

## Effect of Probiotics on the Growth Performance and Survival of Fresh Water Prawn (*Macrobrachium rosenbergii*) Fed on Formulated Diets Supplemented with Probiotics

<sup>1</sup>G. Hajarrooba, <sup>2</sup>N.Y. Hirimuthugoda and <sup>3</sup>K. Radampola

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, University of Jaffna, Ariviyal Nagar, Kilinochchi (44000), Sri Lanka

<sup>2</sup>Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka

<sup>3</sup>Department of Fisheries and Aquaculture, Faculty of Fisheries and Marine Sciences and Technology, University of Ruhuna, Wellamadama, Matara (81000), Sri Lanka

**Abstract:** Two experiments were conducted to study the effects of probiotic Biosyn-AQ (comprising *Lactobacillus sp*, *Bifidobacterium* and *Streptococcus*) on growth and survival of giant freshwater prawn, *Macrobrachium rosenbergii* fed on formulated diets containing probiotics. In experiment-1 (6 weeks), three different dietary treatments; Control diet without probiotics (Commercial feed with 42% Protein [P]) and two formulated diets with probiotics Biosyn-AQ and varying levels of protein; Diet-1 (probiotics 4 g/Kg of feed and 35% P) and Diet 2 (probiotics 4 g/Kg of feed and 33% P) were used in triplicate groups. Post larvae (PL) were stocked at a rate of 40PL/tank (60 cm × 30 cm × 30cm). Feeding was done at 10% of body weight per day and prawns were weighed every 2 weeks. At the end of the 1<sup>st</sup> experiment, Growth parameters; final lengths and weights, length gain (1.83-1.99 cm), weight gain (0.62-0.70 g) and specific growth rate (2.21-2.30) of prawns showed no significant difference between treatments. Significantly lower feed conversion ratios (0.96±0.16, 1.18±0.18) and higher survival (80.00±10.89 %, 62.50±12.5 %) were recorded for control diet and diet-1 respectively. Feed costs for diet 1 and 2 were lower compared to the price of control diet. However, PL fed on diet-1 performed on par with PL fed on protein rich control diet in terms of better growth performance and selected as a basal diet for 2<sup>nd</sup> experiment. In experiment-2 (4 weeks), Basal diet (35% crude protein) was prepared by the supplementation of probiotics at 3 different concentrations ie; 1%, 2% and 3% of the diet. Prawns were stocked at a rate of 20 juveniles / tank. Significantly higher length gain, weight gain, specific growth rate and survival rate were recorded for prawns fed on diets supplemented with 2% and 3% probiotics than those fed on diet supplemented with 1% probiotics fed group. On the other hand, diet with 2% probiotics performed statistically in par with diet with 3% probiotics. Therefore, 2% of probiotics was selected as an ideal concentration in terms of low cost and better growth performance. From both experiments, it can be concluded that diet-1 formulation supplemented with 2% probiotics Biosyn-AQ leads to better growth performance of prawns and can be suggested for promoting sustainable culture of *M. rosenbergii* under Sri Lankan conditions.

**Key words:** Post Larvae · Juveniles · Probiotics Biosyn-AQ

### INTRODUCTION

The giant freshwater prawn (*Macrobrachium rosenbergii*) is an economically important aquatic crustacean species, abundant in the south-southeast Asia and Asia pacific region [1]. Its superior taste, nutritional

value and omnivorous habit have made the species as an excellent candidate for aquaculture [2]. In Sri Lanka, the production of *M. rosenbergii* is based on capture, mainly from reservoirs and flood plains, such as the *villus* of Mahaveli River [3] and freshwater prawn culture gains momentum in the inland fisheries sector today.

**Corresponding Author:** N.Y. Hirimuthugoda, Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka.  
Tel: +940776257331, Fax: +940212060175.

An appropriate feeding is a major factor behind the success in aquaculture. Feed costs constitute 40-60% of operational costs in production of the freshwater prawn [4]. The types of feed used in freshwater prawn farming mainly include live feeds and artificial feeds. At higher stocking densities prawn individuals may be more dependent on artificially prepared diets [5]. The use of probiotics in the culture of aquatic organisms is increasing in demand for more environment friendly aquaculture practices [6]. Use of different commercial probiotics by administering them through feed of *M. rosenbergii* was reported [7]. Growth and survival rates of *M. rosenbergii* fed on the probiotic supplements were significantly greater than those of the controls [8-11].

Development of semi-intensive aquaculture of *M. rosenbergii* as a commercial industry in Sri Lanka is limited mainly due to unavailability of suitable commercial feeds. At present, the widely used commercial feed in prawn farming is poultry feed starter-I, which is of low nutritive value. However some farmers use the commercial feed of *Peneaus monodon* to feed the *M. rosenbergii* post larvae [12]. Developing an artificial feeding regime with probiotics is highly desirable in order to sustain the growth of fresh water prawn farming and exports of Sri Lanka. However such studies are scarce in Sri Lanka.

Therefore, the present study was conducted to evaluate the growth parameters of fresh water prawn fed on formulated diets supplemented with probiotic-Biosyn AQ in aquarium conditions to select an appropriate, cost effective artificial diet formulation combined with best probiotic inclusion level.

## MATERIALS AND METHODS

Post Larvae (PL<sub>20</sub>) of Fresh water prawn were stocked in glass tanks (60 cm × 30 cm × 30 cm) filled with fresh water and were acclimatized to laboratory conditions for 2 weeks (up to PL<sub>35</sub>) before the commencement of experiments. The tanks were cleaned on daily basis by siphoning the tank bottom. Aerators were connected to tanks to provide continuous mild aeration. Small pieces of PVC pipes were put into tanks as a shelter for prawn to minimize the cannibalism among prawns.

**Diet Preparation:** The experimental diets (Diet-1 and Diet-2) were formulated by considering the standard dietary protein requirements of prawns (Table 1).

Probiotics used is Bio-syn AQ comprising *Lactobacillus sp.*, *Bifidobacterium* and *Streptococcus*.

In experiment-1, the ingredients of Diet-1 and 2 were weighted as per formulae, mixed properly; steam cooked for 15 minutes and cooled down to room temperature. Then the feed mixture was mixed with probiotics at the rate of 4g freeze dried powder per kg of feed mixture, made into dough and finally made into pellets by using locally made hand pellet machine. The pellets were dried in a thermostatic oven at 40°C until it reached constant weight and stored in polythene bags at room temperature. Commercial feed for *P. monodon* which is currently used to feed *M. rosenbergii* in grow-out ponds in Sri Lanka was used as the control diet without probiotics. Protein contents of the control and experimental diets were determined by Kjeldhal method. In experiment-2, selected basal diet from experiment-1 was incorporated with 3 different concentrations (1%, 2% and 3%) of probiotic Bio-syn AQ.

## Feeding Experiments

**Experiment-1:** *M. rosenbergii* Post Larvae (PL-35) with the average length and weight of 1.64±0.25 cm and 0.08 g respectively were stocked at a stocking density of 40 PL/tank in glass aquaria (60 cm × 30 cm × 30 cm). Three different dietary treatments; Control diet without probiotics (Commercial feed with 42% Protein [P]) and two formulated diets with probiotics Bio-syn AQ and varying levels of protein; Diet-1 (probiotics 4 g/Kg of feed and 35% P) and Diet 2 (probiotics 4 g/Kg of feed and 33% P) were used in triplicates. The experiment was carried out for 7 weeks.

**Experiment-2:** Prawn juveniles with the average length and weight of 3.75±0.26 cm and 1.02±0.29 g respectively were stocked in glass aquaria in the stocking density of 20 juveniles per tank. Three experimental diets with 1%, 2% and 3% inclusion levels of probiotics Bio-syn AQ were fed to juveniles for a period of 4 weeks.

In both experiments, PLs / juveniles were fed twice a day (8.00 am and 5.00 pm) at a rate of 10% of the body weight. Prawns in each replicate were counted in fortnight interval and 10 randomly selected prawns were weighed and their length measured in every 2 weeks. Uneaten feed, faeces and moult (if any) were siphoned out every day in the morning.

**Evaluation of Growth Parameters:** After the feeding trial, the growth parameters such as weight gain (WG) and length gain (LG), survival rate, specific growth rate (S.G.R) and feed conversion ratio (F.C.R) were individually determined by the following equations [13].

- Weight gain (g) = Mean final prawn weight - Mean initial prawn weight
- Length gain (cm) = Mean final prawn length – Mean initial prawn length

$$\text{Survival rate (\%)} = \frac{\text{No. of total live prawn}}{\text{Total no. of prawn stocked}} \times 100$$

$$\text{S.G.R (\% per day)} = \frac{\log W_2 - \log W_1}{t} \times 100$$

$W_1$  and  $W_2$  = Initial and Final weight (g) respectively  
 $t$  = Total number of experimental days

$$\text{F.C.R} = \frac{\text{Total weight of feed consumed (dry)}}{\text{Biomass gain (wet)}}$$

**Water Quality Analysis:** Water temperature and pH were measured once a day by using thermo meter and pH meter (Model: AD131, U.K) respectively. Dissolved oxygen was measured once a week by using the Field DO meter (Model: YSI Pro-20, USA) whereas turbidity was measured weekly using portable turbidity meter (HACH 2100P, USA).

**Statistical Analysis:** A one-way analysis of variance (ANOVA; SPSS version: 16.0) was used to determine whether significant variation between the treatments existed. Differences between means were determined and compared by the post hoc multiple comparison test (DMRT). All tests used a significance level of  $P < 0.05$ . Data are reported as mean  $\pm$  standard deviation.

## RESULTS

**Water Quality:** Mean values for water quality parameters during experiment-1 and 2 are tabulated in Table 2 and Table 3 respectively. During the both experimental periods, water temperature in glass aquaria ranged from 28-30°C, pH ranged from 6.86 to 7.33, turbidity varied from 1.23 to 2.40 NTU and dissolved oxygen varied from 7.58 to 7.69 mg/l.

**Growth Performance of Fresh Water Prawns:**  
**Experiment 1:** Growth parameters and survival of fresh water prawn post larvae (PL) under different treatments of 1<sup>st</sup> experiment are presented in Table 4.

After 7 weeks, there were no significant differences ( $p > 0.05$ ) in final lengths and weights of PLs fed on control diet, diet-1 and diet-2. It showed that the experimental

Table 1: Proximate composition and cost of formulated diets

Ingredients	Composition	
	Diet 1	Diet 2
Fish meal (g/100g)	25.0	20.0
Soy bean meal (g/100g)	19.5	24.5
Coconut meal (g/100g)	25.0	25.0
Rice bran (g/100g)	10.0	10.0
Wheat flour (g/100g)	14.5	14.5
Vegetable oil (g/100g)	3.0	3.0
Vitamin and mineral premix (g/100g)	3.0	3.0
Total	100	100
Cost/Kg (LKR)	137.00	129.00
Crude protein (%)	35	33
Crude fat (%)	8	8

Table 2: Mean  $\pm$  SD for the water quality parameters in the glass aquaria of *M. rosenbergii* post larvae fed with two experimental diets with probiotics and a control diet during experiment-1

Parameters	Treatments		
	Control diet	Diet-1	Diet-2
Water temperature (°C)	28.42 $\pm$ 0.08 <sup>a</sup>	28.35 $\pm$ 0.07 <sup>a</sup>	28.35 $\pm$ 0.04 <sup>a</sup>
Turbidity (NTU)	1.40 $\pm$ 0.41 <sup>a</sup>	1.23 $\pm$ 0.22 <sup>a</sup>	1.41 $\pm$ 0.27 <sup>a</sup>
pH	6.88 $\pm$ 0.02 <sup>a</sup>	6.87 $\pm$ 0.03 <sup>a</sup>	6.86 $\pm$ 0.02 <sup>a</sup>
Dissolved oxygen (mg/l)	7.58 $\pm$ 0.03 <sup>a</sup>	7.60 $\pm$ 0.01 <sup>a</sup>	7.62 $\pm$ 0.01 <sup>a</sup>

Each value is a mean  $\pm$  SD of three replicate, <sup>a, b, c</sup> within a row, means without a common superscript differ ( $P < 0.05$ )

Table 3: Mean  $\pm$  SD for the water quality parameters in the glass aquaria of post-larvae of *M. rosenbergii* fed with three experimental diets incorporated with different probiotic concentrations (1%, 2% and 3%) during experiment-2

Parameters	Treatments: Level of probiotics		
	1%	2%	3%
Water temperature (°C)	30.05 $\pm$ 0.07 <sup>a</sup>	29.95 $\pm$ 0.07 <sup>a</sup>	30.05 $\pm$ 0.07 <sup>a</sup>
Turbidity (NTU)	1.72 $\pm$ 0.21 <sup>a</sup>	2.07 $\pm$ 0.59 <sup>a</sup>	2.40 $\pm$ 0.18 <sup>a</sup>
pH	7.33 $\pm$ 0.14 <sup>a</sup>	6.91 $\pm$ 0.14 <sup>b</sup>	6.91 $\pm$ 0.03 <sup>b</sup>
Dissolved oxygen (mg/l)	7.63 $\pm$ 0.05 <sup>a</sup>	7.66 $\pm$ 0.04 <sup>a</sup>	7.69 $\pm$ 0.02 <sup>a</sup>

Each value is a mean  $\pm$  SD of three replicate, <sup>a, b, c</sup> within a row, means without a common superscript differ ( $P < 0.05$ )

diets 1 (35% protein) and 2 (33% protein) with probiotics were performed in par with control diet (42% protein) without probiotics. Significantly higher ( $p < 0.05$ ) survival rates were recorded under control diet and diet-1 where as diet-2 showed the lowest survival rate.

Effect of control and experimental diets were not significant on growth parameters such as Percent length and weight gain and SGR of prawn PLs. Length and weight gains varied from 3.47-3.63 cm and 0.62-0.70 g respectively. SGR values found to be 2.21-2.30% (Table 4). In case of FCR, significantly lowest values were observed for control diet and diet-1 compared to the diet-2 fed group of PLs.

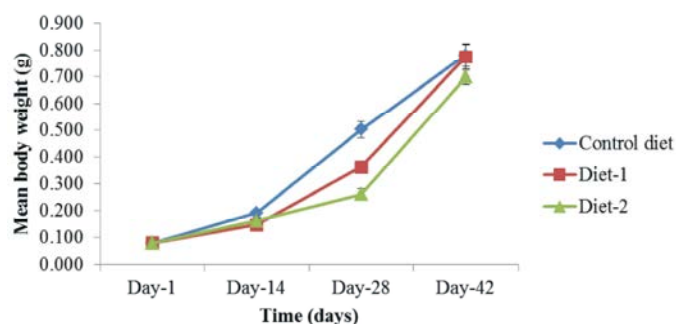


Fig. 1: Mean body weight variations of *M. rosenbergii* fed with a control diet and two experimental diets at different sampling weeks during experiment-1

Table 4: Mean ± SD of growth parameters and survival of post-larvae of *M. rosenbergii* fed on control diet without probiotics and two experimental diets with probiotics during 1<sup>st</sup> experiment

Parameters	Treatments		
	Control	Diet-1	Diet-2
Initial length (cm)	1.64±0.25	1.64±0.25	1.64±0.25
Final length (cm)	3.63±0.46 <sup>a</sup>	3.58±0.37 <sup>a</sup>	3.47±0.038 <sup>a</sup>
Initial weight (g)	0.08±0.00	0.08±0.00	0.08±0.00
Final weight (g)	0.78±0.24 <sup>a</sup>	0.77±0.25 <sup>a</sup>	0.70±0.18 <sup>a</sup>
Survival rate (%)	80.00±10.89 <sup>a</sup>	62.50±12.5 <sup>b</sup>	45.00±6.61 <sup>c</sup>
Length gain (cm)	1.99±0.57 <sup>a</sup>	1.94±0.39 <sup>a</sup>	1.83±0.50 <sup>a</sup>
Weight gain (g)	0.70±0.24 <sup>a</sup>	0.69±0.25 <sup>a</sup>	0.62±0.18 <sup>a</sup>
SGR (% per day)	2.29±0.40 <sup>a</sup>	2.30±0.31 <sup>a</sup>	2.21±0.24 <sup>a</sup>
FCR (g)	0.96±0.16 <sup>b</sup>	1.18±0.18 <sup>b</sup>	2.07±0.66 <sup>a</sup>

Each value is a mean ± SD of three replicate, <sup>a, b, c</sup> within a row, means without a common superscript differ ( $P < 0.05$ )

Table 5: Mean ± SD of growth parameters and survival of post-larvae of *M. rosenbergii* fed on three experimental diets with 1%, 2% and 3% inclusion levels of probiotics during 2<sup>nd</sup> experiment

Parameters	Treatment: Level of probiotics		
	1%	2%	3%
Initial length (cm)	3.75±0.26	3.75±0.26	3.75±0.26
Final length (cm)	4.16±0.54 <sup>b</sup>	4.73±0.62 <sup>a</sup>	4.78±0.061 <sup>a</sup>
Initial weight (g)	1.02±0.29	1.02±0.30	1.02±0.31
Final weight (g)	1.18±0.10 <sup>a</sup>	1.88±0.16 <sup>b</sup>	1.96±0.22 <sup>b</sup>
Survival rate (%)	62.5±3.54 <sup>a</sup>	92.5±3.54 <sup>b</sup>	87.5±3.54 <sup>b</sup>
Length gain (cm)	0.41±0.12 <sup>a</sup>	0.98±0.14 <sup>b</sup>	1.04±0.14 <sup>b</sup>
Weight gain (g)	0.16±0.10 <sup>a</sup>	0.86±0.16 <sup>b</sup>	0.95±0.22 <sup>b</sup>
SGR (% per day)	0.11±0.13 <sup>a</sup>	0.85±0.13 <sup>b</sup>	0.87±0.66 <sup>b</sup>

Each value is a mean ± SD of three replicate, <sup>a, b, c</sup> within a row, means without a common superscript differ ( $P < 0.05$ )

Mean body weights of PLs up to first two weeks were more or less similar and then rapid increases in weights recorded for control diet than experimental diets during 3<sup>rd</sup> week. But the experimental diet fed groups were performed gradually and almost reached the weights of control diet fed groups at the end of 6<sup>th</sup> week (Figure 1).

### Growth Performance of Fresh Water Prawns:

**Experiment 2:** The growth data of prawn juveniles in experiment-2 are given in Table 5. Diet-1 was selected from the experiment-1 and used as a basal diet formulation in experiment-2 with 3 different inclusion levels of probiotics (1%, 2% and 3%).

Mean final lengths and weights of prawn juveniles were significantly higher for diet with 2% (4.73±0.18 cm; 1.88±0.16 g) and 3% probiotics (4.78±0.61 cm; 1.96±0.22 g) when compared to diet with 1% probiotics (4.16±0.54 cm; 1.18±0.10 g). Prawns fed on 2% and 3% probiotic supplemented diets showed significantly higher survival rates. Furthermore, mean values recorded for growth parameters such as length gain, weight gain and SGR were significantly higher for 2% and 3% probiotic supplemented diets than 1% probiotic supplemented diet fed group juveniles. Hence, the effects of different probiotic inclusion levels were not significant on all growth parameters and survival between 2% and 3% probiotic fed groups (Table 5).

### DISCUSSION

**Water Quality:** Growth and survival in prawn larvae are directly related to maintenance of good water quality, food quality and quantity and availability of the prawns of acquire the food [14-16]. Temperature, turbidity, pH and dissolved oxygen recorded in the present study were found to be within the acceptable limits for *M. rosenbergii* reported in various literatures [12, 17-20]. This might be attributed to the supplementation of probiotics with the experimental diets of the current study [21].

**Experimental Diets and Probiotics:** In view of the high cost of cysts and their occasional scarcity, too much dependence on *Artemia* (major live feed) is a major constraint in the expansion of *M. rosenbergii* hatcheries

[22]. Control (42% protein) and the experimental diets (Diet-1: 35% protein; Diet-2: 33% protein) used in the present study meet the protein requirement of fresh water prawns according to a finding that the *M. rosenbergii* required 30-40% protein as optimum in the diet [24]. Likewise there were several literatures [25-28] in agreement with the protein content of the diets used in this study in terms of higher growth for *M. rosenbergii* such as dietary protein levels of 40%, 30-38%, 35% and 25-35%. The protein content in the commercially available poultry feed starter-I (20-21%), which is used to feed the post-larvae of *M. rosenbergii* in semi-intensive pond culture in Sri Lanka at present is less than the minimum required level of 23% [29] whereas in the formulated feeds used in the present study, these values were higher than the required minimum.

**Growth Performance: Experiment-1:** Optimization of growth has received high attention among growers and researchers. Because the size of post-larvae at stocking play an important role in getting the maximum productivity as well as the maximum profit in the aquaculture of *M. rosenbergii* [32]. Performance of diet-1 and diet-2 fed group of PLs were statistically in par with control diet in terms of growth performance regarding all growth parameters; final lengths and weights, length and weight gains and SGR except survival and FCR. Length gains (1.83 to 1.99 cm) in the present experiment-1 were lower than that of length gains (2.65-3.65 cm) whereas the weight gains were more or less similar to the values (0.47-0.66 g) recorded by Muralishankar *et al.* [33] when *M. rosenbergii* fed with different oil supplemented feeds with 40% CP. But higher values for weight gains (0.62-0.70 g) were observed in the present study than that of values (0.21-0.64 g) recorded by Amaraeweera *et al.* [12] when prawns fed with formulated diets (33-35% CP) incorporated with unutilized protein sources such as mussel meat, trash fish and cattle intestine during 90 days.

Higher values observed for specific growth rate indicates that larger size can be attained with lesser time. SGRs observed in the present experiment are supported by SGRs recorded by Amaraeweera *et al.* [12] when prawns fed with formulated diets (33-35% CP) incorporated with unutilized protein sources and higher than those recorded in the feeding experiments with *Lactobacillus*-based probiotics [34], diets enriched with vitamins E and D, cod liver oil and astaxanthin [35], diets supplemented with probiotics Binifit™ [36]. However, the SGRs recorded in the present experiment are lower than those recorded when fed with *Artemia*, *Moina*,

*Tubifex*, clam meat and egg custard [37] might be due to the higher nutritive value, especially the higher content of crude protein of those food items (44-66%) than that of the experimental feeds used in the present study.

Survival rates of the current experiment indicates a range of (45-80%) in which control diet fed group had a significantly higher survival (80%) which may be due to its higher protein content leads to healthier and robust PLs. Survival rates recorded by Hossain *et al.* [38] reported that the survival (%) of the prawns ranged between 46.6 to 66.6 % in monoculture in earthen ponds; Kumar *et al.* [39] reported that the survival rate of *M. rosenbergii* monoculture was 62.4%, Sadek & El-Gayar [40] reported that the survival rates of 62-80% are more or less similar to that of present study. However, survival rates recorded in the experiment-1 were higher than those recorded by several literatures [41-43] which were 11.9%, 10.2% and 30%. Survival rate values attained for control diet and diet-1 were higher than those recorded when fed with *Artemia* (44%) or *Moina* (33%) alone and a diet consisting of 50:50 combinations of *Artemia* and *Moina* (56%) [44]. Diets 1 and diet-2 fed groups showed lower survival rate compared to control diet fed group might be due to slightly lower CP of those diets than control diet, incidence of cannibalism and loss of viability of probiotics. Cannibalism is inevitable in crustacean culture. Therefore, survival cannot be always considered to be directly originated from the nutritional conditions. Long larval phase (Compared to penaeid shrimps) and cannibalistic nature of larvae of freshwater prawn have resulted in poor survival in the commercial hatcheries in India [45]. Venkat *et al.* [34] pointed out that the survival performance of *M. rosenbergii* PL supplemented with *L. acidophilus* and *L. sporogenes* diets with 35% CP had 100% survival. But the results of survival of prawns fed on probiotic Biosyn-AQ supplemented diet-1 and diet-2 of the experiment- 1 has not supported the above finding might be due to loss of probiotic viability and lower adaptation of prawns to glass aquarium conditions.

FCR which is the feed amount needed to increase a single unit of the body weight. Significantly lowest values of FCR and highest values for FCE were obtained for control diet and diet-1 when compared to diet-2. FCRs recorded for control diet and diet-1 in the present study were more or less similar to the values recorded by Parakrama *et al.* [35] in their feeding experiments with vitamins enriched formulated feeds (0.87-1.88). However, present FCR values for control and diet-1 were lower than the values recorded in feeding experiments by Venkat *et al.* [34] with *Lactobacillus*-based probiotics

(2.19-2-75), Amaraeweera *et al.* [12] with unutilized protein sources (2.84-3.22) and Seenivasan *et al.* [10] with different probiotic concentrations (2.36-3.40).

Diet-1 and diet-2 were performed statistically on par with control diet in terms of growth performance regarding all growth parameters except survival and FCR even though; the control diet was highly proteinaceous. As far as the price of the all three diets were concerned, experimental diets (Diet-1: 138/= per kg and Diet-2: 129/= per kg) were cheaper than control diet (250/=). Diet 1 was performed better than diet-2 in terms of survival and FCR. In selecting best diet from the experimental diets, Diet-1 incorporated with probiotics Biosyn-AQ was highly in agreement with the performance of protein rich control diet regarding all the growth parameters and survival and it is cheaper than the control diet as well. Then basal mixture of diet-1 alone has been undergone for second experiment in order to find out the optimum probiotic concentration that leads to maximized growth performance of prawns.

**Growth Performance: Experiment-2:** Probiotics influence digestive processes by enhancing microbial enzyme activity and consequently improving the digestion, absorption and utilization of the feed [46]. According to the results from experiment-2, Diets supplemented with 2% and 3% probiotics fed juveniles showed significantly higher mean values for growth parameters and survival and therefore better growth performance than diet supplemented with 1% probiotics fed juveniles. Higher survival rates of 92.5% for diet with 2% probiotics and 87.5% for diet with 3% probiotics when compared to diet with 1% probiotics (62.5%) were observed. Similar improved survival has been reported in *M. rosenbergii* PL fed with Biogen supplemented diets [8] and diets supplemented with different probiotics [7]. Survival rates of 80-90% for diets with 45% CP supplemented with probiotic Binifitin TM [36], 80-92% for diets (40% CP) supplemented with combined probiotics [9], 80-90% for diets (40% CP) supplemented with *L.sporogenes* [10] were supported the present results. Seenivasan *et al.* [36] reported that the growth parameters (survival, weight gain, SGR, feed conversion efficiency and protein efficiency ratio) were significantly ( $P<0.05$ ) higher in 2% Binifit<sub>TM</sub> incorporated diet fed PL. Although Seenivasan *et al.* [9] were suggested 3% *L.sporogenes* + *B.subtilis* + *S.cerevisiae* incorporated diet fed post larvae (PL) to obtain significantly higher growth parameters and 4% *L. sporogenes* incorporated diet was recommended by Seenivasan *et al.* [11] in order to attained optimized

growth parameters. In the present experiment, results are in agreement with the above findings. Therefore, diet with 2% and 3% probiotics (or >1% probiotics) can be utilized in terms of optimized growth performance of *M. rosenbergii*. But diet with 2% probiotics is strongly recommended from the 2<sup>nd</sup> experiment in accordance with higher survival and economic efficiency due to cost effectiveness than 3% inclusion of probiotics Biosyn-AQ.

Hence, control diet (commercial shrimp feed) and diet-1 supplemented with probiotic Biosyn-AQ which showed improved growth performance and survival of *M. rosenbergii* in experiment-1 can be used instead of currently used poultry feed starter in semi-intensive fresh water prawn nurseries and grow-out ponds. From both experiments, It can be concluded that developing a combination of artificially formulated diet-1 with 2% inclusion level of probiotics Biosyn-AQ can be suggested for promoting sustainable culture of *M. rosenbergii* under Sri Lankan conditions in accordance with better growth parameters and low cost thus leading to higher profits.

## REFERENCES

1. Akand, A.M. and Hasan, M.R, 1992. Status of freshwater prawn (*Macrobrachium spp.*) culture in Bangladesh. In: Freshwater Prawns (ed. by E.G. Silas), pp 33-41. Kerala Agricultural University, Thrissur, India.
2. Chen, S.M. and J.C. Chen, 2003. Effects of pH on survival, growth, molting and feeding of giant freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture*, 218: 613-623.
3. Hettlarachchi, A. and M. Kularatne, 1988. Observations on small scale pond culture of giant fresh water prawn. *Journal of Inland Fisheries*, 4: 25-29.
4. D'Abramo, L.R. and S.S. Sheen, 1991. Nutritional requirements, feed formulation and feeding practices for intensive culture of the freshwater prawn, *Macrobrachium rosenbergii*. *Review of Fisheries Science*, 2: 1-21.
5. Tidwell, J.H., C. Coyle, S.D. Weibel and J. Evans, 1999. Effects and interactions of stocking density and added substrate on production and population structure of freshwater prawns *Macrobrachium rosenbergii*. *Journal of the World Aquaculture Society*, 30: 174-179.
6. Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. *Aquaculture*, 180: 147-165.

7. Shinde, A.N., V.B. Mulye, N.D. Chogale, V.R. Bhatkar, R.D. Bondre and A.S. Mohite, 2008. Effect of different probiotics *Macrobrachium rosenbergii* (De-Man) post larvae. *Aquaculture*, 9: 7-12.
8. Saad, S.A., M.M. Habashy and M.K. Sharshar, 2009. Growth response of the freshwater prawn, *Macrobrachium rosenbergii* (De Man), to diets having different levels of Biogen. *World Applied Science Journal*, 6: 550-556.
9. Seenivasan, C., P. Saravana Bhavan, S. Radhakrishnan and T. Muralisankar, 2012. Effects of probiotics on survival, growth and biochemical constituents of freshwater prawn *Macrobrachium rosenbergii* post larvae. *Turkish Journal of Fisheries and Aquatic Science*, 12: 331-338.
10. Seenivasan, C., P. Saravana Bhavan, S. Radhakrishnan, T. Muralisankar, V. Srinivasan and N. Manickam, 2013. Effect of *Saccharomyces cerevisiae* on survival, growth, biochemical constituents and energy utilization in the prawn *Macrobrachium rosenbergii*. *International Journal of Applied Biology and Pharmaceutical Technology*, 4: 39-47.
11. Seenivasan, C., S. Radhakrishnan, R. Shanthi, T. Muralisankar and P. Saravana Bhavan, 2014. Effect of *Lactobacillus sporogenes* on survival, growth, biochemical constituents and energy utilization of freshwater prawn *Macrobrachium rosenbergii* post larvae. *The Journal of Basic and Applied Zoology*, 67: 19-24.
12. Amaraweera, K.W.R.R., M.J.S. Wijeyaratne and S.C. Jayamanne, 2013. Growth and survival of post-larvae of giant freshwater prawn (*Macrobrachium rosenbergii*) reared using feeds formulated with different sources of protein. *Sri Lanka Journal of Aquatic Sciences*, 18: 17-26.
13. Tekinay, A.A. and S.J. Davies, 2001. Dietary carbohydrate level influencing feed intake, nutrient utilisation and plasma glucose concentration in the rainbow trout, *Oncorhynchus mykiss*. *Turkish Journal of Veterinary and Animal Science*, 25: 657-666.
14. Armstrong, D.A, M.J. Stephenson and A.W. Knight, 1976. Acute toxicity of nitrate to larval of giant Malaysian prawn *Macrobrachium rosenbergii*. *Aquaculture*, 9: 36-46.
15. Aquacop, G.L. and G.B. Magaire, 1983. Effect of pH and salinity on survival, growth and osmoregulation in *P. monodon*. *Aquaculture*, 107: 33-47.
16. Baskerville-Bridges, B. and L.J. Kling, 2000. Larval culture of Atlantic cod (*Gadus morhua*) at high stocking densities. *Aquaculture*, 181: 61-69.
17. Johnson, S.K., 1982. Diseases of *Macrobrachium rosenbergii*. In: New, M.B. (Ed.). In: *Giant Prawn Farming Developments in Aquaculture and Fisheries Science*, 10. Elsevier, Amsterdam, pp: 269-277.
18. New, M.B. and Singholka, 1985. *Freshwater prawn Farming: A manual for the culture of Macrobrachium rosenbergii*. FAO Fisheries Technical Paper No. 225, Rev., 1: 118.
19. Boyd, C. and S. Zimmermann, 2000. Grow-out systems—water quality and soil management. In: New MB and WC Valenti (Eds.) *Freshwater prawn culture: the farming of Macrobrachium rosenbergii*. Blackwell Science, pp: 221-238.
20. New, M.B., 2002. *Farming Freshwater Prawns-A manual for the Culture of the Giant Freshwater Prawn (Macrobrachium rosenbergii)*. FAO Fisheries Technical Paper 428. Food and Agriculture Organization of the United Nations, Rome, Italy, pp: 212.
21. Rajinikanth, T., P. Ramasamy and V. Ravi, 2010. Efficacy of Probiotics, Growth Promoters and Disinfectants in Shrimp Grow out Farms. *American-Eurasian Journal of Agriculture & Environmental Science*, 7(3): 347-354.
22. New, M.B., 1991. Turn of the millennium aquaculture: navigating troubled waters of riding the crest of waves. *World Aquaculture*, 22: 28-49.
23. Asoka, J.M. and M. Hettiarachchi, 2004. Rearing of larvae of giant freshwater prawn *Macrobrachium rosenbergii* up to post-larvae using different diets. *Sri Lanka Journal of Aquatic Sciences*, 9: 57-67.
24. Mitra, G., P.K. Mukhopadhyay and D.N. Chattopadhyay, 2005. Nutrition and feeding in fresh water prawn (*Macrobrachium rosenbergii*) farming. *Aqua feeds: Formulation & Beyond*, 2(1): 17-19. Retrieved from <http://fisheries.tamu.edu/files/2013/09/Nutrition-and-Feeding-in-Freshwater-Prawn-Macrobrachium-rosenbergii-Farming.pdf> [14.12.14].
25. Millikin, M.R., A.R. Fortner, P.H. Fair and L.V. Sick, 1980. Influence of several dietary protein concentrations on growth, feed conversion and general metabolism of the juvenile prawn (*Macrobrachium rosenbergii*). In: *Proceedings of the World Mariculture Society*, pp: 11.

26. Castell, J.D., J.C. Kean, L.R. D'Abramo and D.E. Conklin, 1989. A standard reference diet for crustacean nutrition. I. Evaluation of two formulations. *Journal of World Aquaculture Society*, 20(3): 93-99.
27. Yousef, S. and Al-Hafedh, 2008. Effects of Dietary Protein Level on Growth, Feed Conversion and Protein Efficiency Ratio of Freshwater Prawn, *Macrobrachium rosenbergii*, in Outdoor Concrete Tanks [Abstract]. DOI10.1300/J028v19n01\_05: 51-60.
28. Habashy, M.M., 2009. Growth and Body Composition of Juvenile Fresh water Prawn *Macrobrachium rosenbergii*, Fed Different Dietary Protein/Starch Ratios. *Global Veterinaria*, 3(1): 45-50.
29. Corbin, J.S., M.M. Fujimoto and T.Y.J. Iwai, 1983. Feeding practices and nutritional considerations for *Macrobrachium rosenbergii* culture in Hawaii. In: McVey, J.P., Moore, J.R. (Eds.), *CRC Hand Book of Mariculture*. CRC Press, Boca Raton, pp: 391-442.
30. Fuller, R., 1989. Probiotics in man and animals. *Journal of Applied Bacteriology*, 66: 365-378.
31. Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete, 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology*, 64: 655-671.
32. Reddy, A.K., 1997. Management of freshwater prawn hatcheries and culture possibilities. *Fisheries Chimes*, pp: 32-36.
33. Muralisankar, T., P. Saravana Bhavan, S. Radhakrishnan, C. Seenivasan, N. Manickam and R. Shanthi, 2014. Effects of dietary supplementation of fish and vegetable oils on the growth performance and muscle compositions of the freshwater prawn *Macrobrachium rosenbergii*. *The Journal of Basic & Applied Zoology*, 67: 34-39.
34. Venkat, H.K., N.P. Sahu and K.K. Jain, 2004. Effects of feeding Lactobacillus-based probiotics on the gut micro flora, growth and survival of post larvae of *Macrobrachium rosenbergii* (de Man). *Aquaculture Research*, 35: 501-507.
35. Parakrama M.G.I.S., K.D. Rawat, G. Venkateshwarlu and A.K. Reddy, 2009. Feeding vitamins, antioxidants and cod liver oil enriched formulated feed influences the growth, survival and fatty acid composition of *Macrobrachium rosenbergii* (de Man 1879) post larvae. *Sri Lanka Journal of Aquatic Sci.*, 14: 59-74.
36. Seenivasan, C., P. Saravana Bhavan and S. Radhakrishnan, 2011. Effect of probiotics (Binifit™) on survival, growth, biochemical constituents and energy budget of the freshwater prawn *Macrobrachium rosenbergii* post larvae. *Elixir Aquaculture*, 41: 5919-5927.
37. Indulkar, S. and S.G. Belsare, 2003. Live and inert foods for postlarvae of the giant freshwater prawn *Macrobrachium rosenbergii*. *Israeli Journal of Aquaculture- Bamidgeh*, 55(4): 45-50.
38. Hossain, M.A., M.A.L. Siddique and M.A.H. Miaje, 2000. Development of low-cost feed for culture of *Macrobrachium rosenbergii* in ponds. *Bangladesh Journal of Fisheries Research*, 4(2): 127-134.
39. Kumar, J.S.S, N. Nagamuthu, S. Velliah, N. Nagarathinam and V. Sundararaj, 2000. Production characteristics of *Macrobrachium rosenbergii* and *M. malcolmsonii* under controlled monoculture system. *Journal of Aquaculture in the Tropics*, 15(3): 121-126.
40. Sadek, S.S. and F.M.H. El-Gayar, 1993. Production of the giant freshwater prawn *Macrobrachium rosenbergii* reared in brackish water ponds (Egypt). *Proceedings of the first international symposium on aquaculture technology and investment opportunities*. Ministry of Agriculture and Water, Riyadh, Saudi Arabia, pp: 296-303.
41. Islam, K.S. and Y.S.A. Khan, 1990. Mass production of post larvae of *Macrobrachium rosenbergii* (de Man) at the Prawn Hatchery and Research Centre, Cox's Bazar, Bangladesh. *Bangladesh Journal of Zoology*, 18(1): 53-59.
42. Adisukrenso, S., G.L. Escritor and K. Mintargo, 1982. Mass production of *Macrobrachium* post-larvae in the Brackishwater Aquaculture Development Centre (BADC), Jepara, Indonesia. In: *Giant Prawn Farming* (M.B. New ed.), Elsevier, pp: 143-156.
43. Islam, M.R., S.B. Saha, M.F.A. Mollah and J.A. Rokeya, 2000. Polyculture of *Macrobrachium rosenbergii* (de man) with carps in ponds. *Bangladesh Journal of Fisheries*, 22(1): 39-44.
44. Alam, M.J., K.J. Ang and S.H. Cheah, 1993. Use of *Moina micrura* (Kurz) as an *Artemia* substitute in the production of *Macrobrachium rosenbergii* (de Man) post-larvae. *Aquaculture*, 109(3-4): 337-349.
45. Murthy, H.S., S.K. Satheesha and M.C. Yogeshbabu, 2002. Recent advances in the larval rearing of giant freshwater prawn *Macrobrachium rosenbergii*, *The Fifth Indian Fisheries Forum Proceedings*, AFSIB Mangalore & AOA, Bhubaneswar, India: 9-11.
46. Bomba, A., R. Nemcoa, S. Gancarc-Ova, R. Herich, P. Guba and D. Mudron-Ova, 2002. Improvement of the probiotic effect of micro organisms by their combination with maltodextrins, fructooligosaccharides and polyunsaturated fatty acids. *British Journal of Nutrition*, 88: 95-99.