.,* 1985) yeast based diets provided the incentive to develop a product as a potential substitute for unicellular algae. Such a yeast-based diet has proven to be a valuable algal substitute in the larval culture of marine shrimp (Naessens-Foucquaert *et al.,* 1990). Rumsey *et al*. (1991a and b) found that digestibility of intact brewer's yeast in rainbow trout is significantly lower than that

Corresponding author: **Sarlin, P.J.**
Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, Fine Arts Avenue, Cochin -16, Kerala , India

of disrupted cells. In accordance to this finding, experiments showed 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.

MATERIALS AND METHODS

Microorganisms used

Based on the results of the preliminary feeding experiment on *F. indicus* post larvae, three yeasts were selected for further study. In addition, Baker's yeast *S. cerevisiae* (MTCC36) obtained from Institute of Microbiology (IMTECH) Chandigarh was also included in the study for comparison. The selected marine yeasts (3 Nos.) were identified by IMTECH, Chandigarh: (Table.1). These yeasts are deposited at Microbial Type Culture Collection (MTCC) at IMTECH and the following numbers were assigned.

Table 1. List of yeast strains used for production of SCP

Culture No.	Species	MTCC Number
S 8	*Debaryomyces hansenii*	MTCC 4361
S 100	*Debaryomyces hansenii*	MTCC 4363
S 186	*Candida tropicalis*	MTCC 4366
S 36	*Saccharomyces cerevisiae*	MTCC 36

Proximate composition of the yeast biomass

Biochemical composition of the biomass of 4 yeast cultures (S8, S100, S186 and S36) were analysed.

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^{0}C in a muffle furnace for 5hrs and moisture content by drying in an oven at 80^{0}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 experiment with *Fenneropenaeus indicus* juveniles

Experimental animals: Juveniles of Indian white prawn, (*Fenneropenaeus indicus* H. Milne Edwards) of the size range 0.10- 0.12g were brought to the laboratory from a commercial prawn hatchery in Kannamali, Kochi.

Experimental feed preparation

Powdered ingredients as given in table 2 were mixed well into dough with 100ml water. This was steamed for 10 minutes in an autoclave and pelletised using a laboratory model pelletiser having 1mm die. Pellets were dried in an oven at 50^{0}C for 18hrs. The pellets were broken into pieces of 4-5mm size. Four different feeds were prepared incorporating the biomass of 3 marine yeast strains and the baker's yeast (*S. cerevisiae*) plus the control feed (without the yeast biomass). Water stability of feed was checked by immersing pellets in seawater for 15h and examining stability by visual observation. Feeds were stored in airtight polythene bags at -20^{0}C in a freezer (Fig. 1). A commercially available feed (CF) was also used for the study.

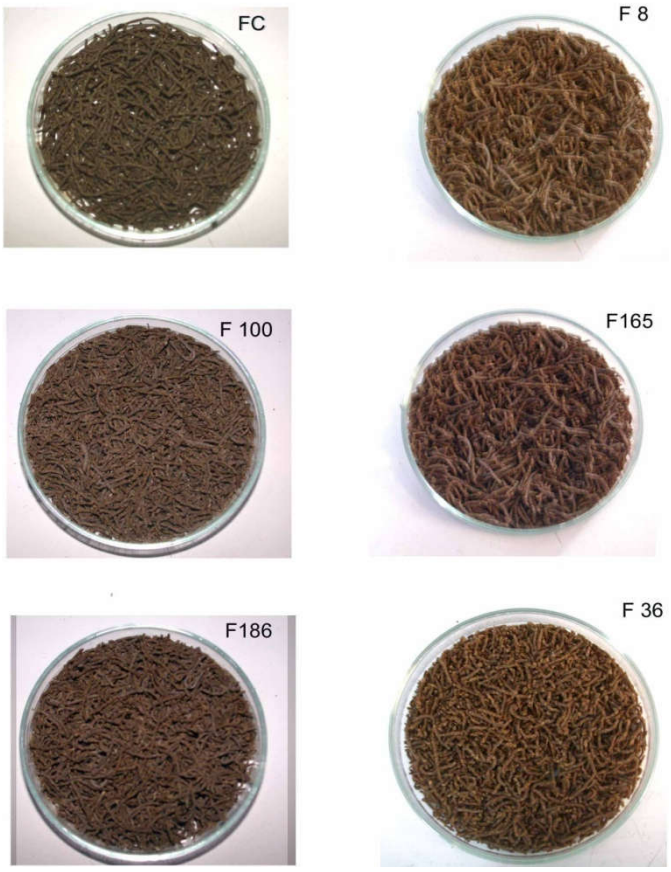

Fig. 1. Experimental diets prepared by incorporating the yeast biomass

Rearing facility

Fiber reinforced rectangular plastic (FRP) tanks of 30L capacity were used for the study (Fig.2). Water quality was monitored daily and was maintained as per Table.3. 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 though air stones. Physiochemical parameters like salinity, nitrogen and dissolved oxygen of the rearing water were estimated daily by following standard procedures (APHA, 1995) (Table.3).

Fig.2. Culture Facilities used for the various feeding experiments

Design of experiment

Juveniles of *Fenneropenaeus indicus* were maintained on prepared control diet for a period of one week. The prawns were then stocked into 30L rectangular fiberglass tanks containing 20L seawater with 20 individuals per tank and

reared on the experimental diets for 28 days. Feeding trials were conducted using triplicate tanks for each treatment.

Feeding schedule

Six different feeds were given to the prawns including four yeast diet, one commercial feed and one control diet. Pre-weighed experimental diets were placed in Petri dishes in the tank. Faecal matter was removed by siphoning twice daily. Uneaten feed was collected twice daily by siphoning and washed gently with distilled water to remove salt and filtered through a pre-weighed filter paper and dried to a constant weight in an electric oven at 80^0C for 24h.

Measurements

The initial body weight of the prawns in each rearing tank was recorded. They were weighed on a precision balance and after they were blotted free of water by tissue paper. The mean weight of all the prawns in a tank was calculated. After 28 days, final weight 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), consumption per unit weight per day (CUD), and protein efficiency ratio (PER) were determined based on the data collected during the experimental period.

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 treatment means. The statistical analysis was performed using the SPSS 11.0 package for windows.

RESULTS

Proximate composition of yeast biomass

Table 2. Proximate composition of yeasts

Culture No.	Protein	Lipid	Carbohydrate
S8	24.72	3.81	24.8
S100	31.51	7.62	28.8
S186	26.54	3.82	27.9
S36	27.52	4.23	25.8

Table 3. Proximate composition of feeds

Feed no.	Protein	Lipid	Fiber	Ash	Moisture	NFE
F 8	44.3	8.8	2.1	6.7	6.6	31.5
F 100	51.9	10.3	2.0	6.3	4.1	25.4
F 186	48.1	8.3	2.0	7.5	4.6	29.6
F 36	45.2	8.2	1.9	5.2	6.3	33.2
CF	48.5	7.2	2.1	6.2	7.3	28.71
Control	47.2	7.9	2.0	5.8	7.2	29.9

Proximate composition of prawn flesh

Prawns fed on various diets exhibited almost the same protein content and the percentage of protein in flesh at the beginning of the experiment was maintained without much change. Protein content was found to be maximum in prawns fed with F8 (73.9%). The percentage of lipid was found to be maximum in prawns fed with feed F8 (12.45%) and carbohydrate with feed F100 (1.07%) (Fig 3).

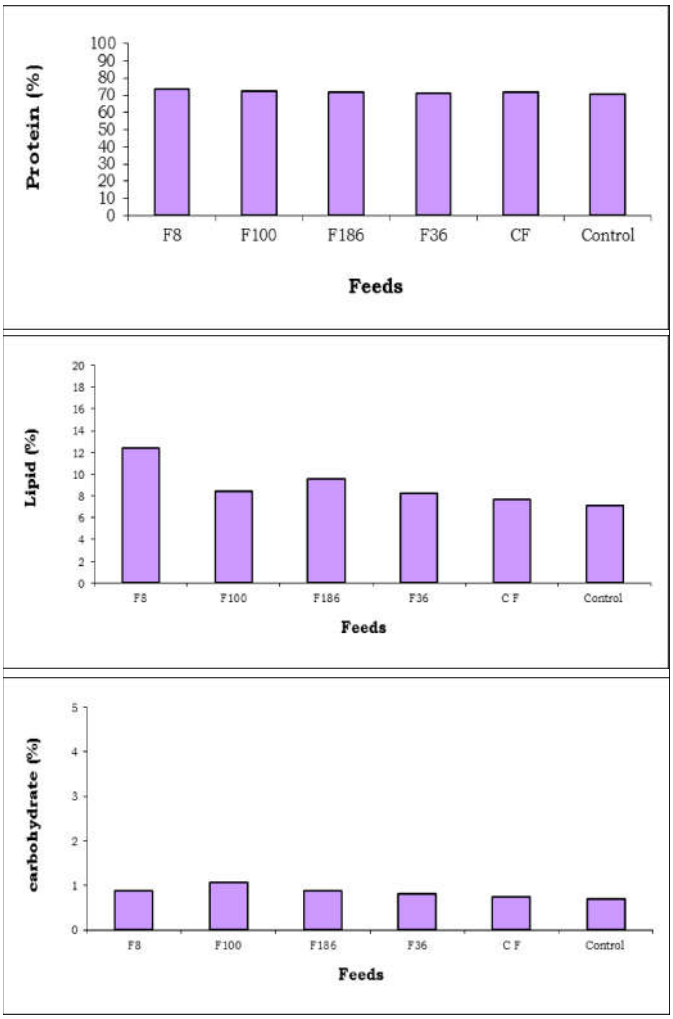

Fig.3. Proximate composition of flesh of *Fenneropeaneusindicus* maintained on different diets

Biogrowth parameters

All the three marine yeast incorporated feeds were superior in performance in terms of the observed biogrowth parameters in prawns compared to both the control feeds and commercial feed. Of the three yeast diets, the feed F8 incorporated with the biomass of S8 (*Debaryomyces hansenii*) gave the best performance in terms of biogrowth parameters followed by F186 and F100 (Table 6). The highest production was recorded in prawns fed with feed F8 (1.07gms) followed by F186 (0.83gms) and the lowest was recorded for control feed (0.42) (Fig.5.6a). Food conversion ratio (FCR) also was found to be the best with feed F8 (1.35), followed by F 186 (1.68) (Fig.4). Table 4: Relative position of various feeds with respect to their performance in terms of bio-growth parameters and percentage survival in *F. indicus*juveniles maintained on experimental diets.

Table 4. Proximate composition of feeds

Parameters	PRO	FCR	SGR	GGE	RGR	PER	CUD
Experimental Feeds	F 8	F 8	F 8	F 8	F 8	F 8	F8
	F 186	F 186	F 186	F 186	F 186	F 186	F 186
	F 100	F 100	F 100	F 100	F 100	F 100	F 100
	C F*	C F	C F	C F	C F	F 36	C F
	F 36	F 36	F 36	F 36	F 36	C F	F 36
	Control	Control	Control	Control	Control	Control	Control

* Commercial Feed

Specific growth rate (SGR) was maximum for prawns fed diet F8 (7.719) followed by F186 (7.454) and the lowest value recorded for control feed (5.12) (Fig.5). Gross growth efficiency (GGE) was found to be maximum with F8 (75.48) followed by F186 (59.48) and the lowest value was recorded for control feed (29.27) (Fig.6).Maximum Relative growth rate (RGR) highest value was recorded for F8 (0.05) followed by F186 (0.049) and the lowest value in control feed (0.037) (Fig.7). Protein efficiency ratio (PER) was found to be the best with F8 (1.70) followed by F186 (1.23) (Fig.8). Consumption per unit weight per day (CUD) was found to be best with F8 (0.07) and F186 (0.08) (Fig.9).

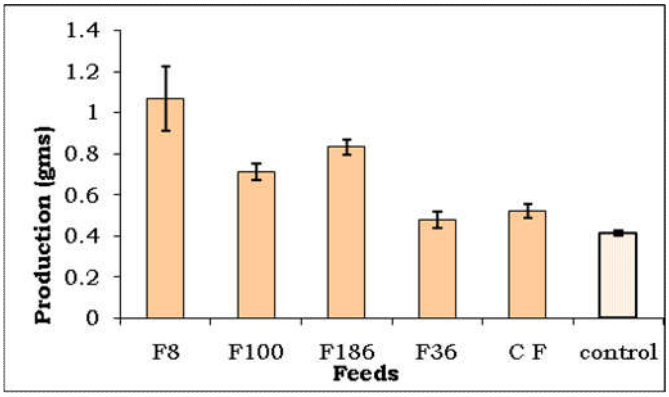

Fig.4. Mean (±S.D) Weight gain (Production) obtained in *F.indicus* post larvae when fed various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05)

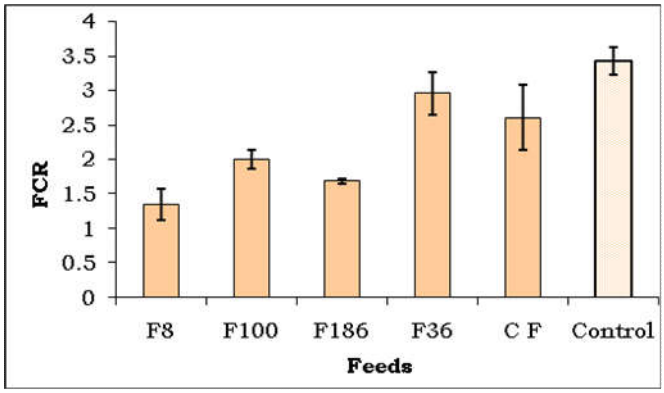

Fig.5. Mean (±S.D) Food Conversion ratio (FCR) obtained in *F.indicus* post larvae when fed various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05).

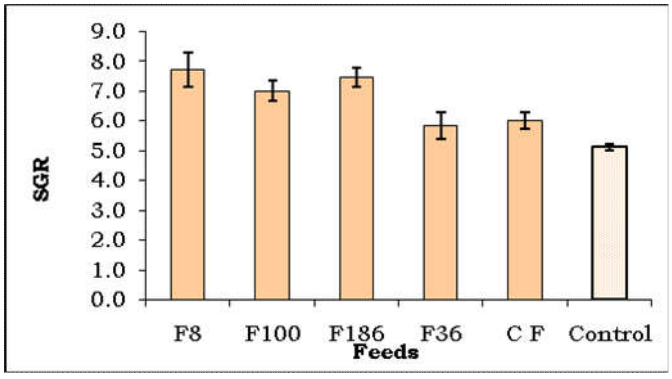

Fig. 6. Mean (±S.D) Specific Growth Rate (SGR) obtained in *F.indicus* post larvae when fed with various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05)

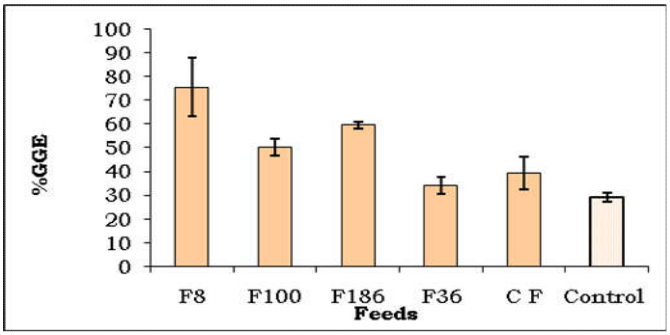

Fig.7. Mean (±S.D) Gross Growth Efficiency (GGE) obtained in *F.indicus* post larvae when fed various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05)

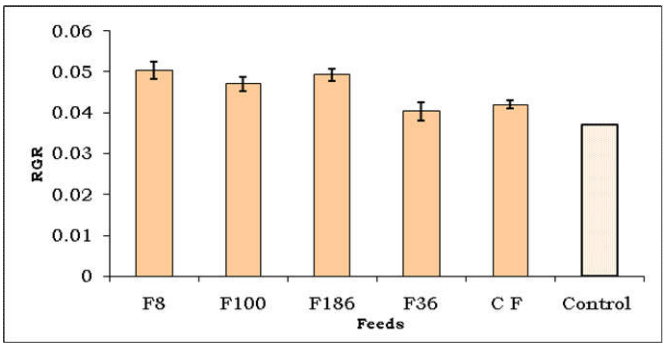

Fig.8. Mean (±S.D) Relative Growth Rate (RGR) obtained in *F.indicus* post larvae when fed various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05)

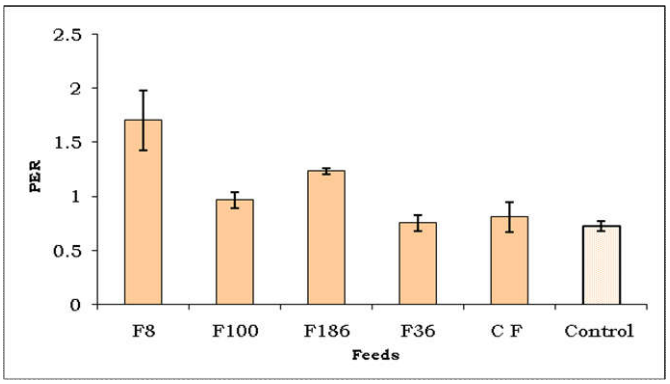

Fig.9. Mean (±S.D) Protein Efficiency Ratio (PER) obtained in *F.indicus* post larvae when fed various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05)

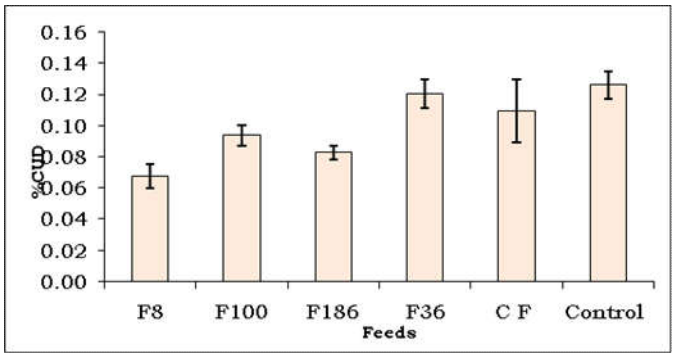

Fig. 10. Mean (±S.D) Consumption per unit weight per day (CUD) of*F.indicus* post larvae when fed various experimental feeds. Data at the same exposure time with different letters are significantly different (p<0.05)

Statistical analysis

Duncan's multiple range analysis of the various growth parameters effected by the different feeds showed that the performance of the feed F8 and F186 were significantly different from other feeds including the control diets.

DISCUSSION

Feeding experiment

Proximate composition of the flesh of prawns did not show any remarkable change when fed on various yeast feeds, commercial feed and the control feed. This shows that all the experimental feeds had the nutritional components in the required proportion and amounts. In the present study three marine yeasts (*Debaryomyces hansenii* S8, *Debaryomyces hansenii* S100, *Candida tropicalis* S186), a Baker's yeast, *Saccharomyces cerevisiae* MTCC 36, a commercial feed (CF) and a control feed were used for the study. The performance of marine yeast incorporated feeds was superior compared to other feeds. A detailed analysis of the proteins, fatty acids, carbohydrates, vitamins, nucleic acids and the mineral content is essential to elucidate the reason for this difference. There is major concern regarding the availability of fishmeal for incorporation in fish diets (Hardy, 1996). Alternative protein sources such as plant feedstuffs are generally not well accepted due to non-palatability and amino acid imbalance. Single Cell Protein (SCP) that includes micro algae, bacteria and yeast are alternative protein sources used in feed ingredients. Among SCP's yeasts have been the most used within aquafeeds (Tacon, 1994). Yeasts have immunostimulatory properties by virtue of their complex carbohydrates and nucleic acid contents (Anderson *et al.,* 1995). Another problem with SCP is their higher content of nucleic acids, 8 to 12% in yeasts (Schulz and Oslage, 1976) mostly in the form of RNA (Rumsey *et al.,* 1991a). Excess supply of dietary nucleic acids cause deposition of uric acid in the body (Schulz and Oslage, 1976). In fishes due to the presence of very active liver uricase, this problem is not usually observed. There are no reports related to this type of disorders in prawns. Investigations have to be undertaken in this area to find out the adverse effects of dietary nucleic acids in prawns, if any.

The application of single cell protein in aquaculture is a relatively recent practice and the interest in such practices is increasing rapidly. Single cell proteins (SCP) include micro algae, bacteria and yeast, and are alternative for conventional protein sources that are frequently used as feed ingredients for fish due to the nutritional values of their nutrients such as proteins, B-vitamins, pigments and complex carbohydrates, such as glucans (Sanderson and Jolly, 1994; Tacon, 1994). Among SCP, yeasts have been the most used within aqua feeds (Tacon, 1994). As a protein source, single cell proteins (SCP) of yeast or bacterial origin appear especially attractive because the protein content and aminoacid composition of these organisms compare well with those of fish meal (Spinelli *et al.,* 1979). Most of the studies performed so far on the use of yeast as food source for crustaceans and fishes were related to the baker's yeast as main source. Studies on the use of marine yeast as main source of protein are limited. In the present study, marine yeasts with better nutritional value were selected and incorporated into the feed of *Fenneropenaeus indicus*. Live micro algae and *Artemianauplii* have been used as essential sources of nutrition in penaeidlarviculture (Cook & Murphy, 1966; Simon, 1978; Tobias-Qunitio& Villegas, 1982). The culture and maintenance of these live food organisms, however, are tedious, labour intensive and expensive. Several attempts have been made to substitute non-living foodstuffs for live food using powdered Soya cake (Hirata *et al.,* 1975), microencapsulated diet (Jones *et al.,* 1979) and micro coated diets (Villegas & Kanazawa, 1980). Throughout this long period, however, reports on developing and using new live food organisms for shrimp larviculture were scarce. Yeast was used as an inexpensive and easily available alternative food for rotifers (James *et al.,*1983 and 1987). Imada (1984) reported rotifers cultured successfully with baker's yeast contained high levels of vitamin B12. Experiments by Abdel Rahman*et al*. (1993) and Chatila, 1994 showed that yeast, *Candida utilis*, is an excellent food for rotifers and Artemia and thus it may be a suitable food for other filter feeders as it has many advantages, such as suitable size range (7-40μm), high nutritive value and simple culture methods. In the present study, the growth and survival of prawns maintained on yeast diets were much higher when compared to the control diet and the commercial feed. 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 protein, lipids, B-vitamins etc. (Mahnken, 1991; van der Meeren, 1991).

Experiments by Hecht and Viljoen, 1982; Dabrowski *et al.,* 1983; Alami-Durante *et al.,* 1991 showed that common carps can utilize a high portion of their dietary protein from the yeasts *Candida tropicalis*, *C. utilis* and *C. lipolytica* with better results than those obtained with soybean or meat and bone meals. In this study also *C. tropicalis* was found to be a good feed supplement for prawns. James *et al*. (1987) reported high production yields in Artemia when fed with yeast, *Candida*. Blanco Rubio (1987) also reported *Torula* yeast (*Candida utilis*) as a promising food for cultivating Artemia. Naessens-Foucquaert*et al.* (1990) reported that yeast-based diets have proven to be a valuable algal substitute in the larval culture of marine shrimp. Among the three marine yeasts, *Debaryomyces hansenii* S8 supported the best biogrowth parameters, in terms of production, FCR, SGR, GGE, PER and CUD followed by S186 (*Candida tropicalis*) and S100 (*Debaryomyces hansenii*). Commercial feed was found to be better in efficiency compared to the Baker's yeast diet and the control diet. Nell *et al*. (1996) evaluated the effect of *D. hansenii*, *C. utilis*, *S. cerevisiae* and *D. capitatus* as dietary supplement in oyster and found it to be inferior to the algal diet. Yeast cell wall constitutes 15-25% of the dry weight of the whole cell and consists of 80-90% complex, difficult to digest polysaccharides (Fleet, 1991). Low digestibility of yeast cell wall may be the reason for the comparatively low nutritional effect with respect to an algal diet.

In the present study algal supplements were not included and the results obtained with marine yeast diets for *Fenneropenaeus indicus* were highly promising. Duncan's multiple range test (Statistical analysis) showed a significant increase ($P<0.05$) in the performance of all the three marine yeast feeds compared to other diets. A detailed analysis of biochemical composition of these yeasts is essential to elucidate the reason for the food value of the marine yeasts. The limited digestibility of yeasts for bivalves has been attributed to their low digestibility (Epifanio, 1979) as well as deficiency or imbalance of nutrients (Urban and Langdon,

1984). The bivalve stomach is well equipped for the digestion of algal carbohydrates by the presence of various carbohydrases (including chitinase and laminarinase) (Reed, 1981). However the enzymes are not necessarily appropriate for an efficient digestion of the polysaccharides composing the cell wall of intact yeast cells (Coutteau *et al.,* 1990). Feed intake depression in rainbow trout could be observed by many workers when fed Brewer's yeast diet (Tacon and Cooke, 1980; Rumsey *et al.,* 1991a and b and Atack and Matty, 1979). On the contrary, Rumsey *et al*. (1992) and Oliva-Teles and Goncalves (2001) noted no negative effects on feed intake at 30% level inclusion of Brewer's yeast for rainbow trout and in sea bass respectively. In the present study also no reduced feed intake could be noticed. This study shows the potential of marine yeasts as a feed supplement in aquaculture. Yeasts are nutritionally rich with proteins, vitamins and carbohydrates. Besides being a nutritional source, yeasts serve as an immunostimulant also by virtue of its high carbohydrate (β, 1-3 glucan) and RNA content. Technology for mass production of the marine yeasts, storage and incorporation into diet has to be developed for application in culture systems.

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