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Use of Brewer's Yeast *Saccharomyces cerevisiae* as Growth Promoter in Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man) Post Larvae

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Abstract

A feeding trial was conducted for 75 days to study the effects of live brewer's yeast (*Saccharomyces cerevisiae*) on growth and body composition in post larvae of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). Five diets were formulated containing optimum protein level of 35.24±0.04. These experimental feeds were supplemented with 0.0% (control diet), 0.1%, 0.2%, 0.5% and 1.0% live cells of *S. cerevisiae.* Weight gain, specific growth rate and protein efficiency ratio tended to increase in the postlarvae fed with either of the live yeast supplemented diets. The significant higher growth ($P<0.05$) performance, tissue protein, ether extract, improved specific growth rate (SGR), feed conversion ratio (FCR) was obtained in post larvae fed 0.5% of *S. cerevisiae* supplemented diets, suggesting that brewers yeast is an appropriate growth promoting feed additives in *M.rosenbergii* post larval feed.

Keywords: *Macrobrachium rosenbergii*, probiotic, yeast.

Introduction

Aquaculture is the fastest growing food-producing sector in the world, with an average annual growth rate of 8.9% since 1970, compared to only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period (Subasinghe, 2005). Freshwater prawn (*Macrobrachium rosenbergii*) farming is rapidly growing sector mainly due to the recent development of culture technologies and their greater environmental sustainability compared to other crustaceans. Because of its large size, tolerance to changes in water quality, ability to cope with handling stress and ability to feed on unconventional feed, it is cultured worldwide (El-Sayed, 1997). The total world farmed *M.rosenbergii* production have increased to 221,174 in 2007 from 82,058 tones in 1998 (FAO, 2009).

Aquaculture require high-quality feeds with high protein content, which should not contain only necessary nutrients but also complementary additives to keep organisms healthy and favor growth (Lara-Flores, 2003). Feed is often the major expense in pond production of freshwater prawns *M. rosenbergii*, representing as much as 40-60% of the operating costs (D'Abramo and Sheen, 1991). To reduce feed costs and improve performance, feed additives including microorganisms have been tested. Some of the most utilized growth-promoting additives are hormones, antibiotics, ionophores, and salts (Klaenhammer and Kullen, 1999). However, some feed additives, such as antibiotics, can cause intestinal disorders (Hinton, 1986) or disease resistance in pathogenic bacteria (Lara-Flores, 2003). Residues of antibiotics in aquaculture products may cause problems to human health (WHO, 2006). Many of the probiotic bacteria alone or in combination have been used as alternative feed additives in aquaculture (Ganguly, 2010; Wang and Wang, 2008; Noh *et al.*, 1994; Ghosh *et al.*, 2005; Appelbaum and Uland, 1979; Saad *et al.*, 2009; Seenivasan *et al.*, 2011; Venkat *et al.*, 2004).

To harness the beneficial effects of probiotics, species-specific microorganisms should be isolated and multiplied to facilitate their establishment in the gut of the host fish. However, it is difficult to isolate and multiply indigenous species-specific microorganisms from fish under farm conditions. Little experimentation has focused directly on live yeast supplementation effects on post larvae of Giant freshwater prawn growth and survival. This study was carried out to evaluate the effect of non- indigenous dietary live brewer's yeast (*S. cerevisiae*), a feeding trial was conducted for 75 days to study the effects of live brewer's yeast (*Saccharomyces cerevisiae*) on

growth and body composition in post larvae of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man).

Materials and Methods

Culture of Post Larvae

The experiment was conducted for 75 days using post larvae obtained from the Central Institute of Fisheries Education (CIFE) hatchery in Mumbai, India. Post larvae were acclimatized for seven days during which they were fed a control diet and supplied continual aeration from a compressed air pump. After acclimation, post larvae (92.66±.85 mg) were stocked in 100-L rectangular tanks at 20 post larvae per tank. The post larvae were fed diets containing 0 (control), 0.1%, 0.2%, 0.5% and 1.0% *S. cerevisiae* (the weight of the rice bran was reduced as the bacteria content was increased) with three replicates per treatment. Equal and sufficient pieces of asbestos sheet and plastic pipe cuttings were provided in every tank as hideouts to minimize cannibalism. Water was exchanged on alternate days. Water temperature, pH, dissolved oxygen, free carbon-dioxide, and carbonate hardness were recorded once a week following methods suggested by APHA (2005). Temperature ranged 26.50-30.3°C, dissolved oxygen 6.0-7.2 mg/L, and total alkalinity 76.5-122 mg/L. Ammonia (NH_3) ranged 0.06-0.14 mg/L and nitrate-N 0.01-0.06 mg/L. Nitrite-N and PO_4 were within optimum ranges: 0.001-0.005 mg/L and 0.06-0.09 mg/l, respectively.

Table 1. Composition of ingredients (% dry weight)

Fish meal	15
Prawn head meal	15
Mustard oil cake	20
Soybean meal	16
Rice bran	18,5
Wheat flour	10
Ragee seed	02
Fish oil	02
*Vit-mineral mix	01
Carboxymethylcellulose	0,5

*Vitamins and minerals mixture added in the experimental diets

Calcium-2.5%, Phosphorous-2.5%, Potassium-0.4%, Salt-0.1%, Chloride-0.1%, Magnesium-0.15%, Iron-3.0 mg, Copper-0.1 mg, Manganese-0.25 mg, Zinc-1.4 mg,

Vitamins-Vitamin A-1,000 IU,Vitamin D-100 IU, Vitamin E-2 IU, Thiamine-0.81 mg, Riboflavin-1.0 mg, Niacin-10.0 mg, Pyridoxine-0.1 mg, Vitamin B_{12}-0.5 mg

*Company name- Pfizer,Mumbai-400102, India Ltd.

Ingredients: Wheat germ, Kaolin, Corn syrup, Pork liver meal, Di-calcium phosphate, Sucrose, Lactose, Safflower oil, Gelatin, Corn starch, Stearic Acid, Niamicinamide, Hydrolyzed vegetable protein, Iron oxide and peptone, Magnesium stearate, di alpha tocopheryl Acetate, Vitamin A, Acetate, Zinc Oxide, Riboflavin, Thiamine mononitrate, Pyridoxine hydrochloride, Cynocobalamin, Manganese sulphate, Copper Acetate monohydrate, Vitamin D_3 supplement, Cobalt sulphate.

Feed Preparation

For each diet, powdered ingredients were thoroughly mixed with water to make dough that was steam-cooked 20 min in a pressure cooker (Table 1). The vitamin/mineral premix was added homogeneously after cooling the dough. *S. cerevisiae* cultures were harvested, washed, and added to the dough together with the vitamin/mineral mix. The live brewer's yeast culture was prepared as follows: Inoculums of *S. cerevisiae* was suspended in one liter flasks containing yeast culture broth, incubated 24 h, and centrifuged at 3000 rpm in a refrigerated centrifuge (Remi, Mumbai, India) to obtain a pellet. The pellet was washed twice with normal saline (0.85%) and weighed. Portions of pellets were added to the feeds to obtain the desired level of yeast. The dough was pressed in a hand pelletizer to obtain 1.5-mm (diameter) feeds that were dried overnight in a hot air oven at 40°C until it reached a constant weight and stored in air-tight containers at room temperature until use. Every 15 days, the viable colony forming units (CFU) per gram feed was determined. To determine the number of CFU of yeast in one gram of feed, 0.1 g of the feed pellet was suspended in 10 ml sterile normal saline (0.85%) and 0.1 ml of this serially diluted sample with added levels of antibiotic to suppress the growth of bacteria were plated in triplicate on yeast agar following the spread plate technique.

Screening for Gut Microbes

Before the start of the feeding trial, isolates from different post larvae organs were subjected to biochemical tests to find out the common microbes residing in the organs of *M. rosenbergii* post larvae. The identification of *S. cerevisiae* isolates was carried out according to conventional yeast identification methods based on the morphology, sporulation and fermentation characteristics, as well as the assimilation of a range of carbon sources (Kreger-van Rij, 1984).

Biochemical Analyses

Proximate compositions of the prawn tissue (carcass) and feeds were analyzed in the Nutrition and Biochemistry Laboratory of the CIFE following standard methods (AOAC, 1995). Moisture was determined by drying weighed samples at 105°C to a constant weight and calculating the difference in weight. Nitrogen contents were analyzed by the Kjeltec system (2200 Kjeltec Auto Distillation, Foss Tecator, Sweden) and crude protein was calculated by multiplying the percent nitrogen by 6.25. Ether extract was designated as crude fat and determined using Soxtec System (1045 Soxtec Extraction Unit, Foss Tecator, Hoganas, Sweden). Diethyl ether (boiling point 40-60°C) was used as a solvent and the weight

of the extract was expressed as a percent of the tissue weight. Ash content was estimated by incinerating samples in a muffle furnace at 600°C for 6 hours. Total carbohydrate (%) was calculated by subtracting protein, fat, moisture, and ash from the total weight. Proximate carcass compositions were analyzed before initiation and after termination of the experiment.

Growth Study

Post larvae from each tank were bulk weighed at the end of experimental duration of 75 days. Growth performance was assessed as percent wt gain = 100(final wt - initial wt)/initial wt, specific growth rate (SGR) = 100(ln final body wt - ln initial body wt)/75 days, feed conversion ratio (FCR) = wt feed given/wt gain, protein efficiency ratio (PER) = wt gain/protein intake, and apparent net protein utilization (ANPU) = 100(final carcass protein - initial carcass protein)/protein fed.

Statistical Analysis

Data were statistically analyzed using SPSS version 16.0. One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (Duncan, 1955) were applied to compare means between treatments.

Results and Discussion

Yeast Survival in Feed

The survival of yeast in experimental feeds affects growth performance of the host. In the present study, the number of colony forming units in the feeds dropped as the storage period of the feed increased (Table 2). Gabriel Aguirre-Guzman (2002) also observed reduced counts of agglomerated *S. cerevisiae* in pelleted shrimp feeds, though the level of reduction may vary according to the procedure applied and temperature and moisture level used for drying.

Gut Microbes

Pseudomonas spp. *Citrobacter* spp. *Aeromonas* spp., *Enterobacter* spp., and *Proteus* spp., of bacteria were commonly occurring in gills of post larvae. *Citrobacter* spp. *Aeromonas* spp., *Enterobacter* spp., *Plesiomonas* spp., and *Streptococcus* spp., were commonly occurring bacteria observed in hepatopancreas. *Pseudomonas* spp., *Citrobacter* spp., *Pantoea* spp., *Streptoccus* spp., *Sphingomonas* spp., *Micrococcus* spp., were most common bacterial genera observed in haemolymph of post larvae of *M.rosenbergii.* Commonly observed bacterial genera in gut of post larvae were *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Streptococcus* spp., *Klebsiella* species. The occurrence of bacteria may vary according to the water quality characteristics used in rearing of post larva and bacterial load present in feed supplied to the early larval stages. Bacterial composition in pond water, hatchery and larvae, rearing water has been studied by Lalitha and Surendran (2004); and Uddin and Al-Harbi (2005), also reported some similar genera as observed in this study. Colony characteristics and biochemical tests showed that *S. cerevisiae* was not present among the indigenous micr-oflora in the post larvae.

Biochemical Composition of Feeds and Carcasses

Biochemical compositions and brewer's yeast content contents of the diets are presented in Table 3. Protein content of the diets were varying between 35.23±0.04 to 35.24±0.10 percent. Lipid content of diets were varying between 5.63±0.05 to 5.66±0.02.The diets prepared were statistically similar in their protein and lipid and carbohydrates values.

After the feeding trail the proximate composition of the post larvae shows that the carcass protein and lipid content in post larvae fed the supplemented feeds are containing significantly higher protein and lipid amount($P<0.05$) compared to the post larvae fed control diets. Highest crude protein and crude lipid content were found in post larvae fed with 0.5% yeast supplemented feed. Moisture was lowest in the post larvae fed with 0.5% yeast supplemented diets. There was no specific trend observed for carbohydrate contents among the different treatments. Ash content were almost similar and were not differing significantly ($P<0.05$) among post larvae fed the different test and control feeds. Similarly, significantly improved basic carcass biochemical constituents was previously observed in *M.rosenbergii* post larvae fed with live *L. sporogenes* (Prasad *et al.,* 2012); bio-encapsulated *L. sporogenes* (Seenivasan *et al.,* 2011) and yeast *S. cerevisiae* (Lara-Flores *et al.,* 2010).

Growth Performance

Probiotics have beneficial effects in shrimp aquaculture and commercially available microbes provided through feeds are used to improve growth

Table 2. *Saccharomyces cerevisiae* counts in different feeds after 15 days interval

Day					
0(x10^5 CFU/100g±SD)	0.0	0.1% Y	0.2% Y	0.5% Y	1.0% Y
15 (% Survival)	0.0	66.66±5.61	65.60±4.16	65.83±3.65	66.67±2.54
30 (% Survival)	0.0	35.20±2.72	35.84±5.68	35.32±4.15	34.44±3.02

Y:Yeast (*Sacchacromyces cerevisiae*)

(Gomezgil, 1995). Various species of yeast and different yeast containing products have been fed to fish and crustaceans for assortment of reasons. Dietary yeast has been reported to improve the growth of fish species also (Noh *et al.,* 1994; Oliva-Teles and Goncalves, 2001; Lara-Flores *et al.,* 2003; Li and Gatlin; 2003, 2004).In addition to probable immunostimulant properties (Siwicki *et al.,* 1994; Nakano *et al.,* 1995). In the present study, all the four yeast supplemented diets showed better result than with control diet (Table 4). The highest weight gain percentage, SGR, PER, and ANPU and lowest FCR ($P<0.05$) were obtained with the 0.5% diet followed by the post larvae fed with 0.2%,1.0% and 0.1% yeast supplemented diet. Similarly, improved growth with *L. acidophilus* and yeast *S. cerevisiae* have been reported in Koi Carp (Dhanraja *et al.,* 2010). Suralikar and Sahu (2001) also reported improved growth in post larvae of *M. rosenbergii* when fed with *L. cremoris* supplemented diets (in *M. amazonicum* juveniles fed with *S.cerevisiae* and yeast derivatives inclusion diet (Hisano *et al.,* 2008). Lara- Flores *et al.* (2003) also observed similar results of best growth performance in all the yeast supplemented diets in Nile Tilapia (*Oreochromis niloticus*). In Indian major carp species *Mrigala (Cirrhinus mrigala)* fingerlings similar results of improved the weight gain, FCR, SGR, and PER were observed when *L. coagulans* and *S. cerevisiae* were added to feed (Swain *et al.,* 1996). Barnes *et al.* (2006) also observed improved growth performance in rainbow trout fed with yeast supplemented diets.

All the diets containing brewer's yeast showed better growth and feed utilization capacity. Significantly higher ($P<0.05$) effect were achieved at 0.5%, this can be analyzed by comparing other parameters such as SGR, FCR, PER, and ANPU, are presented in Table 5. The PER and ANPU indicate that supplementing feeds with yeast significantly improves the protein utilization capacity of *M. rosenbergii* post larvae. This contributes to optimizing protein use for growth, significant since protein is the most expensive feed nutrient. Better efficiency of protein uptake may be due to better digestion and assimilation of nutrients in the gut by the supplemented micro-flora, possibly by virtue of essential and non essential amino acids contained in yeast (Appelbaum, 1979; Shcherbina *et al.,* 1987; Cheng *et al.,* 2004). Yeast may also be improving fish nutrition indirectly by producing polyamines (Vazquez- Juarez *et al.,* 1993; Tover *et al.,* 2002 ; Tovar- Ramirez *et al.,* 2004).

In conclusion, feeding non-indigenous *S. cerevisiae* through prepared feed improves growth performance, feed utilization capacity and nutritional profile of *M. rosenbergii* post larvae. Therefore,0.5% live *S. cerevisiae* can be incorporated in feed formulations for better growth performance of *M.rosenbergii* post larvae.

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Table 3 . Proximate composition (Mean ± SD) of diet (% dry matter basis) *Sacchacromyces cerevisiae* supplemented diets and Control diets

Proximate composition	0.0% Y	0.1% Y	0.2% Y	0.5% Y	1.0% Y
Crude protein	35.24±0.06	35.23±0.04	35.24 ±0.1	35.25± 0.09	35.23 ± 0.1
Crude lipid	5.63 ± 0.30	5.63 ± 0.11	5.65 ± 0.06	5.66 ± 0.02	5.63 ± 0.05
Carbohydrate	44.62±0.28	44.64 ± 0.20	44.61±0.01	44.61±0.01	44.58 ± 0.01
Ash	14.49±0.03	14.50±0.03	14.51±0.01	14.51± 0.02	14.52 ± 0.02
Energy (kcal/100 g)	392.94±1.5	392.88±0.50	392.87±0.75	393.03±0.90	392.87±0.50

Y: Yeast (*Sacchacromyces cerevisiae*)

Table 4. Proximate composition of *M. rosenbergii* Post Larvae (% dry matter basis) tissues fed with *Sacchacromyces cerevisiae* supplemented diets and control diets

	0.0% Y	0.1% Y	0.2% Y	0.5% Y	1.0% Y
Moisture	71.67 ± 0.54^{b}	71.34 ± 0.45^{ab}	71.24 ± 0.80^{ab}	69.68 ± 1.45^{a}	70.53 ± 0.50^{ab}
CP	58.82 ± 0.24^{a}	59.40 ± 0.081^{b}	59.74 ± 0.98^{c}	60.50 ± 0.21^{d}	59.72 ± 0.13^{c}
EE	3.56 ± 0.06^{a}	3.93 ± 0.14^{b}	4.09 ± 0.10^{c}	4.64 ± 0.04^{d}	4.11 ± 0.03^{c}
Ash	15.67 ± 0.57^{a}	15.23 ± 1.03^{a}	15.52 ± 0.52^{a}	15.08 ± 0.43^{a}	15.09 ± 0.08^{a}
TC	21.94 ± 0.39^{d}	21.43 ± 0.89^{bc}	20.63 ± 0.39^{ab}	19.77 ± 0.46^{a}	21.07 ± 0.04^{c}
OM	84.32 ± 0.52^{ab}	84.76 ± 1.03^{a}	84.48 ± 0.52^{a}	84.91 ± 0.43^{a}	84.90 ± 0.08^{a}
Energy Kcal/100g	14.91 ± 0.10^{a}	$15.06\pm.20^{ab}$	15.05 ± 0.11^{ab}	15.23 ± 0.06^{b}	15.12 ± 0.015^{ab}

(*Values with identical superscript letters in the same row are not significantly different ($P<0.05$)

OM - Organic matter = 100-Ash, CP- Crude prtotein =(N%x6.25), EE- Ether extract, TC=Total Carbohydrate. Y:Yeast (*Sacchacromyces cerevisiae*)

Table 5. Growth performance of *M. rosenbergii* fed experimental diets for 75 days (mean±sd) supplemented with *Saccharomyces cerevisiae* supplemented diets

Performance	0.0%Y	0.1%Y	0.2%Y	0.5%Y	1.0%Y
Initial weight (mg)	92.33±3.56[a]	92.66±3.01[a]	94.00±2.17[a]	91.66±2.75[a]	92.66±2.51[a]
Final weight (mg)	421.48±2.74[a]	461.11±2.93[b]	486.48±2.31[d]	477.96±5.56[c]	455.37±2.56[b]
Live weight gain (%)	356.64±9.36[a]	397.89±13.74[bc]	417.71±11.73[bc]	421.84±9.65[d]	391.61±11.78[b]
SGR (% per day)	2.02±0.02[a]	2.14±0.03[bc]	2.19±0.03[c]	2.19±0.30[c]	2.12±0.03[bc]
FCR	1.95±0.05[c]	1.72±0.02[b]	1.67±0.04[ab]	1.61±0.03[a]	1.73±0.02[b]
PER	1.45±0.03[a]	1.64±0.02[bc]	1.72±0.04[bc]	1.76±0.10[cd]	1.63±0.04[b]
ANPU	22.36±2.04[a]	25.00±6.89[ab]	32.06±1.01[c]	32.42±5.25[c]	32.22±0.48[c]
Survival (%)	90	90	90	90	90

*Value with identical superscript letters in the same row are significantly different (P<0.05)

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