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RESEARCH ARTICLE

INFLUENCE OF COMMERCIAL PROBIOTICS ON WATER QUALITY IN FRESHWATER PRAWN MACROBRACHIUM ROSENBERGII CULTURE PONDS

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ABSTRACT

The present study was conducted for period of 11 months to investigate the water quality and physico chemical parameters in the freshwater prawn *Macrobrachium rosenbergii* culture ponds, (Control and Experiment). The prawn groups of (mean length 12.8 \pm 1.1 mm and mean weight 1.2 \pm 0.2 mg) were fed with two different types of artificially prepared feed Viz, in control pond (Commercial prawn feed) and in experiment pond (Probiotic feed). Water temperature, pH, dissolved oxygen, NH₃ and H₂S were found better in trials with probiotics. Moreover, daily usage of probiotics in experiment pond was the best among trials. The best water quality was recorded in animal group fed with supplementary animal feed.

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INTRODUCTION

Giant freshwater prawn (*Macrobrachium rosenbergii* deMan, 1879) is an important commercial species thank to its delicious meat and lower cholesterol and high protein content. The freshwater prawn *Macrobrachium rosenbergii* was the first species to be studied extensively and farmed commercially which is indigenous in the whole of South and South East Asian countries as well as Northern Oceania and Western Pacific islands. It has been transferred extensively within its natural range and has been introduced into many

countries where its farming has been established (Nandlal and Pickering, 2005). Among all the freshwater prawn, scampi is the largest known species and grows to a maximum size of 750 gm. Recently, the use of probiotics to improve and maintain healthy environment for prawn culture has become popular. Probiotics was used to supply beneficial bacterial strains to rearing water that will help to increase microbial species composition in the environment and to improve water quality. Probiotics is 'living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition' Coeuret et al. (2004). Most probiotics are supplied as live supplements in food, which must have the ability to survive passage through the intestinal tract (Fuller, 1992 and Verschuere et al., 2000b).

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Benefits of Probiotic application in Aquaculture: The probiotic bacteria are useful in the aquaculture by the way of following criteria:

- Production of inhibitory compounds
- Competition for adhesion sites
- Competition for nutrients
- Source of nutrients and enzymatic contribution to digestion
- Enhancement of immune response
- Influence on water quality
- Interaction with phytoplankton
- Antiviral activity (Balcazar (2003)

This study aimed to figure out the roles of probiotics in improving and maitaining water quality in larval rearing water of giant fresh water prawn (*Macrobrachium rosenbergii*) applying modified static green water system. Our main purposes were to evaluate the influence of probiotic on water parameters for the growth and the development of larvae and its sensitivity to bacterial pathogens.

MATERIALS AND METHODS

Pond location and Postlarval stocking: The present study was conducted to investigate the quality of water parameters of culture ponds of the giant freshwater prawn, Macrobrachium rosenbergii, by adding probiotic in the diet by semi-intensive culture practice. 60,000 post larval prawn/0.6 ha (mean length 12.8 ± 1.1 mm and mean weight 1.2 ± 0.2 mg) were divided into two groups as control and experiment pond in Vishnuvakkam village, Tiruvallur Dist, Tamilnadu, India.. The experiment was conducted from February 2008 to December 2008. The Commercial prawn feed applied in control pond whereas the Probiotic mixed feed in experimental pond. The stocking density of *M. rosenbergii* in control pond and probiotic experiment pond was $1.3/m^2$.

Probiotic feeding: The known quantity of Commercial prawn feed and the Lactact (*Lactobacillus sporogens*) 10g/kg, Thionil (mixture of bacterial culture) 20g/kg and Mutagen (C.P. Aquaculture (India) 15g/kg were mixed with water and to this 30 ml of affinity gel also mixed and kept for 10 minutes, dried in the shade for 20-30

minutes, and then feed was broadcasted as per feeding schedules (Table.1).

Physical and chemical parameter of water analysis: Physical and chemical parameters of water samples of both the ponds were analysed weekly and monthly samples during the culture period. The physical and chemical parameters such as odour, colour, transparency (Secchi disc), water level, pH (C.P. pH kit), salinity (Refractometer), dissolved oxygen (C. P. DO kit), temperature (Mercury thermometer-atmospheric and water) were analysed weekly in the culture farm. Various parameters of water analysis were analysed in the laboratory by adopting standard procedures of APHA (1995).

RESULTS

Physico chemical parameters of pond water: (weekly analysis): The weekly analysis of colour, odour, DO, pH and Temperature of control and probiotic applied pond from 3rd February to 21st December 2008 was recorded. Control pond shows light green, thick green, greenish brown, golden yellow, dark green colours during the culture period. Earthy odours were smelled in the beginning of the culture, after that odourless and sandy odour was noticed. The recorded dissolved oxygen ranges between 3.0 - 5.5 ppm during the culture period. The recorded pH ranges between 7.4 - 8.9 during the culture period. The temperature was varied between 26 - 34°C in probiotic experiment pond. The't' test values, correlation and ANOVA values for DO, pH and temperature of control and probiotic experiment pond were presented in table 2a,b.

Monthly analysis: Monthly recorded values of physical and chemical parameters of control and probiotic experiment pond were presented in table 3. The resulted values of physical and chemical parameters of control and probiotic experiment pond were found to be statistically significant at various levels. Normal pH ranges were appeared in both the ponds where as the alkalinity pH showed fluctuated. Higher alkalinity was recorded in the month of December in both the ponds. Total hardness of the water shows fluctuation during the study period. Nutrient such as calcium, magnesium, sodium, potassium, sulphates showed normal

S.no	Period	I	Feed broad cast tin	ne (hrs)	Quantity of Feed broad cast(Kg)
1.	5.2.08 - 20.2.08	6.30-7.30	17.00	-	0.6
2.	21.2.08 - 30.2.08	6.30-7.30	17.00	-	1.0
3.	31.3.08 - 3.4.08	5.30-6.30	16.00-16.30	22.30-23.00	1.5
4.	4.4.08 - 25.4.08	5.30-6.30	16.00-16.30	22.30-23.00	2.0
5.	26.4.08-25.5.08	5.30-6.30	16.00-16.30	22.30-23.00	3.0
6.	26.5.08-25.7.08	5.30-6.30	16.00-16.30	22.30-23.00	5.0
7.	26.7.08 - 31.8.08	5.30-6.30	16.00-16.30	22.30-23.00	7.0
8.	1.9.08 - 28.10.08	5.30-6.30	16.00-16.30	22.30-23.00	10.0
9.	29.10.08-23.12.08	5.30-6.30	16.00-16.30	22.30-23.00	4.0

Table.1.Feeding schedule of *Macrobrachium rosenbergii* during the culture period in control and probiotic experimental pond

 Table- 2a. T-test values of DO, pH and temperature of control and Probiotic experiment pond of freshwater prawn M. rosenbergii culture (On the spot values)

Parameters	Ponds	Mean \pm SEM*	T-test value	P-value
DO	Control	3.808 ± 0.102	37.106	0.000
	Experimental	5.000± 9.375E-02	53.336	0.000
pН	Control	8.293 ± 5.254	157.854	0.000
	Experimental	7.717 ± 3.867	202.289	0.000
Temperature	Control	30.893 ± 0.258	119.512	0.000
1	Experimental	28.702 ± 0.342	83.767	0.000

: Mean sample 47 Significance at the 5 % level (P<0.05)

 Table 2b. Correlation (r- value) and ANOVA (F-value) of DO, pH and temperature of control and Probiotic experiment pond of freshwater prawn M. rosenbergii culture

Parameters	(r-value)	F-value	p-value	
DO	0.048	2.737	0.032*	
pН	0.069	0.520	0.722	
Temperature	0.394	1.082	0.393	

range. However, fluoride showed higher range in the month of April, June, July, August, September, October (1.5 ppm) in control pond. Phosphorous, iron and nitrate showed normal range in all the months. Chloride, Free ammonia, Nitrate and Sulphate content of the water shows fluctuation in control and probiotic experiment pond during the study period. The't' test values of control and probiotic experiment pond were presented in table 3a. In the present experiment, very high mean difference values were recorded in total dissolved solids, calcium and fluoride showed significant and some values are found to be non significant. The positive and negative correlation co-efficient (r) values of physic-chemical parameters of control and experiment pond are presented in the table. 4b. Most of the parameters showed high correlation except fluoride (0.013) and magnesium (0.149).

DISCUSSION

Physico- chemical parameters of pond water:

Colour of the pond water: The observed colour in the present study may be (1) reddish brown, (2) light or bright green, (3) dark green resulted is due to the growth of *Chaetoceras*, Navicula. Skeletonema, Cvclotella, Svnedia, Achnathes, Amphora, Euglena and Chlorella (table.3). The present study was supported by Wang et al. (2005) who observed (combinations of Bacillus. Saccharomyces cerevisiae. Nitrosomonas and Nitrobacter) a brownish-green water color in commercial probiotic applied ponds of P. vannamei.

Total dissolved Oxygen content: The oxygen level in the studied period was higher in probiotic applied pond than the control group with

Pa	raameters			Feb		Ν	/lar	Ap	or	Ma	ıy	Jı	ine		July		Aug		Sep		Oct		No	v	Dec
			(С	Е	С	E	С	E	С	E	С	E	С	E	C	E	Ċ	E	Ċ	E	Ċ	E	С	E
	pН		7	.6	7.9	8.2	8.2	8.5	8.4	8.5	8.4	8.5	8.5	8.2	8.2	8.8	8.2	8.8	8.5	8.5	8.5	8.2	7.6	8.2	7.9
Alk	alinity pH		12	2.0	12.0	16.0	16.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.00	16.00	16.00	16.00	16.00	12.00	12.00	16.00	16.00
Electrica	l conducti	-vity	16	550	1180	1680	1190	1750	1680	1940	2000	1885	2260	2030	2250	1950	2230	1860	2140	1855	1920	1850	1865	1780	1780
Total di	issolved so	lids	11	30	1130	1220	1125	1300	1150	1250	1420	1350	1530	1365	1615	1265	1565	1260	1460	1255	1355	1210	1260	1150	1280
Tur	bidity(cm)		3	34	24	35	25	25	45	28	42	30	60	38	45	35	50	30	35	15	35	18	45	20	40
Total A	lkalinity (p	pm)	4	15	50	50	65	70	65	75	70	80	70	85	70	90	80	110	100	110	100	120	110	125	120
	ardness (pp	m)		45	225	220	220	235	220	240	210	290	190	220	170	180	180	160	160	140	140	190	160	210	190
	cium (ppm)			04	45	104	30	77	38	69	25	69	45	77	30	64	25	67	27	62	32	104	40	104	45
	esium (ppn	n)		13	38	48	42	32	45	28.	44	28.	55	32.	42	26.	36	29	33	27	37	43	42	48	48.
Sod	lium (ppm)		2	24	24	25	24	20	20	23	22	22	22	20	21	18	17	20	20	22	23	24	27	22	24
Phosphorus (ppm) Iron (ppm)	0.29	0.28	0.36	0.34	1.0	_	1.05 2.6	0.30 3.0	0.25	0.98	1.08 2.8	1.0		1.08 2.8	1.58 1.30	1.48 1.8	2.02	2.00	2.12	-			0.27 2.1	0.36 0.90	0.35
Fluoride (ppm)	1.20	0.15	1.40	0.25	1.5	50	0.15	0.70	0.15	1.5	0.15	1.5	50 (0.15	1.50	0.15	1.50	0.15	1.50	0.1	.5	1.20	020	1.40	0.25
Chloride (ppm)	358	341	392	376	27	1	251	298	285	292	275	29	3 2	251	246	218	284	263	285	26	1 :	365	341	382	376
Free Ammonia (ppm)	0.24	0.20	0.32	0.21	1.0)4	0.80	0.56	0.44	0.10	0.09	1.0	07	1.02	1.05	0.90	1.01	0.70	1.03	0.8	30	0.24	0.12	0.32	0.25
Nitrate (ppm)	3	2	3	2	3		2	3	2	4	3	4	-	3	5	3	5	3	4	3	4	4	3	4	3
Sulphate (ppm)	40.	30	15	12	13		13	9	8	10	10.	13		11	13	12	17	14	18	15	4	40	35	15	18
Potas	ssium (ppm)	2	20	20	20	20	18	22	22	22	22	18	18	18	16.	16	18	18	20.	20	20	22	20	22

Table-3: Physical and Chemical parameters of control and probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (February – December 2008)

C- Control

E-Experiment

Parameters	Ponds	Mean \pm SE •	t- values	p-value
pН	С	8.363 ± 0.102	81.960	0.000
-	Е	$8.209 \pm 8.990 \text{E-}02$	91.310	0.000
Alkalinity	С	13.090 ± 0.414	31.574	0.000
pH	Е	13.454 ± 0.608	22.112	0.000
Electrical	С	1839.090 ± 34.946	52.625	0.000
conductivity	Е	1863.181 ± 116.798	15.952	0.000
Total Dissolved	С	1250.454 ± 21.944	56.982	0.000
solids	Е	1353.636 ± 53.746	25.186	0.000
Turbidity	С	28.000 ± 2.304	12.152	0.000
•	Е	40.545 ± 3.171	12.783	0.000
Total Alkalinity	С	81.818 ± 6.683	12.242	0.000
,	Е	87.272 ± 8.100	10.774	0.000
Total hardness	С	211.818 ± 12.796	16.552	0.000
	Е	188.636 ± 9.047	20.849	0.000
Calcium	С	81.909 ± 5.454	15.016	0.000
	Е	34.727 ± 2.442	14.219	0.000
Magnesium	С	34.909 ± 2.633	13.256	0.000
8	Е	42.000 ± 1.848	22.717	0.000
Sodium	С	21.818 ± 0.644	33.873	0.000
	Е	22.181 ± 0.807	27.487	0.000
Potassium	С	19.454 ± 0.545	35.667	0.000
	Е	19.818 ± 0.629	31.466	0.000
Phosphorus	С	0.950 ± 0.211	4.489	0.001
1	Е	0.927 ± 0.206	4.486	0.001
Iron	С	1.600 ± 0.220	7.248	0.000
	Е	2.163 ± 0.182	11.853	0.000
Fluoride	С	1.354 ± 7.43 E- 02	18.230	0.000
	Е	0.172 ± 1.236 E–02	13.969	0.000
Chloride	Ē	315.09 ± 14.962	21.058	0.000
	Е	294.36 ± 16.467	17.875	0.000
Free ammonia	Ē	0.634 ± 0.121	5.222	0.000
	Ē	0.502 ± 0.104	4.807	0.001
Nitrate	Ē	3.818 ± 0.226	16.868	0.000
1.111.000	Ĕ	2.636 ± 0.152	17.331	0.000
Sulphate	Ē	18.454 ± 3.309	5.576	0.000
Surprise	Ē	16.181 ± 2.579	6.274	0.000

Table-3a. Levels of physical and chemical parameters of control and probiotic experiment pond (T-test analysis)

• : Mean of 11 samples

Significant at 1% level (P<0.01)

Table-3b. Correlation co-efficient (r-value) of physical and chemical parameters of control and probiotic experiment pond

Parameters	Correlation (r-value)	p-value
pН	0.638	0.035•
Alkalinity pH	0.812	0.002†
Electrical conductivity	0.915	0.000†
Total dissolved solids	0.667	0.025•
Turbidity	- 0.063•	0.853
Total Alkalinity	0.958	0.000†
Total hardness	0.711	0.014•
Calcium	0.553	0.078
Magnesium	0.149	0.661
Sodium	0.881	0.000†
Potassium	0.500	0.117
Phosphorus	0.999	0.000†
Iron	0.805	0.003†
Fluoride	0.013	0.969
Chloride	0.989	0.000
Free Ammonia	0.980	0.000†
Nitrate	0.864	0.001†
Sulphate	0.973	0.000†

significant level (P<0.05). In the present study, the mean value of the DO concentration was 3.80 mg/l, 5.00 mg/l in control and experimental pond respectively with recommended ranges for fresh water prawn culture. The present study was corroborated to the work of Hossain and Paul (2007) who reported 5.1 - 8.2 mg/l in low cost diet on farm trial of *M. rosenbergii* culture.

pH: In the present study, the resulted mean pH $(8.363 \pm 0.102 \text{ and } 8.209 \pm 8.990)$ was recorded in control and probiotic experimental pond respectively (table.3a). The present investigation was supported by Sadek and Moreau (2000) found pH 8.2 ± 0.25 in *M. rosenbergii* culture pond.

Temperature: In the present study, the range of temperature fluctuation during the study period between 26 to 34° C and the noticed mean temperature for control was ($30.893 \pm 0.258^{\circ}$ C) and for probiotic experimental pond ($28.702 \pm 0.342^{\circ}$ C) (table.3a), which are favourable for the normal growth of prawn. Das *et al.* (2006) who reported the temperature variation 27 - 31° C between the control and probiotic applied pond which are favourable for the growth of *M. rosenbergii*. Deeseenthum *et al.* (2007) also reported temperature ranges between 21 - 35° C favourable for *M. rosenbergii* culture in probiotic mixed culture Bacillus KKUU 2 and KKUU3 applied pond and control pond.

Total solids: In the present observations, the higher levels of total solids were noticed in probiotic experiment pond (1353.636 \pm 53.746) than that of control pond (1250.454 \pm 21.944) which was found to be significant (P<0.005) (table.4a). The present study was further supported by Mohanty (2009) who recorded 363 ppm total suspended solids in their *M. rosenbergii* with carps in phased harvested system in India.

Alkalinity pH: In the present study, the levels of alkalinity pH mean were 13.09 ± 0.41 and 13.45 ± 0.60 noticed in control and probiotic experiment pond respectively. The present study was supported by Sadek and Moreau (2000) who reported the concentration of alkalinity similar to the present results.

Total Hardness and Alkalinity: In the present report, the recorded mean hardness was 211.81 ppm and 188.63 ppm in control and probiotic experimental pond respectively (table.3a). The present study was supported by Sadek and Moreau (2000) reported 1250 –4115 mg/l higher hardness level. In the present study, the noticed level of mean alkalinities of 81.818 ± 6.683 ppm in control pond and 87.272 ± 8.100 ppm in probiotic experiment pond. Ranjeet and Kurup (2002) recorded normal alkalinity level (40 – 87 ppm) in their *M. rosenbergii* monoculture experiments in coconut garden of Kuttanad, Kerala, India.

Ammonia: Levels of free ammonia observed in the present study are within the normal values in most of the monthly analysis but in mid of the culture period, the free ammonia content showed higher value (table.3) due to heavy phyto and zooplankton population and fast organic degradation. Similar reports were given by Danaher *et al.* (2007) and Mohanty (2009), recorded various level of ammonia on different culture method of M. *rosenbergii.*

Nitrate: In the present study, the mean of total nitrate content was 3.818 ppm and 2.636 ppm in control and probiotic experiment pond, respectively. The present study was supported by Asaduzzamann *et al.* (2008) and Mohanty (2009) who were recorded different ranges in their culture ponds of *M. rosenbergii.*.

Chloride: In the present study, higher levels of chlorides were recorded in control pond (mean 294.36 ppm) than probiotic experimental pond (mean 315.09 ppm) (table. 3a). Boyd and Zimmermann (2000) suggested <250 ppm of chloride level for freshwater prawn culture. Similar results reported by Quareshi *et al.* (2000) who recorded high chloride content (394.9 ppm) in *M. rosenbergii* culture pond.

Calcium: In the present study, the mean values of 81.909 and 34.727 ppm of calcium were recorded in control and probiotic experiment pond respectively (table. 3a). Wudtisin and Boyd (2006) reported 55 ± 45 , 39 ± 16 and 34.5 ± 16.1 ppm of average values of calcium in 42 catfish, 40 freshwater prawn and 18 carp ponds in Thailand, respectively.

Magnesium: The mean of total magnesium concentrations recorded in the present study are 34.909 and 42 ppm in control and probiotic experiment pond respectively which are within the

range (20 - 200 ppm). According to Wudstisin and Boyd (2006) the magnesium concentrations normally were above 5mg/l, with averages 11.6 - 15.0 mg/l in catfish, prawn and carp ponds.

Sodium, Potassium and Sulphate: In the present study, the resulted mean value of sodium, potassium and sulphate were 22.81, 19.45 and 18.45 ppm recorded in control pond whereas 22.18, 19.81 and 16.18 ppm in probiotic experimental pond respectively as per the recommended amount. New (2002) also reported 0.26 - 30.0 ppm, 0.01 - 4.9 ppm and 0.1-2.60 ppm, sodium, potassium and sulphate ranges in *M. rosenbergii* culture respectively.

Phosphorus: In the present experimental study, the resulted total phosphorus mean values are 0.950 ± 0.211 ppm and 0.927 ± 0.206 ppm for control and probiotic experiment pond respectively. New (2002) reported 0.003 - 4.4 ppm of total phosphorus value in *M. rosenbergii* culture. This present study was supported by Wang *et al.* (2005) who also reported the reduction of nitrogen and phosphorus in commercial applied probiotic in shrimp culture.

Iron: In the present study, the mean of total iron content was recorded 1.60 and 2.16 ppm in control and probiotic experiment pond respectively. The values recorded in the probiotic experiment pond showed higher iron content compared to New (2002), <1 ppm value. Adhikari *et al.* (2007) studied the impact of manganese and iron in water on survival, growth and feeding of juvenile *M. rosenbergii.*

Fluoride: In the present investigation, resulted high fluoride mean values in control pond (1.354 ppm) whereas in probiotic experiment pond showed low (0.172 ppm). This observed value was within the normal range (Boyd and Zimmermann, 2000) for the freshwater prawn culture. In the present experiments of DO, pH and temperature found to be statistically significant (P<0.05) in both the pond. The eighteen studied water parameters values found to be statistically significant (P<0.01) in the both ponds of which the turbidity value (-0.063) showed negative correlation co-efficient (table.4b). According to the results of this study, all factor of water quality parameters were at optimum level in the experimental pond compared with the control.

Conclusion

In the present study, the site selected for freshwater prawn M. rosenbergii farming is located near the border of AP which is famous place for aquaculture and is suitable climatic condition (temperature, quantum and reasonably of rainfall, evaporation, sunlight and wind speed etc.,) of the availability of water and free from pollution and flooding. The resulted physico-chemical parameter of water was found to be favourable for the growth of the M. rosenbergii in both ponds. Temperature, pH and DO are critical factors for the growth of M. rosenbergii. This probiotic application technology ensures desired soil and water pH and detoxification of soil and water (Ramakrishna, 2010). The probiotic applied technology is ecofriendly improve the water quality and it makes water suitable for aquaculture.

The probiotic product is used in the present study; it contains some of enzymes, vitamins, minerals etc. The enzymes break down the pollutants ant where present in the pond and reduced BOD, COD, TSS, Phosphates, nitrates, potash, sulphate etc. these enzymatic reaction converts organic matter into gases. The gases also help in multiplication of algae, bacteria and viruses. The probiotic treatment rejuvenates aquatic life. The present study was undertaken in outdoor earthen pond trails and the finding is confirmed that the probiotics are beneficial to improve water quality, enhance the growth and yield of M. rosenbergii. In future, the application for probiotics looks bright. The field of probiotics intended for aquaculture animals is now attracting considerable attention and numbers of commercial products are available particularly directed for prawn larval culture.

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