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Monitoring the effect of water and feed probiotics on the growth potentials in fresh water prawn *Macrobrachium rosenbergii*

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ABSTRACT

Probiotic use in Aquaculture is increasing as producers attempt to improve pond soil and water quality, enhance survival and improve the growth of cultured species. In the present investigation an attempt has been made to know the effect of water, feed probiotics individually and combined effect of both water and feed probiotics were studied as fresh water prawn *Macrobrachium rosenbergii* as the candidate species of culture activity in Nellore District of Andhra Pradesh. The probiotics either individually or in combined action, induced the best growth potentials in fresh water prawn *M. rosenbergii* compared to control pond where in which only commercial feed C. P. Brand was broad casted. The use of water probiotics and feed probiotics in the culture activity indicated that, probiotics play a vital role in inducing growth and survival rates by maintaining good water quality parameters throughout the culture operation. Probiotic treatment offers a promising alternative to the use of antibiotics in prawn culture activity.

INTRODUCTION

The Animal production for human consumption is rapidly increased, which is mainly depending upon terrestrial and aquatic farm animals. *Macrobrachium rosenbergii* known as the giant freshwater prawn or scampi is a species of fresh water shrimp native to the Indo-Pacific and Northern Australia. *M. rosenbergii* is an important commercial species due to property as food supply as well as a valuable expert product. In India, giant freshwater prawn distributes mainly in the Southern region where environmental conditions are most favorable for the growth of scampi.

Increasing demand of this species for domestic consumption and export markets has increased remarkably scampi cultured systems with large scale, high stoking density and intensive feeding. Consequently, cultivation of this economic species is being expanded to culture on rice fields, Orchard gardens, Pens along river banks. Hence, disease is inevitable in these controllable culture models.

The growth of the shrimp Aquaculture industry increased the need to intensify farming practices to maximize the profits. Problems of diseases often accompanied this intensification as environmental conditions deteriorated and brought the decline of the industry. Pressure to ensure production led to reliance on Antibiotics and Chemotherapeutic agents. The regular and indiscriminate use of Antibiotics and Chemotherapeutic agents in Aquaculture has led to problems of drug resistance (Karunasagar et al., 1994; Balcazar et al., 2006; 2007). The use of preventive and environmentally friendly approaches, namely Antibacterial peptides, probiotics and prebiotics are becoming increasingly important on Aquaculture, particularly in light of new trends toward organic production systems (Soltanian et al., 2007; Decamp et al., 2008). The original definition of probiotics is organisms and substances contributing to intestinal microbial balance (Parker, 1974). The probiotics are a live microbial feed supplement, which beneficially affects the host by improving its intestinal balance. Moriarty et al (2005) proposed to extend the definition of probiotics in Aquaculture to microbial "water additives". Probiotics can also be defined as microbial cells administered through the gastrointestinal tract with the aim of improving health of the hosts (Gate Scoupe, 1999). The present investigation is aimed to study the impact of different types of probiotics i.e., water probiotics and feed probiotics on the growth patterns of fresh water prawn *Macrobrachium rosenbergii*.

MATERIALS AND METHODS

The Post larvae of fresh water prawn *M. rosenbergii* (PL15) were purchased from Local Hatcheries, Near Allur, Nellore Dist., Andhra Pradesh, India and were stocked in different ponds, adjacent to each other. The study was conducted in the Aquaculture ponds located in Ramayapatnam, 16 kms away from Kavali. The PL's were first acclimatized and stocked in to the culture ponds. The Experimental pond water had these physicochemical parameters. pH 7.2 ± 0.2, Total dissolved solids 0.98 ± 0.05 g/L; Dissolved oxygen 7.3 ± 0.20 mg/L, BOD 33 ± 2 mg/L, COD 135 ± 12 mg/L and Ammonia 0.03 ± 0.005 mg/L.

Feeding Trails

Macrobrachium rosenbergii of 0.32 ± 0.05 g were selected for present investigation and were stocked at the rate of 15 Pcs/M^2 in the culture ponds. Water probiotics namely PROBAC-BC and Feed probiotics ZYMETIN were selected and used in the present study. The feed probiotic ZYMETIN was added to the feed 5 g/kg, whereas PROBAC-BC was added as following.

Days of culture	Quantity Added
7	200
21	600
35	900
50	1050
65	1200
85	1750
100	1900
115	2200
130	1800
180	1000
210	1200
250	1000
300	750
320	700

The Four Experimental ponds were selected in the present study. The Ist pond was the Control pond wherein which C. P. Brand Feed is broad casted throughout the culture period. 2nd pond was fed with C.P.Brand feed and Water probiotics were used. 3rd pond was fed with Probiotic feed was used. The 4th pond was fed with Probiotic feed and Water probiotics were used. The experimental period was 320 days. The feeding was adjusted to two times a day is 6.00 AM and 6.00 PM. The daily ration was given at the rate of 10% of the body weight of animals with two equal halves throughout the culture period.

Determination of Growth Parameters

At the end of the 320 days of culture period the growth parameters such as Survival rate (SR), Weight gain (WG), Specific growth rate (SGR), Feed conversion Ratio (FCR), Protein Efficiency Ratio (PER) were individually determined by following equations for both male and female sex.

$$SR: \frac{\text{Total Number of Live Animals}}{\text{Total Number of Animals stocked}} \times 100$$

$$WG: \frac{\text{Weight of the Animal (G)-Weight of the Animal (G)}}{\text{At the end of the culture period at the time of stocking}}$$

$$FCR: \frac{\text{Total Amount of Feed broadcasted (Kgs)}}{\text{Total Biomass (weight) of the prawns (Kgs)}}$$

$$PER : \frac{\text{Total weight gain (G)}}{\text{Total Protein consumed (G)}}$$

$$SGR : \frac{(\text{Log } W_2 - \text{Log } W_1)}{T} \times 100$$

Where
W_1 = Weight of the Animal at the starting point of the culture period.
W_2 = Weight of the Animal at the end of the culture period.
T = Total number of experimental days.

Analysis of Gut Micro flora

The Bacteriological analysis of the gut flora of the prawn was carried out after the completion of the experiment. The wet weight of each prawn was determined before the dissection. The prawns were dissected to remove the gut, after washing the dorsal surface area of the prawn with sterile distilled water. The entire gut was homogenized in 9 ml of the diluents (Phosphate Buffer 0.01M) and mixed with a Vortex mixer and used as 10^{-1} dilution. Each sample was serially diluted, using Pour plate method, poured into four different media. The Total plate count was determined using Tryptone Soya Agar (TSA) media. The *Lactobacilli* count was determined using MRS Agar media. The inoculated plates were incubated at $35^0 \pm 2^0$C for 3 days. The Anaerobic count was determined using Anaerobic agar media. The inoculated plates were incubated at 35 ± 2^0C for 3 days in anaerobic condition. The Total coliform count was determined using the most probable number method (MPN). Bacterial cultures were isolated and identified through standard methods (Holt et al., 1996).

Biochemical Parameters

Biochemical parameters including Catalase and Peroxidase activity (Ohkawa 1979), Superoxide dismutase (Misra & Fridovich, 1972) and Protein (Lowry et al., 1954) were determined by following standard procedures.

The data obtained in the present investigation was subjected to Statistical treatment using SPSS.

RESULTS AND DISCUSSION

The Growth rates of Freshwater prawn *Macrobrachium rosenbergii* was monitored after subjecting to the treatments with Water probiotics and Feed probiotics for a culture period of 320 days. *M. rosenbergii* were cultured in four different ponds, subjected to different treatments. The first pond was treated as control pond, where commercial feed obtained i.e., C.P.Brand feed was broad casted. The second pond was treated with commercial feed application along with Water probiotics were used. The Third pond where Probiotic feed was broad casted. In the case of fourth pond both Probiotic feed and Water probiotics were used. The results obtained in the

present investigation were presented in Tables 1 and 2. In the present investigation male freshwater prawn recorded maximum growth rates compared to female prawn. After 320 days of culture operation, the male prawn recorded 93.92 g compared to 63.18 g in the case of female prawn in the control pond. Both male and female prawns recorded maximum growth rates in ponds treated with water probiotics, feed probiotics and both water and feed probiotics (Tables 1 and 2) compared to control pond male and female prawns fed with commercial C.P.Brand feed. Male prawn recorded weights at the end of culture operation as control (93.92 g), pond treated with water probiotics (100.65 g), pond treated with feed probiotics (101.82 g) and pond treated with both feed and water probiotics (102.37 g). In all the four ponds, the growth increment was statistically significant ($P<0.001$). The FCR values obtained in the present investigation also ranged from 1.82 to 2.06. The minimum FCR recorded with pond treated with both water and feed probiotics as 1.82, followed by 1.92 recorded with pond treated with only feed probiotics, 1.94 recorded with pond treated with water probiotics and 2.06 recorded with pond where commercial feed was broad casted. The daily growth rates recorded in all the four ponds are following results 0.2925 g with control pond, 0.3135 g with pond treated with water probiotics, 0.3172 g with pond treated with feed probiotics and 0.3189 g with pond treated with both water and feed probiotics. The percent survival values obtained in the present investigation for male freshwater prawn ranges 85.2 to 93.2%. The Protein Efficiency Ratio (PER) values also recorded to be 1.34 with pond treated with commercial C.P.Brand feed and 1.31 with pond treated with water probiotics, 1.29 with pond treated with feed probiotics and 1.27 with pond treated with both water and feed probiotics. Similarly after the completion of 320 days of culture operation, the female prawn recorded maximum weight i.e., 67.29 g with pond treated with feed and water probiotics, 67.07 g with pond treated with feed probiotics, 66.16 g with pond treated with water probiotics and 63.18 g with pond treated with commercial feed application. The weight gain in the female prawn recorded was statistically significant ($P<0.001$). The FCR values recorded as 2.01 with pond applied with commercial C.P.Brand feed and 1.90 with pond treated with water probiotics, 1.88 with pond treated with feed probiotics and 1.79 with pond treated with both feed and water probiotics. The percent survival values also ranges than 84.3 to 92.3%. The PER values recorded to be 1.28 with pond treated commercial feed application, 1.23 with pond treated with water probiotics, 1.22 with pond treated with feed probiotics and 1.20 with pond treated with both feed and water probiotics. The Gut Microbial load of both male and female freshwater prawns were estimated and presented in Table 3. Total plate count, Total coliforms, Total faecal coliforms and Facultative anaerobes were found to be significantly reduced with the treatment with water probiotics, feed probiotics and both feed and water probiotics compared to control pond. But, *Lactobacillus sp* were significantly increased in the gut of both male and female fresh water prawn in ponds treated with water and feed probiotics compared to control pond. The Antioxidant enzyme activities in midgut gland tissue of freshwater Prawn of both male and female sex were assayed and presented in Table 4. The Super oxide dismutase (SOD), Catalase and Glutathione Peroxidase (GPX) activities of mid gut gland showed an

significant elevation in water, feed probiotics treated pond animals compared to control pond animals.

In the last decade, the consumption of Aquatic products has been increased substantially, but the world fishery production was decreased and hence the productions of Aquatic products through controlled conditions have been come into lime light. The probiotics are known to play an important role in carrying out a wide variety of functions including modulation of mucosal and systemic immunity, improving microbial balance by preventing colonization of undesirable bacteria in the intestinal tract (Gatesoupe, 1999; Ravi et al., 1998; Saha et al., 1988; Suralikar Sahu, 2001)

The common probiotics used in pond management are live Bacterial inocula (Non-Pathogenic organisms) rich in extracellular enzymes claims about the potential benefits of probiotics in Aquaculture ponds enhances decomposition of organic matter, reduction of Nitrogen and Phosphorous concentrations, enhances the availability of Oxygen, reducing the Blue-Green Algae, controls Ammonia, controls Nitrate and Hydrogen sulfide, enhances the production rates.

The sustainability and the success of Aquaculture depend on the quality of soil, water. seed selected and feed used. A good quality of soil, water and seed and feed plays an important role in the successful yield under skillful management practices. The ponds often accumulate with uneaten feed materials excreta, molted shells, dead algae and surface run of organic matter carried by wind and water. When all the above mentioned materials remain un-degraded or partially degraded in reduced oxygen condition and toxic gases such as H_2S and NH_3 will be produced. These gases give rise to stress to the cultured organisms resulting in the loss of appetite sluggishness, gulping for oxygen etc and ultimately results in the reduction of growth patterns. Due to usage of water and feed probiotics in the present investigation, the primary principle for acceleration of organic matter decomposition by probiotics and the function of C:N ration management by heterotrophic bacteria was carried out successfully (Gatesoupe, 1999). In the present investigation, Ammonia, H_2S and CO_2 contents were decreased consequent upon the usage of water probiotics in the culture ponds, whereas the dissolved oxygen content of the water was increased (Rangappa, 2011). The probiotics used in the present investigation known to enhance the quantity of heterotrophic Bacteria in the culture environment, which in turn maintains the C:N ratio in the ponds. The results obtained in present investigation also gains support from the earlier reports in the culture *Penaeus monodon*, *Litopenaeus vannamei* (Porubcan, 1991a; Prabhu et al., 1999; Rangappa, 2011), so it is very clear that both water probiotics and feed probiotics are capable of maintaining good quality and inducing best growth potentials.

In the present investigation both male and female freshwater prawns showed a similar kinds of response to the probiotic treatments. Among the three different types of treatments adopted in the present investigation, the treatment with water probiotics and feed probiotics yielded good results in terms of productivity and growth potentials. In between the two treatments ponds treated with feed probiotics yielded good results in turns of growth compared to ponds treated with water probiotics. The freshwater prawns responded well for all the probiotic treatments, but the maximum

effect for induction of growth was recorded with ponds treated with both water and feed probiotics. The average body weight of the harvested prawns of probiotics treated and control prawns (Table 1 and 2) showing the difference was statistically significant. The Average daily growth rates recorded in the present investigation also emphasizing that probiotic treatment clearly increasing the growth potentials. Results showed that all probiotic-supplemented ponds and diets resulted in higher growth in prawns than prawns fed with control diet i.e., C.P.Brand commercial diet. This result was very inspiring in prawn culture with probiotics as the size of the prawn was directly related to better foreign exchange earnings. Several authors reported that probiotic treatments improved growth rates in prawn and crab culture activity (Maeda & Nagami 1989; Wang et al., 2005; Wang & Xu, 2006; Wang, 2007; Saad et al., 2009; Yu et al., 2009; Zhang et al., 2009). The average survival rates recorded are also appears to be relatively more in probiotic treated ponds compared to control pond prawns. Here in this study with the application of probiotics, survival rate of prawns has been found to be more compared to the control ponds, which is similar to the report of (Garriquees and Arevalo, 1995). Maeda & Liao (1992a) also found higher survival and molt rates of prawn larvae of *P.monodon* by treating the pond with soil probiotics.

Organisms life depends upon oxygen as the final acceptor of electrons in mitochondrial electron transport, but the process also generates toxic metabolites, Reactive oxygen species (ROS), and leak from mitochondria into the cytoplasm where they cause cellular damage by oxidizing a variety of biologically important molecules, including DNA, Proteins, Lipids and Carbohydrates. Aerobic organisms possess a baseline status of antioxidant system, involved in a variety of detoxification reactions, to assure the maintenance of a balance between production and removal of reactive oxygen species (ROS) and other pro-oxidants. These ROS include Super oxide anion radical (O_2), hydrogen peroxide (H_2O_2) and highly reactive hydroxyl radical (OH). As a consequence of the reactivity of ROS and their potential to damage cells and tissues, majority of organisms balance the production of these radical with a wide variety of cellular antioxidant defenses. Prominent among these antioxidants are the enzymes Superoxide dismutase (SOD), Catalase, Glutathione Peroxidase (GPX). SOD catalyses the conversion of superoxide anion radical to H_2O_2, Catalase reduces H_2O_2 to water. Glutathione Peroxidase acts on conjunction with other enzymes on H_2O_2 and to terminate Lipid Peroxidation. Under normal conditions i.e., without the influence of stress conditions, a balance exists between the generation of ROS and other pro-oxidants and their detoxification and removal by Antioxidant defense mechanisms. A number of studies have demonstrated the potential for ROS generation, antioxidant enzyme and the radical scavenging responses and oxidative damage in species of invertebrates including Molluscs (Livingstone, 2001; Livingstone et al., 2000). However, few studies have been undertaken on Crustaceans and little is known about such mechanisms. In the present investigation CAT, SOD and GPX activities, the key enzymes were found to indicate the involvement of detoxification of ROS species. In the present investigation, all the antioxidant enzymes are playing key role in the detoxification of ROS species and inducing the best growth potentials.

The present investigation may be concluded that the probiotics either water or feed probiotics play a vital role in inducing growth, survival and disease resistance of the aquatic animals by maintaining good water quality parameters throughout the culture period. Probiotic treatment offers a promising alternative to the antibiotics for prawn culture activity in Andhra Pradesh. By using both water and feed probiotics jointly the growth potentials of fresh water prawn was considerably elevated and which in turn results in good yields. In Andhra Pradesh sustainable prawn culture activity with a wide variety of probiotics is increasing and unemployment can be minimized through this sector. The joint action of both water probiotics and feed probiotics is needed to unravel the mode of action of probiotics on fresh water prawn and its effect on growth response and other related parameters.

Table.1: Growth rates of fresh water prawn *Macrobrachium rosenbergii* treated with different types of Probiotics.

Parameter	Control	With Water probiotics	With Feed probiotics	With WP+FP
Initial weight (g)	0.32±0.05	0.32±0.05	0.32±0.05	0.32±0.05
Final weight (g)	93.92±1.96	100.65±2.17	101.82±2.73	102.37±1.59
Weight gain (g)	93.60	100.33	101.50	102.05
" T" test	P<0.001	P<0.001	P<0.001	P<0.001
Daily growth rate (g)	0.2925	0.3135	0.3172	0.3189
FCR	2.06±0.06	1.94±0.07	1.92±0.13	1.82±0.07
% Survival	85.2±2.1	89.1±2.3	91.2±2.5	93.2±2.4
PER	1.34±0.05	1.31±0.04	1.29±0.06	1.27 ±0.05
SGR				

The values are mean ±SD of six individual observations.
WP+FP: Water probiotics + Feed probiotics.
PER: Protein efficiency ratio.
FCR: Food conversion ratio.
SGR: Specific growth rate.

Table.-2: Growth rates of female fresh water prawn *Macrobrachium rosenbergii* treated with different types of Probiotics.

Parameter	Control	With Water probiotics	With Feed probiotics	With WP+FP
Initial weight (g)	0.32±0.05	0.32±0.05	0.32±0.05	0.32±0.05
Final weight (g)	63.18±1.54	66.16±2.15	67.07±2.40	67.29±2.95
Weight gain (g)	62.86	65.84	66.75	66.97
'T'test	P<0.001	P<0.001	P<0.001	P<0.001
Daily growth rate (g)	0.1964	0.2058	0.2086	0.2093
FCR	2.01±0.08	1.90±0.07	1.88±0.05	1.79±0.03
% Survival	84.3±2.2	88.4±2.4	89.3±2.3	92.3±2.5
PER	1.28±0.04	1.23±0.03	1.22±0.04	1.20±0.03

The values are mean ± SD of six individuals' observations.
WP+FP: Water probiotics + feed probiotics
PER: Protein efficiency ratio.
FCR: Food conversion ratio.
SGR: Specific growth rate.

Table. 3: Gut microbial load of fresh water prawn *Macrobrachium rosenbergii* treated with different types of Probiotics.

Micro organisms	Control	WP+FP used ponds 120 DOC (cells/g)	WP+FP used ponds 240 DOC (cells/g)
Male prawn:			
Total plate count	5.6×10^{8}	2.5×10^{8}	2.2×10^{8}
Total coli forms	1586	1113	1034
Total fecal coli forms	345	148	140
Facultative anaerobes	6.8×10^{7}	3.1×10^{7}	3.0×10^{7}
Lacto bacillus sps	1.7×10^{6}	4.6×10^{7}	4.9×10^{7}
Female prawn:			
Total plate count	5.3×10^{8}	2.3×10^{8}	2.1×10^{8}
Total coli forms	1579	1109	950
Total fecal coli forms	341	146	138
Facultative anaerobes	6.5×10^{7}	2.9×10^{7}	2.7×10^{7}
Lacto bacillus sps	1.58×10^{6}	4.52×10^{7}	4.7×10^{7}

Table.-4: Anti oxidant enzymes activities in mid gut gland tissue of fresh water prawn *Macrobrachium rosenbergii.*

Enzyme	Control	Final		With WP+FP
		With Water probiotics	With Feed probiotics	
Male prawn				
SOD	20.42±2.21 PDC	24.34±1.98 +19.20	25.43±1.67 +24.54	26.23±2.21 +28.45
Catalase	40.18±2.16 PDC	44.23±2.46 +10.01	45.26±2.18 +12.64	50.28±2.17 +25.14
GPX	6.14±0.68 PDC	8.14±0.75 +31.71	8.85±0.82 +43.20	9.84±0.83 +59.20
Female prawn				
SOD	19.22±0.95 PDC	22.54±1.23 +17.19	23.92±0.98 +24.48	24.63±1.24 +28.13
Catalase	37.16±1.78 PDC	41.51±2.03 +11.71	43.18±2.13 +16.21	47.13±2.35 +26.83
GPX	4.66±0.69 PDC	6.37±0.84 +36.69	7.38±0.93 +58.37	8.54±0.95 +83.26

The values are mean ± SD of six individual observations.
WP+FP: Water probiotics + Feed probiotics.
All values are statistically significant at P<0.001.
PDC: Percent deviation over control.

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