

# Efficiency of probiotics on the growth of the green tiger shrimp *Penaeus* semisulcatus (de Haan, 1844)

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# Abstract

The efficiency of probiotics in supplementation with fresh feed namely clam meat and pellet feed (Irawan-300-Grower) on the growth and survival of the green tiger shrimp *Penaeus semisulcatus* was studied in captivity. In each feed, one control (without probiotics) and one experiment (with probiotics containing *Lactobacillus* spp., *Streptococcus* spp., lipase and protease) were maintained. The experimental period was two intermoult duration (40 days). Water temperature, salinity, pH and dissolved oxygen were in the range of 27.5 to 31 °C; 25 to 30 psu; 7.8 to 8.2 and >4.5 ml/l respectively. Phosphate, nitrate, silicate and ammonia were estimated in the control and experimental shrimps. Phosphate and silicate concentrations were higher in experimental animals receiving pellet feed. The maximum weight gain of 5.20 g and length of 16.3 mm were found in experimental females fed with pellet feed. Low weight gain of 2.42 g and length of 3.7 mm were observed in control males fed with clam meat. Maximum (95%) survival was recorded in experimental females fed with pellet feed and minimum (60%) in control males fed with clam meat.

Keywords: Probiotics, Penaeus semisulcatus, pellet feed, clam meat

## Introduction

Disease outbreak caused mainly by bacteria, virus, fungi or a combination of these etiological agents is attributed for inconsistent production and disturbance in shrimp farms. To tide over this, various antibiotics and chemicals are being used in shrimp farms. As these are found not environment-friendly, their use has been banned. Probiotics is a microbial culture (in the form of spores and dormant organisms) that is administered to enter the gastrointestinal tract and kept alive (Gatesoupe, 1999) or a live microbial feed supplement which beneficially affects the host animal by improving the intestinal microbial balance (Fuller, 1989). A large number of studies have been conducted using probiotics in the black tiger shrimp Penaeus monodon (Allen and Maguire, 1992; Ravi et al., 1998; Uma et al., 1999; Prasad, 2002; Ghoshal et al., 2006) while such information is scarce on the green tiger shrimp Penaeus semisulcatus. In recent years, in view of the viral

disease in *Penaeus monodon* culture, shrimp farmers are on the search for alternate species. In the present study, an attempt has been made to ascertain the efficiency of addition of probiotics with feed on the growth and survival of the green tiger shrimp *Penaeus semisulcatus*.

# Material and methods

*Penaeus semisulcatus* were collected from Vellar estuary (11° 29'N lat.; 79° 46'E long.), Tamil Nadu, using cast net. Immediately after collection, the shrimps were brought to the laboratory and acclimatized. Later, the healthy individuals were seggregated by size and sex, and stocked separately. The average initial size range of the control and experimental male shrimp used for the experiment was 105-107 mm in length and 8-10 g in weight, and of female was 105-110 mm in length and 10-12 g in weight. Commercial probiotics (ZYMETIN) with the composition of the *Streptococcus faecalis*, *Bacillus mescenteri*, *Clostridium butricum*, protease, lipase and beer yeast were used for the

experiment. There were four sets of experiments with and without probiotics in both the sexes. The stocking density was 4 animals per tank. In one set, commercial pellet feed (Irawan-300-Grower) and in another set fresh feed (clam meat) were used both in control and experimental tanks. The probiotics (5 g) was mixed with clam meat/pellet feed in the experimental tank. Initially feed was given at the rate of 2% body weight and was increased subsequently to 5% as the animal increased its feed intake. The faecal matter and uneaten feed were siphoned-out daily. The weight of every moulted shrimp was recorded in the control and experimental tanks using an electronic balance. After measurement, the animals were tagged with thread for easy identification. The period of study was two intermoult duration (40 days).

During the study period, the water quality parameters such as salinity, temperature, dissolved oxygen, pH and ammonia and nutrients (nitrate, phosphate and silicate) were analyzed once in ten days. Temperature was measured using a standard centigrade thermometer; salinity using a refractometer (Atago, Japan); dissolved oxygen by the Winkler's method (Strickland and Parsons, 1972); pH using pH pen (Elico model); nitrate, phosphate and silicate following standard methods proposed by Wood *et al.* (1967) and Murphy and Riley (1962), and ammonia following the method proposed by Strickland and Parsons (1972).

#### Results

*Water quality and nutrient analysis*: During the study, the temperature varied between 27.5 and

 $31^{\circ}$  C; salinity from 25 to 30 psu; dissolved oxygen remained above 4.5 ml/l and the pH from 7.8 to 8.2 (Table 1).

The concentration of nutrients and ammonia in the pellet feed and clam meat experiments are given in Figs. 1- 3. In the case of pellet feed, the mean and SD values of nitrate (0.0009 ppm  $\pm$  3.3 x 10<sup>-4</sup>), phosphate (0.0005 ppm  $\pm$  2.2 x 10<sup>-4</sup>), silicate (0.0018 ppm  $\pm$  6.1 x 10<sup>-4</sup>) and ammonia (0.0018 ppm  $\pm$  6.4 x 10<sup>-4</sup>) showed that the phosphate and silicate concentrations were higher in experimental females, while in the control, nitrate and ammonia were higher in females and males respectively.

The mean and SD values of nitrate (0.0011 ppm  $\pm$  4.8 x 10<sup>-4</sup>), phosphate (0.00053 ppm  $\pm$  1.5 x 10<sup>-4</sup>), silicate (0.002 ppm  $\pm$  6 x 10<sup>-4</sup>) and ammonia (0.00435 ppm  $\pm$  1.6 x 10<sup>-3</sup>) in the clam meat experiment showed that the silicate and nitrate were higher in experimental females and control females respectively. As observed in the pellet feed, the ammonia value was higher in the experimental males.

*Growth analysis*: The maximum weight gain of 5.20 g was found in the experimental females fed with pellet feed and the minimum of 2.42 g was in the control males fed with clam meat. The maximum length gain of 16.3 mm was also recorded in the experimental females fed with pellet feed and the minimum length of 3.7 mm was noticed in the control males fed with clam meat. Among the sexes, females receiving pellet feed showed better growth in terms of weight and length. The details

Table 1. Water quality parameters (mean ± SD) in the control and experimental tanks

Feed	Parameters	Con	trol	Exper	iment
	-	Males	Females	Males	Females
Pellet feed	Temperature (°C)	$29~\pm~0.89$	$29.4 \pm 1$	$30 \pm 0.89$	$28.6 \pm 1.2$
	Salinity (psu)	$27.2 \pm 1$	$28.8 \pm 0.7$	29.6± 0.37	27± 0.73
	pН	$7.94 \pm 0.04$	$7.9 \pm 0.08$	$8.04 \pm 0.04$	$8.04 \pm 0.04$
	Dissolved oxygen (ml/l)	$5.22 \pm 0.16$	$5.44 \pm 0.36$	$4.94 \pm 0.36$	$5.76 \pm 0.18$
Clam meat	Temperature (°C)	$27.8 \pm 0.74$	$28.8 \pm 0.74$	$29.5 \pm 0.33$	$28.7 \pm 1.07$
	Salinity (psu)	$27.1 \pm 0.8$	$29.6 \pm 0.3$	$28.8 \pm 1$	$29.2 \pm 0.7$
	pН	$7.94 \pm 0.04$	$8.06 \pm 0.04$	$7.98 \pm 0.07$	$8.04 \pm 0.04$
	Dissolved oxygen (ml/l)	$5.4 \pm 0.3$	$5.92 \pm 0.33$	$5.36 \pm 0.58$	$6.12 \pm 0.29$

0.0035 0.003 0.0025

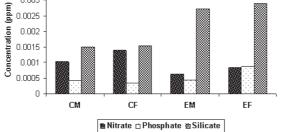


Fig. 1. Nutrients in the control and experimental animals fed with pellet feed (CM: Control Male; CF: Control Female; EM: Experimental Male; EF: Experimental Female)

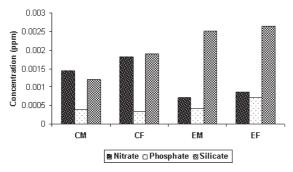


Fig. 2. Nutrients in the control and experimental animals fed with clam meat (CM: Control Male; CF: Control Female; EM: Experimental Male; EF: Experimental Female)

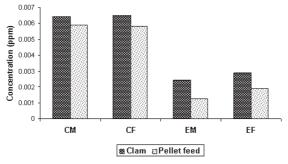


Fig. 3. Ammonia in the control and experimental animals fed with clam meat and pellet feed (CM: Control Male; CF: Control Female; EM: Experimental Male; EF: Experimental Female)

are given in Table 2. The maximum survival (95%) was recorded in experimental females fed with pellet feed and the minimum (60%) in control males fed with clam meat.

Table 2. Growth (Mean $\pm$ SD) of		ntrol and experim	nental male and f	emale P. semisul	control and experimental male and female P. semisulcatus fed with pellet feed and clam meat for 40 days	llet feed and clan	n meat for 40 di	tys
		Pellet feed	eed			Clam meat	neat	
Parameters	Control	rol	Experiment	ment	Control	01	Exper	Experiment
	Male	Female	Male	Female	Male	Female	Male	Female
Initial length (mm)	$106 \pm 0.4$	$106.9 \pm 0.04$	$105.5 \pm 0.03$	$107.7 \pm 0.09$	$110.4 \pm 0.14$	$109.2 \pm 0.04$	$107 \pm 0.58$	$107.6 \pm 0.28$
Length after first	100 5 - 0.01		112 - 0.04	116 - 0.04	110 · C11	1111 - 0.00	FC 0 - 3 C11	210 - 0711
	100.7 ± 0.04	109.2 ± 0.12	110 ± 0.04	110 ± 0.04	112 ± 0.14	111.1 ± 0.09	10.0 I C.211	$114.2 \pm 0.10$
Final length (mm)	$111.1 \pm 0.04$	$112.1 \pm 0.09$	$120.2 \pm 0.16$	$124.3 \pm 0.04$	$114.1 \pm 0.09$	$113.2 \pm 0.18$	$118.1 \pm 0.09$	$121.2 \pm 0.14$
Initialweight(gg)	$10.08 \pm 0.05$	$10.13 \pm 0.02$	$9.86 \pm 0.04$	$10.3 \pm 0.004$	$10.54 \pm 0.02$	$10.86 \pm 0.04$	$10.21 \pm 0.04$	$10.3 \pm 0.004$
Weight after first								
inter-moult (g)	$11.54 \pm 0.02$	$11.62 \pm 0.01$	$11.65 \pm 0.02$	$12.6 \pm 0.4$	$11.73 \pm 0.02$	$12.22 \pm 0.01$	$11.77 \pm 0.25$	$12.26 \pm 0.01$
Final weight (g)	$13.01 \pm 0.009$	$13.13 \pm 0.02$	$13.43 \pm 0.02$	$15.47 \pm 0.05$	$12.95 \pm 0.03$	$13.34 \pm 0.2$	$13.14 \pm 0.1$	$14 \pm 0.12$
Length gain (mm)	$5.1 \pm 0.08$	$5.06 \pm 0.12$	$14.1 \pm 0.3$	$16.3 \pm 0.23$	$3.7 \pm 0.04$	$4.13 \pm 0.09$	$10.5 \pm 0.18$	$13.4 \pm 0.12$
Weight gain (g)	2.899	3.004	3.556	5.204	2.422	2.75	2.98	3.93
Growth in								
length (%)	4.41	4.85	13.79	15.30	3.34	3.84	9.85	12.52

#### Discussion

The water quality parameters monitored during the present study were within normal range. Nutrients such as nitrate, phosphate and silicate levels were found to be higher in the experimental animals fed with pellet feed. As regards growth, females and males fed with pellet feed in supplementation with probiotics gained considerable weight of 5.20 g and 3.55 g respectively. Control females and males fed with pellet feed attained a weight gain of 3.00 g and 2.89 g respectively. The shrimps fed with clam meat supplemented with probiotics attained a weight gain of 3.93 g and 2.98 g in females and males respectively. The control female and male fed with clam meat attained a weight gain of 2.75 g and 2.42 g respectively. Based on the results obtained in the present study, the female shrimps fed with pellet feed supplemented with probiotics showed the highest weight gain than the shrimps fed with clam meat. This is in agreement with Ghoshal et al. (2006) who studied the effect of probiotics on the black tiger shrimp Penaeus monodon production in culture ponds. Their study revealed that the shrimps fed with pellet feed supplemented with probiotics gave better growth and production.

Similar type of study was conducted earlier by Ravi *et al.* (1998) who studied the influence of probiotics on the growth of the Indian white shrimp *Penaeus indicus.* In their experiment, there was no significant difference in the water quality parameters between the experimental and control tanks. In the present study also, there was no significant difference between the experimental and control tanks. Ammonia levels were found to be less in the experimental tanks.

Koshio *et al.* (1989) reported that high growth rate is related to fast molt frequency. In the present study also, the shrimps in the experimental tanks were found to moult faster with higher growth than the control. Uma *et al.* (1999) studied the effectiveness of a commercial probiotics feed supplement (Lacto-sac) in order to improve the growth of *Penaeus indicus*. Their study revealed that the growth and survival of juveniles noticeably

improved with the addition of probiotics in the feed, which is similar to that of the present findings. Gatesoupe (1999) reported improvement in the digestive activity with the addition of probiotics because of synthesis of vitamins. Sambhu and Jayprakash (2001) proposed a new herbal product Livol (IHF-1000), which was found to be a potent growth promoter to stimulate the growth of white shrimp. Irianto and Austin (2002) reported that inclusion of probiotics in feed not only promotes growth rate but also inhibits the proliferation of pathogens by stimulating the non-specific immune response. In general, the lactic acid bacteria have the ability to attach with the gut epithelium and establish there. By their presence, they saturate the adhesion receptors and prevent the pathogenic bacteria from attachment and thereby prevent the incident of diseases (Vine et al., 2004).

In conclusion, the female shrimp fed with pellet feed with probiotic supplementation led to faster growth rate compared to shrimp fed with clam meat. Therefore, the probiotic treatment could be regarded as an effective method for enhancing the growth of the green tiger shrimp, *P. semisulcatus*.

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