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EFFECT OF PROBIONTS ON THE GROWTH AND FOOD UTILIZATION OF CLOWN FISH, *AMPHIPRION SEBAE (*BLEEKER (1853))

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ABSTRACT : To evaluate the efficiency of different probionts (*Lacto bacillus* and yeast) on clown fish, *Amphiprion sebae* three different pelleted diets were formulated with 30% protein. Two diets were supplemented with probionts such as 2% *Lacto bacillus* and 2% yeast without probiont was used as control. The water stability of these diets was studied and the leaching percentage during 6 hrs period was 17.15 to 18.25%. The specific growth rate of control diet fed fish has the lowest value of 3.65 to 4% in probionts supplemented diets fed fishes. The gross production efficiency was also higher in probionts supplemented diet fed groups than control group.
Key words : Probiotics, aquaculture, clownfish, energetic, supplementary diet

INTRODUCTION

Anemone fishes are one of the most popular attractions in the international marine aquarium fish trade. They are well known for their symbiotic relationship with sea anemones. The anemone fishes, which are brightly coloured with interesting behavior and their ability to adapt to captivity are mainly responsible for this popularity. Anemone fishes represent some of nature‘s finest pair formation, protandry and have a superb strategy for egg security (Wilkerson, 1998). There are few reports, which say that this species has been bred in captive conditions in Philippines (Alava and Gomes, 1989) and Australia (Job *et al.,* 1997).

Aquaculture is one of the fast growing systems in the world, which has emerged as an industry possible to supply protein rich food throughout the world (Prassad, 1996). Currently varieties of marine and brackish water species of finfishes and shellfishes are cultured in over one million hectares for food in several Asian Countries (Broak, 1991). Presently, aquaculture is facing heavy production loss both in hatcheries and grows out systems due to disease outbreak. In many land animals, growth stimulating microorganisms incorporated in the feed are reported to have beneficial effects. Since, the microorganisms or probiotics are found to have the capability of improving the water quality, their application in aquaculture has gained momentum. Probiotics, live microbial food supplements that beneficially affect the host by improving its intestinal microbial balance, are quickly gaining interest as functional foods in the current era of self-care and complementary medicine. Further, feed related soluble and solid waste accumulation is also posing environmental problems in aquaculture. Use of probionts has been proposed as a measure to maintain healthy environment in aquaculture and to prevent occurrence of disease (Lipton, 1998).

The probiotics effect was observed by Douillet (1993) and Douillet and Langdon (1994) in enhancing survival and growth of *Crassostrea gigas* larvae. The effect of probiotics containing lactic acid bacteria in the feed was investigated by Gildberg *et al., (*1997) on the fry of Atlantic cod. Otta *et al.,* (1993) studied its effect on the growth of *P. monodon.* Sharma and Bhukhar (2000) recorded significant effect of Aquazyn TM-1 commercial probiotics on the water quality and growth of *Cyprinus carpio.*

The positive effects of probiotic administration to finfish growth and development are well documented (Gatesoupe 2008, Wang, Li and Lin, 2008). Generally, probiotic administration during early developmental stages is most effective, frequently resulting in greater than an order of magnitude increase in survivorship (Gatesoupe, 2008). The false percula clownfish (*Amphiprion ocellaris*) has a high demand for aquarium trade, but its production in captivity faces critical bottlenecks during early life stages. The stresses in captive rearing conditions frequently result in high mortality rates (Benetti *et al.,* 2008) and growth abnormalities leading to high incidence of skeletal deformities (Fernandez *et al.,* 2008 and Koumoundouros *et al.,* 2002**)**. Probiotics are well known to positively impact fish welfare (Kesarcodi-Watson *et al.,* 2008) by reducing the general stress response and promoting growth, as well as increasing survivorship overall (Wang Li and Lin, 2008). Among the lactic acid bacteria (LAB), *Lactobacillus rhamnosus*, a LAB species originally intended for human use, is well known to have probiotic properties in humans (Reid, 1999) as well as in teleosts (Nikoskelainen *et al.,* 2001). To date, although a few studies have delineated the effects of this *Lactobacillus* species on immune modulation in fish (Nikoskelainen *et al.,* 2003 and Panigrahi *et al.,* 2007), no studies have reported its effect on larval fish survivorship, growth, and development.

In the present investigation, an attempt was made to study the impact of *Lactobacillus* and yeast, a commercial probiotics supplemented in the feed on the growth and food utilization of the commercially important marine ornamental clownfish, *Amphiprion sebae.*

MATERIALS AND METHODS

The juveniles of clown fish, *Amphiprion sebae* were collected from the Gulf of Mannar with the help of SCUBA divers and they were acclimated to the ambient laboratory conditions. Based on the suitability, different ingredient was selected for feed formulation (Immanuel *et al.,* 2003) (Table 1). The feeds were formulated following the square method (New, 1987). Three different types of experimental diets (A, B & C) with 30% protein were compounded separately by mixing different ingredients at various proportions. Then the probionts such as *Lacto bacillus* and yeast were added as feed additives at 2% concentration in feed A and B respectively. Feed C was used as a control, without addition of probionts. The water stability of the formulated experimental diets was tested over a period of 6 hrs by the method of Jayaram and Shetty (1981) and Immanuel *et al.,* (1997). After acclimatization, the healthy fishes were weighed individually and the initial weight ranged from 0.731 to 0.802 g. They were reared at the rate of 6 numbers/12 l water and fed at *ad libitum.* The leftover food and fecal matters were removed and dried at 80ºC in an oven. Four replicates were maintained for each feed randomly. During the experiment, which lasted 30 days, water quality was maintained. The bioenergetic parameters were calculated following the modified IBP formula of Petrusewicz and Mac Fadyen (1970). Then the results obtained were subjected to statistical analysis, following the procedures given in Zar (1974).

RESULTS AND DISCUSSION

Incorporation of probiotics into aquaculture feeds first used the commercial preparations designed for land animals. *Bacillus toyoi* isolated from soil substantially reduced the mortality of Japanese eel, which were infected by *Edwardsiella* species (Kozasa, 1986).

Table 1. Percentage composition of ingredients in the diets.

Ingredients (%)	Diets		
	Lactobacillus (A)	**Yeast (B)**	**Control (C)**
Fish meal	22.38	22.38	21.32
Sea weed (ulva)	15.36	15.36	18.08
Groundnut oil cake	15.36	15.36	18.08
Rice bran	13.30	13.30	10.68
Wheat bran	12.30	12.30	13.92
Tapioca powder	15.30	15.30	13.92
Vitamin and mineral mixture	2.00	2.00	2.00
Cod liver oil	2.00	2.00	2.00
probionts	2.00 (*Lactobacillus)*	2.00 (Yeast)	---

The above feed additive increased the growth rate of yellowtail. The same strain of *B. toyoi* used by (Kozasa, 1986) was later tested on rotifer (*Brachionus plicatilis*), which was left to filter the spores for two hours (Gatesoupe, 1989). The treatment increased the growth rate of larval turbot. Commercial preparations with live lactic acid bacteria have also been introduced in the medium of live food organisms for rearing of flat fish. Some of these treatments increased the production of rotifer and growth of Japanese flounder. These trials with commercial probiotics for land and aquatic animals were important to show the interest of bacterial additive in aquaculture feed but the survival of these probiotics microbes was uncertain in gastro intestinal tract of aquatic animal (Gatesoupe, 1999).

As a measure of feed stability, the leaching percentage in the formulated pelleted diets was studied (Table 2). From the result, it is inferred that the leaching of diets varied from 3.8 to 9.5% up to 4 hrs of exposure and upon further increase in exposure time, the leaching percentage was high. During 6 hr period, the leaching percentage varied from 17.15 to 18.42%.
It is obvious from the experiments that weight gain was highest with the feed containing Yeast, as probiotics while it was lowest in case of control. During the experimental period of 30 days, the highest survival rates of 77% and 81.1% were recorded for those fishes fed with diets A and B respectively. But the survival rate of fishes fed with diet C was only 67.76% (Table 4). The observed growth improvement in the present study may be due to the supply of essential nutrients and enzymes important in digestion process (Kennedy *et al*., 1998) or due to alteration in host mechanism (Deeth, 1984).

Table 2. Water stability of different diets in different hours. Each value (mean ± SD) is a mean of three individual estimates.

Diets	Initial Amount (g)	1 hour		2 hour		4 hour		6 hour	
		Final Amount (g)	**Leaching (%)**	**Final Amount (g)**	**Leaching (%)**	**Final Amount (g)**	**Leaching (%)**	**Final Amount (g)**	**Leaching (%)**
Lactobacillus (A)	1.0	0.943 ± 0.03	5.700	0.928 ± 0.005	7.2	0.911 ± 0.03	8.900	0.828 ± 0.005	17.15
Yeast (B)	1.0	0.975 ± 0.005	2.5	0.961 ± 0.007	3.9	0.922 ± 0.004	7.800	0.817 ± 0.005	18.25
Control (C)	1.0	0.962 ± 0.01	3.8	0.935 ± 0.05	6.5	0.905 ± 0.013	9.500	0.815 ± 0.005	18.42

(3.92%) and low (2.98%) in control. At the same time, in the fishes that received probiont-B added diet, the SGR value was 3.46% (Table 3).

The absorption efficiency of clown fish, *A. sebae* fed on control and experimental diets was not altered much and ranged between 82.24 and 84.26%. The one-way ANOVA showed that the variation in absorption efficiency among the diets was not statistically significant (P > 0.05) (Table 4).

Table 3. Specific Growth Rate (%) of *A. sebae* fed with different probiont supplemented diets. Each value (mean ± SD) is a mean of four replicates.

Diets	Initial Weight (g)	Final Weight (g)	Specific Growth Rate (%)	Survival (%)
Lactobacillus (A)	2.25	5.53	3.92	77±2.7
Yeast (B)	2.30	5.26	3.46	81±3.062
Control (C)	2.15	4.85	2.98	67.76±2.0

Table 4. Energy budget of *A. sebae* fed with different probiont supplemented diets. Each value (mean ± SD) is a mean of four replicates.

S. No.	Parameters	Feed Types		
		Lactobacillus *(A)*	Yeast (B)	Control (C)
1.	Absorption efficiency (AE) (%	82.24	84.260	83.275
2.	Gross Production Efficiency (K1) (%)	12.5	16.66	5.714
3.	Net Production efficiency (K2) (%)	15.152	20.00	6.897

Gross production efficiency was high in group B (16.67%). Fishes fed with control diet displayed low followed by 12.51% in group B with the value of 5.71% (Table 4). The one way ANOVA revealed that the variation in the gross production efficiency of clown fish, *A. sebae* fed with different diets was highly significant (P < 0.05). Similarly, the net production efficiency of fishes fed on control and experimental diets varied between 6.89 and 20.0% (Table 4).

Only limited number of studies has been carried out on the influence of probiotics on fish. Addition of probionts such as yeast in the diet increased the growth rate by accelerating the secretion of certain enzymes namely amylase, alkaline phosphatase etc. in post embryonic *Labeo rohita* (Das, 1975). The present study shows considerable weight gain in clown fish, *A. sebae* fed with probiont supplemented diets than control diet. The percentage increase in weight was 20 and 25% in *Lactobacillus* and yeast supplemented diet fed fishes than control diet. Similarly, Gatesoupe (1991) recorded the improved growth rate of the fish larvae turbot (*Scopthalmus maximus*) in the hatchery when treated with probiotics.

The present observation is in congruence with the findings of Paulmony (1996). He reported that the probiont yeast supplemented diet significantly influenced the growth, food conversion ratio and specific growth rate of *Cyprinus carpio*. A high percentage (123.46%) increase in growth was achieved for those fishes fed with 6% yeast supplemented diet. A similar result was reported by Singh *et al.,* (1980) in *Labeo rohita*.

CONCLUSION

Under certain aquaculture conditions, stress results in the production of corticosteroids making the cultured animals immuno-compromised and making them more susceptible to diseases. The probionts administered through diet might choose binding sites in the intestine, preventing colonization by pathogens. So far results with probiotics to reduce disease prevalence among commercially produced finfish have been disappointing. However, the principles behind their use remain sound and their full potential needs to be explored further. The potential probiotics in culture and production of fisheries needs considerable effects of research before their large scale production and commercialization.

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