“Efficacy of enzymatic and bacterial probiotic on growth and survival of giant freshwater prawn ***Macrobrachium rosenbergii*** **(De Man)”**

M.F.Sc. (Inland Fisheries) Thesis

by
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“Efficacy of enzymatic and bacterial probiotic on growth and survival of giant freshwater prawn ***Macrobrachium rosenbergii*** (De Man)”

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CERTIFICATE-I

This is to certify that the thesis entitled **"Efficacy of enzymatic and bacterial probiotic on growth and survival of giant freshwater prawn *Macrobrachium rosenbergii* (De Man)"** submitted in partial fulfillment of the requirements for the degree of **"Master of Fisheries Science (Inland Fisheries)"** of the Indira Gandhi Agricultural University, Raipur, is a record of the bonafide research work carried out by **PRAVIN VASANTRAO CHAVAN** under the guidance and supervision. The subject of the thesis has been approved by Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate awarded etc.) or has been published/ published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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Member: Dr. R. R. Saxena _____

CERTIFICATE-II

This is to certify that the thesis entitled **"Efficacy of enzymatic and bacterial probiotic on growth and survival of giant Freshwater prawn *Macrobrachium rosenbergii* (De Man)"** submitted by **PRAVIN VASANTRAO CHAVAN** to the Indira Gandhi Agricultural University, Raipur in partial fulfillment of the requirements for the degree of **M.F.Sc. (Inland Fisheries)** in the **Department of Fisheries** has been approved by the Student's Advisory Committee after oral examination in collaboration with the external examiner.

Date: **EXTERNAL EXAMINER**

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Department of Fisheries, IGAU, Raipur **(Pravin Chavan)**

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CHAPTER - I

INTRODUCTION

Aquaculture of finfish, crustaceans, molluscs, and algal plants is one of the fastest-growing food-producing sectors, having grown at an annual rate of almost 10% from 1984 to 1995 compared with 3% for livestock meat and 1.6% for capture fisheries production. Large number of commercially important finfish and shellfish are under culture practice in freshwater, brackishwater and seawater. Among the freshwater crustacean species, farming of freshwater prawns is getting immense popularity world over. Freshwater prawns are grouped under the genus *Macrobrachium* and *Palaemon* of the class Crustacea. Commercially important prawns come under genus *Macrobrachium*, which comprise of more than 150 species (Brown, 1991). About 25 species of *Macrobrachium* are listed from the Indian waters (Dwivedi and Reddy, 1984; Reddy and Kohli, 2000). Among them, *Macrobrachium rosenbergii*, commonly known as 'Scampi' in the international market and popularly known as "Giant freshwater prawn" and *Macrobrachium malcomsonii* (Indian river prawn) are suitable for Indian freshwater aquaculture.

The Scampi production of India through aquaculture has shown a great leap during the last four years, increased from 7140 metric tons in the year 1999-2000 to 30,450 metric tons in the year 2002-2003 (Bojan, 2004), but disease is now considered to be the limiting factor in the culture sub sector (Lin, 1995, Subasinghe, 1997). Hence, it is necessary to supply disease free seed, quality feed and to provide good environment for

success in culture. Antibiotics have sometimes been used to reduce disease as the Swann committee restricted the use of antibiotics as growth promoters, leaving these antibiotics only for use in the treatment of disease (Swann, 1969). However, indiscriminate use has in some cases led to increased antibiotic resistance and problem of tissue residues and trade issues (Vasudevan, 2000; John, 1999). Vaccines are successfully used in other livestock industries but there are no vaccines currently available for most of the fish disease in this region. So prevention of disease and improvement in the growth of finfish and shellfish are important at aquafarms. Therefore, it is essential to address the issues related to the effective farm management practices. In the recent days, “probiotics” and enzymes are in use in various aquafarm management practices to maintain environment, health and growth of different farming aquatic organisms.

Probiotics are cultured product or live microbial feed supplements, which beneficially affects the host by improving its intestinal balance and health of the host (Fuller, 1989). The first probiotic discovered long time ago was *Lactobacillus* sp., the lactic acid producing bacteria. They were thought to prevent colonization of the gut by other disease causing bacteria - a process known as competitive exclusion. Presently the range of probiotics extends well beyond the *Lactobacillus* sp. to include *Bacillus* sp. *Vibrio* sp., *Pseudomonas* sp., *Bifidobacterium* sp., enzymes, yeasts and algae. Some of the commercial probiotics currently available include Aqualact, Porbe-la, Lacto-sacc, Epicin, Biogreen, Environ, Wunupuo-15

and Epizyme. Feed probiotics are applied with the feed and a binder (egg or liver oil) that secure optimal use of the feed by aiding in its digestion, that improve water quality, and/or that stimulate the immune system and improves the growth of the host. The characteristic feature of a probiotic microbe is that it should be able to colonize the gastro-intestinal tract, but the intestinal microflora in aquatic animals changes rapidly with the constant influx of microbes coming from water and food. The microbial community of the gut can therefore be considered to be transient in nature. This transience allows the extension of the probiotic concept to the use of microbial preparations in aquaculture.

. Considering the recent successes of the alternative approaches, the Food and Agriculture Organization of the United Nations (Subasinghe, 1997) defined the use of probiotics for the improvement of aquatic environmental quality as major areas for further research in disease control in aquaculture.

So, this study aims to provide the efficacy of the bacterial and enzymatic probiotics on growth and survival of giant freshwater prawn *M. rosenbergii* (De Man) with the following objectives:

1. To study the various enzymatic and bacterial probiotic formulations available for use in *Macrobrachium rosenbergii* rearing.
2. Selection of suitable proboitic for use in present study.
3. Efficacy of proboitic for increasing growth of *M. rosenbergii.*
4. Efficacy of proboitic for increasing survival of *M. rosenbergii*.
5. Effect of proboitic on biochemical composition of *M. rosenbergii*.

CHAPTER - II

REVIEW OF LITERATURE

2.1 Effect of probiotics on survival in aquaculture

The research of probiotics for aquatic animals is increasing with the demand for environment-friendly aquaculture. Generally, probiotics are applied in the feed or added to the culture tank or pond as preventive agents against infection by pathogenic bacteria and growth improvement and for reducing mortalities. Several researchers studied the use of probiotics in aquaculture giving promising results as follows

Gatesoupe (1990) was able to improve the survival of larval turbot (*Scopthalmus maximus*) by daily addition of lactic acid bacteria could be retrieved in large amounts from the turbot larvae and a significant reduction of larval mortality was observed when the larvae were challenged with a pathogenic *Vibrio* on day 9.The hypothesis was that the lactic acid bacteria would act as a microbial barrier against the pathogenic *Vibrio* and might curb the invasion of turbot larvae by the pathogen.

Kumari *et. al.* (2004) used immuplus, a polyhedral commercial formulation to modulate the immune system of commercially important giant freshwater prawn *M. rosenbergii*. The prawns were fed with basal diet supplemented with Immuplus at 1 g/kg feed for 4 weeks. Results showed that the phenoloxidase activity (Po), haemagglutination and lysosome activities were significantly elevated in Immuplus-fed prawn upto 3 weeks of feeding. The total protein level in Immuplus –fed prawn increased up to 2nd week of feeding. Incorporation of Immuplus at the rate

of 1 g/kg feed in the diet of prawn for 3 weeks may be beneficial in raising the immune status of prawn.

Garcia de la Banda *et. al.* 1992 added lactic acid bacteria (*Streptococcus lactis* and *Lactobacillus bulgaricus*) to B*rachionus* and *Artemia* used in turbot larval feeding. In a single experiment without triplicates, 55% survival was found on day 17 when living lactic acid bacteria had been added and 66% survival was found with disabled ones, as opposed to 34% in the control group. Apparently, the bacterial cells, alive or disabled, provoked improved survival of the turbot larvae.

Gatesoupe (1991) introduced spores of *Bacillus* strain IP5832 into the culture medium of rotifers, which were fed to turbot larvae. A decrease in the proportion of members of the *Vibrionaceae* in the rotifers was observed, and the mean weight of the turbot larvae on day 10 was significantly improved with the spore-fed rotifers, when an experimental infection was performed with the spore-fed rotifer. When an experiment was performed with an opportunistic *Vibrio* sp. mortality was observed in all treatments but the mean survival of the spore fed rotifers on day 10 was significantly higher than that of control group (31 and 10%, respectively).

Gatesoupe (1997) studied sidophore production and the probiotic effect of *Vibrio* type E on trout larvae. The main effect of rotifer enrichment with this strain was to improve the survival of the larval turbot after a 48-hours challenge with the pathogenic *Vibrio* type p

In experiments performed by Gildberg *et. al*. (1995), Atlantic Salmon (*Salmon salar*) fry given a diet supplemented with a *lactic acid*

bacterium was challenged with cohabitant fishes infected with *Aeromonas salmonicida* through intraperitonial injection. Mortality was recorded during the next 4 weeks. It was seen that in spite of the lactic acid bacteria given as supplements in the dry feed could colonize the intestine, but protection against *A. salmonicida* infection could be detected. Contrary to the expectations, the highest mortality was recorded with fish given the diet containing lactic acid bacteria.

Joborn *et. al.* (1997) studied the ability of *Carnob acterium* strain KI to colonize the intestinal tract of Rainbow trout (*Oncoryncus mykiss*) (13 to16 cm) and inhibit two common fish pathogens *V. anguillarium* and *A. salmonicida* in mucus and fecal extract. This was demonstrated in vitro in both the mucus and the fecal extract. Furthermore, *carnobacterium* cells remained viable in the intestinal tract, since considerate densities (10 CFU/g) were found in the fecal pellets till at least 4 days after the last feeding. However, a sharp decrease of 3 log units was observed after 3 days once the treatment was stopped.

Olsson *et. al.* (1998) found that the growth of *V. anguillarum* in fecal extracts from turbot juveniles was inhibited by *Carnobacterium* cells. It was concluded that the turbot intestinal tract and feces can serve as an enrichment site for *V. anguillarum* and that the use of intestinal bacteria with inhibitory activity against *Vibrio sp*. might be used to decrease the load of fish pathogenic *Vibrio* sp. in turbot hatcheries.

Maeda and Liao (1992) reported the use of soil bacteria strain, PM-4 that promoted the growth of *Penaeus monodon* nauplii probably

acting as a good food source. This strain also showed an in vitro inhibitory effect against *V. anguillarum* strain. When added to tanks inoculated with diatoms and rotifers, the strain resulted in 57% survival of the larvae after 13 days, while without the bacterium Maeda and Lio (1994) produced similar data in another study, but the effect was attributed to strain NS-110.

Naogami and Maeda (1992) isolated a bacterial strain from a crustacean culture pond which improved the growth of crab (*Portunus triberculatus*) larvae and repress the growth of other pathogenic bacteria especially *Vibrio* sp, but would not kill or inhibit micro algae in seawater when it was added into the culture water. Among the bacteria population present in the culture water of the crab larvae, the number of *Vibrio* sp and pigment bacteria decreased and even became undetectable when the bacteria was added to the culture water. The production and survival rate of crab larvae were greatly increased by the addition of the probiotic into the culture water. They also suggested that the bacteria might improve the physiological state of the crab by serving as a nutrient source during its growth. This bacterium may have a good effect on the crab larval culture as a biocontrolling agent in the future.

Austin *et. al.* (1992) reported a kind of microalgae (*Tetraselmis suecica*), which can inhibit pathogenic bacteria of fish. *Tetraselmis suecica* was observed to inhibit *Aeromonas hydrophila*, *A. salmonicida* and *Serrstia liqueficants*, *Vibrio anguillarum* and *Yersinia ruckeri* type 1, when used as a food supplement, the algal cell inhibited laboratory induced

infection in Atlantic salmon. When used therapeutically, the algal cells and their extract reduced mortalities caused by *A. salmonicida, S. liquuefaciens, V. anguillarum, V. salmonicida* and *Yersria ruckeri* type 1. They suggested that they may become bioactive compound in the algal cells and there appears to be a significant role for *Tetraselmis* in the control of fish disease.

Smith and Davey (1993) reported that a florescent strain *pseudomonad* bacterium could competitively inhibit the growth of fish pathogen *A. salmonicida*. Their result shows that the fluorescent *Pseudomanad* is capable of inhibiting the growth of *A. salmonicida* in culture media and that this inhibition is probably due to competition for free iron in a challenged test of the Atlantic salmon by *A. salmonicida*, a statistically significant reduction in the frequency of stress induced infection in the group of fish bathed in the bacterium fluorescent pseudomonad compared to the controlled growth was observed.

Austin *et. al.* (1995) reported a probiotic strain of *V. alginuliticus* which didn't cause any harmful effect in Salmonidies by using the cross streaking method the probiant was observed to inhibit the fish pathogens. When the freeze-dried culture supernatant was added to the pathogenic bacteria such as *V. ordalli, V. anguillarum, A. salmonicida*, and *Y. ruckeri*, showed a rapid or steady decline in the number of culturable cells, compared to the controls. Their results indicated that application of probiant to Atlantic salmon culture led to reduction in mortalities when

challenged with *A. salmonicida* and to lesser extent *V. anguillarum* and *V. ordalli*. The observation with this probiotic *Vibrio* is encouraging.

Maeda and Naogami (1989) reported some aspects of the biocontrolling method in aquaculture. In their studies bacterial strengths possessing *Vibrio* static activity, which improved the growth of prawn and crab larvae, were observed. By applying these bacteria in aquaculture, a biological equilibrium between competing beneficial and deleterious microorganisms was produced, and results show that the population of *Vibrio* species which frequently causes egg scale damage to the larval production decreased. Survival rate of the crustacean larvae in these experiments was much higher than those without the addition of bacterial strains. They hoped that addition of these strains of bacteria would repress the growth of *Vibrio* sp. fungi and other pathogenic micro- organisms. Their data suggests that controlling the aquaculture ecosystem using bacteria and protozoa is quite possible and if this system is adopted it will maintain the aquaculture environment in better condition, which will increase production of fish and crustaceans.

Verschure *et. al.* (1999) selected 9 bacterial strains that positively influenced the growth and survival of juveniles of the brine shrimp *Artemia* cultures as a larval food for other sp. All 9 strains were able to delay the death of the *artemia* when experimentally infected with the pathogenic *Vibrio proteolyticus*, C. W. 8T2, although large differences were found among the strains. While all *artemia* in exenic control died within 2 days after the infection. The survival rates of the *artemia* culture inoculate

before hand with strains LVS 8 or a mixture of 9 strains showed more than 80% survival after 4 days. Further more the growth of *V. proteolyticus* CW8T2 in the *Artemia* culture water was considerably slowed in the presence of LVS8. (Verschure *et. al.,* 2000).

2.2 Effect of probiotics on water quality and growth

The transience of aquatic microbes may legitimate the extension of the probiotic concept to living microbial preparations used to treat aquaculture ponds. Moriarty (1998) proposed to extend the definition of probiotics to microbial “water additives”.

In 1991, Porubcan reported two attempts of bacterial treatments to improve water quality and yield of *Penaeus monodon*. They are floating biofilters pre-inoculated with nitrifying bacteria decreased the amounts of ammonia and nitrite in the rearing water. This treatment increased shrimp survival (Porubcan, 1991a) and the introduction of *Bacillus* sp. in proximity to pond aerators reduced chemical oxygen demand, and increased shrimp harvest (Porubcan, 1991b). Several commercial products have sought to exploit the same idea that bacteria which improve water quality may be beneficial to animal health (e.g. “bacteria”, Aquatic Warehouse, San Diego, CA; ”Biostart”, Advanced microbial Systems, Shakopee, MN; ”BRF-1A,BRF-13A, PB-32, PBL-44”, Enviro-Reps International, Camarillo, CA; ”Liqualife”, Cargill, Animal Nutrition Division; ”microbial and enzymic products”, Alliance Bioremediation and Composting, Encinitas, CA: ”Pond-proVC”, Biomanagement Systems, Wellington point, Australia; ”probiotics”, Contessa, ZB Industries, San Pedro, CA).These products are referred to

as “probiotics” and most of them contain nitrifying bacteria and/or *Bacillus* sp. These two kinds of bacteria are quite different. The nitrifying bacteria have strict ecological niches, and they have not been detected in the gastrointestinal tracts of animals. The strains of *bacillus* sp. used as probiotics for terrestrial livestock have telluric origins, and they are not autochthonous in the gastrointestinal tract, but they may be active during intestinal transit (Gourneir-Chateau *et. al*., 1994). Moreover there are many reports of isolation of *Bacillus* strains from fish (Hamid *et. al*., 1978; Strom and Olafsen, 1990; Nedoluha and westhoff, 1995; Sadhukhan *et. al*., 1997; Kennedy *et. al*., 1998; Sugita *et. al*., 1998), crustaceans (Austin and Allen, 1982; Sharmila *et. al*., 1996; Sugita *et. al*., 1996a), and bivalves (Sugita *et. al*., 1981). Queiroz and Boyd (1998) confirmed that a commercial innoculum of *Bacillus* sp. increased survival and production of channel catfish, but these authors focused on their water quality criteria, which were poorly affected by the treatment. Kennedy *et. al* (1998) isolated a strain of *Bacillus subtilis* from the common snook, *Centropomus undecimalis*. The inoculation of this strain into the rearing water resulted in the apparent elimination of *Vibrio* sp. from whole larvae of snook, after decreasing salinity. Moriarty (1998) noted an increase of prawn survival in ponds where some strains of *Bacillus* sp. were introduced. This treatment decreased the proportion of the pathogenic luminous *Vibrio* sp. in the sediments, and to a lesser extent, in the water. The strains of *Bacillus* were selected because of their antibiotic activity against luminescent *Vibrio* sp., but the author emphasized the multiplicity of the possible probiotic

effects, e.g. enzymatic excretions competition for nutrients and for space. These various mechanisms of action might prevent the emergence of resistant strains, a well-known risk of antibiotic treatments. The actual data of Moriarty (1998) showed the inhibitory activity of *Bacillus* sp. against luminous *Vibrio* sp. in pond sediment, but the effect on prawn survival might be due either to a probiotic effect, or to an indirect effect on animal health. For instance, the degradation of organic matter by *Bacillus* sp. might improve water quality.

The probiotic treatments may be considered as methods of biological control, the so-called “biocontrol” that termed the limitation or the elimination of pests by the introduction of adverse organisms, like parasites or specific pathogens. Maeda *et. al*. (1997) proposed to designate as biocontrol the methods of treatments using” the antagonism among microbes through which pathogens can be killed or reduced in the aquaculture environment”. In this acceptation, the pond treatment proposed by Moriarty (1998) is disputably relevant to biocontrol, as well as many other microbial treatments, including those whose target organisms are not animals, but concept refers to the treatment of pollutants or waste by the use of microorganisms that break down the undesirable substances. The same concept is sometimes named bioaugmentation (Moriarty, 1997; 1998).

In China, the studies on probiotics in aquaculture were focused on the photosynthetic bacteria. Qiao Zhenguo *et. al*. (1992) has studied three strains of photosynthetic bacteria used in prawn (*P. chinensis*) diet

preparation and their effect. Addition of the bacteria in the food or culture water was found to improve the growth of the prawn and the quality of the water. Cui Jingjin *et. al.* (1997) showed that the water quality of the pond treated with photosynthetic bacteria was remarkably improved, the fouling on the shell of the larvae was reduced, the metamorphosis time of larvae was 1 day or even earlier, and the production of post-larvae was more than that of control.

2.3 Application of commercial probiotics

The first trials of incorporation of probiotics into aquaculture feeds used commercial preparations designed for land animals. Spores of *Bacillus toyoi* isolated from soil reduced the mortality of Japanese eel, which were infected by *Edwardsiella* sp., (Kozasa, 1986). The same feed additive increased the growth rate of yellowtail (Kozasa, 1986). Spores may be easily incorporated into compound food, but their fate in the gastrointestinal tract of fish was not followed in these experiments. It would be particularly interesting to know whether the spores may germinate in the gut, depending on the transit time and rearing temperature. The same strain of *B. toyoi* used by Kozasa (1986) was later tested on rotifer, *Brachionus plicatilis*, which were left to filter the spores for 2 h (Gatesoupe, 1989). This treatment increased the growth rate of larval turbot, but the microbiota study was neither made in the larvae nor in the rotifers. Later, a study was performed with the food additive “Paciflor 9” containing spores of Bacillus IP5832 by counting and characterizing bacteria associated with spore-fed rotifers and turbot larvae (Gatesoupe, 1993). Most spores of

Bacillus sp. were filtered by the rotifers within less than half an hour, but the number of cultivable cells of the strain decreased sharply in the rotifers 1 h after introduction of spores into the water. These spores were thus recorded alive in the rotifers, but for a period probably too short to allow an actual probiotic effect, according to definition. Many *Bacilli* produce antibiotics, especially in the sporulation process (Brock, 1974), and some antibiotics may be produced by proteolysis of the vegetative cells (Vitkovic and Sadoff, 1977). When rotifers fed with spores, the decrease of the Vibrionaceae normally dominant in the rotifers might be due to such a release of antibiotic from the cells of *bacillus* sp.(Gatesoupe,1993). Few cultivable cells of the strain of *Bacillus* sp. were recovered from turbot fed for five days with the spore treated rotifers. This treatment increased the resistance of turbot larvae exposed to pathogenic *Vibrio* sp.

Commercial preparations with live lactic acid bacteria have also been introduced into the medium of live food organisms for larval flatfish. Some of these treatments increased the production of rotifers and the growth of turbot and Japanese flounder (Gatesoupe, 1989, 1991; Gatesoupe *et. al*., 1989). Other commercial preparations of *streptococcus faecium* improved the growth and feed efficiency of Israeli carp (Noh *et. al*., 1994; Bogut *et. al*.,1998). *Escherichia coli* disappeared from the intestinal microbiota of carp after 14 days of feeding with the probiotic preparation (Bogut *et. al*., 1998).

Surlikar and Sahu (1997) reported that the use of probiotic strain *Lactobacillus lactis* sub-species *cremoris* showed better growth performance of *Macrobrachium rosenbergii* postlarvae.

Khairnar *et. al.* (2000) applied probiotic 'Epicin' (Epicore Network Inc., Canada) at 0.20 ppm in the larval rearing tanks of *Macrobrachium rosenbergii*, which reduced ammonia and nitrite and maintained water quality parameters within optimum range. The average survival achieved was 39% as against 21% in the control group.

2.4 Role of enzyme supplements

Phytase is already used in swine and poultry feeds to increase phosphorus availability in grains and oilseeds by dephosphorylation of myo-inositol hexakisphosphate (phytate) (Cromwell *et. al*. 1993). Studies with catfish (Jackson *et. al*. 1996; Eya and Lovell, 1997; Li and Robinson, 1997) and trout (Cain and Garling, 1995; Rodehutscord and Pfeffer, 1995; Vielma *et. al*. 2000) demonstrate the effectiveness of phytase at increasing phosphorus availability in fish, although these studies also demonstrate the significance of rearing water temperature on effectiveness and optimum dietary phytase level. Li and Robinson (1997) found that the cost of adding phytase to catfish feeds was nearly equal to the savings associated with eliminating dietary supplementation with inorganic phosphorus.

Mixtures of proteases may be used to increase the digestibility of protein in rendered products. Such products would contain enzymes that hydrolyze connective tissue and skin, two components of rendered

products that are difficult for fish to digest. Another category of enzyme supplements is that break down fiber and certain carbohydrates found in protein sources from grains and oilseeds. One such product, designed specifically for use in high-wheat feeds for poultry, contains endo-xylanase, which breaks down pentose sugars. A similar product breaks down glucans found in wheat, barley, triticale and rye, releasing glucose. To date, these products have been only used in poultry and swine diets, but it is likely that they will be effective in diets for tilapia, catfish, scampi and perhaps shrimp.

Studies in poultry show that supplementing feeds with a glycanase increases the performance of the birds when their diet contained low metabolizable energy (Choct, Hughes, *et. al.* 1995 #20771). Supplementation with the enzyme significantly increased solubilization of non-starch polysaccharides in the intestine of the birds. Enzymes that break down non-starch polysaccharides must be tested in fish to determine if nutritional value, specifically energy availability, is increased in soybean meal-containing diets when enzyme supplements are used.

CHAPTER - III

MATERIAL AND METHODS

` This chapter deals with the materials required and methods followed for conducting the experiment on “Efficacy of enzymatic and bacterial probiotic on growth and survival of giant freshwater prawn *Macrobrachium rosenbergii* (De Man)”.

3.1 Site of the Experiment

The experiment was conducted over a period of 90 days from 2nd November 2004 to 2nd February 2005 in the Aquaculture Division of Central Institute of Fisheries Education, Mumbai. Feeding trial was conducted at the Hatchery complex of Aquaculture Division of Central Institute of Fisheries Education, Mumbai.

3.2 Experimental containers

The experiment was conducted in 12 plastic flat bottom circular tubs each of 50-litre capacity. The 12 tubs were arranged in four series each of three. All the 12 tubs were filled with filtered borewell water.

3.3 Seed source

The giant freshwater prawn seed was brought from the commercial freshwater prawn hatchery, which is located at Chiplun, Ratnagiri District, Maharashtra. The seed was 15 to 20 days old i. e. post larvae 15-20 (PL-15 to PL-20). They were acclimatized for seven days in a circular tank (1000 l) with proper aeration till their use in the feeding trial during which they were fed practical diet.

3.4 Glassware and chemicals

Neutral glassware of Borosil was used throughout the experiment. Chemicals of various companies viz. Sigma, SRL, Hi-Media, Qualigens, and Merck etc. were used.

3.5 Experimental Design

The experimental design included four distinct experimental groups (T_1, T_2, T_3 and T_4) each group is having 3 replications so that overall setup follows a completely randomized design (CRD) with 12 uniform sized tubs of 50 litres capacity. The details of the experimental setup are as follows:

T_1 - Control feed i.e. feed without probiotics

T_2 - feed with bacterial and enzymatic probiotic supplementation @ 100mg/kg of feed.

T_3 - feed with bacterial and enzymatic probiotic supplementation @ 150mg/kg of feed.

T_4 - feed with bacterial and enzymatic probiotic supplementation @ 200mg/kg of feed.

The tanks were cleaned with bleaching powder and potassium permanganate and washed with borewell water before the feeding trial.

3.6 Physico-chemical Parameters of Water

The water quality parameters viz. temperature, pH, dissolved oxygen, free carbon dioxide, ammonia-N, nitrite-N, nitrate-N, total alkalinity and phosphate-P were recorded on the day after siphoning at an interval of every 20 days (APHA, AWWA, WEF, 1992).

Plate no. 1: Experimental set-up

3.6.1 Dissolved oxygen and temperature

The dissolved oxygen and temperature of water was measured by membrane electrode method using dissolved oxygen meter (Merck, Germany) for all the experimental tubs during the morning hour (around 7:00 am).

3.6.2 Free carbon dioxide

Free carbon dioxide was measured by titrimetric method (APHA-AWWA-WEF, 1998) and calculated by using the formula:

$$CO_2 \text{ (mg/l)} = \frac{A \times N \times 44 \times 1000}{\text{Volume of sample (ml)}} \times 100$$

Where, A= Volume (ml) of titrant (NaoH)

N= Normality of titrant (N/44)

3.6.3 pH

The pH was measured by a digital pH meter (LABINDIA Ltd.) for all the experimental tubs.

3.6.4 Ammonia-N

Ammonia-N concentration was measured spectrophotometrically at 640 nm by Phenate method (APHA- AWWA-WEF, 1998) and compared with the standard graph.

3.6.5 Nitrite-N

Nitrite nitrogen of water was estimated using colorimetric method (APHA-AWWA-WEF, 1998), the optical density was measured at 543 nm and compared with the standard graph.

3.6.6 Nitrate-N

Nitrate nitrogen of water was determined using cadmium reduction method (APHA-AWWA-WEF, 1998). The optical density was measured at 543 nm and compared with the standard graph.

3.6.7 Total Alkalinity

Total alkalinity was estimated by titrimetric method (APHA, 1998) by titrating against standard H_2SO_4 and phenolphthalein and methyl orange as indicator.

3.6.8 Phosphate-P

The phosphorus content of water was determined by ascorbic acid method. The optical density was measured at 880 nm and compared with the standard graph.

3.7 Formulations and preparations of experimental diet

The feed ingredients, soybean meal, wheat flour, mustard oil cake, rice polish, de-oiled rice bran, fishmeal, cod liver oil, vitamin C, Vitamin B (Becosules) were taken for feed formulation. All the ingredients were procured from the Mumbai local market. 33% protein feed was prepared as an experimental diet by the following method:

All the ingredients except vitamins were mixed in a big plastic bowl. To this mixture water was added to prepare dough. The dough was then left for 1 hr. to conditioning followed by steaming for 20 minutes in a pressure cooker. Vitamin C and vitamin B were mixed after cooling. Pellets were prepared by hand pelletizer having 1.5 mm diameter size holes. Finally the pellets were air dried for 30 minutes and kept in oven at 60°C

till complete drying. After drying the pellets were packed in airtight containers (table no.1).

Table no.1: Composition of experimental diet of 33% crude protein

Ingredients	Composition (%)
Soybean meal	40.60
Fish meal	5.0
Wheat flour	10.0
Mustard oil cake	14.0
Rice bran	23.0
Fish oil	6.0
Vitamin C and Vitamin B	0.7 each

3.8 Presentation of the bacterial and enzymatic probiotic mixture (Hydroyeast)

The bacterial and enzymatic probiotic mixture (Hydroyeast) is a new commercial mixture, manufactured by Agranco Corp., USA. The mixture is provided as in powdered form. The details of the mixture are as follows

What is Hydroyeast?

Hydroyeast aquaculture is powder blend of live active yeast, enzymes, probiotics, and oligo-sacharides (B-Glucans and Manans), specially designed will be shrimp and fish rations, will be the purpose of improving feed efficiency, will be helping in disease prevention and it will be improving shrimp and fish quality as best as possible.

Mode of action

The active live yeast increments total bacterial count in the digestive tract, specially the cellular population. Additionally, helps in conditions of stress and in the assimilation of carbohydrates.

Enzymes break down proteins, carbohydrates, fats and fibers, making nutrients lives available in the digestive tract.

Probiotics help in maintaining balanced microflora. Provide competitive exclusion, synthesize enzymes and promote passive immunity.

Oligosaccharide immune stimulant agent it binds specifically the receiving molecule on surface of phagocytes. When the molecule is engaged, the cells become lives activate, killing and digesting bacteria. At the same times, they secrete single molecules, which stimulate the formation of white blood cells.

All components in Hydroyeast aquaculture work in synergism, incrementing to their respective efficacies, resulting in the common end of incrementing volume and quality of production.

Characteristics

Protein: 38% min., Phosphate-Dry Base: 1.6%, Moisture: 8.0%, Fiber: 8.0%, Ash: 4.6%, Fat: 1.0%, Thiamine(B1): 20 mcg/g, Riboflavin (B2): 40 mcg/g, Niacine: 390 mcg/g, Panthotenic Acid: 125 mcg, Choline 3,000 mcg/g, Color: Tan, Texture: 8 mesh (table no.2).

Table no.2: Ingredients of enzymatic and bacterial probiotic mixture

Ingredient	units/kgmin	Yeast and probiotic	*cfu/ kg min.
Oligosaccharide	50,000ppm	--	--
Enzymes	--	**Active Live Yeast**	5,000,000,000,000
Amylase	3,750,000	*Lactobacillus acidophilus*	22,500,000,000
Protease	500,000	*Bifedobact. thermophilum*	22,500,000,000
Cellulase	200,000	*Bifedobat. longhum*	22,500,000,000
Pectinase	100,000	*Streptococcus faecium*	22,500,000,000
Xylanase	10,000	--	--
Phytase	3,000	--	--

* cfu : colony forming units

Approvals

ALL ingredients are approved by FDA as listed in the American Feed Control Officials, official manual publication, 1998 edition.

Thus, it is assumed that Hydroyeast should be able to provide positive effects directly to the farmed scampi/prawn as well as indirectly by improving its environment. From these general considerations, the Hydroyeast is an interesting probiotic mixture. No studies have been carried out regarding possible probiotic effects of Hydroyeast on scampi/prawn. Thus, the present study is undertaken to evaluate the Hydroyeast as a probiotic candidate for intensive prawn farming.

3.9 Proximate analysis

Proximate analysis of prawn was estimated by using standard methods. Crude protein, moisture, ash, lipids and crude fiber were analysised after completion of the experimental period by the following methods

3.9.1 Moisture

The moisture content of experimental prawn muscle was determined by taking the known sample weight in a petridish and drying it in the hot air oven and measuring the final weight of the sample. After obtaining the final weight, the moisture content was calculated by using the following formula

$$\text{Moisture (\%)} = \frac{\text{Wet weight – dry weight}}{\text{Wet weight of the sample}} \times 100$$

3.9.2 Crude protein

The crude protein was determined by using micro-kjeldhals method where the nitrogen content was obtained and from that value the crude protein was calculated using the following formula

Crude protein= nitrogen % × 6.25

3.9.3 Lipid

The lipid content of the tissue was obtained by using Soxtec system using diethyl ether with boiling point 40-60°C as the solvent and the lipid content was calculated according to the following formula:

$$\text{Lipid content} = \frac{\text{Weight of ether extract}}{\text{Wet weight of the sample}} \times 100$$

3.9.4 Crude fiber

Crude fiber of the sample was obtained by using Fibretec system (Model M,1027 hot extraction, Tecator).In this method, a known amount of the sample was digested with 0.128 M H_2SO_4 followed by 0.313 M NaOH and finally washed in cold extraction unit by acetone(5-10 ml).After drying the filtrate, the sample with the crucible was kept in muffle furnace at 400 °C for 6 hrs for making it into ash. The crude fiber was calculated using the following equation:

$$\text{Crude fiber (\%)} = \frac{\text{Weight of the digested residue} - \text{weight of the ash}}{\text{Wet weight of the sample}} \times 100$$

3.9.5 Ash

Ash content was estimated by using vetrosil crucible. The sample was placed in it and kept in a muffle furnace at 55°C.

$$\text{Ash content} = \frac{\text{Weight of the ash}}{\text{Wet weight of the sample}} \times 100$$

3.10 Growth parameters

The growth parameters of the *M*. *rosenbergii* were assessed by taking their body weight at 15 days interval. The animals were kept starved overnight before body weight was measured. The prawns were anaesthetized with clove oil ($50\mu l^{-1}$) before taking weight to minimize handling stress. After taking weight, the prawns were treated with 0.3% salt water to relieve from handling stress. The growth performance was assessed by using the following formulae

3.10.1 Body weight gain

Individual body weight gain (g) = Final weight (g) – Initial weight (g)

3.10.2 Percentage weight gain

$$\text{Percent weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

3.10.3 Specific growth rate

$$\text{Specific growth rate (SGR)} = \frac{\text{Log}_e(\text{Final weight}) - \text{Log}_e(\text{Initial weight})}{\text{Experimental period in days}} \times 100$$

3.10.4 Feed conversion ratio (FCR)

$$\text{Feed conversion ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (Wet weight gain)}}$$

3.10.5 Feed efficiency ratio (FER)

$$\text{Feed efficiency ratio} = \frac{\text{Net weight gain (dry weight)}}{\text{Feed given (dry weight)}}$$

3.10.6 Protein efficiency ratio (PER)

$$\text{Protein efficiency ratio} = \frac{\text{Net weight gain (wet weight)}}{\text{Crude protein fed}}$$

3.11 Survival

Survival percentage was calculated at the 15 days interval throughout the experiment by counting the number of prawns in each tub and is calculated as follows

$$\text{Survival (\%)} = \frac{\text{Total number of prawns harvested}}{\text{Total number stocked}} \times 100$$

3.12 Cleaning and siphoning

Experimental tubs were cleaned manually by siphoning the water along with fecal matter and left over feed every day and the same was replaced by 50% of fresh chlorine free bore well water.

3.13 Fatty acid analysis

3.13.1 Isolation of lipids

Total lipids were extracted from the tissues of the prawn as per the method described by Folch *et*. *al*. (1957). About 1 g of the tissue was weighed and a 10 % homogenate was made using chloroform: methanol (2:1) mixture and were transferred to a stoppered measuring cylinder and sufficient amount of chloroform: methanol mixture was added to it for a period of 24-48 hrs. The cylinder was shaken intermittently. It was then filtered through whatman filter paper –I. The residue was then transferred to another stoppered measuring cylinder and required amount of chloroform: methanol mixture was added and was kept aside. The above procedure was repeated once more and the filtrate was collected. The pooled filtrate was then taken in another stoppered measuring cylinder and 1/5 volume of 0.85 % of NaCl solution was added. It was then kept for

6 hrs. at room temperature. Two layers get separated out; the lower phase contains the nonlipid contaminants. The upper layer was decanted after 4-6 hrs. The lower layer containing the lipids and chloroform were transferred to a conical flask and chloroform was evaporated by passing N_2 gas. The lipid that was extracted by this method was taken out of the flask and was used for further analysis.

3.13.2 Esterification or methylation for fatty acid analysis

To increase the volatility before Gas chromatography (GC) analysis, the lipid extracted by the above-mentioned procedure was typically saponified and the fatty acids thus liberated were esterified with boron trifluroid methanol (BF_3 methanol) reagent to form fatty acid methyl esters (FAME)(AOAC, 1995).

The reaction was conducted in a round bottom flask. To the sample (lipid from prawn tissue), about 20 ml of 1 N methanolic NaoH was added to avoid the bumping. The condenser was attached and the flask was kept for reflux for about 15-20 minutes until the fat globules disappeared. Then BF_3 methanol reagent was added and the boiling was continued for about 5 minutes. After cooling, the solution was extracted with adequate amount of petroleum ether in a separating funnel. The gas liberated was removed by inverting the funnel.

After the petroleum ether extraction, the solution was extracted again twice with distilled water so as to remove excess BF_3 or to neutralize the acidic medium formed by the BF_3 complex. Further it was again extracted with 30 ml of saturated sodium chloride. This was done to

remove unreacted fatty acids from the sample solution. The upper layer was extracted and passed over anhydrous sodium sulphate so as to trap the water molecules left, if any, in the sample. Finally, a clean and clear solution or extract was collected in a beaker and evaporation on a water bath till any tiny droplets of the yellow coloured liquid appeared at the bottom. These droplets were esters of fatty acids, which were further diluted with chloroform and stored in 15 ml glass vials sealed with paraffin tape to make air proof. The methyl esters were used for analysis of fatty acid through gas chromatography.

3.13.3 Gas chromatography analysis

Fatty acid methyl esters (FAME) were analyzed using a gas chromatography equipped with a flame ionization detector. The FAME was separated on a capillary column, coated with silica. The samples were diluted with chloroform at the rate of 10%. The injection volume was 0.5 ml.

3.13.4 GLC column specification

Fused silica capillary column of 30 m length, 0.25 mm ID and 0.20 mm thickness was used for the analysis of the respective FAME. The column used was packed with SP-2330.

3.13.5 GLC operating conditions

The temperature of the injector and the detector was maintained at 250°C during the operation. The column was operated on a temperature programme of initial temperature 180°C for a period of 5 minutes. Nitrogen was used as the carrier gas at the rate of 1 ml / minute.

Fatty acids were identified by comparison with retention times of references standards consisting of a mixture of saturated and unsaturated fatty acids.

3.14 Statistical Analysis

The data were statistically analyzed by statistical package SPSS version 11. Efficacy of enzymatic and bacterial probiotic on growth, survival, water parameters and their interaction were analyzed by one way analysis of variance (ANOVA). Comparison between two treatments was done by Duncan's Multiple Range Test (DMRT). Comparison among all the treatment was done by one way ANOVA. Comparison was made at the 5% probability levels.

CHAPTER-IV

RESULTS AND DISCUSSION

A study was conducted to see the efficacy of bacterial and enzymatic probiotic on growth and survival of the giant freshwater prawn *M. rosenbergii* (De Man). Feeding trial was conducted for a period of 90 days fed with four probiotic-coated diets. Experimental setup was with four treatments and three replications for each treatment.

Probiotics available in market

There are wide ranges of probiotics available in the market. During this study it has been observed that there are very few probiotics which, are popular and used frequently in the aquaculture area; namely Biogreen (J.V. Marine, USA), Epicin (Epicore Networks Inc. Canada), Hydroyeast (Agranco ltd. USA). Hydroyeast is found suitable among all above-mentioned probiotics, as it contains bacteria, enzymes, yeast and oligosaccharide. Therefore, it has been selected for this study. The results of Hydroyeast application in rearing of *M. rosenbergii* postlarvae are discussed below

4.1 Physico-chemical parameters of water

Physical and chemical parameters of water influence the ability of prawns and shrimps to perform physiological functions, including growth, disease resistance, reproduction and tolerance to extreme environmental conditions. Aquaculture species are in more intimate contact with their own waste than other terrestrial species, because the water in which they live is

also, necessarily, self-contaminated (Tomasso & Brune, 1991). Similar to humans and terrestrial animals (Holzapfel, *et. al*., 1998), it can be assumed in aquaculture that the intestinal microbiota does not exist as entity by itself but that there is a constant interaction with the environment and the host functions. Many researchers have already investigated the relationship of the intestinal microbiota the aquatic habitat and food, so quality of environment in which they live has mere importance in aquaculture. Cahill (1990) summarized the results of these investigations on fishes, giving evidence that the bacteria present in the aquatic environment influence the composition of the gut microbiota and vice versa. The genera present in the intestinal tract generally seem to be those from the environment or the diet, which can survive and multiply in the intestinal tract (Cahill, 1990). However, it can be claimed that in aquaculture systems the immediate ambient environment has a much larger influence on the health status than with terrestrial animals or humans.

Much more than terrestrial animals, aquatic farmed animals are surrounded by an environment that supports their pathogens independently of the host animals, and so (opportunistic) pathogens can reach high densities around the animal (Moriarty, 1998). Surrounding bacteria are continuously ingested either with the feed or when the host is drinking. This is especially the case with filter feeders, which ingest bacteria at a high rate from the culture water, causing a natural interaction between the microbiota of the ambient environment and the live food.

Probiotics could also be active on the gills or the skin of the host but also in its ambient environment by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment (Fuller, 1989). Chinese researchers have done some studies on the probiotics to improve the shrimp culture water, and achieved remarkable results (Li Zhuojia *et. al*., 1997).

4.1.1 Temperature

Data pertaining to the temperature present in the experimental tanks through out the experimental period is depicted in the table no. 3. No Significant difference (P>0.05) was found among the treatment groups. As temperature is environmental factor, there is no co-relation between probiotic feeding and temperature.

The giant freshwater prawn *M. rosenbergii* is poikilothermic in nature. Thus temperature is a crucial factor which influences growth, moulting and reproduction of this species. In the present experiments the over all ambient water temperature ranged from 22.4°C to 25.0°C, which was below the optimum temperature. The optimum range of temperature for scampi culture is 29°C to 31°C (New and Singholka, 1985), which is higher than the present experiment. Temperature of 23-27°C was reported to have negative impact on growth and survival of *M. rosenbergii* (Indulkar, 1996).

Table no.3: Water temperature of different experimental groups

Treatments	Replication*			Mean±SE
	A	B	C	
T_1	22.6	22.4	22.2	22.40± .12
T_2	22.8	22.7	22.4	22.63± .12
T_3	22.7	22.5	22.6	22.60±.06
T_4	22.6	22.6	22.9	22.70± .10

Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

Temperature

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.150	3	.050	1.622	.260
Within Groups	.247	8	.031		
Total	.397	11			

4.1.2 pH

Data pertaining to the pH in the experimental tanks through out the experimental period is depicted in the table no. 4. Significant difference ($P<0.05$) was found among the treatment groups. The lowest pH content (7.13±0.03) was found in T_1 treatment group and the highest pH content (7.50 ±0.00) was found in T_3 treatment group. There is no significant difference ($P>0.05$) between T_1, T_2 and T_4 and also there is no significant difference between T_2, T_3 and T_4. The pH was found in the optimum range of 7.5-8.0 (New and Singholka, 1982). It means probiotic treatment balances the pH of the treatment group. Same results were obtained by Chinese researchers (Li Zhuojia *et. al*., 1997).

pH is a key indicator of water quality in the experimental tanks. The ratio of unionized and ionized ammonia alters the pH value. The value of pH increases with the increase in unionized ammonia (NH_3), which is toxic to scampi if the concentration is greater than 0.1 mg L^{-1}. Low pH is associated with decreased inorganic carbon, which has been shown to affect metabolism of calcium and several other minerals (Wickens, 1984). In the case of freshwater crayfish, low pH causes softness of shell. This is due to the shell of the crayfish being composed of calcium carbonate, which reacts with acid.

Table no.4: pH of water in different experimental groups

Treatments	Replications*			Mean ± SE
	A	B	C	
T_1	7.2	7.1	7.1	7.13^{a}± 0.03
T_2	7.0	7.5	7.3	7.27^{ab}± 0.14
T_3	7.5	7.5	7.5	7.50^{b}± 0.00
T_4	7.4	7.3	7.4	7.37^{ab}± 0.03

Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.217	3	.072	4.127	.048
Within Groups	.140	8	.018		
Total	.357	11			

4.1.3 Dissolved oxygen (DO)

Data pertaining to the Dissolved oxygen present in the experimental tanks throughout the experimental period is depicted in the table no. 5. No Significant difference ($P>0.05$) was found among the treatment groups.

Dissolved oxygen plays an important role for the living organisms in maintaining the metabolic balance, specially in the aquatic environment its solubility needs to range between 5 ppm to 10 ppm more particularly for the better growth and survival of rearing organisms. The DO level to be maintained at 75 percent saturation level (New and Singholka, 1985).

Dissolved oxygen (DO) is the most important parameter, which affects growth of prawn indirectly as it influences the feed consumption and metabolism. The desired DO level was maintained through efficient aeration system and by daily exchange of 50 % water, thus the growth of scampi was not affected by the DO.

4.1.4 Free carbon dioxide (CO_2)

It is an association of carbon and oxygen, which in water forms carbonic acid and acts as important limiting factor for rearing organisms, if its solubility concentration exceeds more than 3 mg L^{-1}. The free carbon dioxide was absent in the experimental tanks throughout the experimental period. Thus, no adverse effect was seen on the experimental animals.

Table no.5: Dissolved oxygen of water in different experimental groups

Treatments	Replications*			Mean ±SE
	A	B	C	
T_1	6.3	6.2	6.3	6.27 ±0.033
T_2	6.1	6.3	6.3	6.23 ±0.067
T_3	6.3	6.4	6.4	6.37 ±0.033
T_4	6.3	6.5	6.5	6.43 ±0.067

Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.076	3	.025	3.033	.093
Within Groups	.067	8	.008		
Total	.143	11			

4.1.5 Total Alkalinity

Data pertaining to the Total alkalinity present in the experimental tanks through out the experimental period is depicted in the table no. 6. No Significant difference ($P>0.05$) was found among the treatment groups.

Alkalinity of water is expressed as the concentration of calcium carbonate ($CaCO_3$) in mg L^{-1} (ppm). The alkalinity determines the pH of water. Water with low alkalinity has low buffering action, leading to wide fluctuations in pH value. High alkalinity raises the pH and leads to mortality of scampi.

As pH of the experiment was in optimum level the total alkalinity didn't have any adverse effect on experimental animal.

4.1.6. Ammonia nitrogen (NH_4^+-N)

Data pertaining to the ammoniacal nitrogen present in the experimental tanks through out the experimental period is depicted in the table no. 7. Significant difference ($P<0.05$) was found among the treatment groups. The lowest ammoniacal nitrogen content (0.19±0.075) was found in T_4 treatment group and the highest ammoniacal nitrogen content (0.45±0.054) was found in control group. It means ammoniacal nitrogen can be considerably kept checked by probiotics application.

Ammonia is the second most important water quality parameter after dissolved oxygen as it is one of the most toxic substances to the culturable organisms. The ratio of unionized to ionized ammonia in culture medium is related to pH. The rise of pH and temperature of water

Table no.6: Total alkalinity of water in different experimental groups

Treatment	Replications*			Mean ±SE
	A	B	C	
T_1	127.1	135.6	136.2	132.97±2.94
T_2	130.5	133.5	129	131.00±1.32
T_3	123.5	126.6	133.1	127.73±2.83
T_4	137.6	133.4	127.4	132.80±2.96

Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	53.149	3	17.716	.870	.495
Within Groups	162.873	8	20.359		
Total	216.022	11			

Table no.7: Ammoniacal nitrogen in different experimental groups

Treatments	Replications*			Mean±SE
	A	B	C	
T_1	0.42	0.37	0.55	0.45^{c} ± 0.054
T_2	0.38	0.41	0.32	0.37^{bc} ±0.026
T_3	0.35	0.22	0.23	0.27^{ab} ±0.041
T_4	0.25	0.28	0.04	0.19^{a} ± 0.075

Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.115	3	.038	4.630	.037
Within Groups	.066	8	.008		
Total	.181	11			

increases the concentration of unionized ammonia (NH_3-N). Ionized ammonia (NH_4^+-N) is comparatively not as toxic as it is unable to pass through the gill membrane of the prawn. Ammonia concentration in the culture media should not exceed 1.0 ppm of NH_4^+-N and 0.1 ppm of NH_3-N (New and Singholka, 1982). Maintaining sufficient dissolved oxygen level, which helps in oxidation of ammonia to harmless nitrate by nitrifying bacteria, can easily eliminate the ammonia. Ammonia level can get increased due to excretion and uneaten food accumulated at the tank bottom. The maximum acceptable ammonia levels for long-term exposure are between 0.05 and 0.1 mg NH_3-N l^{-1}.

Studies by the Chinese researcher Li Zhuojia *et. al*., (1997) corroborates our results, wherein they added photosynthetic bacteria into the water, which could eliminate the NH_3-N, H_2S and organic acids, and other harmful materials rapidly, improved the water quality and balanced the pH. The heterotrophic probiotics may have chemical actions such as oxidation, ammonification, nitrification, denitrification, sulphurification and nitrogen fixation. When these bacteria were added into the water directly or through feed, they could decompose the excreta of fish or prawns, remaining food materials, remains of the plankton and other organic materials to CO_2, nitrate and phosphate along with the improvement of the growth. These broken-down inorganic salts provide the nutrition for the growth of micro algae, while the bacteria grow rapidly and become the dominant group in the water and/or intestinal tract, inhibiting the growth of the pathogenic microorganisms. The photosynthesis of the micro algae

provide dissolved oxygen for oxidation and decomposition of the organic materials and for the respiration of the microbes and the cultured animals. This kind of cycle may improve the nutrient cycle and it can create a balance between bacteria and micro algae and maintaining a good water quality environment for the cultured animals.

Same observations were made by Khairnar *et. al.* (2000) after application of probiotic 'Epicin' (Epicore Network Inc., Canada) at 0.20 ppm in the larval rearing tanks of *Macrobrachium rosenbergii*, which reduced ammonia and nitrite and maintained water quality parameters within optimum range.

Barik (2004) found reduced levels of ammonia and nitrites after inoculation of nitrifying bacteria i. e. bacterial probiotic into culture.

4.1.7 Nitrite nitrogen (NO_2-N)

Nitrite is highly toxic to the prawn. Nitrite (NO_2-N) is an intermediate product in the bacterial oxidation of ammonia to nitrate (NO_3-N), a process called nitrification.

$2NH_3 + 3O_2 = 2NO_2 + 2H + 2H_2O$; $2NO_2 + O_2 = 2NO_3$

The safe level of nitrite nitrogen to the prawn should be maintained at less than 0.1 ppm. The acute toxicity of nitrite is known to be affected by water pH. The nitrite toxicity increases with increasing the pH. The nitrite is maintained at safer level by daily exchange of water. Nitrite-N concentration was recorded in the range of 0.069-0.81 mg L^{-1}, which is

within the permissible limits for pond aquaculture (Boyd and Tucker, 1998).

Data pertaining to the nitrite nitrogen present in the experimental tanks through out the experimental period is depicted in the table no. 8. No Significant difference ($P<0.05$) was found among the treatment groups. It means there was no effect of enzymatic and bacterial probiotic on the nitrite nitrogen content.

4.1.8 Nitrate nitrogen (NO_3-N)

Data pertaining to the nitrate nitrogen present in the experimental tanks through out the experimental period is depicted in the table no. 9. Significant difference ($P>0.05$) was found among the treatment groups. The lowest nitrate nitrogen content (5.78±0.07) was found in T_3 treatment group and the highest nitrate nitrogen content (8.32±0.49) was found in control group. It was found within the safe limit.

It is one of the better steps in nitrogen cycle, which is a complex and well-buffered gaseous type. The safe limit of nitrate nitrogen to the prawn is <20 ppm. Nitrate level in a productive pond can be within 0.06-5.0 mg L^{-1} (Boyd and Tucker, 1998).

It means probiotic feeding may improve water quality and provide sufficient nutrients in aquaculture. Same results were found by Chinese researchers (Li Zhuojia *et. al*., 1997).

Same observations were made by Khairnar *et. al.* (2000) after applying probiotic ‘Epicin’ (Epicore Network Inc., Canada) at 0.20 ppm in

Table no.8: Nitrite nitrogen in different experimental tanks

Treatments	Replications*			Mean± SE
	A	B	C	
T_1	0.064	0.075	0.07	0.07 ±0.003
T_2	0.087	0.084	0.068	0.08 ±0.006
T_3	0.082	0.06	0.11	0.08 ±0.014
T_4	0.089	0.084	0.07	0.08 ±0.006

Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.539	.669
Within Groups	.002	8	.000		
Total	.002	11			

Table no.9: Nitrate nitrogen in different experimental tanks

Treatments	Replications*			Mean± SE
	A	B	C	
T_1	7.74	7.9	9.31	8.32^{b}±0.49
T_2	8.98	7.51	6.59	7.69^{b}±0.69
T_3	5.7	5.73	5.92	5.78^{a}±0.07
T_4	8.06	7.66	6.89	7.54^{b}±0.34

Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.621	3	3.540	5.516	.024
Within Groups	5.135	8	.642		
Total	15.756	11			

the larval rearing tanks of *Macrobrachium rosenbergii*. Barik (2004) also found improved levels of nutrients after inoculation of nitrifying bacteria in to culture system, which supports our results.

4.1.9 Phosphate (PO_4-P)

Phosphorus is one of the major nutrients that are required by the biota, which occurs in the form of phosphates. The high concentration of phosphorus indicates eutrophication of aquatic bodies.

Data pertaining to the phosphate phosphorus present in the experimental tanks through out the experimental period is depicted in the table no. 10. Significant difference ($P<0.05$) was found among the treatment groups. The lowest phosphate phosphorus content (0.0317±0.00240) was found in T_3 treatment group and the highest phosphate phosphorus content (0.0673 ±0.00837) was found in T_4 treatment group which non similar with T_1 and T_2 group. It was found within the optimum range 0.005-0.2 mg/l (Boyd, 1998).

When bacteria were added into the water directly or through feed, they could decompose the excreta of fish or prawns, remaining food materials, remains of the plankton and other organic materials to CO_2, nitrate and phosphate along with the improvement of the growth. These inorganic salts provide the nutrition for the growth of micro algae, while the bacteria grow rapidly and become the dominant group in the water and/or intestinal tract, inhibiting the growth of the pathogenic microorganisms. The photosynthesis of the micro algae provide dissolved oxygen for oxidation and decomposition of the organic materials and for the

Table no.10: Phosphate-phosphorus in different experimental tanks

Treatment	Replications*			Mean ±SE
	A	B	C	
T_1	0.06	0.068	0.065	$.064^{b}$±.0023
T_2	0.064	0.049	0.049	$.054^{b}$± .0050
T_3	0.033	0.035	0.027	$.032^{a}$±.0024
T_4	0.067	0.053	0.082	$.067^{b}$±.0083

Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	3	.001	9.817	.005
Within Groups	.001	8	.000		
Total	.003	11			

respiration of the microbes and the cultured animals. This kind of cycle may improve the nutrient cycle.

4.2 Proximate Composition of prawn tissues

Proximate composition of the tissue of prawn larvae on % dry matter basis is given in table no. 11.The highest % of protein was found in the T_1 group (68.5 %) and the lowest in the T_4 group (67%). The lipid content showed very high variation among groups and the highest content was shown by T_4 group with (7.5%) and lowest in T_1 group (4.3%). Total carbohydrate content did not show much variation in the percent. Ash content was found to be highest in the group T_1 (14.1%) and lowest in the group T_4 with (13.0%).

Table no.11: Proximate composition (% dry matter basis) of the *M. rosenbergii* tissues fed by different concentration of probiotic coated diet.

Components	% (Dry matter basis)			
	T_1	T_2	T_3	T_4
Crude protein	68.5	67.6	67.24	67.0
Crude fat	4.3	7.0	6.0	7.5
Total carbohydrate	13.1	12.2	13.4	12.5
Ash	14.1	13.2	13.36	13.0

4.3 Growth Parameters

4.3.1 Final weight gain

Data pertaining to body weight of the prawn during the experimental period is depicted in the table no. 12 and graphically represented in figure no. 1. Significant difference ($P<0.05$) was found among the treatment groups. The lowest Body weight gain (1.77±0.072) was found in T_1 treatment group and the highest Body weight gain (3.38 ±0.135) was found in T_3 treatment group.

Same observation was made by Surlikar and Sahu (1997) when they used probiotic strain of *Lactobacillus lactis* sub-species *cremoris* which showed better growth performance of *Macrobrachium rosenbergii* postlarvae. Venkat *et. al.* (2004) also found similar results after feeding Lactobacillus- based probiotic to the postlarvae of *M. rosenbergii* (De Man), which confirms our results.

In the present studies significantly higher growth was recorded in larvae fed diets supplemented with enzymatic and bacterial probiotics. Although differences between probiotic treatments were not significantly different (T_3 & T_4) a consistent trend towards increased weight was observed for T_3 diet. Likewise, feeding turbot larvae with bioencapsulated Lactic acid bacteria (LAB) and *B. toyoi* improved the weight of larvae significantly (Gatesoupe, 1991). And also Maeda and Liao (1992) also reported the use of soil bacteria strain, pM-4 that promoted the growth of *Penaeus monodon* probably acting as a good food source. It can be

Table no.12: Final weight gain in different experimental tanks

Treatments	Replications*			Mean ± SE
	A	B	C	
T_1	1.74	1.67	1.91	1.77^{a}± 0.07
T_2	2.34	2.29	2.57	2.40^{b}± 0.09
T_3	3.21	3.38	3.61	3.40^{c}±. 12
T_4	2.49	2.61	2.67	2.58^{b}± 0.06

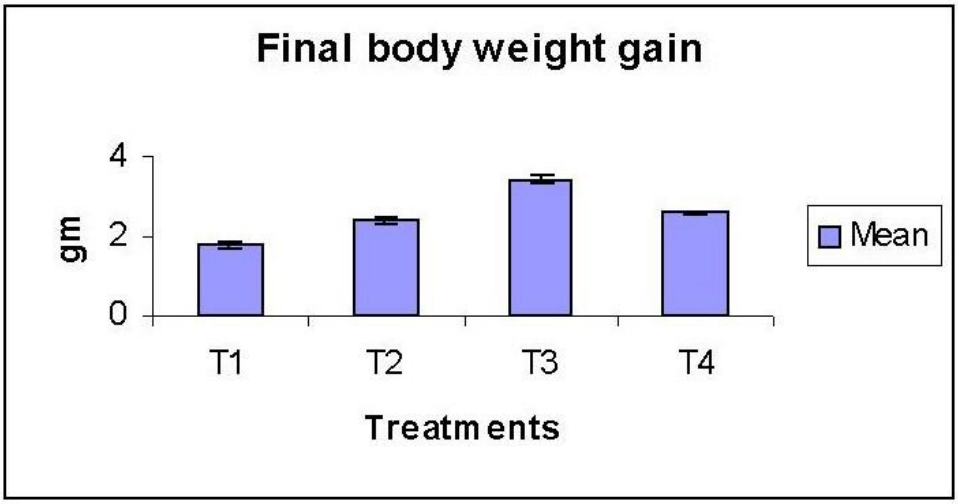
Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.048	3	1.349	62.596	.000
Within Groups	.172	8	.022		
Total	4.221	11			

Figure no.1: Final weight gain in different experimental tanks



concluded from above results that the higher growth is because of enzymatic and bacterial probiotic feeding.

LAB is responsible for fermentation in gastro-intestinal tract. They act on complex carbohydrates like starch and cellulose and split them into simpler compounds for absorption besides synthesizing many vitamins of B complex group and vitamin K in the GI tract.

Naogami and Maeda (1992) isolated a bacterial strain from a crustacean culture pond, which improved the growth of crab (*Portunus triberculatus*) larvae. They also suggested that the bacteria might improve the physiological state of the crab by serving as a nutrient source during its growth.

Ravi *et. al.* (1998) studied the influence of probiotics on growth of Indian white prawn *Penaeous indicus*.

Smith and Davey (1993) reported that florescent strain pseudomonad bacteria can competitively inhibit the growth of fish pathogen *A. salmona*.

Also Maeda and Naogami (1989) reported improved growth of prawn and crab larvae in their studies. Spores of *Bacillus toyoi* isolated from soil used as feed additive increased the growth rate of yellowtail (Kozasa, 1986). Gatesoupe, 1989, 1991, Gatesoupe *et. al*., 1989 made same observations, which supports our findings.

Commercial preparations of *streptococcus faecium* improved the growth and feed efficiency of Israeli carp (Noh *et. al*., 1994; Bogut *et. al*., 1998).

It has been observed that application of microbial phytase has beneficial effect on fish growth (Debnath D., 2003). In the present study phytase application also showed better growth rate in *M. rosenbergii*.

Mixtures of proteases may be used to increase the digestibility of protein, which helps to improve growth. Enzymes hydrolyze connective tissue and skin, which are difficult to digest to fishes or crustaceans. Enzymes break down fiber and certain carbohydrates found in protein sources from grains and oilseeds. Xylanase breaks pentose sugars, and from glucans it releases glucose.

Enzyme supplementation increases solubilization of non-starch polysaccharides in the intestine of the birds, which was also tested in fishes, wherein increase in the energy availability was observed (Hardy, R., 2000). So enzyme supplementation also helps in improvement of growth.

Use of enzyme supplements is one potential aspect of ingredient utilization that will increase the nutritional value.

4.3.2 Specific Growth Rate (%)

Data pertaining to specific growth rate present in the experimental tanks through out the experimental period is depicted in the table no. 13 and graphically represented in figure no.2. Significant difference ($P<0.05$) was found among the treatment groups. The lowest specific growth rate (1.77±0.072) was found in T_1 treatment group and the highest (3.38 ±0.135) in T_3 group. There was no significant difference ($P>0.05$) between T_2 and T_4 groups.

Table no.13: Specific growth rate in different experimental tanks

Treatments	Replications*			Mean ± SE
	A	B	C	
T_1	1.01	0.98	1.01	1.01^{a}±0.01
T_2	1.13	1.08	1.12	1.11^{b}±0.01
T_3	1.12	1.31	1.26	1.27^{c}±.02
T_4	1.12	1.18	1.14	1.14^{b}± .02

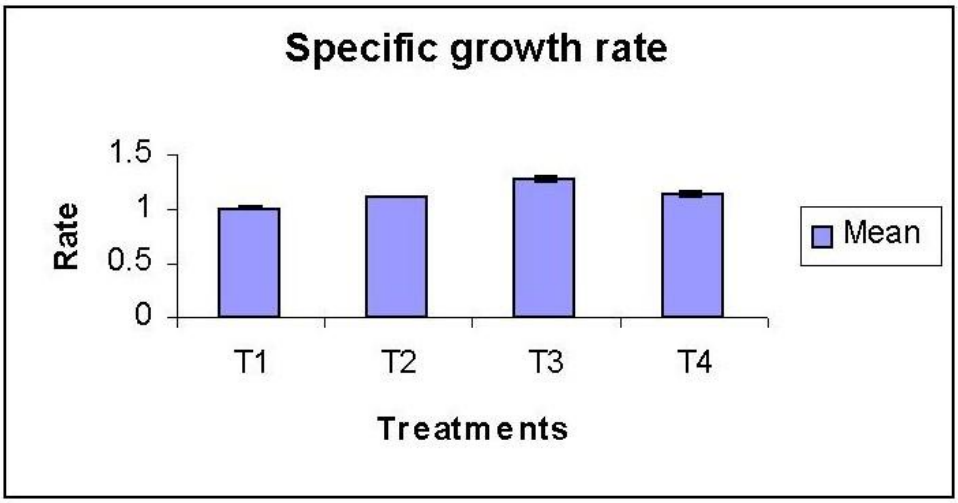
Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.109	3	.036	47.717	.000
Within Groups	.006	8	.001		
Total	.115	11			

Figure no.2: Specific growth rate in different experimental tanks



Same results were found by Venkat *et. al*. (2004). Surlikar and Sahu (2001) also found higher Specific growth rate after probiotic bacteria feeding to *M. rosenbergii* post larvae.

Spores of *Bacillus toyoi* isolated from soil used as feed additive increased the growth rate of yellowtail (Kozasa, 1986). The same strain *was* used by Kozasa (1986) later on rotifer, *Brachionus plicatilis*, which were left to filter the spores for 2 h (Gatesoupe, 1989). This treatment increased the growth rate of larval turbot.

4.3.3 Feed Conversion Ratio (FCR)

Data pertaining to feed conversion ratio presented in the experimental tanks through out the experimental period is depicted in the table no. 14 and graphically represented in figure no. 3. Significant difference ($P<0.05$) was found among the treatment groups. The lowest (2.16± .01) in T_3 treatment group and the highest Feed conversion ratio (2.87± .02) was found in T_1 treatment group. There was no significant difference ($P>0.05$) between T_2 and T_4 treatment groups.

Uma *et. al.* (1999) observed a significant improvement in FCR of shrimp larvae when fed with *L. plantarum* bioencapsulated in *Artemia*. Similar observations were made by Surlikar and Sahu (2001) when feeding probiotic *L. cremoris* at 8.5 x 10^{11} CFU g^{-1} diet to post larvae of *M. rosenbergii.* However in the present study the enzyme supplementation is responsible for lower FCR along with probiotic supplementation.

Table no.14: Feed conversion ratio in different experimental tanks

Treatments	Replications*			Mean ± SE
	A	B	C	
T_1	2.82069	2.895808	2.88377	2.87^{c}±.02
T_2	2.492308	2.447162	2.530739	2.49^{b}±.02
T_3	2.175701	2.16213	2.154017	2.16^{a}±.01
T_4	2.501205	2.413793	2.516854	2.48^{b}±.03

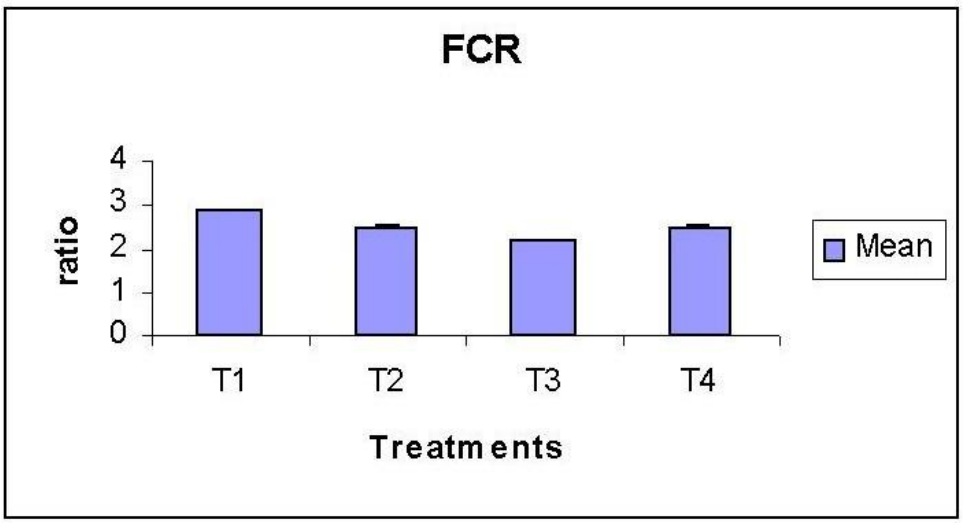
Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.744	3	.248	150.74	.000
Within Groups	.013	8	.002		
Total	.757	11			

Figure no.3: Feed conversion ratio in different experimental tanks



4.3.4 Feed Efficiency Ratio (FER)

Data pertaining to feed efficiency of the experimental groups is depicted in table no. 15 and graphically represented in figure no. 4. Significant difference ($P<0.05$) was found among the treatment groups. The lowest feed efficiency ratio (.3463±.01) was found in T_1 treatment group and the highest Feed efficiency ratio (.4637±.01) in T_3 group. There is no significant difference ($P>0.05$) between T_2 and T_4 groups. Similar results were found to Uma *et. al*. (1999), which confirms our results.

4.3.5 Protein Efficiency Ratio (PER)

Data pertaining to Protein efficiency ratio of the experimental tanks group is depicted in table no. 16 and graphically represented in figure no.5. Significant difference ($P<0.05$) was found among the treatment groups. The lowest protein efficiency ratio (0.0534±.002) was found in T_1 treatment group and the highest (0.103 ±0.0.003) in T_3 group. There is no significant difference ($P>0.05$) between T_2 and T_4 treatment groups.

Protein efficiency ratio was significantly higher than the control group. Better efficacy of protein uptake may be due to better digestion and assimilation of the nutrients in the gut by the modified gut flora. Bacteria, by virtue of their extracellular enzymes, have been reported to play an important role in the process of digestion in turbot larvae (Munilla, Moran, Stark & Barbour 1990).

Uma *et. al*. also found same results so it may be concluded that highest PER is due to probiotic supplementation.

Table no. 15: Feed efficiency ratio in different experimental tanks

Treatments	Replications*			Mean ± SE
	A	B	C	
T_1	0.345327	0.346768	0.346768	$.35^{a}$±.01
T_2	0.408637	0.395141	0.395141	$.40^{b}$±.01
T_3	0.462507	0.464249	0.464249	$.467^{c}$±.01
T_4	0.414286	0.397321	0.397321	$.40^{b}$±.01

Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.021	3	.007	174.51	.000
Within Groups	.000	8	.000		
Total	.021	11			

Figure no.4: Feed efficiency Ratio in different experimental tanks

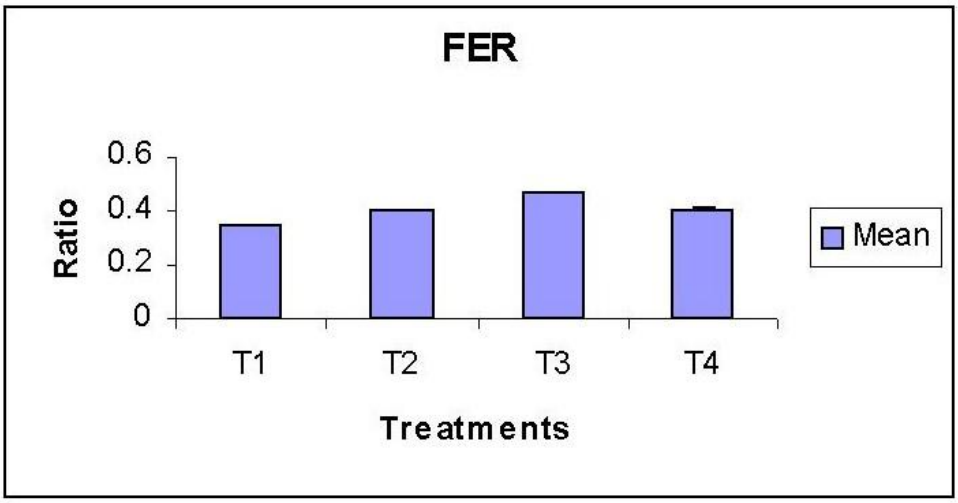


Table no.16: Protein efficiency ratio of different experimental groups

Treatments	Replications*			Mean ± SE
	A	B	C	
T_1	0.052	0.050	0.058	$.0534^{a}$±0.002
T_2	0.070	0.069	0.078	$.0723^{b}$±0.003
T_3	0.095	0.102	0.109	$.1030^{c}$±0.00351
T_4	0.074	0.079	0.081	$.0785^{b}$±. 00160

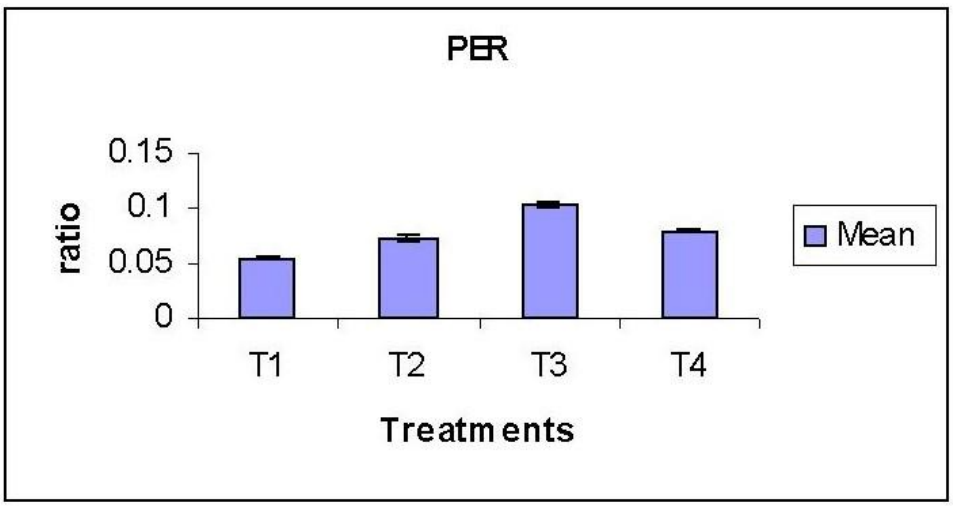
Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.004	3	.001	62.596	.000
Within Groups	.000	8	.000		
Total	.004	11			

Figure no.5: Protein efficiency ratio in different experimental tanks



4.4 Survival (%)

Data pertaining to the survival percentage of the experimental group is depicted in table no. 17 and graphically represented in figure no.6. Significant difference ($P<0.05$) was found among the treatment groups. The lowest Survival percentage (56.6±1.67) was found in T_1 treatment group and the T_4 and T_2 treatment groups and the highest (75 ±2.89) in T_3 treatment group.

Gatesoupe (1990) was able to improve the survival of larval turbot (*Scopthalmus maximus*) by daily addition of lactic acid bacteria.

Garcia de la Banda *et. al*. 1992 also found probiotic bacteria are effective in enhancing the survival after addition of lactic acid bacteria (*streptococcus lactis* and *Lactobacillus bulgaricus*) to B*rachionus* and *Artemia* used in turbot larva feeding. Gatesoupe (1991) also found higher survival than control in turbot larvae fed by Bacillus spore fed rotifers. Gatesoupe (1997) studied sidophore production and the probiotic effect of *Vibrio* type E on turbot larvae. The main effect of rotifer enrichment with this strain was to improve the survival.

Austin *et. al.* (1995) also reported reduced mortalities when challenged with *A. salmonicida* and to lesser extent *V. anguillarum* and *V. ordallii*.

Maeda and Naogami (1989) reported survival rate of the crustacean larvae in the experiments was much higher than those without the addition of beneficial bacterial strains.

Table no.17 Survival in different experimental tanks

Treatments	Replications*			Mean ± SE
	A	B	C	
T_1	55	60	55	56.67^{a}±1.67
T_2	60	65	60	61.67^{a}±1.67
T_3	75	70	80	75.00^{b}±2.89
T_4	55	60	55	56.67^{a}±1.67

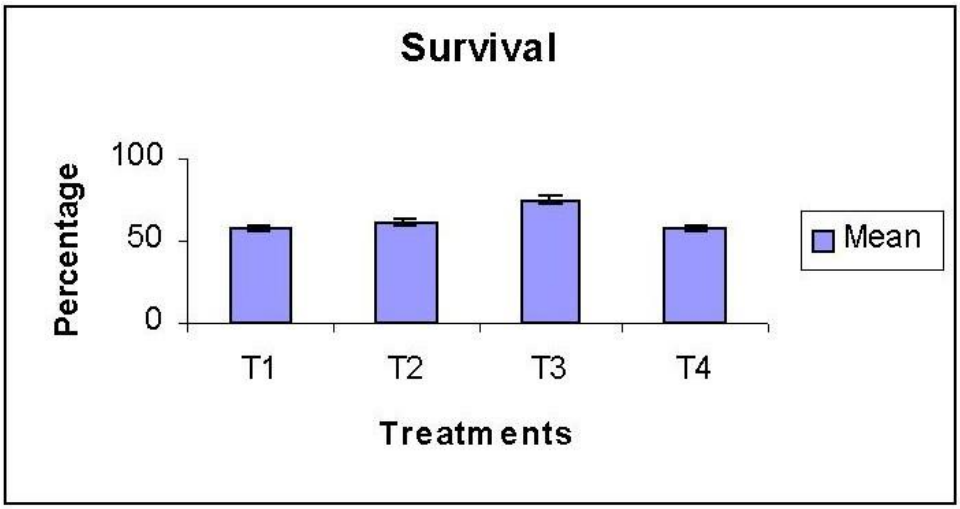
Figures having the same super scripts do not vary significantly (P>0.05)

*Indicates mean values per experimental unit

ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	675.000	3	225.000	18.000	.001
Within Groups	100.000	8	12.500		
Total	775.000	11			

Figure no.6: Survival in different experimental tanks



Queiroz and Boyd (1998) confirmed that a commercial innoculum of Bacillus sp. increased survival and production of channel catfish.

Moriarty (1998) noted an increase of prawn survival in ponds where some strains of Bacillus sp. were introduced.

Gildberg *et. al*. (1995) recorded highest mortality in fishes given the diet containing lactic acid bacteria.

Venkat *et. al*. (2004) also found higher survival and found no adverse effect of probiotic on survival, but in the present case highest survival was found in T_3 group.

All above findings confirm that probiotics increase survival and other growth parameters. So from the above findings it may be concluded that the probiotic Hydroyeast improves the survival of *M. rosenbergii* post larvae in the present studies.

4.5 Fatty acid Profile

Table no.18: Fatty acid composition of the tissues of the *M. rosenbergii* fed with the different experimental diets

Fatty Acids	Treatments			
	T_1	T_2	T_3	T_4
Myristic acid, C14:0	5.5715	12.56	12.55	12.54
Palmitic acid, C16:0	29.0409	16.26	16.25	16.28
Palmitoleic acid, C16:1,n-9	7.6744	17.20	17.20	17.19
Stearic acid, C18:0	9.7715	19.81	19.80	19.83
Oleic acid, C18:1,n-9	21.6727	20.60	20.58	20.63
Linoleic acid, C18:2,n-6	5.1984	21.90	21.90	21.91
Linolenic acid,C18:3,n-3	1.4245	26.58	26.57	26.57
Arachidonic acid,C20:4,n-6	5.3659	28.08	28.07	28.07
Eicasopentaenoic acid, C20:5,n-3	0.2367	30.24	30.25	30.20
Docosahexaenoic, C22:6,n-6	1.8416	31.68	31.68	31.66

Myristic acid (C14:0) content of T_1, T_2, T_3, T_4 group were 5.5 %, 1.66%, 2.22%, and 1.62 % respectively. Palmitic acid (C16:0) content in T_1 T_2, T_3, T_4 group were 29.04%, 20.87%, 21.68%, and 19.53 % respectively.

Palmitoleic acid (C16: 1n-9) content in T_1 group was 7.67%, T_2 group was 2.79%, T_3 group was 3.06%, and T_4 group was 2.99 %.

Stearic acid (C18:0) content in T_1 group was 9.77%, T_2 group was 7.55%, T_3 group was 7.57%, T_4 group was 7.47 %.

Linoleic acid (C18: 2n-6) content in T_1 group was 5.19%, T_2 group was 15.30%, T_3 group was 19.04%, T_4 group was 17.07 %.

Linolenic acid (C18:3n-3) content in T_1 group was 1.42%,T_2 group was 2.70%, T_3 group was 3.03%, T_4 group was 4.01 %.

EPA (C20: 5n-3) content in T_1 group was 0.23%,T_2 group was 0.34%, T_3 group was 0.58%, T_4 group was 0.76 %.

DHA (C22: 6n-6) content in T_1 group was 1.84%, T_2 group was 2.39%, T_3 group was 3.29%, T_4 group was 3.28 %.

n-3 PUFA , n-6 PUFA levels found higher in T_3 group as compare to that of T_1, T_2, T_3 treatment groups.

Fatty acid profiles from tissues of prawns exhibiting superior weight gains may indicate when dietary requirements are met (D'Abramo and Sheen, 1993).

The dietary level of fatty acids (FA) has for long deserved special attention by researchers (D'Abramo, 1997). Certain FA is considered to be

essential for crustaceans and require an external source (Sorgeloos and Leger, 1992;Merican and Shim, 1996; Glencross and Smith, 1999). The 18C polyunsaturated fatty acids (EFA), since crustaceans posses a limited ability to synthesize de novo the polyunsaturated Linoleic (18:2n-6,LOA) and linolenic (18:3n-3,LNA) acids (Kanazawa, Teshima, Ono and Chlayondeja 1979 b; Kanazawa, Teshima and Tokiwa 1979 c).

Additionally, crustaceans also have a need for dietary highly unsaturated fatty acids (HUFA) because of their inefficient or inability to convert these 18C PUFA to 20C and 22 C HUFA, such as arachidonic (20:4n-6, ARA), eicasopentaenoic (20:5n-3,EPA) and docosahexaenoic (22:6n-3,DHA) acids, of their respective families (Kanazawa, Teshima and Ono 1979 a; Kayama, Hirata, Kanazawa, Tokiwa and Saito, 1980).

Crustaceans require dietary lipids as a source of essential fatty acids (EFA) and other lipid classes like phospholipids, sterols and carotenoids. The qualitative requirements of penaeids for EFA are well documented (Merican and Shim, 1996).HUFA such as eicosapentaenoic (20:5 n-3,EPA) and docosahexaenoic (22:6n-3,DHA) acids are important nutrients and are considered as EFA because of the limited ability of crustaceans to elongate and desaturate shorter -chain polyunsaturated fatty acids (PUFA) to HUFA. In fact, crustaceans have a limited ability to bio-synthesize de novo the n-3 and n-6 families of fatty acids (Kanazawa *et. al.* 1979). However, many studies have demonstrated the greater value of HUFA compared to PUFA for shrimps (Merican and Shim, 1996; Kanazawa *et. al.*,1979; Xu *et. al.*, 1993).In studies with many kinds

of shrimp, it has been shown that 18:3n-3 has greater EFA value than that of 18:2n-6. With *Penaeus japonicus* longer chain n-3 HUFA, such as EPA and DHA had greater EFA value than 18:2n-6 or 18:3n-3(Kanazawa *et. al.*, 1979; Kanazawa *et. al.*,1979).

4.5.1 Correlation between fatty acid composition and growth

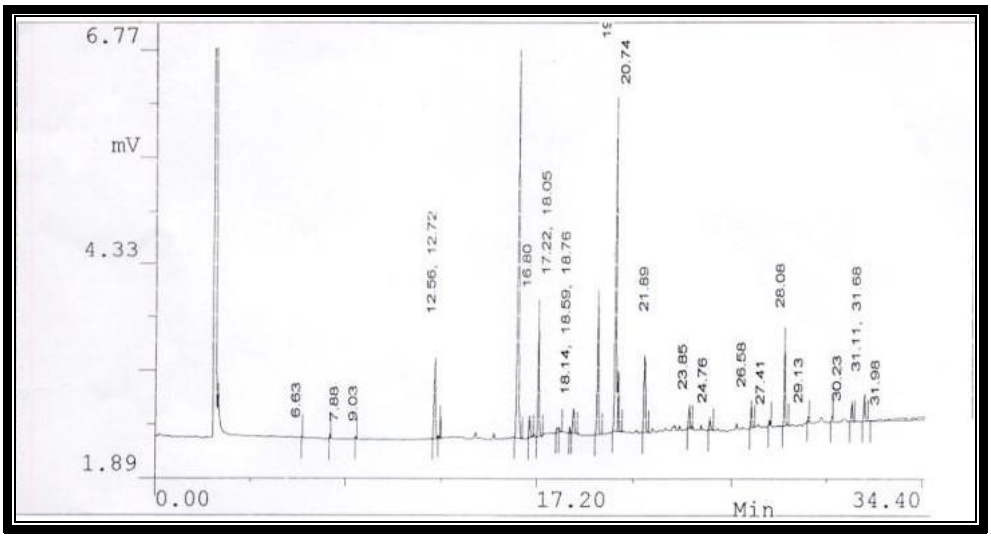
Growth performance reflects the enhancement of unsaturated fatty acid content of the post larvae of prawn. which was boosted by the feeding of probiotic coated feed. Highest growth was found in the groups with higher DHA and arachidonic acid composition indicates that the above fatty acids have direct correlation with the growth enhancement as suggested by Rhomdhane *et. al*. (1995). In the same type of study proposed higher growth and moulting rate in relation to the feeding of the HUFA to *M. rosenbergii* larvae. The importance of arachidonic acid for larval welfare was demonstrated lately and this n-6HUFA was shown to have associated with fish larval growth (Bessonart *et. al.*, 1999).

4.5.2 Correlation between the fatty acid composition and survival of the post larvae

Survival was highest in those groups, which had higher fatty acid content in terms of DHA and arachidonic acid. It indicates that the fatty acid content in the tissue have direct correlation with the increase in survival rate as proposed by Koven *et. al.* (2001), who reported that arachidonic acid had the role in improvement of stress in fish as well as the osmoregulatory capacity.

Plate no.2: Fatty acid profile of the tissue *M*.rosenbergii fed with T_1 diet.

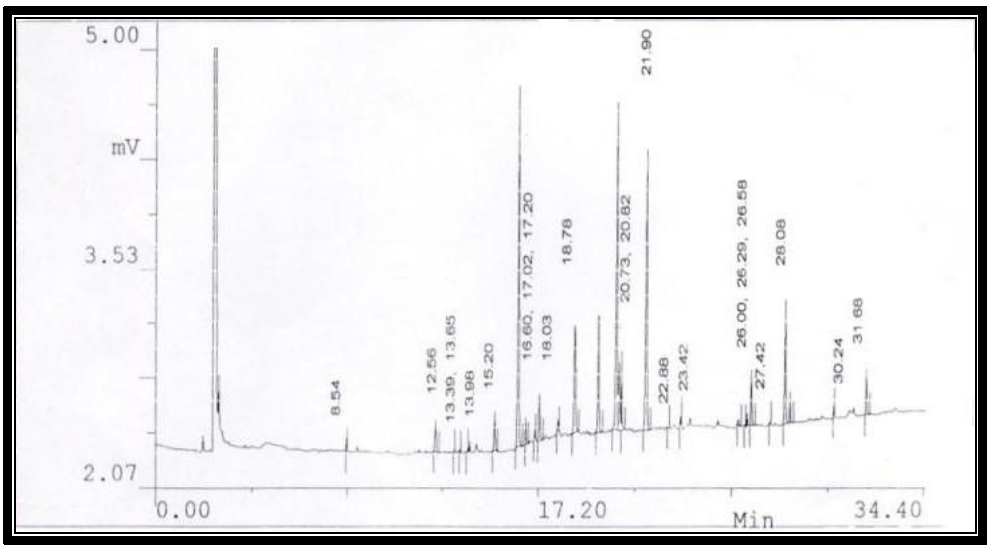
Chromatogram



Retention time	Area	Area %	Pk Ty	Fatty acid name
12.56	61345	5.5715	BB	Mystiric acid,C14:0
16.31	319756	29.0409	BB	Palmitic acid,C16:0
17.22	84499	7.6744	BB	Palmitoleic acid,C16:1,n-9
19.83	107589	9.7715	BB	Stearic acid,C18:0
20.63	238628	21.6727	BV	Oleic acid,C18:1,n-9
21.89	57237	5.1984	BB	Linoleic acid,C18:2,n-6
26.58	15685	1.4245	BB	Linolenic acid,C18:3,n-3
28.08	59082	5.3659	BB	Arachidonic acid,C20:4,n-6
30.23	2606	0.2367	BB	EPA,C20:5,n-3
31.68	20277	1.8416	BB	DHA,C22:6,n-6
Saturates[1]	44.38	[1] sum of C14:0, C16:0, C18:0 [2] sum of C18:3, n-3, C20:5,n-3, C22:6,n-6 [3] sum of C18:2, n-6, C20:4,n-6 [4] ratio between Σn-3PUFA and Σn-6PUFA		
n-3 PUFA[2]	3.49			
n-6 PUFA[3]	10.55			
n-3/n-6[4]	0.3308			
EPA+DHA	2.07			

Plate no.3: Fatty acid profile of the tissue *M. rosenbergii* fed with T_2 diet.

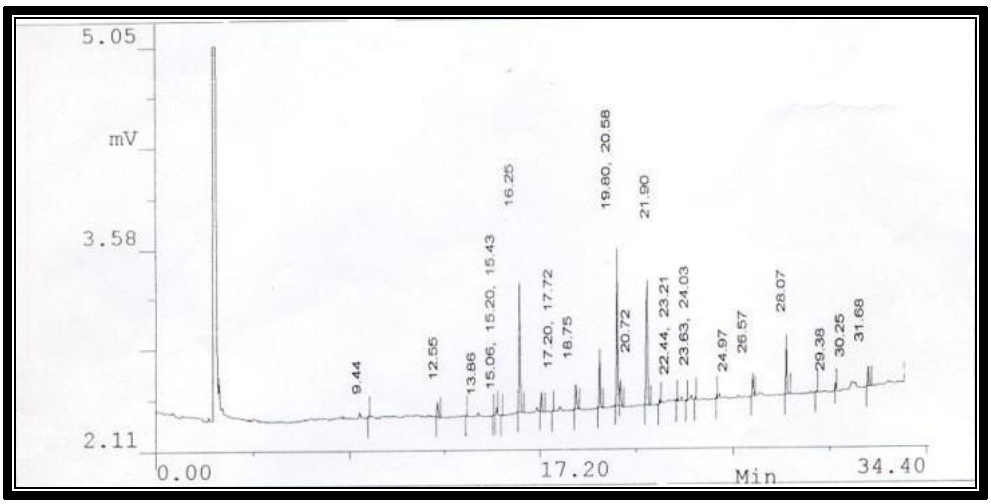
Chromatogram



Retention time	Area	Area %	Pk Ty	Fatty acid name
12.56	11808	1.6639	BB	Mystiric acid,
16.26	148127	20.8733	BB	Palmitic acid
17.20	19837	2.7953	BB	Palmitoleic acid
19.81	53639	7.5585	BB	Stearic acid C18:0
20.60	133944	18.8748	BV	Oleic acid,C18:1,n-9
21.90	108603	15.3038	BB	Linoleic acid,C18:2,n-6
26.58	19214	2.7075	BB	Linolenic acid,C18:3,n-3
28.08	54716	7.7103	BB	Arachidonic acid,C20:4,n-6
30.24	2424	0.3416	BB	EPA
31.68	16980	2.3927	BB	DHA
Saturates[1]	30.08	[1] sum of C14:0, C16:0, C18:0 [2] sum of C18:3, n-3, C20:5,n-3, C22:6,n-6 [3] sum of C18:2, n-6, C20:4,n-6 [4] ratio between Σn-3PUFA and Σn-6PUFA		
n-3 PUFA[2]	5.43			
n-6 PUFA[3]	23			
n-3/n-6[4]	0.236087			
EPA+DHA	2.73			

Plate no.4: Fatty acid profile of the tissue *M.rosenbergii* fed with T_3 diet.

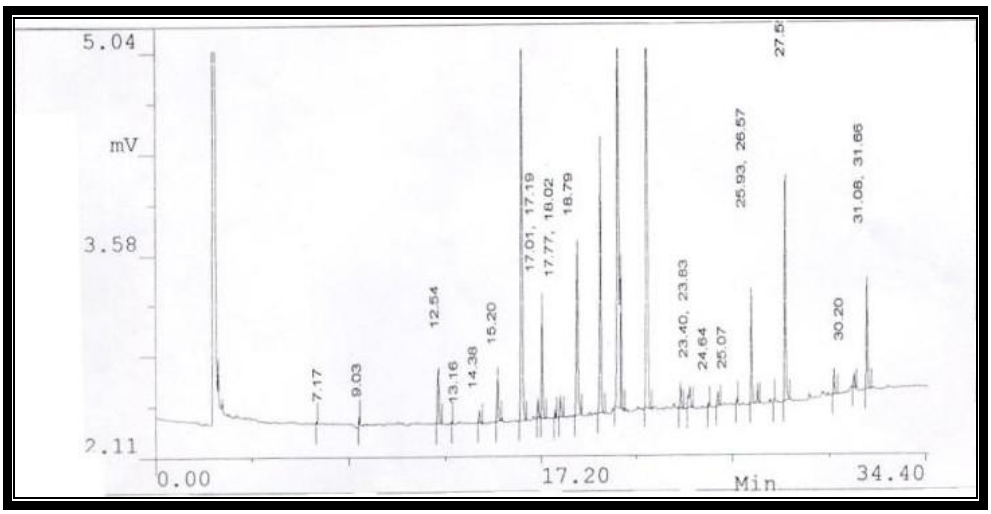
Chromatogram



Retention time	Area	Area %	Pk Ty	Fatty acid name
12.55	6578	2.2296	BB	Mystiric acid,
16.25	63968	21.6813	BB	Palmitic acid
17.20	9032	3.0613	BB	Palmitoleic acid
19.80	22343	7.5729	BB	Stearic acid C18:0
20.58	62761	21.2722	BV	Oleic acid,C18:1,n-9
21.90	56179	19.0413	BB	Linoleic acid,C18:2,n-6
26.57	8953	3.0345	BB	Linolenic acid,C18:3,n-3
28.07	23092	7.8268	BB	Arachidonic acid,C20:4,n-6
30.25	1703	0.5772	BB	EPA
31.68	9720	3.2945	BB	DHA
Saturates[1]	31.47	[1] sum of C14:0, C16:0, C18:0 [2] sum of C18: 3, n-3, C20:5, n-3, C22:6,n-6 [3] sum of C18:2, n-6, C20:4,n-6 [4] ratio between Σn-3PUFA and Σn-6PUFA		
n-3 PUFA[2]	8.15			
n-6 PUFA[3]	26.86			
n-3/n-6[4]	0.303425			
EPA+DHA	4.08			

Plate no.5: Fatty acid profile of the tissues of *M. rosenbergii* fed with T_4 diet

Chromatogram



Retn. Time	Area	Area %	Pk TY	Fatty acid name
12.54	25934	1.6219	BB	Mystiric acid, C14:0
16.28	312357	19.5350	BB	Palmictic acid,C 16:0
17.19	47896	2.9954	VB	Palmitolic acid,C16:1,n-9
19.83	119535	7.4758	BB	Stearic acid,C18:0
20.63	344509	21.5458	BV	Oleic acid,C18:1(n-9)
21.91	273089	17.0791	BB	Linoleic acid,C18:2(n-6)
26.57	64231	4.0170	BB	Linolenic acid,C18:3(n-3)
28.07	114369	7.1527	BB	Arachidonicacid,C20:4(n-6)
30.20	12245	0.7658	BB	EPA, C20: 5(n-3)
31.66	52560	3.2871	BB	DHA, C22: 6(n-3)

Saturates[1]	28.61	[1] sum of C14:0, C16:0, C18:0 [2] sum of C18: 3, n-3, C20:5, n-3, C22:6,n-6 [3] sum of C18:2, n-6, C20:4,n-6 [4] ratio between Σn-3PUFA and Σn-6PUFA
n-3 PUFA[2]	8.05	
n-6 PUFA[3]	24.22	
n-3/n-6[4]	0.33237	
EPA+DHA	4.04	

Chapter- V
SUMMARY CONCLUSIONS AND SUGGESTIONS

FOR FUTURE RESEARCH WORK

India is a rich country blessed with enormous wealth of natural resources but both terrestrial and aquatic resources are declining due to various anthropogenic stresses. Alternatively we should go in for culture production. As culture production has many impediments specially disease and water quality we might use probiotics along with effective management practices are crucial. In aquaculture one measure that might be of assistance is the use of “probiotics”. The research of probiotics for aquatic animals is increasing with the demand for environment-friendly aquaculture.

Therefore, feeding trials were conducted for a period of 90 days on *Macriobrachium rosenbergii* postlarvae. The feeding rate varied from 8-10%, which was adjusted, based on the daily observation. Weight of prawn in each experimental unit was checked every 20 days to adjust the feeding rate. Prawns were fed twice a day. Water quality was regularly monitored every 15 days to ensure good environment for prawn rearing.

Water temperature, dissolved oxygen, total alkalinity, nitrite nitrogen did not vary significantly ($P>0.05$) by the supplementation of enzymatic and bacterial probiotics (Hydroyeast) through feed at the rate of 150 mg/kg of feed. CO_2 was absent. pH, ammonia nitrogen, nitrate nitrogen, phosphate phosphorus final body weight gain, specific growth rate, FCR, PER, FER and survival rate vary significantly ($P<0.05$) varied with the

supplementation of enzymatic and bacterial probiotics(Hydroyeast) at the rate of 150 mg/kg of feed .

n-6 PUFA,EPA and DHA content were found higher with the supplementation of enzymatic and bacterial probiotics (Hydroyeast) at the rate of 150 mg/kg of feed .

Based on the results of the present study, the following conclusion can be made:

- The administration of Hydroyeast through feed at 150 mg/kg of feed was found to be effective and safe.
- Hydroyeast @ 150 mg/kg of feed reduce ammoniacal nitrogen, and provide nitrate nitrogen, Phosphate may be due to its capacity to decompose the excreta of prawns, remaining food materials and other organic materials to CO_2 , nitrates, phosphates.
- Hydroyeast @ 150 mg/kg of feed can be recommended in prawn farming to improve production since it enhances the growth rate of freshwater prawn *M. rosenbergii.*
- Hydroyeast @ 150 mg/kg of feed appears to increase the survival of freshwater prawn *M. rosenbergii.*
- Hydroyeast at 150 mg/kg of induces more n-3 PUFA to be deposited in the tissues of prawn, which might have enhances the growth and also enhances the survival of postlarvae.
- From above findings it can be concluded that Hydroyeast probiotic is better than other probiotics supplementations at 150mg/kg of feed dose.

Suggestions for future work:

There are a series of works, which need to be done in future. They are as follows:

- Individual bacterial effect should have to be detected on growth, water quality and survival.
- Individual digestive enzymes should be analyzed quantitively and qualitatively and effect of particular enzyme should be studied.
- Microbiological work on Hydroyeast should have to be studied.
- Use of Hydroyeast in disease resistance has to be studied.

“Efficacy of enzymatic and bacterial probiotic on growth and survival of giant freshwater prawn *Macrobrachium rosenbergii* (De Man)”

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ABSTRACT

A feeding trial was conducted for 90 days to study the efficacy of enzymatic and bacterial probiotic on the growth and survival of giant freshwater prawn *Macrobrachium rosenbergii* (De Man) postlarvae (PLs). During the trials physico-chemical parameters were analysed for the culture water and at the end of the experiment, estimated the proximate composition of PLs. Four iso-nitrogenous (33%) and iso-caloric diets were prepared and coated with various concentrations of enzymatic and probiotic supplementation (Hydroyeast), Control feed i.e. feed without probiotics (T_1), and test diets with bacterial and enzymatic probiotic supplementation @ 100mg/kg (T_2), @ 150mg/kg (T_3) and @ 200mg/kg of feed (T_4) and fed to the PLs. Two hundred and forty *Macrobrachium rosenbergii* postlarvae were distributed randomly into 12 experimental units (20 PLs in each unit) that were divided into four distinct treatments with three replicates each. The nitrogen and phosphate content of the cultured water decreased with bacterial and enzymatic probiotic supplementation @ 150mg/kg of feed (T_3) in the diet. Temperatures, dissolved oxygen and free CO_2 were not influenced by the probiotic supplementation. Survival rate of PLs was higher with T_3 diet. T_3 diet influenced specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency ratio (FER), protein efficiency ratio (PER). Final body weight gain was also found higher with T_3 diet. Linoleneic acid and percentage of PUFAs increased with the T_3 diet. n-3 PUFA, n-6 PUFA, EPA and DHA found higher in the prawn tissues fed with bacterial and enzymatic probiotic supplementation @ 150mg/kg in diet (T_3 diet).

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