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Effects of feed-based microbial supplements regulate vibrio proliferation and growth enhancement in *Litopenaeus vannamei* reared under higher salinity conditions

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Abstract

Probiotics are considered one of the most important components of shrimp aquaculture. Because of its many positive effects including disease prevention, water quality management and growth performance. The experiment was designed to study the effects of probiotic on vibrio colony and growth performance of *Litopenaeus vannamei* cultured with feed supplementation at higher salinities. A 60 days experiment trial was carried out at different higher salinities (45, 50, 55 and 60 ppt) with probiotic. The important water quality parameters were analyzed such as temperature, pH, total ammonia nitrogen, nitrite. It was found to be in optimum range after few weeks in all treatments and control but the effect was better in control (45 ppt) and treatment 1 (50 ppt). Green and yellow colony (CFU/ml) was also found lower in control and T-1 compared to other treatment groups. The highest and lowest value of yellow colony was found in the range from 82.33 ± 2.962 to 9.33 ± 2.027 in control and T-3 respectively. However, the level of the green colony was insignificant in all groups. The growth of *L. vannamei* was found better in control and T-1 compared to the rest of the treatments. Hence it can be concluded that 45 and 50 ppt with probiotics is most suitable for culture as compared to other higher salinities.

Keywords: *L. vannamei*, higher salinity, probiotics, vibrio colony

1. Introduction

Aquaculture is the fastest growing food sector in the world which provides nutrition, food security, income and livelihoods for over hundreds of millions of people presently contributing around 47% of world total food fish production. The world aquaculture production was 171 million tonnes. The global aquaculture production was recorded at 80 million tonnes during year of 2016 (Anon., 2018c) [3]. World per capita fish supply reached a new record high of 20.3 kg in 2016. World population is growing rapidly and by 2050 it is expected to cross 9 billion and hence food production has to be doubled to fulfil the demand. Supply of nutritionally balanced and high quality protein food to growing population is a major challenge front of the world. Indian aquaculture is undergoing rapid developments towards achieving the goal of blue revolution and holding the second rank in the world aquaculture. India has showcased phenomenal growth in fisheries production rising from 0.75 MT in 1950-51 to around 12.60 million tonnes in 2017-18 (Muruganandam *et al.*, 2019) [16]. The scope of vertical expansion and shows the hope to provide nutritional security to rapidly growing populations all around the world. Shrimp farming is the key player which has changed the face of aquaculture around the globe. Indian shrimp aquaculture industry has undergone remarkable transformations during the last 10 years. The export of *L. vannamei* shrimp has improved from 3, 29,766 MT to 4, 02,374 MT in 2017-18 with a growth of 22.02% in quantity and 24.74% in USD value (Anon., 2018b) [2].

Litopenaeus vannamei is the most widely cultured shrimp in Central and South American countries, China, India and Thailand and accounts for around 90% of the shrimp population in the Western hemisphere (Frias-Espicueta *et al.*, 2001; McGraw *et al.*, 2002; Saoud *et al.*,

2003)^[9, 14, 19]. The geographical diversity of this species is mostly in the area of brackish water. This species mainly expose to where annual rainfall and evaporation cycles are wide changes in temperature and salinity in season. It is cultured an extensive range of salinity from 0 ppt to 35 ppt. After WSSV outbreak in *P. monodon* shrimp industry, Govt. of India allow the culture of exotic shrimp *Litopenaeus vannamei* in 2009 after reviewing the result of pilot project initiated by Coastal Aquaculture Authority of India (CAA). At present *L. vannamei* occupied a major part of India and being cultured in freshwater, brackish water and inland saline water. In India, *L. vannamei* culture is going on at very low salinities (~0-2 ppt) in Godavari and Krishna districts of Andhra Pradesh and Thanjavur district of Tamil Nadu and very high salinities (50-60 ppt) in Tamil Nadu and Gujarat (CIBA, 2017)^[17].

India has a total brackish water area of 1.24 million ha and only 20% of it is being utilized. After West Bengal, Gujarat has the second largest area of brackish water resource. In Gujarat, shrimp culture is increasing popularity day after day. About 15,000 ha have recently been used for brackish water shrimp farming, and it is expected to expand further. In Gujarat, the major shrimp farming areas are South Gujarat (Valsad, Navsari, Surat and Bharuch), in which Surat is taking lead in brackish water aquaculture. Bhavnagar, Amreli and Jamnagar of Saurashtra coast recently catch attention and found suitable for shrimp culture.

Global warming has emerged as a serious issue worldwide. It is further predicted that global warming and a consequent increase in water temperature could impact aquaculture significantly. Exceeding temperature beyond optimal range has a direct impact on salinity due to increased evaporation. Salinity change is highly correlated with many other water parameters such as pH, dissolved oxygen, alkalinity, hardness, nitrite TAN etc. These water quality parameters directly affect growth and survival of organism especially in stressful hyperosmotic environment. Many parts of Gujarat and Tamilnadu faces sever salinity rise which reaches up to 60 ppt during extreme summer.

Probiotics are being applied extensively in shrimp farming to control the outbreak of disease. Several farmers are using probiotics at higher salinity too. But whether probiotics show their beneficial effects at higher salinity (especially >40 ppt) or not is still under the scanner.

Therefore, the experiment was designed to study effect of probiotic on the water quality parameters, vibrio count, growth of *Litopenaeus vannamei* reared with feed supplementation at higher salinities.

2. Materials and Methods

The materials used and methodology adopted for present research work is described as following.

2.1 Experimental Details

The study was conducted at Ratnakar Aquaculture farm, Rajula, District Amreli, Gujarat, India for 60 days in square plastic tanks with water volume of 1000 L in each tank. Experimental tanks were washed with disinfectant potassium permanganate solution (5 ppm) and sun-dried before the start of the experiment. All the tanks were half filled (around 500 liters) with water of desired salinity by mixing creek water and brine (Helm and Bourne, 2004)^[10]. Tank water was further disinfected by bleaching powder @

60 ppm. Water used for the culture was collected from the creek and brine water was collected from salt pan located at Victor village, Rajula. The experiment used shrimps of average size 2.9-3.0 gm. which were brought from the ponds of Ratnakar Aquaculture farm at Rajula and were transferred into the tank with different salinities (according to treatment) for acclimatization for 10 days before the start of research. The experiment included one control and three treatment groups in triplicates following Completely Randomized Design (CRD) such as 45 ppt salinity to rear shrimps with probiotic supplementation (control), 50 ppt salinity to rear shrimp with probiotic supplementation (T-1), 55 ppt salinity to rear shrimp with probiotic supplementation (T-3), 60 ppt salinity to rear shrimp with probiotic supplementation (T-3). All the experimental tanks supplied with 4 air stone-hoses type of diffuser system connected to 2 HP blowers for vigorous aeration. Aeration was provided throughout the experimental duration except during feeding and sampling. After 10 days of preparing the experimental tanks, only healthy and active shrimps mean initial weight of 3.03 g., 2.52g, 3.00g and 3.21 g, respectively were randomly distributed (stocked) in the experimental tanks with stocking density 45 numbers per tank. Animals were fed at the rate of 5% of their body weight during the experiment with commercially available feed (CP) having crude protein 35%, crude fat 5%, fiber 4%, and moisture 11%). Feeding frequency was four times a day similar to actual shrimp farm at 07:00 PM (morning), 11:00 PM, 3:00 AM (afternoon) and 7:00 AM (evening). Feed Probiotic (*Bacillus* spp) was incorporated into the feed along with binder @ 10gm/ kg. The same probiotic added feed was used in all treatments and control. Zero water exchange system (with intermittent water addition of desired salinity to maintain water volume in all tank) were followed during the whole experimental period of 60 days. Water was not exchanged throughout the experiment except for the compensation of evaporation loss.

2.2 Assessment of physico-chemical parameters

Different water quality parameters such as dissolved oxygen, salinity, pH and temperature were measured at regular intervals. pH and temperature were measures by using pH meter and thermometer (Hanna, Portugal). The salinity of water was measured using refractometer (Atago, Ltd, Japan) and expressed in ppt. The concentration of dissolved oxygen was determined weekly using the Winkler titration method and expressed as mg/l. Total ammonia nitrogen (TAN) and nitrite were measured weekly following standard protocol. Total ammonia concentration was measured weekly by using spectrophotometer at a wave length of 640 nm following Phenate method (APHA, 1998)^[4] and compared with the standard graph. The concentration of ammonia was expressed in mg/l. Nitrite level of water was measured as per Phenate method (APHA, 1998)^[4] at 543 nm using a spectrophotometer and it was compared with the standard graph of nitrite nitrogen concentration. The concentration of nitrite was expressed in mg/l.

2.3 Determination of fish growth and survival

To determine the growth performance of shrimp under experiment randomly 30% of shrimp were sampled from each tank treatment fortnightly without sacrifice. The weight of animal was measured by using electronic balance. The utmost care was taken while sampling to minimize stress on

the animal. The growth parameters like weight gain (%), feed conversion ratio (FCR) and survival (%) were determined as follows:

$$\text{Weight gain (\%)} = \frac{(\text{FW}-\text{IW})}{\text{IW}} \times 100/$$

$$\text{FCR} = \frac{\text{Dry weight of feed given (DW)}}{\text{Body weight gain (WW),}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final fish count}}{\text{Initial fish count}} \times 100$$

Where, FW = Final body weight, IW = Initial body weight, DW = Dry weight of feed and WW = Wet weight of fish.

2.4 Microbiological Parameters of water

Microbiological parameters were measured weekly following standard protocol. Vibrio count of water sample of each tank was estimated using TCBS culture media following the standard microbiological protocol of Vibrio culture. Green and yellow colonies were counted after 24 hours of incubation at 36 °C and presented as cfu/ml.

2.5 Statistical Analysis

The results were presented as mean $\pm$ standard error (SE). Prior to statistical analysis, all data were passed the test of normality (Shapiro-Wilk test) and homogeneity (Barlett's test). The data were then statistically analyzed by completely randomized experimental design (one-way analysis of variance, one-way ANOVA). If a significant interaction was detected between the main effects, one-way ANOVA was carried out followed by Duncan test to determine significant difference among treatments. Differences were considered significant at $P < 0.05$ and extremely significant at $P < 0.01$. Statistical analysis was carried out using SPSS version 24.

3. Results

3.1 Physico-chemical Parameters of water

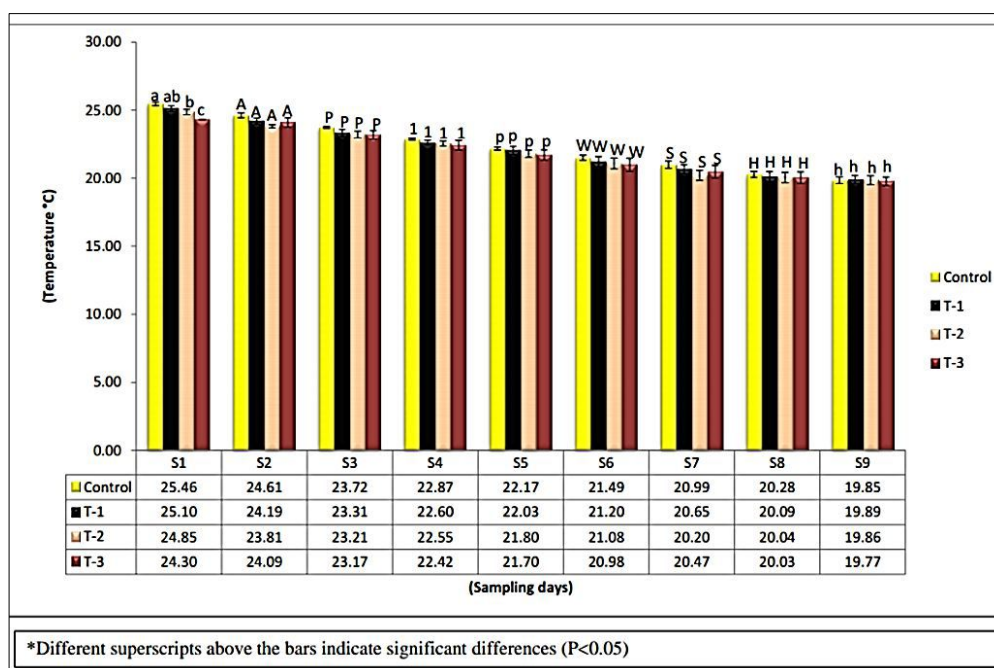
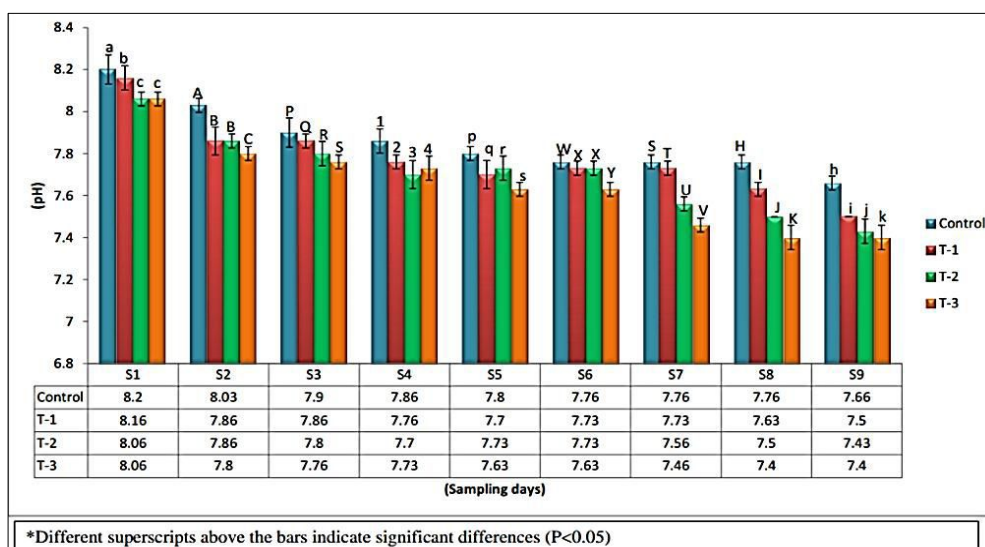
During the experiment, the water quality parameters were measured at regular intervals and presented comprehensively in table no. 1 (control and T-1) and table no. 2 (T-2 and T₃). Temperature plays a critical role in shrimp physiology and affects final growth performance hence monitored regularly. In present experiment, temperature is observed during the experiment was found to decrease from start (25.46 ± 0.128 °C) to the end (19.76 ± 0.304 °C). A decrease in temperature during the experimental period was observed in all tanks due to the onset of winter. Observations are presented in table 1 & 2 and graphically illustrated in fig. 1 The pH levels of water were found well within the desired range of shrimp culture throughout the experimental period in control all the treatments. The range of pH recorded during the experiment was 7.4 ± 0.057 to 8.2 ± 0.057 . Water pH was gradually decreased from day to day. Values of pH recorded throughout the experiment and presented in table 1 and 2 and graphically illustrated in fig. 2. Although DO level in water was within desired range for shrimp culture. A constant dissolve oxygen level of 5.00 ± 0.00 to 6.00 ± 0.00 mg/l is maintained during the experimental period in all the tanks. It was observed routinely and aerations were adjusted accordingly to maintain the optimum level. Constant salinity in each tank according to treatment and control (45, 50, 55 and 60 ppt) was maintained throughout the experimental period. It was precisely monitored and actions were executed in case of any fluctuations observed. Total Ammonia Nitrogen (TAN) concentration is critical for shrimp aquaculture and known to affect it significantly. TAN was estimated weekly and observations are presented in table no. 1 and 2 and the same is illustrated graphically in fig. 3. The range of the total ammonia nitrogen during the experiment was 0.10 ± 0.000 to 0.40 ± 0.100 mg/l. Nitrite was estimated weekly during the experimental period and observations are presented in table no. 1 and 2 and the same is illustrated graphically in fig. 4. The range of the nitrite during the experiment was 0.05 ± 0.000 to 0.20 ± 0.043 mg/l. TAN and Nitrite were gradually decreased last month at the end of the experiment.

Table 1: Physico chemical and microbiological parameters of water observed during the experiment in control (45 ppt) and T-1(50 ppt)

Treatment	DOC (Days)	Sampling	Temperature (°C)	pH	TAN (ppm)	Nitrite (ppm)	Yellow colony	Green colony
Control	1	S1	25.46 ± 0.128	8.20 ± 0.057	0.30 ± 0.000	0.15 ± 0.000	31.33 ± 3.095	0.33 ± 0.033
	8	S2	24.61 ± 0.176	8.03 ± 0.033	0.23 ± 0.008	0.13 ± 0.004	40.33 ± 4.137	0.66 ± 0.033
	15	S3	23.72 ± 0.073	7.90 ± 0.152	0.26 ± 0.001	0.13 ± 0.005	40.00 ± 5.930	0.66 ± 0.066
	23	S4	22.87 ± 0.058	7.86 ± 0.033	0.23 ± 0.007	0.13 ± 0.006	51.00 ± 4.072	1.00 ± 0.077
	30	S5	22.16 ± 0.123	7.80 ± 0.152	0.16 ± 0.001	0.07 ± 0.008	68.33 ± 8.860	1.00 ± 0.077
	38	S6	21.49 ± 0.187	7.76 ± 0.033	0.30 ± 0.000	0.15 ± 0.001	10.33 ± 0.583	0.00 ± 0.000
	45	S7	20.98 ± 0.268	7.76 ± 0.033	0.16 ± 0.003	0.08 ± 0.004	9.33 ± 0.409	0.00 ± 0.000
	52	S8	20.28 ± 0.220	7.76 ± 0.033	0.10 ± 0.000	0.05 ± 0.002	17.33 ± 0.870	0.33 ± 0.033
	60	S9	19.84 ± 0.249	7.66 ± 0.033	0.13 ± 0.002	0.06 ± 0.001	22.33 ± 1.005	0.33 ± 0.033
T-1	1	S1	25.10 ± 0.204	8.16 ± 0.066	0.30 ± 0.000	0.15 ± 0.000	38.00 ± 3.371	0.00 ± 0.000
	8	S2	24.18 ± 0.226	7.86 ± 0.033	0.33 ± 0.004	0.15 ± 0.003	32.33 ± 1.089	0.33 ± 0.033
	15	S3	23.31 ± 0.251	7.86 ± 0.033	0.23 ± 0.003	0.11 ± 0.005	43.66 ± 5.197	0.00 ± 0.000
	23	S4	22.60 ± 0.175	7.76 ± 0.033	0.23 ± 0.009	0.11 ± 0.006	49.66 ± 3.310	0.66 ± 0.033
	30	S5	22.03 ± 0.300	7.70 ± 0.057	0.30 ± 0.000	0.15 ± 0.006	38.66 ± 2.382	0.00 ± 0.000
	38	S6	21.19 ± 0.388	7.73 ± 0.033	0.30 ± 0.002	0.15 ± 0.002	22.33 ± 1.562	0.00 ± 0.000
	45	S7	20.64 ± 0.313	7.73 ± 0.033	0.13 ± 0.001	0.07 ± 0.003	23.33 ± 1.487	0.66 ± 0.066
	52	S8	20.09 ± 0.376	7.63 ± 0.033	0.13 ± 0.001	0.05 ± 0.004	24.00 ± 1.886	0.00 ± 0.000
	60	S9	19.89 ± 0.293	7.50 ± 0.000	0.10 ± 0.000	0.05 ± 0.002	9.33 ± 0.409	0.00 ± 0.000

Table 2 Physico chemical and microbiological parameters of water observed during the experiment in T-2 (55 ppt) and T-3 (60 ppt)

Treatment	DOC (Days)	Sampling	Temperature (°C)	pH	TAN (ppm)	Nitrite (ppm)	Yellow colony	Green colony
T-2	1	S1	24.85 ± 0.190	8.06 ± 0.033	0.30 ± 0.000	0.15 ± 0.000	53.00 ± 5.854	0.00 ± 0.000
	8	S2	23.80 ± 0.113	7.86 ± 0.033	0.40 ± 0.005	0.20 ± 0.004	35.66 ± 3.864	0.00 ± 0.000
	15	S3	23.20 ± 0.254	7.80 ± 0.057	0.26 ± 0.001	0.13 ± 0.004	56.33 ± 4.976	0.00 ± 0.000
	23	S4	22.54 ± 0.187	7.70 ± 0.057	0.23 ± 0.000	0.13 ± 0.006	68.33 ± 6.805	0.33 ± 0.033
	30	S5	21.79 ± 0.269	7.73 ± 0.033	0.23 ± 0.001	0.11 ± 0.008	78.33 ± 7.632	1.00 ± 0.077
	38	S6	21.08 ± 0.390	7.73 ± 0.033	0.26 ± 0.001	0.13 ± 0.005	15.33 ± 1.588	0.00 ± 0.000
	45	S7	20.20 ± 0.378	7.56 ± 0.033	0.13 ± 0.001	0.07 ± 0.002	36.00 ± 2.582	0.33 ± 0.033
	52	S8	20.04 ± 0.386	7.50 ± 0.000	0.13 ± 0.002	0.08 ± 0.001	43.66 ± 3.477	0.66 ± 0.066
	60	S9	19.85 ± 0.319	7.43 ± 0.066	0.13 ± 0.001	0.07 ± 0.004	23.33 ± 2.783	0.33 ± 0.033
T-3	1	S1	24.30 ± 0.000	8.06 ± 0.033	0.30 ± 0.000	0.15 ± 0.000	41.66 ± 4.168	0.00 ± 0.000
	8	S2	24.09 ± 0.353	7.80 ± 0.000	0.33 ± 0.004	0.16 ± 0.005	61.66 ± 4.702	0.00 ± 0.000
	15	S3	23.17 ± 0.303	7.76 ± 0.033	0.30 ± 0.000	0.15 ± 0.004	82.33 ± 5.962	0.00 ± 0.000
	23	S4	22.42 ± 0.353	7.73 ± 0.033	0.26 ± 0.008	0.13 ± 0.007	53.66 ± 5.020	0.66 ± 0.013
	30	S5	21.69 ± 0.378	7.63 ± 0.033	0.23 ± 0.002	0.11 ± 0.009	67.00 ± 5.383	0.00 ± 0.000
	38	S6	20.98 ± 0.477	7.63 ± 0.033	0.10 ± 0.000	0.05 ± 0.005	44.00 ± 3.358	0.00 ± 0.000
	45	S7	20.47 ± 0.465	7.46 ± 0.233	0.13 ± 0.001	0.07 ± 0.002	36.00 ± 2.163	0.00 ± 0.000
	52	S8	20.03 ± 0.433	7.40 ± 0.057	0.10 ± 0.000	0.05 ± 0.006	45.33 ± 4.009	0.00 ± 0.000
	60	S9	19.76 ± 0.304	7.40 ± 0.057	0.13 ± 0.002	0.08 ± 0.004	25.00 ± 3.110	0.00 ± 0.000

**Fig. 1** Weekly variation of temperature during the experiment**Fig 2:** Weekly variation of pH during the experiment

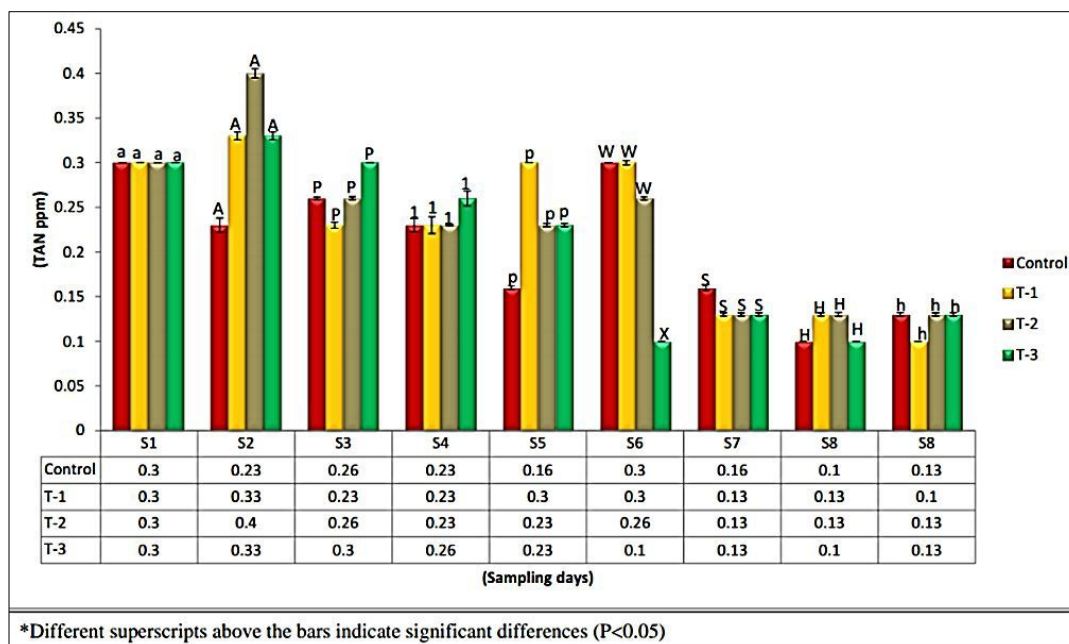


Fig 3: Weekly variation in TAN during the experiment

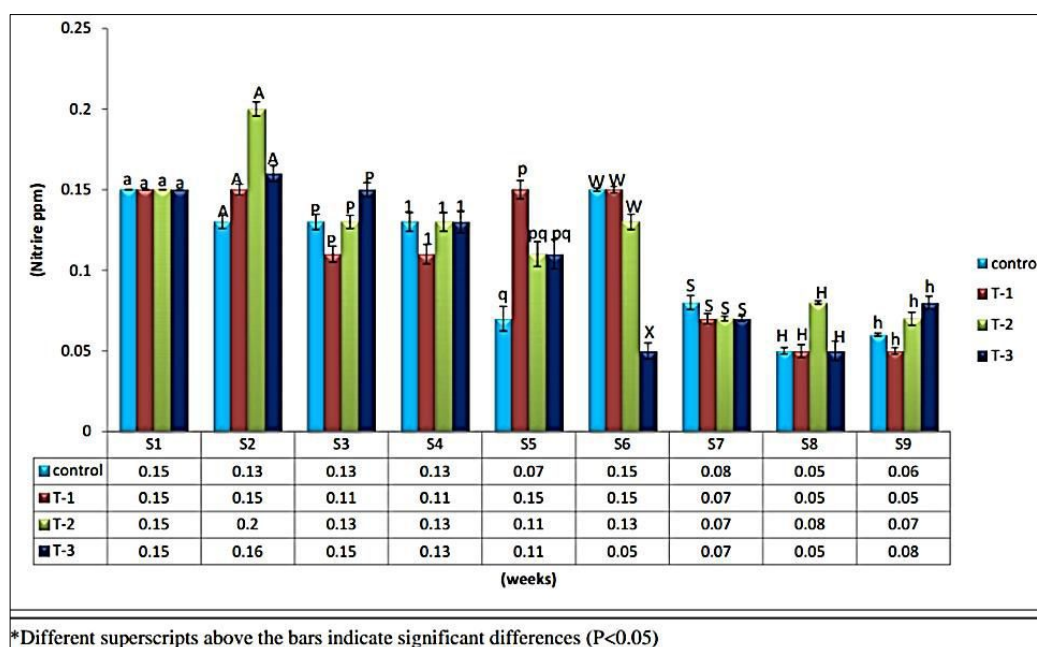


Fig 4: Weekly variation in nitrite during the experiment

3.2. Growth of experimental shrimp

The present study shrimp reared showed higher growth performance in control as compared to treatments (T₁, T₂ and T₃). Average body weight gain (ABWG) of shrimp reared under control and treatments are presented in table-3 and fig 15. Shrimp showed the highest average body weight

gain in control (3.96 ± 0.439 g) and minimum in T-3 (2.57 ± 0.001 g). Control showed better growth performance (ABWG) compared to treatments. There was significant ($P < 0.05$) difference of body weight (g) of shrimp found in control and treatments groups (T₂ and T₃) (Fig 14). Final weigh gain was also illustrated in fig 6.

Table 3: Growth performance and survival of *L. vannamei*

	Initial Weight	Final Weight
Control	3.04	7.06
T ₁	2.52	6.23
T ₂	3.00	5.63
T ₃	3.21	5.78

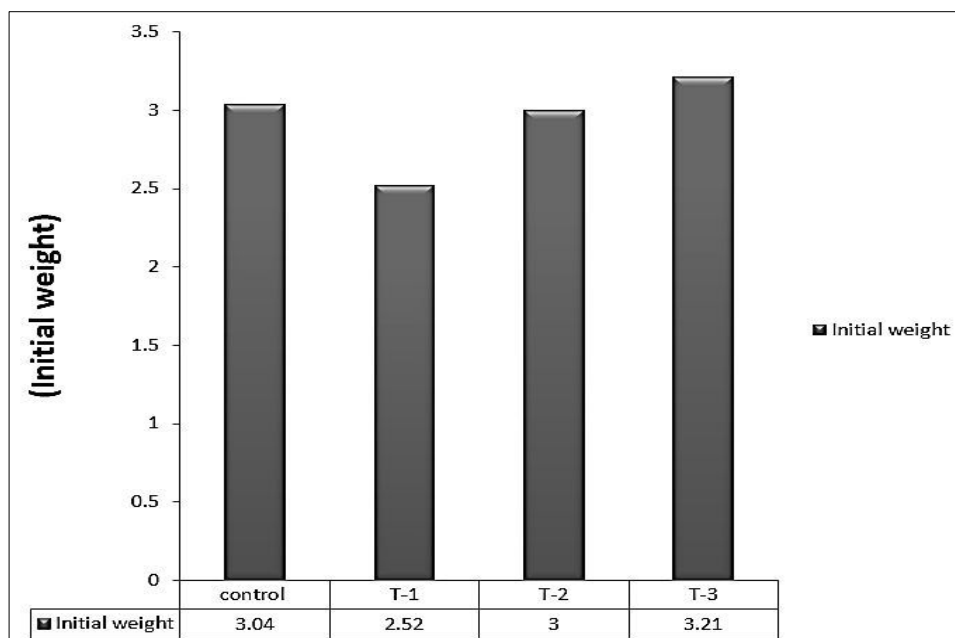


Fig 5: Initial body weight gain during the culture period

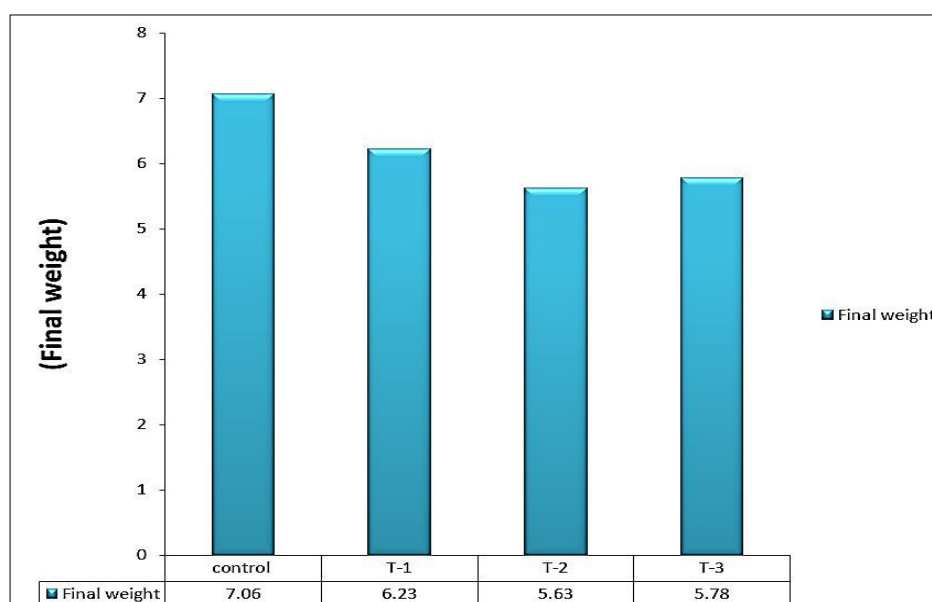


Fig 6: Final body weight gain during the culture period

3.3 Microbiological parameter

At the end of experiment, *Vibrio* count is significantly important microbiological parameters presented in table 1 and 2. the highest and lowest value of yellow colony was found in the range from 82.33 ± 2.962 to 9.33 ± 2.027 in control and T-3 respectively. The mean value of yellow colony in control, T-1, T-2 and T-3 were found to be 32.25 ± 3.219 , 32.35 ± 2.368 , 45.55 ± 4.396 and 50.73 ± 4.130 respectively and presented in figure 8. Yellow colony was found to be maximum during initial phase of the experiment in T-3 at S3 and minimum in control, T-2 at S7, S9. Here decrease in yellow colony along with decrease in salinity

was observed. At the end of experiment, yellow colony decrease as compared to initial phase of research. This decrease may be due to effect of probiotics.

Green and yellow colony (CFU/ml) was also found lower in control and T-1 compared to other treatment groups. The range of the green colony during the experiment was 0.00 ± 0.000 to 1.00 ± 0.577 cfu/ml. The mean value of green colony in control, T-1, T-2 and T-3 were found to be 0.48 ± 0.039 , 0.18 ± 0.148 , 0.29 ± 0.027 and 0.07 ± 0.001 respectively presented in figure 9. However, the level of the green colony was insignificant in all groups.

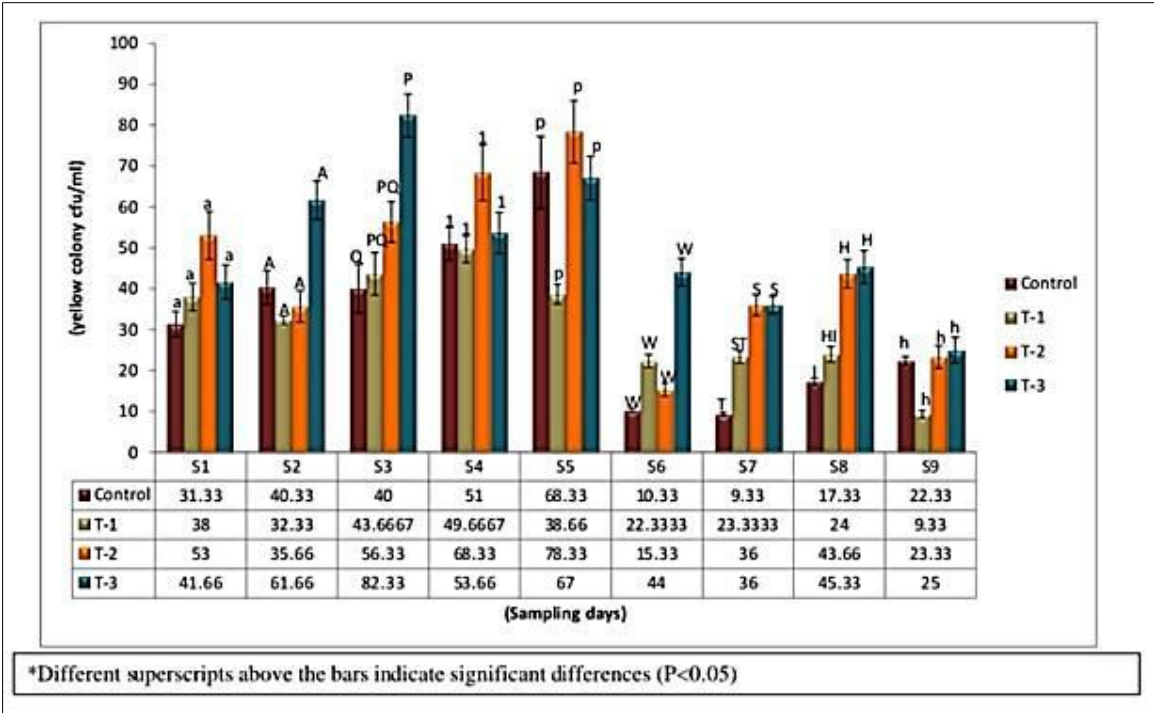


Fig 7: Weekly variation in yellow colony forming unit during the experiment

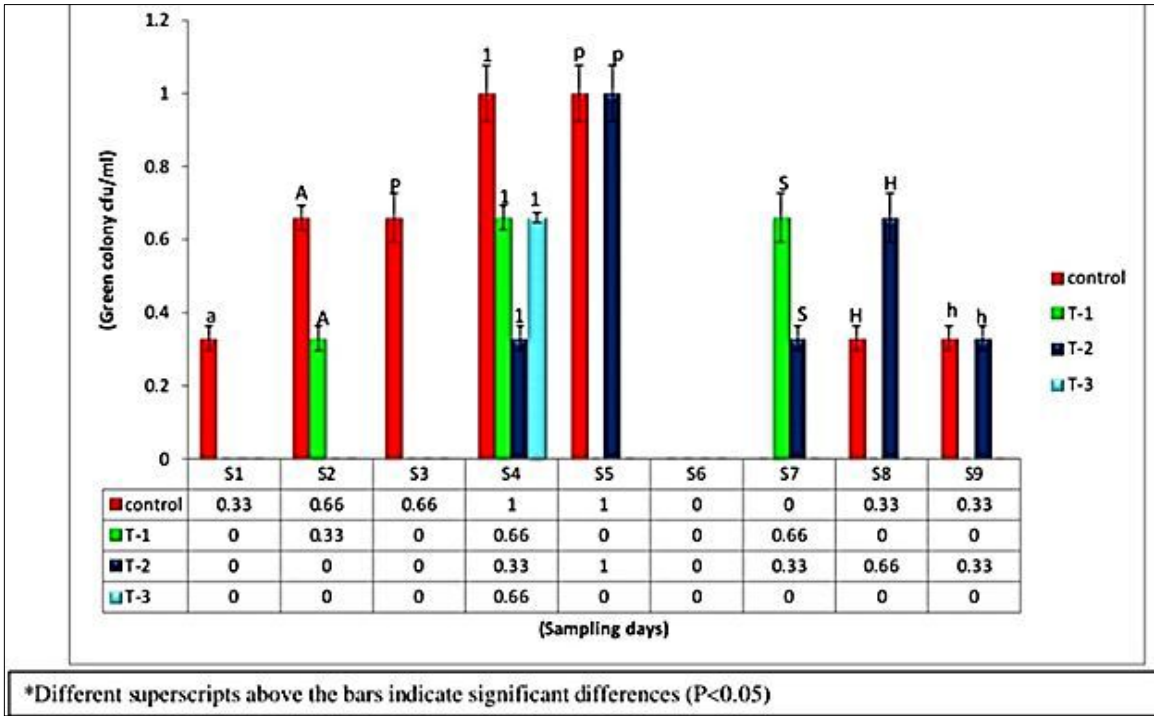


Fig 8: Weekly variations in green colony forming unit during the experiment

4. Discussion

Temperature is one of the most critical parameters in aquaculture systems affecting animal physiology as well as hydrological parameters. Hence it has a direct role on growth performance of the animal. In present experiment, temperature is found to decrease from start to the end due to the onset of winter. The optimal range of temperature for this species is between 30 and 34 °C. At 20 °C or lower growth virtually stops (Ravichandran *et al.*, 2012) [18]. Similar trends were observed here as the growth performance was poor towards the end of the experiment due to a decrease in temperature. There was no significant difference ($P > 0.05$) in temperature between different

treatments and control on different days of sampling except first sampling. pH is also considered as one of the important parameters which affect the physiology as well as soil water chemistry. pH in the range of 7.5-8.5 is considered as the most suitable for aquaculture. Stable day night pH with fluctuation not more than 0.5 is considered the most appropriate for shrimp farming (Ravichandran, 2012; Saraswathy, 2016) [18, 20]. In the present study except for few occasions pH was reported in the above said range. Mean pH value in all the treatments and control was between 7.5-8.0. Further the pattern of fluctuation in pH during the experimental period was found to be almost similar in all the treatments and control. The temperature decrease in also

corresponds to a pH also decrease. Hence in the present experiment, a clear decrease in pH was observed from the beginning towards the end. TAN concentration is critical for shrimp aquaculture and known to affect it significantly. Most of the efforts in shrimp farm are invested towards minimizing the level of TAN in water. TAN concentrations below 0.1 ppm are recommended in shrimp culture (CIBA, 2017) [7]. Here TAN level was found to be in the range of 0.10 ± 0.000 to 0.40 ± 0.100 mg/l. The mean value of total ammonia nitrogen in control, T-1, T-2 and T-3 were found to be 0.20 ± 0.002 , 0.22 ± 0.002 , 0.23 ± 0.001 and 0.20 ± 0.002 respectively. It is reported that the shrimp can tolerate the increased level of total ammonia nitrogen at higher salinity (Kır, M. and Öz, O., 2015) [12]. Hence here it can be further concluded that the growth observed even at higher TAN level was due to the same. The lower level of TAN was found in control which may be due to the better action of probiotics applied. Kim *et al.*, (2005) [11] reported that *Bacillus* strain has got potential to reduce TAN through nitrification and denitrification. The present study also indicates that TAN level decreased along with culture period which may be due to the better action of probiotic after acclimatization at higher salinity. Nitrite level < 1 mg/l (Ravichandran *et al.*, 2012) [18] is recommended for shrimp farming. Here nitrite level is reported below 1 ppm in all treatments and control. Here the decrease in the level of nitrite was observed from beginning till the end which may be due to better effect of probiotic. Dissolved oxygen is critical for aquaculture and level of 4.5 ppm or more is recommended for shrimp aquaculture (Ravichandran *et al.*, 2012) [18]. A level of 5.00 to 6.00 mg/l was maintained throughout the experimental period in all the tanks.

Bray *et al.*, (1994) [6] reported that *L. vannamei* grows the best in salinities below the isosmotic point. The different optimum salinities for growth in different species generally correspond to the differences in their osmotic capacities. Species with higher hyper-OC grow better in the lower salinities, whereas species with lower hyper-OC grows better in the highest salinities. The results obtained in our experiment showed that *L. vannamei* growth performance in treatment 1 was comparable with control without significant difference. Poor growth of shrimp in T₃ as compared to control may be attributed due to high saline water, as reported by Zhu *et al.*, (2004) [26] where they observed poor growth performance of *L. vannamei* juveniles at higher salinities. This result is in agreement with the finding of several researchers (Menz and Blake, 1980; Pante, 1990; Bray *et al.*, 1994; and McGraw *et al.*, 2002) [15, 17, 6, 14]. It may be due to low salinity and the effect of probiotics which provide a conducive environment for the better growth of the organisms. Our investigation was supported by the report of improved growth by use of gut probiotic in the culture of *P. monodon* by Shailender *et al.*, (2012) [21]. Kumar *et al.*, (2013) [13] reported that the use of *B. subtilis* as feed probiotics in shrimps improved growth performance. In present study, green colony was found to be ≥ 1 cfu/ml in all treatments and control which may be due to effect of probiotic activity as well as higher salinity. However, the level of the green colony was insignificant in all groups. The Soundarapandian *et al.*, (2010) [23] also reported higher green colony at 28 ppt as compared to seawater (35 ppt) in *P. monodon* culture. Balcázar *et al.*, (2007) [5] observed that application of *Bacillus subtilis* UTM 126, as feed probiotics in *L. vannamei* culture suppresses the proliferation of *Vibrio*

parahaemolyticus. Vieira *et al.*, (2010) [24] also reported similar effect of *Lactobacillus plantarum* (feed probiotic) on total bacterial count and *Vibrio* spp. and modified digestive tract providing increased resistance against *V. harveyi* infection in *L. vannamei*. No single symptoms of vibriosis disease were observed at the end of the experiment

Yellow colony was found to be maximum during initial phase of the experiment. In this studied decrease in yellow colony along with decrease in salinity. This decrease may be due to effect of probiotics. Further it was observed that decrease is more significant at lower salinity which may be due to better action of probiotics at lower salinity. Similar observations of reduced level of yellow colony after application of probiotics were made by several authors (Wang *et al.*, 2005; Far *et al.*, 2009) [25, 8]. Sivakumar *et al.*, (2012) [22] reported antibacterial activity of *Lactobacillus acidophilus* against pathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio Harvey* and *Vibrio alginolyticus*

5. Conclusion

White legs shrimp *L. vannamei* reared in higher salinities using probiotic as feed supplement for the sixty days resulted in better growth performance and survival at 45 to 50 ppt compared to other higher salinities. In addition, probiotic shows better effect at salinity up to 50 ppt as compared to beyond that. Hence farmers can be recommended to maintain a salinity of 50 or lower in the pond for a better harvest. The probiotic had also beneficial effect on yellow and green vibrio colony. Furthermore, better convenient environment due to probiotic helped to maintain the quality of water which had contributed to the better performance of the reared organism. Further research can be performed on the identification of different strains of *Vibrio* Spp. found at higher salinities and also identification of another probiotics bacterium which can show its effect at such higher salinities. Different probiotics combinations can be also identified through research for better performance at higher salinities

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