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Effect of *Lactobacillus sporogenes* on survival, growth, biochemical constituents and energy utilization of freshwater prawn *Macrobrachium rosenbergii* post larvae

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Abstract The present study was conducted to investigate the optimization of probiotic, *Lactobacillus sporogenes* on survival, growth, biochemical constituents and energy utilization of the freshwater prawn *Macrobrachium rosenbergii* post larvae (PL). Experimental diets were the same in all, except for the variation in probiotic levels. The probiotic *L. sporogenes* was used at 0%, 1%, 2%, 3% and 4% inclusion in the experimental diets. These diets were fed to *M. rosenbergii* PL for a period of 90 days. The food index parameters, such as SR, WG, SGR, FCE and PER were significantly ($P < 0.05$) higher in 4% *L. sporogenes* incorporated diet fed PL, whereas the FCR was significantly ($P < 0.05$) lower in 4% *L. sporogenes* incorporated diet fed PL. This indicates the fact that this feed produced higher growth rate than that of other experimental diets. Similarly the proximate composition of the total protein, total free amino acid, total carbohydrate, and total lipid content was significantly ($P < 0.05$) higher in 4% *L. sporogenes* incorporated diet fed PL. However, insignificant differences were recorded in ash and moisture contents between control and experimental groups. Energy utilization parameters, such as feeding rate, absorption rate, conversion rate and excretory rate were significantly ($P < 0.05$) higher in 4% *L. sporogenes* incorporated diet fed PL. Statistically insignificant differences were recorded in metabolic rate between control and experimental groups. This indicates that there were no differences in energy loss between control and experimental groups. However, *L. sporogenes* incorporated diet fed PL produced better growth performance.
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Introduction

Macrobrachium rosenbergii is a species of aquaculture importance owing to its high fecundity, rapid growth, wide range of salinity and temperature tolerance, disease resistance as well as its superior taste and high commercial value (Johnson, 1982;

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New, 1995; Roustaian et al., 2001). *M. rosenbergii* is highly preferred for culturing due to its fast growth rate, tolerance to wide fluctuations of temperature, salinity and resistance to major diseases. The use of probiotics in the aquatic organisms is increasing with the demand for more environment-friendly aquaculture practices (Gatesoupe, 1999). A probiotic is generally defined as a live microbial food supplement, which improves the balance of the host animal's intestinal flora (Fuller, 1989). However in aquaculture, probiotics can be administered either as a food supplement or as an additive to water (Moriarty, 1998). Probiotics in aquaculture have been shown to have several modes of action, competitive exclusion of pathogenic bacteria through the production of inhibitory compounds, improvement of water quality, enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Gomez Gill et al., 2000; Verschuere et al., 2000). Probiotics have been shown to be effective in a wide range of species for the promotion of growth enhanced nutrition, immunity and survival rate. Venkat et al. (2004) has worked out different modes of administration of probiotics to *M. roesenbrgii* PL either through feed or as bioencapsulated in *Artemia*. Shinde et al. (2008) have reported the use of different commercial probiotic supplements administered through feeds to PL of *M. rosenbergii*. Saad et al. (2009) have reported the use of Biogen® a probiotic for *M. rosenbergii* PL. However, the nutritional effects of probiotic bacteria, especially the effects of the bacteria on energy utilization have not been evaluated in aquaculture. Therefore, this study attempted to investigate the effect of probiotic, *L. sporogenes* on the survival, growth, biochemical constituents and energy utilization performance of *M. rosenbergii* PL.

Materials and methods

The post larvae of freshwater prawn, *M. rosenbergii* (PL 15) were purchased from a Happy Bay Annexe, Kanchipuram, Tamil Nadu, India and were stocked in a cement tank (1000 L) filled with freshwater. The PL were acclimated at ambient laboratory conditions for 15 days (up to PL 30) and starved for 24 h before the commencement of the feeding experiment. The experimental water had these physicochemical parameters: pH 7; total dissolved solids 0.90 g/L^{-1}; dissolved oxygen 7.20 mg/L^{-1}; BOD 30.00 mg/L^{-1}; COD 125.00 mg/L^{-1}; ammonia 0.028 mg/L^{-1}.

Diet preparation

The composition of the experimental diets is given in Table 1. The probiotic *L. sporogenes* (Uni-Sankyo Ltd., Maharashtra, India) was incorporated into the test diets at five different concentrations individually 0% (control), 1%, 2%, 3% and 4%, respectively. Feed formulation was done basically by "Spearson's square-method" using determined values of 40% protein content (Table 1). The proportion of each ingredient required was calculated precisely providing allowance for the premix. The dough was steam cooked and cooled to room temperature. After that, different concentrations of *L. sporogenes* were mixed with the dough and the feeds were pelletized separately with a locally made (Kolkata, India) hand pelletizer. The pellets were dried in a thermostatic oven (M/s Modern Industrial, Mumbai, India) at 40 °C until the pellets reached a constant weight and then stored in airtight jars at room temperature. The biochemical constituents of the experimental diets were determined, total protein content (Lowry et al., 1951), total free amino acids (Moore and Stein, 1948), total lipids (Folch et al., 1957), total carbohydrate (Roe, 1955), ash and moisture contents (APHA, 2005). These diets were freshly produced after 30 days to ensure high probiotic viability throughout the duration of feeding trail. In the control feed, no *L. sporogenes* was found throughout the duration of the feeding trail.

Feeding experiment

M. rosenbergii (PL-30) with the length and weight range of 1.34 ± 0.20 cm and 0.18 ± 0.04 g, respectively, were used for feeding experiment. Forty PL for each feed in triplicate were maintained in plastic tanks with 20/L water. The PL was maintained at the stocking density of 2/L. One group served as control, which was devoid of probiotics (0%). The experimental groups were fed with the respective concentration of *L. sporogenes* incorporated diets. The feeding was adjusted daily at 6:00 am and 6:00 pm. The daily ration was given at the rate of 10% of the body weight of PL with two equal halves, throughout the experimental period. The unfed feed, faeces and moult, if any, were collected after the respective hours of feeding. The feeding experiment was prolonged for 90 days; mild aeration was given continuously in order to maintain the optimal oxygen level.

Determination of food indices

After the feeding trial, food index parameters such as survival rate (SR), weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR), feed conversion efficiency (FCE) and protein efficiency rate (PER), were individually determined by the following equations.

$$\text{Survival rate}(\%) = \text{Total No. of live animals}/\text{Total No. of initial animals} \times 100$$

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{SGR}(\%) = \log w_2 - \log w_1/t \times 100(\text{where}, w_1 \& w_2 = \text{Initial and final weights, respectively (g), and } t = \text{Total number of experimental days})$$

$$\text{Condition factor (CF)} = \text{Prawn weight (g)}/\text{Prawn length (cm)} \times 100$$

$$\text{Feed conversion rate (FCR)} = \text{Total Feed intake(g)}/\text{Total weight gain of the prawn (g)}$$

$$\text{Feed conversion efficiency (FCE)} = \text{Biomass (g)}/\text{Total Feed intake (g)} \times 100$$

$$\text{Protein efficiency rate (PER)} = \text{Total Weight gain of PL (g)}/\text{Total Protein consumed (g)}$$

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