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Comparative Effect of *Pediococcus acidilactici* as Probiotic and Vitamin C on Survival, Growth Performance and Enzyme Activities of White Leg Shrimp (*Litopenaeus vannamei*)

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Abstract: A 90 days feeding trial was conducted to investigate and compare the effects of dietary probiotic, *Pediococcus acidilactici* and vitamin C on water quality, survival rate, growth performance and enzyme activities of Pacific white shrimp *Litopenaeus vannamei*. Shrimps (with initial weight of 2.63±0.07 g) were grown and fed by probiotic (initial concentration of 1×10^9 cfu g^{-1}) and vitamin C diets in separate Earthen ponds (1 g kg^{-1}). At the end of feeding trial, survival rate, growth parameters and enzyme activities were assessed. The best Survival Rate (SR), Weight Gain (WG), Feed Conversion Ratio (FCR), Specific Growth Rate (SGR) and Protein Efficiency Ratio (PER) were observed in shrimps fed *P. acidilactici*-diet (T.2) ($p<0.05$) than shrimps fed vitamin C (T.3) and control diet (T.1). Similarly, significantly better results were registered in T.3 compared to T.1 (T.2>T.3>T.1). Amylase, protease, lipase and alkaline phosphatase activities were significantly higher ($p<0.05$) in T.2 compared to T.3 and T.1. Amylase and alkaline phosphatase activities were recorded higher in vitamin C-treatment rather than control ($p<0.05$). Trypsin and chymotrypsin activities in shrimp were not significantly different ($p>0.05$) among probiotic, vitamin C treatments and control. The results from this study suggest that *P. acidilactici* supplemented could significantly increase survival rate, growth parameters and some digestive enzyme activities of *P. vannamei*. Proximate compositions in shrimp muscle are calculated. Researchers found that crude fat had the lower rate in T.2 and T.3 rather than T.1 ($p<0.05$). Ash had significantly higher in T.2 compared to T.1 and T.3 ($p<0.05$) whereas there were no different significant in crude protein and moisture among the treatments ($p>0.05$). These findings demonstrate potential for *P. acidilactici* to enhance the survival, growth performance and some enzyme activities in white shrimp.

Key words: *Litopenaeus vannamei*, *Pediococcus acidilactici*, probiotic, vitamin C, growth, enzyme activity

INTRODUCTION

White leg shrimp, *Litopenaeus vannamei* is distributed along the pacific coasts of Central and South America and is farmed as the main species in some tropical countries extensively, like South of Iran. Along with development of shrimp industry, the overuse of various antibiotic drugs and disinfectants in farms to increase efficiency and prevent the outbreaks has caused the evolution of resistant strains of bacteria (Esiobu *et al*., 2002). In recent years, the use of proper natural supplements instead of chemotherapeutics has been considered because of sympathetic effects to the environment. Use of probiotics or effective bacteria that control pathogens is considered as an appropriate alternative to antibiotic treatment.

A probiotic is an entire microorganism or components of a microorganism that is beneficial to the health of the host (Irianto and Austin, 2002). The major aim of using probiotics is to maintain or reestablish a favorable relationship between friendly and pathogenic microorganisms that constitute the flora of intestinal or skin mucus of aquatic animals (Ali, 2000). The use of probiotic bacteria is now commonly accepted in shrimp farming as feed additives or are brought into pond water (Leyva-Madrigal *et al*., 2011; Wang and Gu, 2010; Liu *et al*., 2010).

Pediococcus acidilactici is a species of gram positive coccus and being able to colonize the digestive tract (Klaenhammer, 1993). Studies showing such effects on aquatic species have generally assessed *Pediococcus acidilactici* (Castex *et al*., 2008, 2009).

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According to Niu *et al*. (2009) vitamin C is an essential nutrient for penaeid shrimp. It relieves in the retention and growth of shrimp and works in the organism as a cofactor for some biochemical reactions (Wang and Xu, 2006). Fishes and crustaceans do not have the enzyme L-gulonolactone oxidase which is responsible for synthesis of vitamin C, thus they are unable of biosynthesis of ascorbic acid (Wilson, 1973).

The principal objective of this study was to examine and compare the growth parameters, digestive enzyme activities and muscle composition of the white shrimp in response to the probiotic *Pediococcus acidilactici* and vitamin C supplemented in the diet as an option to upgrade the efficiency of shrimp farming in Iran.

MATERIALS AND METHODS

Experimental procedure and determination of water quality conditions: The shrimps *P. vannamei* used in the experiments were reared intensively in Earthen ponds (1 Hac) in Abadan, South of Iran. The ponds were stocked with 20 days Post-Larvae (PL20) at a density of 30 pL/m^2. It usually takes 3 and 4 weeks for the post-larvae to reach 1 g. Thereafter, the growth rate is estimated to be approximately 1-1.2 g per week. The experiment was conducted about 90 days in 2013 from June to September. Shrimps were fed four times a day with commercial pellets (Havourash Co., Boushehr, Iran). The feeding rate was determined according to the weekly calculated body weight and amount of residual meal in the feeding tray 2 h after feeding. The water quality parameters such as temperature, pH and salinity were measured using thermometer, pH meter and refractometer, twice a day, respectively. Dissolved oxygen and Total Ammonia-N (TAN) were analyzed immediately after sampling following the methods described by Clesceri (1998).

Bacterial strain and vitamin C and shrimp feed: The probiotic was prepared commercially and tested, Bactocell$^+$ (Lallemand Animal Nutrition) that formulated with *P. acidilactici* MA 18/5M (Institute Pasteur, Paris, France). The treatment applied at an initial concentration of 1×10^9 cfu g^{-1}. Ascorbic acid was procured from E-MERCK Darmstadt, Germany. The basal diet formulation is given in Table 1. At first, the probiotic concentration in the feed was checked by counting bacteria strains on MRS plates using serial dilution. The concentrations used in the experiment 1 g kg^{-1} of the probiotic and vitamin C was coated on the pellets using 2% of fish oil as a carrier. This procedure was done daily. One group served as the control (T.1) and was fed un-supplemented diet and the other two groups were fed by *P. acidilactici* (T.2) and vitamin C (T.3)-supplemented during the entire experimental period.

Table 1: Formulation and chemical proximate composition of the basal diet

Ingredients	Percentage
Soybean meal	20
Fish meal	30
Squid meal	6
shrimp's head meal	5
Wheat flour	25
Wheat bran	7
Soy lecithin	2
Cholesterol	1
Vitamin-mineral mix	4
Crude protein	34
Crude lipid	8
Crude fiber	4
Ash	14
Moisture	10

Vitamin-mineral premix: (mg kg^{-1}) vitamin B_{12}: 0.2; nicotinic acid: 70; riboflavin: 65; pantothenic acid: 150; menadione: 40; folic acid: 7.5; biotin: 0.9; thiamin hydrochloride: 20; pyridoxine: 40; thiamin: 25; inositol: 350; astaxanthin: 60; choline chloride: 20.0; vitamin C: 200, vitamin A: 5000 IU; vitamin D_3: 1500 IU; vitamin E: 5000 IU. Nicotinamide: 6 g; choline chloride: 75 g; calcium pantothenate: 0.5 g; Mn: 15 g; Zn: 5 g; Fe: 4 g; Cu: 1 g; iodine: 0.3 g; Co: 0.3 g

Measurement of survival and growth: A total of 100 shrimps were randomly sampled from each pond every week to determine wet weight, total length and carapace length. At the end of experiment, the survival rate was determined. Survival Rate (SR), Weight Gain (WG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) were calculated according to the method of Felix and Sudharsan (2004):

$$SR\ (\%) = \left(\frac{\text{Final numbers}}{\text{Initial numbers}}\right)\times 100$$

$$WG\ (\%) = \left(\frac{\text{Final weight (g)-Initial weight}}{\text{Initial weight}}\right)\times 100$$

$$FCR = \frac{\text{Total feed given}}{\text{Weight gain}}$$

$$SGR\ (\%) = \left(\frac{\text{ln final weight-ln initial weight}}{\text{Experimental days}}\right)\times 100$$

$$PER = \frac{\text{Net weight gain}}{\text{Protein in feed applied}}$$

At the end of period, 100 shrimps (average weight = 18.5 g) were collected from each treatment for enzyme assays. The shrimps transported to the laboratory under controlled conditions (continuous aeration and reduction of stress) and kept in aquariums. To prevent external pollution whereas removing organs, the surface of shrimps was cleaned using 70% ethanol. Afterward, shrimps were dissected to remove gut and immediately frozen at -70°C in sterile tube until enzyme assays were done.

Protein and enzyme assays: The total soluble protein content was measured in diluted homogenates by the Bradford Method (Bradford, 1976) using bovine serum albumin as a standard. The α-amylase activity was assayed by Worthington (1991) Method using 1% soluble starch (SIGMA) as substrate in phosphate buffer 20 mM and react it with 3,5-dinitrosalicylic acid. One unit of enzymatic activity was defined as 1 mg of maltose liberated in 15 min at 37°C. Total protease activity was assayed according to a method modified from that by Anson (1938) using casein (Merck) as the substrate and react it with Folin-Ciocalteurs phenol reagent (Merck, Germany) and tyrosine (Fluka, Switzerland) was used as a standard. Lipase activity was determined based on the measurement of fatty acid release due to enzymatic hydrolysis of triglicerides in stabilized emulsion of olive oil (Fluka) (Worthington, 1991). Trypsin was assayed by its amidase activity using N-Benzoyl L-Arginine Ethyl Ester (BAEE) as substrate following the method by Garcia-Carreno *et al*. (1994). Assays were initiated by the addition of sample supernatant and the release of N-Benzoyl L-Arginine was measured at 253 nm over 15 min. A positive control of 3 mg mL^{-1} trypsin (SIGMA) was used. Chymotrypsin was measured by using Benzoyl-L-Tirosine Ethyl Ester (BTEE) as substrate. The reaction rate is determined by measuring an increase in absorbance at 256 nm resulting from the hydrolysis of substrate (Erlanger *et al*., 1961). The alkaline phosphatase activity was assayed by Walter and Schutt (1974) Method, utilizing diethanolamine and p-Nitrophenyl Phosphate, then record the increase in 405 nm for 5 min. Enzyme activities were measured as the change in absorbance using a Shimadzu 160-UV spectrophotometer and expressed as specific activity (U/mg protein).

Approximate composition of experimental diet and muscle: In the termination of experiment, 30 samples from each pond were homogenized and analyzed. The proximate composition of the experimental diet and the muscle contents were determined following the standard methods by AOAC (2005). The moisture content was determined by drying at 105°C to a constant weight and the difference in weight of the sample indicated the moisture content. Protein content was determined by the Kjeldahl Method (AOAC, 2005). Crude lipid content was performed by acid digestion prior to petroleum ether extraction (40-60°C) in a Soxtec System (AOAC, 2005). These analyses were performed in triplicate. Ash content was determined by incinerating the samples in a muffle furnace at 600°C for 6 h. Crude fiber was determined based on the weight loss on ignition of the oven dried residue remaining after sequential digestion of a sample with H_2SO_4 and NaOH solution using Fibretec (Tecator, Sweden).

Statistical analysis: Differences among the means were tested for statistical significance using one-way Analysis of Variance (ANOVA) followed by Duncan's multiple range tests. A significance level of $p<0.05$ was used in the current study. All statistics were performed using SPSS Version 21. Drawing the figures was done in excel.

RESULTS

Physicochemical parameters: The water quality parameters during experimental period are shown in Table 2. Due to continuous aeration, the dissolved oxygen level was held constant. At the second month, the temperature was increased and then slightly decreased according to the nearing fall season. The salinity and pH were shown slightly reduced. And Total Ammonia-N (TAN) level had increased. There was no obvious effect of probiotic on the quality of water in the treatment groups.

No significant difference among the treatments was observed in D.O during the experimental period whereas other items have significant difference ($p<0.05$) between various months but there was no significant difference ($p>0.05$) among the treatments was shown in treatments during the experimental period (Table 2).

Growth performance: Growth performances of *P. vannamei* over the time period are presented in Table 3. The average initial weight was registered in

Table 2: The average physicochemical parameters in the experimental period (mean±SE)

Groups	Parameters	Dissolved oxygen (mg L^{-1})	Temperature (°C)	Salinity (ppt)	pH	Total ammonia-N (μg L^{-1})
1st month	T.1	7.2±0.290^{a}	26.66±1.93^{b}	23.6±1.510^{c}	8.41±0.151^{c}	101.31±8.500^{a}
	T.2	7.0±0.410^{a}	25.92±1.75^{b}	23.1±1.110^{c}	8.5±0.1450^{c}	104.34±9.200^{a}
	T.3	6.9±0.310^{a}	26.05±1.55^{b}	23.9±0.900^{c}	8.44±0.100^{c}	100.00±10.01^{a}
2nd month	T.1	6.8±0.160^{a}	27.78±2.11^{c}	19.68±1.51^{b}	8.22±0.110^{b}	104.10±7.520^{a}
	T.2	7.0±.0.27^{a}	27.53±1.96^{c}	19.01±1.59^{b}	8.2±0.2230^{b}	105.12±8.320^{a}
	T.3	7.0±0.190^{a}	28.05±1.51^{c}	19.23±1.23^{b}	8.25±0.170^{b}	102.15±7.960^{a}
3rd month	T.1	6.9±0.230^{a}	25.14±1.35^{a}	18.23±1.02^{a}	8.14±0.090^{a}	110.01±7.320^{b}
	T.2	6.7±.0410^{a}	25.55±1.22^{a}	17.95±1.25^{a}	8.15±0.110^{a}	112.21±9.850^{b}
	T.3	6.8±0.620^{a}	25.17±2.01^{a}	18.5±1.650^{a}	8.12±0.210^{a}	108.54±7.610^{b}

The means with no superscript letter in common per factor indicate significant difference

Table 3: Growth performance of *P. vannamei* reared on the experimental and control condition

Groups	Growth parameters	Weight (g)	WG (%)	FCR	SGR	PER	Length (cm)	Carapace length (cm)	Survival rate (%)
1st month	T.1	7.087 ± 0.04^{aA}	171.530 ± 5.660^{aA}	2.02 ± 0.12^{bA}	3.33 ± 0.12^{aC}	2.55 ± 0.48^{aA}	4.278 ± 0.2^{aA}	1.51 ± 0.2^{bA}	-
	T.2	8.170 ± 0.11^{cA}	215.444 ± 7.150^{cA}	1.70 ± 0.25^{aA}	3.83 ± 0.14^{cC}	3.17 ± 0.55^{cB}	4.431 ± 0.1^{bA}	1.60 ± 0.3^{cA}	-
	T.3	7.931 ± 0.06^{bA}	200.416 ± 4.540^{bA}	1.97 ± 0.22^{bA}	3.67 ± 0.20^{bC}	2.71 ± 360^{bB}	4.25 ± 0.24^{aA}	1.49 ± 0.3^{aA}	-
2nd month	T.1	12.686 ± 0.48^{bB}	386.050 ± 10.30^{bB}	2.17 ± 0.41^{cB}	2.64 ± 0.31^{bB}	2.66 ± 0.54^{bB}	9.08 ± 0.26^{aB}	1.96 ± 0.3^{aB}	-
	T.2	13.940 ± 0.38^{cB}	438.220 ± 9.780^{cB}	1.91 ± 0.25^{aC}	2.81 ± 0.41^{cB}	3.61 ± 0.14^{cC}	9.89 ± 0.45^{bB}	2.11 ± 0.2^{cB}	-
	T.3	11.840 ± 0.23^{aB}	348.480 ± 10.52^{aB}	2.07 ± 0.14^{bB}	2.5 ± 0.460^{aB}	2.17 ± 0.35^{aA}	8.99 ± 0.49^{aB}	2.01 ± 0.3^{bB}	-
3rd month	T.1	16.611 ± 0.11^{aC}	536.430 ± 6.450^{aC}	2.21 ± 0.15^{cC}	2.06 ± 0.04^{aA}	2.71 ± 0.13^{aC}	13.33 ± 0.63^{bC}	2.51 ± 0.2^{aC}	84.1 ± 4.12^{a}
	T.2	20.087 ± 0.21^{bC}	675.550 ± 7.870^{bC}	1.73 ± 0.17^{aB}	2.28 ± 0.08^{bA}	3.51 ± 0.17^{bB}	14.10 ± 0.59^{bC}	2.75 ± 0.2^{bC}	91.0 ± 3.54^{c}
	T.3	16.844 ± 0.12^{aC}	538.030 ± 8.180^{aC}	2.15 ± 0.11^{bC}	2.06 ± 0.06^{aA}	2.75 ± 0.09^{aB}	13.08 ± 0.56^{aC}	2.54 ± 0.2^{aC}	85.6 ± 3.41^{b}

The means with no superscript letter in common per factor indicate significant difference. Lowercase and uppercase letters indicate difference between the treatments and the months, respectively

treatments (T.1 = 2.610±0.2; T.2 = 2.59±0.17 and T.3 = 2.64±0.12 g). At the beginning of the trial there were no different significant between groups in initial weights ($p>0.05$).

In the 1st month, researchers had differed significant in the body weight between the groups ($p>0.05$) so that in the 1st and 2nd month, researchers had calculated the lowest rate in T.1 and T.3, respectively. In the last month, T.2 had differed significantly ($p<0.05$) to two others groups whereas T.1 and T.3 did not differ significantly to each other ($p>0.05$). The same results in body weight were found for weight gain. In the 1st and 2nd month, the minimal WG were investigated in T.1 and T.3, respectively. Whereas T.2 showed the maximum weight gain in all periods ($p<0.05$). The best FCR was observed in T.2 which was significantly lower from control and T.3. Similarly, the best performances in SGR and PER were registered in T.2 compared to two other groups. In T.2, SGR and PER were shown differed significant compared to T.1 and T.3 ($p<0.05$) in all periods. In the 1st 2 months, there was statistically significant difference between groups whereas in the last month was found no significant difference between T.1 and T.3.

The length had differed significantly in the 1st month among the groups that this condition was observed in the whole experimental period ($p<0.05$). Similarly, significantly different in the carapace length was registered in the 1st and 2nd month ($p<0.05$). Although in the last month, T.2 had differed significantly to T.1 and T.3. At the end of experiment, the survival rate in T.2 (91±3.54) was significantly higher ($p<0.05$) than T.3 (85.6±3.41) and T.1 (84.1±4.12). Moreover, T.3 had differed significant to the control ($p<0.05$).

Digestive enzyme activities: At culture of 90 days, 5 pancreatic (α-amylase, protease, lipase, trypsin and chymotrypsin) and 1 intestine (alkaline phosphatase) enzyme activities were studied. Specific activity of amylase in the gastrointestinal tract was significantly higher ($p<0.05$) in T.2 (1.381±0.15 U mg^{-1} protein) compared with control (0.722±0.1 U mg^{-1} protein) and T.3 (0.758±0.09 U mg^{-1} protein) (Fig. 1) and there was

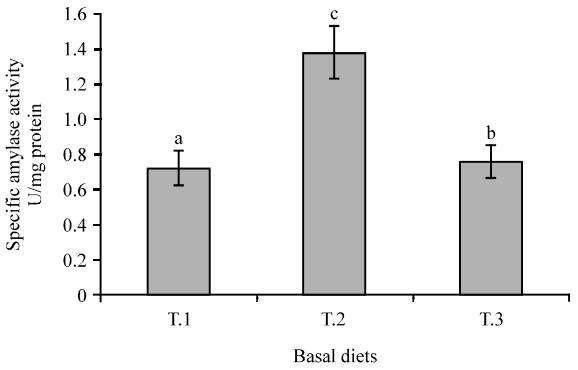

Fig. 1: Specific amylase activity in *P. vannamei* fed with basal diet (T.1), *Pediococcus acidilactici* (T.2) and vitamin C (T.3) at the end of 90 days culture. Error bars represent mean±SE. Superscripts are significantly different ($p<0.05$)

differed significantly among T.3 and T.2. T.2 treatment (1.34±0.06 U mg^{-1} protein) showed a statistically significant increase in the protease activity in comparison to T.1 (1.01±0.1 U mg^{-1} protein) and T.3 (1.04±0.05 U mg^{-1} protein) ($p<0.05$) but there was no significant difference between T.1 and T.3 (Fig. 2). Similarly, significantly higher lipase activity was registered in T.2 (0.06±0.006 U mg^{-1} protein) compared to T.3 (0.035±0.005 U mg^{-1} protein) and T.1 (0.03±0.007 U mg^{-1} protein) ($p<0.05$), respectively while no significant difference was noticed among T.1 and T.3 ($p>0.05$) (Fig. 3). Trypsin and Chymotrypsin activities in the gastrointestinal tract of vannamei in the treated and control groups were relatively similar (Fig. 4 and 5). There was no significant difference ($p>0.05$) in activities of both enzymes between the treated and control groups, even lower trypsin and chymotrypsin activities were observed in T.2 (Trypsin in T.1 = 0.0142± 0.001, T.2 = 0.141±0.002, T.3 = 0.141±0.003 U mg^{-1} protein and chymotrypsin in T.1 = 0.177±0.0009, T.2 = 0.176±0.002, T.3 = 0.0178±0.0008 U mg^{-1} protein) than that of the control and T.3.

Alkaline phosphatase activity was significantly higher ($p<0.05$) in T.2 (12.3±1.3U mg^{-1} protein) compared

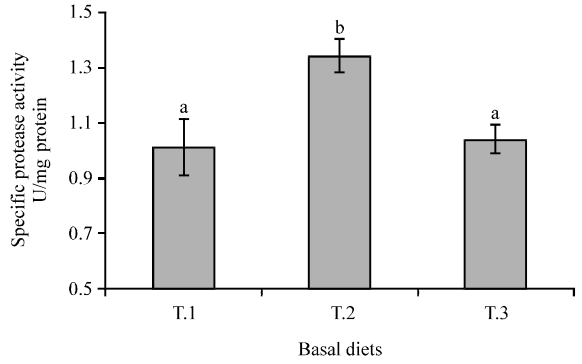

Fig. 2: Specific protease activity in *P. vannamei* fed with basal diet (T.1), *Pediococcus acidilactici* (T.2) and vitamin C (T.3) at the end of 90 days culture. Error bars represent mean±SE. Superscripts are significantly different ($p<0.05$)

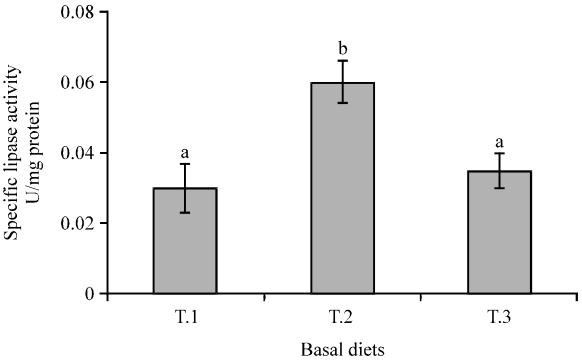

Fig. 3: Specific lipase activity in *P. vannamei* fed with basal diet (T.1), *Pediococcus acidilactici* (T.2) and vitamin C (T.3) at the end of 90 days culture. Error bars represent mean±SE. Superscripts are significantly different ($p<0.05$)

with T.3 (7.1±2U mg^{-1} protein) and control (5.5±0.4U mg^{-1} protein), respectively (Fig. 6). Furthermore, there was slightly differed significant among T.3 and T.2 ($p<0.05$).

Proximate composition of *P. vannamei*: The results of proximate composition are presented in Table 4. Non-significant difference was found in crude protein among the groups ($p>0.05$). The group that received probiotic cells had a significantly lower 1.83±0.11 ($p<0.05$) crude fat composition in the muscle than that of the control (2.08±0.12) and vitamin C (2.03±0.14) groups. However, there was no significant difference ($p>0.05$) in crude fat composition between T.1 and T.3 shrimp. T.2 treatment (1.41±0.21) showed a statistically significant increase in the ash content in comparison to T.1 (1.3±0.14) and T.3 (1.34±0.22) ($p<0.05$) but there was no significant

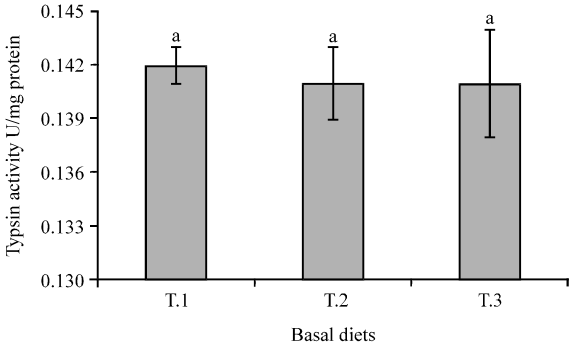

Fig. 4: Specific trypsin activity in *P. vannamei* fed with basal diet (T.1), *Pediococcus acidilactici* (T.2) and vitamin C (T.3) at the end of 90 days culture. Error bars represent mean±SE. Superscripts are significantly different ($p<0.05$)

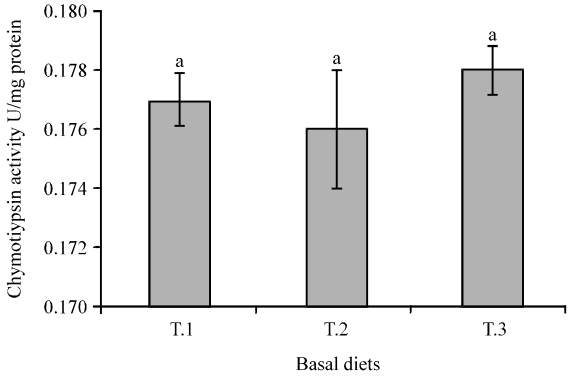

Fig. 5: Specific chymotrypsin activity in *P. vannamei* fed with basal diet (T.1), *Pediococcus acidilactici* (T.2) and vitamin C (T.3) at the end of 90 days culture. Error bars represent mean±SE. Superscripts are significantly different ($p<0.05$)

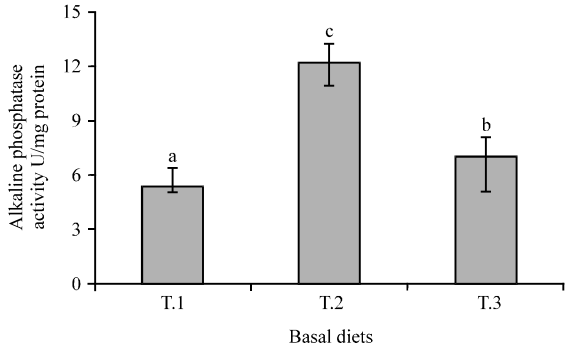

Fig. 6: Alkaline phosphatase activity in *P. vannamei* fed with basal diet (T.1), *Pediococcus acidilactici* (T.2) and vitamin C (T.3) at the end of 90 days culture. Error bars represent mean±SE. Superscripts are significantly different ($p<0.05$)

Table 4: Proximate composition (%) in the muscle of *P. vannamei*

Items	T.1	T.2	T.3
Crude protein	18.53±0.83[a]	19.52±0.58[a]	18.50±1.01[a]
Crude fat	2.08±0.12[b]	1.83±0.11[a]	2.03±0.14[a]
Ash	1.30±0.14[a]	1.51±0.21[b]	1.34±0.22[a]
Moisture	77.01±1.50[a]	75.65±0.81[a]	76.91±1.48[a]

difference between T.1 and T.3 ($p>0.05$). As for the muscle compositions of moisture, there was no significant difference ($p<0.05$) among all the groups.

DISCUSSION

In most researches, the explanation for the mechanisms of act of probiotics is largely based on laboratory experiments and studies that have been conducted in earthen are scare whereas *in vivo* physiology might be different from the metabolic process *in vitro*. The use of probiotics for improvement the microbiota is a beneficial action in aquaculture that cause to improvement of nutrition of host species via the production of supplemental digestive enzymes and raise the growth and feed efficiency, prevention of intestinal disorders and digestion of anti-nutritional parameters existent in the components (Balcazar *et al*., 2007).

It was reported that the use of probiotics in *P. vannamei* ponds had favorable effects on water quality and probiotics could significantly reduce the concentrations of nitrogen and phosphorus in pond water compared with the control (Wang *et al*., 2005). All the environmental variables during the trial were within the suitable range for the culture of white shrimp. Indeed, there was no distinct effect of probiotic on the water quality during the study. This result may be explained by the good water quality (by continuous aeration) in this study in contrast to theirs. In fact, the dissolved oxygen quantities, pH values and concentrations of ammonium were kept in acceptable ranges (Boyd and Tucker, 1998) which is consistent with the results by Zhou *et al*. (2009).

The bacteria survive and endure the conditions of the shrimp digestive tract which is an important feature, since it is accepted that probiotic activity is often linked to the assumed viability of the strain inside the digestive tract of the host (Panigrahi *et al*., 2005). It was clear from this study that the application of probiotic, *P. acidilactici* had beneficial effects on the survival rate of *P. vannamei* compared to the control and vitamin C treatments ($p<0.05$) were found during 90 days trial. The enhancement of growth parameters and promotion some digestive enzymes were registered in the T.2 treatment than in the control (T.1) and vitamin C treatment (T.3) that is consistent with the results by Liu *et al*. (2010) and Javadi *et al*. (2011). Although, the exact mechanism of action is not well understood. One of the explanations could be related to the action of competitive exclusion by which probiotics may create a hostile environment for pathogen colonization. In some cases, probiotics have not shown any positive effects on growth parameters or survival rate or any promising result on the cultural condition (Alavandi *et al*., 2004).

The mean value observed for survival rate in T.3 (vitamin C treatment) was 85.6±3.41 which is calculated more than other studies on *P. vannamei*. For example, Ferraz *et al*. (2012) were reported the survival rate was 81.96%. However in a study on *P. vannamei* fed by enriched artemia with vitamin C, the survival rate was reported 96.1±1.6 (Wang and Xu, 2006). In a study, Ferraz *et al*. (2012) that studied the effect of vitamin C on *P. vannamei*, FCR, SGR and PER were recorded 1.41, 1.28 and 2.02, respectively that had observed the better SGR and PER in the study. Although, it has been proven that vitamin C is less important than increasing growth and survival.

Probiotics may improve digestive activity by synthesis of vitamins, cofactors or by improving enzymatic activities (Gatesoupe, 1999). These properties could have contributed to the weight increase seen in T.2 compared to T.1 and T.3 (Table 4) by improving some enzyme activity in the shrimp digestive tract. In this study, α-amylase and alkaline phosphatase activities had the highest rate in the T.2 treatment that treated with probiotic, *P. acidilactici* and then in T.3 (vitamin C) were shown differ significant to T.1 (control) (T.2>T.3>T.1), however, in other enzyme activities there was no significant difference between T.3 and T.1. Non-significant difference was found in trypsin and chymotrypsin activities among the groups. The presence of *P. acidilactici* from the faeces indicated that it reached to the digestive system of shrimps but this does not confirm that the bacteria colonized in the gut.

Bacteria, particularly members of the genus *Bacillus* secreted a wide range of exoenzymes (Moriarty, 1998). It is likely that the low rate of increase in enzyme activities was due to use of *Pediococcus* sp. It is not possible to distinguish between activity due to enzymes synthesized by the shrimp and that due to enzymes synthesized by the probiotic strains colonized in the digestive tract. However, the exogenous enzymes produced by the probiotic would represent at most only a small contribution to the total enzyme activity of the gut (Ziaei-Nejad *et al*., 2006).

The observed increases in specific activities of digestive enzymes in probiotic treatments may have led to enhanced digestion and increased absorption of food which in turn contributed to the improved survival and growth including improved Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR).

In this study, the higher level of total digestive enzyme activity was recorded where the better growth performances were observed compared to control. Similar results have been reported by Ziaei-Nejad *et al.* (2006) who observed a higher digestive enzyme activity in *Fenneropenaeus indicus* treated with *Bacillus* sp. than the controls.

Proximate compositions in shrimp muscle are affected by several factors such as species, growth stage, feed and season (Karakoltsidis *et al.*, 1995). In present study, the proximate contents found for *P. vannamei* was within the range of other shrimp species (Turan *et al.*, 2011). The protein was found as the major constituent, indicating that shrimp muscle can be a good source of amino acids. It has been reported that protein content of shrimp ranged between 17 and 21% depending on shrimp species (Sriket *et al.*, 2007). In this study, the protein content was about 19% and found not statistically significant difference between the different treatments. Similar findings were recorded by Silva and Chamul (2000). Lipids are highly efficient as sources of energy and they contain twice the energy of carbohydrates and proteins. (Okuzumi and Fujii, 2000). In this investigation, there was a significant higher different in T.1 compared to two other groups. In the present study, the ash content was significantly higher in T.2 compared with T.3 and T.1, respectively furthermore, there was slightly differed significant among T.3 and T.2 (T.2>T.3>T.1). Ash content of shrimp is generally 1-1.5%. Yanar and Celik (2006) and Gunalan *et al.* (2013) calculated the amount of ash in white shrimp were 1.47 and 1.2%, respectively. These values are very close to the findings of the present study. Moisture of fresh shrimp is generally reported as 75-80% (Yanar and Celik, 2006). In the present study, the moisture was recorded 75.65-77.01% that was reported 76.2% in *P. vannamei* by Gunalan *et al.* (2013).

The data on composition muscle in the current study showed the probiotic and vitamin C had good affects. Finally, *P. acidilactici* had higher efficiency than vitamin C although, it did not cause to significant changes in protein content.

CONCLUSION

Most studies have been performed in laboratory and controlled conditions that is very different to Earthen ponds. It is suggested that more studies conducted on various probiotics in the ponds, if the results are successful, the probiotics is recommended to use in grow-out ponds for increase the efficiency.

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