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An ex vivo Loom to Evaluate the Brewer's Yeast *Saccharomyces cerevisiae* in Clownfish Aquaculture with Special Reference to *Amphiprion percula* (Lacepede, 1802)

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Abstract

This study was conducted to assess the role of dietary commercial brewer's yeast, *Saccharomyces cerevisiae* on the growth factors (final weight, specific growth rate (SGR) and feed conversion ratio (FCR)), survival rate, haematological parameters and serum metabolic products (glucose and total protein) of the clown fish, *Amphiprion percula*. Feeding trial was carried out for 90 days to study the effect of brewer's yeast on the young ones of clown fish, *A. percula*. Fifty young ones (avg. wt 1.345±0.03 g) were randomly distributed in each treatment and fed with different diets, prepared with six incremental levels (0%, 0.5%, 1%, 2%, 3%, and 5% of the feed) of brewer's yeast except the control. Haematology and serum parameters were studied before pathogen challenge and they were significantly higher ($P<0.05$) in 2% yeast supplemented group. Survival (%) of the fish, after challenging with *Streptococcus* sp., was also higher in the yeast fed groups. Results of our short-range study give an idea about the role of yeast in marine ornamental aquaculture.

Keywords: Clownfish, *Amphiprion percula*, growth performance, fish pathogen, challenging study.

Introduction

India is a rich country blessed with enormous wealth of marine resources, compare with its counterparts of the world. On one hand, our aquatic resources are dwindling and we are losing precious genetic resources and on the other hand neither do we have a contingency plan nor an appropriate technology for culture of most of the endemic cultivable ornamental species. Aquaculture is the answer to meet food security, restore biodiversity and also for trade. Ornamental fish rearing is an important component of the fishery industry in several nations. For the economic benefits of aquaculture production, particularly on ornamental organisms, a conscious effort to develop it in these areas is necessary. Among the coral reef fishes, clown fishes are considered to be most popular attractions of aquarists and they play a major role in the aquarium trade in view of their bright colour, interesting display behaviour and their ability to adapt in captive conditions (Ajith Kumar *et al.*, 2010). Among the different species of clowns, the true clownfish, *Amphiprion percula* has a high demand in aquarium trade but its production in captivity poses severe problems especially in its early life stages (Dhaneesh, 2009). Stresses in captive rearing conditions frequently result in higher mortality rates and growth abnormalities (Can, 2013). Probiotics are well known to make positively impact to fish welfare (Kesarcodi-Watson *et al.*, 2008) by reducing the general stress response and promoting growth (Suzer *et al.*, 2008).

Generally, probiotic administration during early developmental stages is most effective, usually resulting in greater than an order of magnitude increase in survivorship (Gatesoupe, 2007). It has also been reported that in captive rearing, higher mortality occurs frequently (Benetti *et al.*, 2008) and growth abnormalities lead to higher incidence of skeletal deformities (Fernandez *et al.*, 2008).

Brewer's yeast is a natural feed additive, which positively influences the non-specific immune responses of many aquaculture species (Thanardkit *et al.*, 2002). β Glucans, which are most commonly found in the cell wall of yeasts are generally considered as the main factor for the immunological mechanism (Can *et al.*, 2012). Brewer's yeast, *S. cerevisae* contains various immunostimulating compounds such as β glucans, nucleic acids as well as mannon oligosaccharides which have the capability to enhance the immune responses (Abdel- Taw wab *et al.*, 2008) as well as growth performance (Li and Gatlin, 2005) of various fish species. In view of above mentioned factors, we incorporated the probiotic

strain *S. cerevisae* in the clown fish (*Amphiprion percula*) juvenile's regular diet to augment the growth rate and survivorship. The resistance of fish can be evaluated by analyzing the survival rate after experimental infection (Wasson and Kelly, 1990) as well as hematological and immunological parameters (Martins *et al.*, 2009; Can *et al.*, 2012) We have also evaluated the response of probiotic treatment through morphometric measurements such as total length and body weight.

Materials and Methods

Study Location

The present study was carried out in the Brood Stock Bank for Marine Ornamental Fish Facility, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India.

Experimental Animal

Hatchery bred clown fish juveniles, *A. percula* (1.345±0.03 g) were obtained from the marine ornamental fish hatchery of the Centre of Advanced Study in Marine Biology. The fishes were kept an indoor glass tanks for two weeks for acclimatisation to laboratory conditions.

Experimental Design

Experimental trials were carried out with six glass tanks (300 L) and three replicates for treatment and control was maintained, followed fishes were accommodated at an initial density of 50 fishes per tank. Theses fishes were fed three times daily at 6:00, 12:00 and 18:00 h with the formulated diet. Feeding rate was 5% of total body weight (g) and properly regulated. Every seventh day, 30% of the water was siphoned out and supplied with running estuarine water (23-25ppt) filtered through a Ultra Violet light and bio filters. Water temperature was maintained at 25-26°C with a photoperiod of 12 h light and 12 h darkness. Weekly measurements were made for ammonia, nitrite and pH. Dissolved oxygen level was maintained at 6 mg L^{-1}. Each tank was supplied with compressed air via air-stones using air pumps. Settled fish wastes were siphoned out daily without much loss of water. Fishes in each tank were group-weighed every 2 weeks and dead fishes were removed.

Diet Preparation

Feed was formulated (ingredients [g kg^{-1}]: fish meal - 250, rice bran - 160, groundnut oil cake - 480, binder - 100, vitamin mix - 10) with different levels of commercially available yeast, *S. cerevisiae* were supplemented at 0.0 (control), 0.5, 1.0, 2.0, 3.0 and 5.0 g kg^{-1} diet. The paste was passed through a grinder and pelleted in a paste extruder. The diets were air-dried and stored in plastic bags at -2°C for further use (Simi Rose Andrews *et al.*, 2011).

Chemical Analysis of Diet

The test diet for each treatment was analyzed adopting the standard methods of AOAC (2011) for moisture, crude protein, fat and ash. Moisture content was estimated by drying the samples to constant weight at 85 °C in a drying oven.

Feed Performance Analysis

Growth performance of the fish was determined and feed utilization was calculated using the following standard formula (Mohsen Abdel-Tawwab *et al.*, 2008).

Weight gain = Final weight (g) - Initial weight (g)

Specific Growth Rate (SGR) = 100 (ln W_2 - ln W_1)/T

where W_1 and W_2 are the initial and final weight, respectively, and

T is the number of days in the feeding period

Feed Conversion Ratio (FCR) = feed intake (g) /weight gain (g)

Protein Efficiency Ratio (PER) = weight gain (g) /protein intake (g)

Apparent Protein Utilization (APU; %) = 100 × (protein gain (g) / protein intake (g))

Haematological Measurements

At the end of feeding trials, five fishes of each experimental group were randomly selected. They were anesthetized, and approximately 0.2 ml of blood was collected from the caudal vasculature using a 1-ml syringe and 27-gauge needle and allowed to clot at room temperature and, centrifuged at 1,400 g for 10 minutes. Serum aliquot was taken using micropipette and stored at -80ºC till further analysis. For total protein concentration and total immunoglobulin were found with the modifying method of Panigrahi *et al.*, (2005). Total hemolytic complement was determined adopting the method of Nonaka *et al.*, (1981).

Bacterial Challenge Study

In this study, a bacterial pathogen, *Streptococcus* sp. (isolated from clown fishes) was collected from the marine ornamental fish hatchery of the Centre of Advanced Study in Marine Biology. The bacterium was cultured and collected by a modified method of Simi Rose Andrews *et al.* (2011) and it was used for the susceptibility study in *A. percula*. At the end of experiment, fishes of each experimental diet were exposed to an estimated LD50 dose of the pathogen. For this, 20 fishes from each tank were infected with virulent *Streptococcus* sp. The pathogen were grown

in Tryptic Soy Broth on 24 hours at 28°C and diluted in sterile PBS to give 1 x 10^6 CFU per ml of tank water. Fishes from each tank were challenged by immersion in 10 L of this solution for 30 minutes with aeration and returned to their respective tanks for observation. Fishes were monitored for disease signs for 10 days, and dead fish were removed daily. After 21st day of post-challenge, surviving asymptomatic fish were bled as before from the caudal vasculature. The blood was immediately transferred to Triptic soy agar plates and colony-forming units were counted after 24 h incubation at 26°C. The results were analysed by pooling of treatment data.

Statistical Analysis

Data were subjected to one-way ANOVA to test the effect of dietary commercial brewer's yeast on growth performance, weight gain, hematology and serum biochemistry of the clown fish, *A. percula*. When significant differences were detected (P<0.05), Duncan's multiple range test was used to compare mean values among the dietary treatments. Data have been expressed as mean values ±S.D. All statistical analyses were made using the statistic software SPSS version 16.0 as described by Dytham (1999).

Results

Proximate composition of the experimental diets given in Table 1 and the growth performance of the experimental fishes is given in Table 2. When formulating the diets, the intention was to have similar crude protein content in all diets. The body weight gain of experimental animal was recorded at interval of 2 weeks. The final weight of the fish, weight gain and specific growth rate increased significantly (P<0.05) with the increase in dietary yeast level. It was observed that the optimum growth was obtained at 2g yeast in kg^{-1}. Results also indicated that among the offered diets to the fishes, brewer's yeast exhibited greater weight gain than those of control diet (Figure 1). There was no significant difference in triplicate experimental growth (P>0.05) able fish offered with various inclusion levels of yeast.

Total erythrocyte, haemoglobin, glucose, total protein and globulin levels of *A. percula* are shown in Figure 2. There was significant difference (P<0.05) in the total erythrocyte count and haemoglobin concentration among the treatment groups. Irrespective level of yeast, the erythrocyte count and haemoglobin concentration was significantly (P<0.05) higher in 2% yeast fed group compared to the other concentrations. Lowest value was recorded in control and 0.5% is supplemented groups. Level blood parameters were significantly higher in 2% yeast supplemented group compare to control, 0.5%, 1%, 3%, 4% and 5% yeast supplemented groups.

Clown fish fed with diets supplemented with yeast (2%) showed positive influence of resistance to *Streptococcus* sp. (Figure 3). In the disease challenged fishes 37% mortality was observed after 20 days, where the fishes fed the diet with 5% yeast. The survival rate of the fish fed with the diet containing brewer's yeast was significantly lower (P<0.05) than the fish fed with basal diet after the same period. The bacterial count, after incubation with the fish sera has also decreased with the increase of the yeast level in fish diets. The lowest bacterial count was noted in the fish fed with 5.0 g yeast/kg, whereas the highest count was recorded in fish fed with control diet.

Table 1. Proximate composition of the formulated feed used in the experiments

Sample no	Moisture (%)	Carbohydrate (mg g^{-1})	Protein (mg g^{-1})	Lipid (mg g^{-1})	Ash (%)
Control	70.23 (± 0.23)	19.792	45.09 (±0.64)	23.6 (± 0.23)	10.84 (± 0.23)
0.5%	72.29 (± 0.41)	47.346	45.08 (±0.75)	29.6 (± 0.61)	11.12 (± 0.52)
1%	76.37 (± 0.73)	67.914	45.47 (±1.33)	34.0 (± 0.35)	12.23 (± 0.42)
2%	74.13 (± 0.24)	39.778	45.48 (±1.54)	27.6 (± 0.42)	12.42 (± 0.42)
3%	72.49 (± 0.11)	16.299	45.81 (± 0.98)	27.4 (± 0.32)	13.54 (± 0.65)
5%	71.22 (± 0.14)	49.267	44.58 (±0.45)	31.6 (± 0.41)	10.72 (± 0.52)

Table 2. Growth parameter and feed utilization of *A. percula* fry fed diet containing different level of yeast

Particulars	Control	0.5%	1%	2%	3%	5%
Initial weight(g)	1.345±0.03	1.345±0.03	1.345±0.03	1.345±0.03	1.345±0.03	0.3425±0.19
Final weight (g)	3.12±0.23	3.75±0.14	3.86±0.17	4.51±0.11	3.71±0.21	3.13±0.22
Weight gain (g)	1.775±0.22	3.41±0.12	3.55±0.18	4.14±0.13	3.47±0.17	2.78±0.21
Survival	92.3±0.38	95.12±0.31	96.5±0.22	99.34±0.13	99.11±0.21	97.3±0.30
SGR(g/day/fish)	3.1±0.014	3.788±0.071	3.944±0.092	4.6±0.091	3.855±0.72	3.088±0.003
FCR	0.071±0.02	0.058±0.07	0.056±0.11	0.048±0.07	0.057±0.04	0.071±0.09
PER	0.062±0.031	0.075±0.012	0.078±0.031	0.092±0.064	0.077±0.21	0.061±0.012

SGR - Specific Growth Rate; FCR - Feed Conversion Ratio; PER - Protein Efficiency Ratio

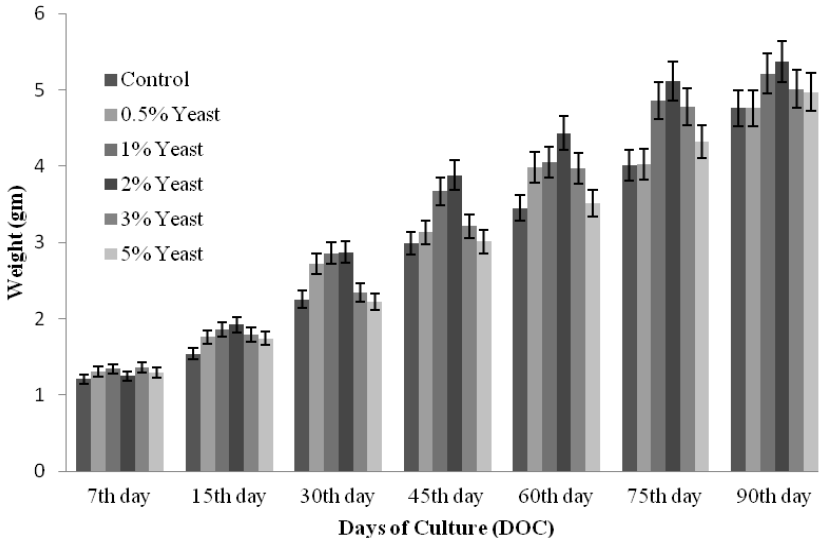

Figure 1. Weight improvement of *A. percula* during experimental period.

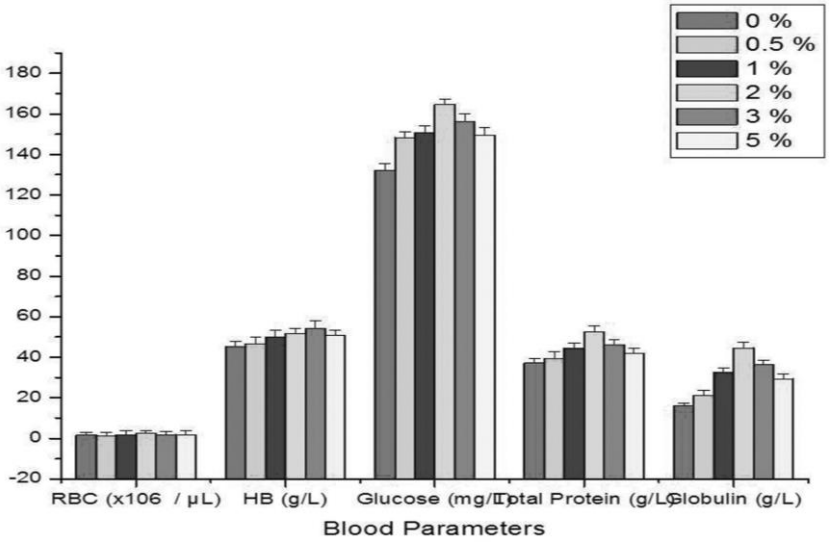

Figure 2. Effect of different sources and levels of yeast on serum parameters of *A. percula*.

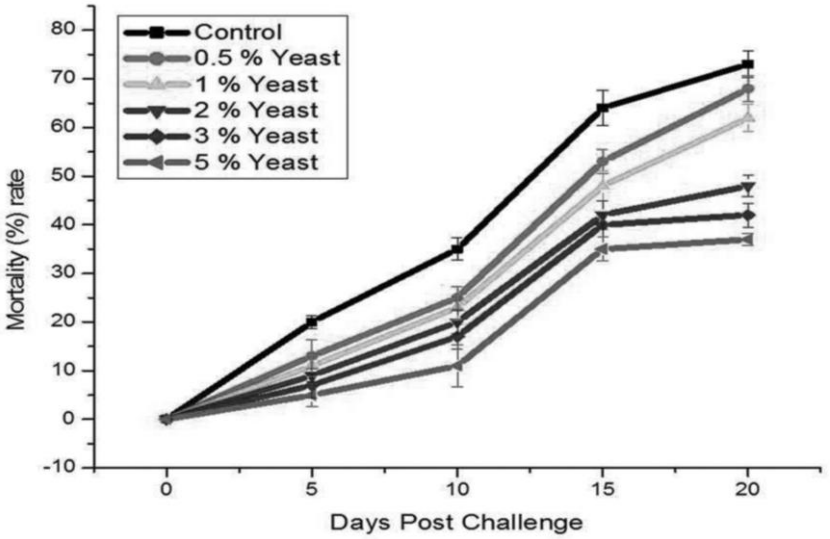

Figure 3. Mortality (%) of different experimental groups after challenge with *Streptococcus* sp. (mean ± SE) (N = 3)

Fishes showed typical symptoms of *Streptococcus* infection including extreme erratic swimming, haemorrhages around mouth and damages in fin and dark reddish pigmentation.

Discussion

Single cell proteins (SCPs) are the alternatives to conventional protein sources that are frequently used as feed ingredients for fish. They are gaining more attention in animal feed industry due to their immune boosting property (Siwicki *et al.*, 1994; Li and Gatlin, 2004 and 2005). In this regard, present study is an attempt to identify potent natural immunostimulants for the successful rearing of the ornamental clown fish, *A. percula*.

Brewers' yeast, *S. cerevisiae* has been recognized to have potential as a substitute for live feed in the production of certain fishes (Nayar *et al.*, 1998). In the present study also supplementation of the commercial live yeast, *S. cerevisiae* improved the growth, feed utilization and disease resistance of *A. percula*. This is in agreement with studies on Catla carp (Mohanty *et al.*, 1996), Hybrid striped bass (Li and Gatlin, 2004 and 2005) and Japanese flounder (Taoka *et al.*, 2006). Similar results have been obtained when *S. cerevisiae* was added to fish diet for Israeli carp (Noh *et al.*, 1994) and Nile tilapia (Lara-Flores *et al.*, 2003).

Improved fish growth and feed utilization observed in the present study could be possible due to the improved nutrient digestibility. In this regard, Tovar *et al.* (2002), Lara-Flores *et al.* (2003) and Waché *et al.* (2006) have found that the addition of live yeast, improved the diet and protein digestibility and this might explain the better growth and feed efficiency.

Weight gain observed in the fishes suggests that the yeast supplementation plays a role in enhancing the feed intake with a subsequent enhancement of fish body composition. The better intake of yeast supplemented diets by the fishes (1.0-5.0 mg kg^{-1} diet) might have been due to the increased fish appetite resulting in a higher feed intake and therefore improved growth due to the higher feed intake, nutrient utilization and higher nutrient digestibility.

Changes in the biochemical composition often provide with vital information, useful for health assessment and management of the cultured fish (Cnaani *et al.*, 2004; Řehulka *et al.*, 2004). In addition, results of immune response assays demonstrated that brewer's yeast can be administered for relatively long periods without causing immunosuppression. In the present study, fish fed diets containing 1.0–5.0 $g.kg^{-1}$ yeast exhibited higher RBCs, Hb, and Ht values, whereas glucose, protein and globulin values increased upto 2.0 $g.kg^{-1}$ yeast diet, and decreased afterwards. These results suggest that there is an improvement of fish health when fed with the yeast supplement. Moreover, these serum parameters will be of considerable diagnostic value in fish, as they relate to general nutritional status as well as the integrity of the vascular system and liver function, as observed by Schaperclaus *et al* (1992).

In the present study, fish mortality was observed 10-days after the infection of *Streptococcus* sp. and the bacterial count in the fish sera decreased significantly with the increase of yeast level in the diets. This suggests that the yeast supplementation could increase the nonspecific immune system of *A. percula* resulting in a fish resistance to *Streptococcus* sp. infection. This finding is supporting the study of Taoka *et al.* (2006) who investigated the effect of live and dead probiotic cells on the non-specific immune system of Nile tilapia. They found that the probiotic treatment enhanced the non-specific immune parameters such as lysozyme activity, migration of neutrophils and plasma bacteriocidal activity, resulting in improved resistance to *Edwardsiella tarda* infection.

This could be possible because of the fact that the bakers' yeast is a source of nucleic acids and β-1, 3-glucans that can effectively enhance immune functions, as reported in the African catfish (Yoshida *et al.*, 1995), Atlantic salmon (Engstad *et al.*, 1992), and rainbow trout (Jørgensen *et al.*, 1993; Siwicki *et al.*, 1994). Present study concludes that the brewer's yeast positively enhances the growth performance, feed utilization of clown fish and the disease resistance to *Streptococcus* sp. infection at the optimum level of 2.0 g kg^{-1} diet.

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