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RESEARCH ARTICLE

EFFECT OF PROBIOTIC BACTERIUM ON GROWTH AND BIOCHEMICAL PARAMETERS OF SHRIMP *LITOPENAEUS VANNAMEI*

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ABSTRACT

The effect of probiotics on growth performance and biochemical activity of the shrimp *Litopenaeus vannamei* was investigated. *Lactobacillus sp*. was added to shrimp basal diets as probiotics at three concentrations: 5%, 10% and 15% probiotic bacteria was added to basal diet

Twelve aquaria with three replicates for each parameter and the Control group were used. After three weeks, shrimp receiving the diets supplemented with probiotics showed significantly better growth performance than those fed the basal diet (Control). The significant ($p<0.01$) increase in glucose levels were observed in 5% and 10% and 15% groups. Significant difference in triglyceride levels were not observed between control, 5% and 10% probitic treated groups. The 15% group displayed a higher level 43.25 ± 1.71mg/dl and showed significant difference with control. Lactic acid levels were significantly ($P<0.01$) increased in 10% and15% treated groups. No significant difference was observed in DNA levels but RNA levels were increased in all treated shrimp and significant difference ($P<0.01$) was observed in 10% group. The 10% probiotic treated group showed highest protein levels and all the treated groups showed significant difference when compared to control.

INTRODUCTION

Various strategies have been investigated to change the composition of the gut microbiota for better digestion, growth, immunity and disease resistance of the host (Burre *et al*., 2007). The dietary supplementation of these beneficial microbes is a novel approach to improve nutritional value and also as an alternative to drugs which have adverse effects. These beneficial microbes are usually referred as probiotics. According to current definition probiotics are live microorganisms which when administered in adequate amounts confer a health beneficial on the host (FAO, 2001). These beneficial microbes which after administration can able to colonize and multiply in the host's gut and give numerous beneficial effect by modulating the biological system (Cross ML., 2002).

Probiotics are used for quite long time in aquaculture. The effects of probiotics in fish and shrimp have been reported (Wang *et al*., 2005; Wang and Xu., 2006, Vo minh Son *et al*., 2009). But from few years they become an integral part of the culture practice for growth improvement disease resistance bacteria and many microbial compounds such as lipo polysaccharides, peptide glycans and β glucans have been reported in shrimp as stimulators of cellular functions (Vargas-Albores *et al*., 1998). These probiotic bacteria also have their effects against viruses like WSSV and *Vibrio spp*. (Itami *et al*., 1998; Gullian *et al*., 2004). However there are very few reports or little study on effects of probiotics on growth performance and biochemical parameters in the shrimp *Litopenaeus Vannamei*. So this study was aimed to investigate the effects of different concentrations of probiotic bacteria on growth and different biochemicals like glucose, lactose, triglycerides, nucleic acids and protein levels of shrimp *Litopenaeus vannamei* which is one of the most cultured and valuable shrimp in Andhra pradesh, India.

MATERIALS AND METHODS

Bacteria

The *Lacto bacillus sps* bacteria isolated from shrimp cultured pond in Nellore district, Andhra Pradesh, India (Sandeepa and Ammani,2014). Bacteria were grown in sterile conditions using MRS broth until final density reaches to $1X10^6$ cells per ml. These cells were mixed with a commercial gel (Him C, Himalaya chemicals) to attain different concentrations (5%, 10%, 15%) for using as feed supplement to study its effect on biochemical parameters.

Diets and experimental design

Healthy shrimp (wt of approximately 6-8 gms) was collected from a commercial farm and bring to lab immediately. The collected shrimp were acclimated to culture conditions for one week in tanks containing 50% filtered sea water and 50%

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freshwater which is UV treated to bring salinity to 20ppm and continuous aeration was given.

Each tank stocked with 6 healthy shrimp. For each parameter to study a group of 24 animals were taken and acclimatized to laboratory conditions for a period of one week. These animals were divided into 4 groups each consists of 6 animals, all groups were run in triplicate. Three diets containing different doses (5%, 10%, 15%) of probiotic mixture were prepared. Keeping one group as control the remaining three groups were fed probiotic supplemented feed ($1X10^6$ @10gm gel /kg feed, 50gms, 100gms, 150gms of gel was added per Kg feed to make 5%,10% and 15%) . The feed was given to each group for a period of three weeks. For control group the feed was supplied only by mixing with commercial gel without probiotic. The feeding experiment was done up to three weeks, in this feeding trial all shrimps were fed diets four times a day at 4% of the body weight. During the experiments 30% of seawater was exchanged daily to maintain the water quality,and the water temperature was maintained at 28 ± 1^0C the pH at 7.5-8.4 and the salinity was maintained at 20ppm.

After three weeks of feeding hemolymph (0.8µl) of individual shrimp (probiotic treated and virus challenged) was drawn from the ventral sinus in the first abdominal segment with a sterile 1-ml syringe (26-gauge hypodermic needle). The syringe was loaded with 200µL of pre cooled anti coagulant solution (10mM Tris Hcl, 250mM sucrose, 100m sodium citrate, at pH 7.6). More anticoagulant was added to hemolymph to make equal volume ratio of hemolymph to anticoagulant. This anti coagulated hemolymph was centriguged at 300Xg for 10 min at 4^0C to separate plasma and hemocytes. Then these were immediately frozen in liquid nitrozen and thawed once for assays.

Growth performance

The weight of shrimps were determined at the start and at the end of the 28 day experiment the Mean weight, Weight gain, Daily weight gain (DWG) and relative gain rate (RGR), Specific growth rate (SGR) was calculated as

$$DWG = \frac{\text{Total weight gained}}{21\ \text{d}}$$

$$RGR = \frac{\text{Finalweight(g)} - \text{Initialweight(g)}}{\text{Initial weight (g)}} \times 100\%$$

$$SGR = \frac{(\ln W_t - \ln W_0)}{21\text{d}} \times 100$$

W_t= Final weight after experiment; W_0= Initial weight

Biochemical analysis of haemolymph

Commercial kits were used for glucose ŽGOD-PAD, Merck-74039., lactate ŽSigma-cat. 73510., triacylglycerol ŽGPO-PAP, Merck, cat. 14354., determinations, adapted to a microplate using 20 ml of plasma and 200 ml of enzyme chromogen reagent. Absorbance was recorded on a microplate reader ŽBIO-RAD model 550 and concentrations were calculated from a standard solution of substrate.

Estimation of protein

Plasma was further diluted 1:500 for protein determination by the Bradford Ž1976. technique adapted to a microplate method using commercial chromogen reagent ŽSigma, cat. 610. and bovine serum albumin as a standard.

Estimation of nucleic acids

Nucleic acid was extracted from methanol insoluble tissue residue by the method of Schneider (1957), and the contents of DNA and RNA were assayed following the method of Burton (1956) and Ceriotti (1955) respectively.

Statistical analysis

The data were expressed as the arithematic mean ± standard deviation and were analyzed by one- way –ANOVA. Least significant difference test used to determine the differences among the data with SPSS statistical software (SPSS Inc., USA). Levels of $p< 0.01$and $p<0.05$ was considered as significant.

RESULTS

No significant difference was observed for initial weight between the control and treatment groups. After 28 days of experiment the mean weight of each group was increased significantly ($P<0.01$) when compared to control. The 10% probiotic treated shrimp showed highest mean weight (9.13±0.14 gms) than other groups (Table-1). The Daily Weight Gain (DWG), Relative gain Rate (RGR) specific growth rate (SGR) values in all treated groups were significantly higher than those the control. But the mean values of DWG and RGR were not significantly different among treated group (fig-1).

The significant ($p<0.01$) increase in glucose levels were observed in 5% and 10% and 15% groups. Among three groups 10% treated groups showed high glucose levels 26.45±0.43mg/dl when compared to control 25.35±1.23 mg/dl (fig-2). Significant difference in triglyceride levels were not observed ($p>0.05$) between control and probitic treated groups of 5% and 10%. But triglyceride levels were increased in all probiotic treated groups when compared to control groups. The 15% group displayed a higher level 43.25 ± 1.71mg/dl (fig-3) and showed significant difference with control. Lactic acid levels were significantly ($P<0.01$) increased in 10% and15%

Table1 Growth performance of shrimp *Litopenaeus vannamei* after 21 days of feed with 5%, 10% and 15% probiotics containing diet

Group	Initial weight (g)	Final weight(g)	Weight gain (g)	DWG (g/d)	RGR (%)	SGR(%/day)
Control	5.74 ± 0.04^a	7.24 ± 0.07^b	1.50 ± 0.03^a	0.07 ± 0.01^a	26.1 ± 0.06^a	5.90 ± 0.25^a
5%	5.86 ± 1.54^a	7.61 ± 0.61^b	1.75 ± 0.87^b	0.083 ± 0.05^b	29.86 ± 0.12^b	5.95 ± 0.01^b
10%	6.10 ± 0.58^a	9.12 ± 0.14^b	3.02 ± 0.44^b	0.143 ± 0.36^b	49.5 ± 0.83^b	7.16 ± 0.02^b
15%	5.63 ± 0.32^a	8.34 ± 0.11^b	2.71 ± 0.21^b	0.129 ± 0.1^b	48.13 ± 0.03^b	7.01 ± 0.30^b

DGW- Daily gain weight, RGR- relative gain rate, SGR-specific growth rate,Values are expressed as mean±SD three replicates were set for four tests . Means in the rows with different superscripts are significantly different at ($p<0.01$).

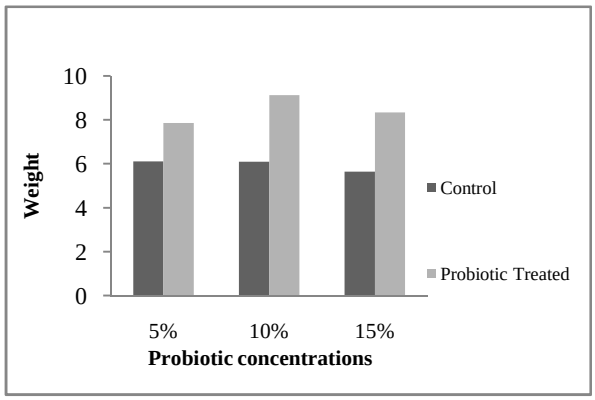

Fig-1 Effect of different concentrations of probiotic bacteria on growth of *Litopenaeus vannamei*

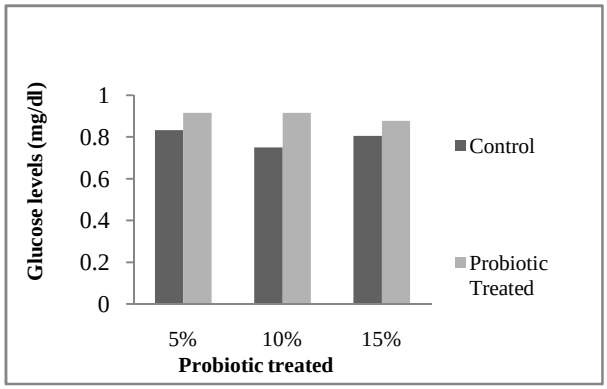

Fig-2 Effect of different concentrations of probiotic bacteria on glucose

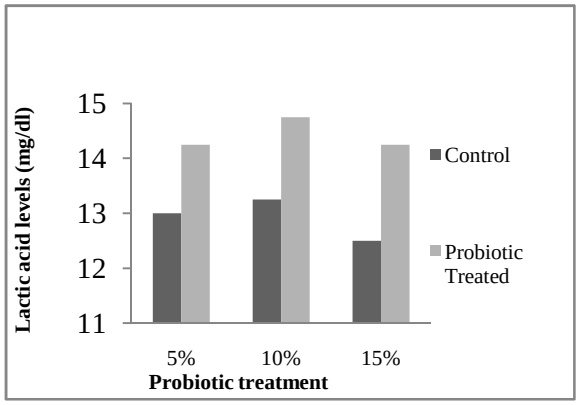

Fig-3 Effect of different concentrations of probiotic bacterium on Lactic acid levels

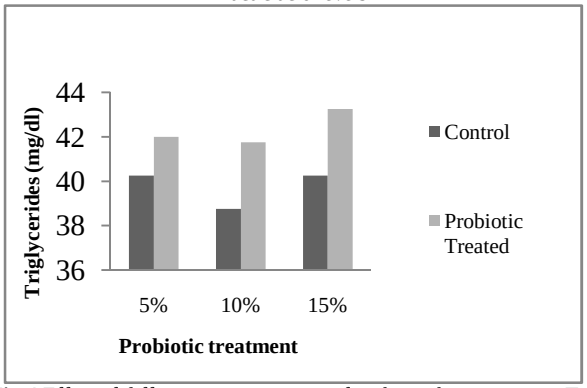

Fig-4 Effect of different concentrations of probiotic bacterium on Try acyl glycerol levels

treated groups . Among the three groups high lactic acid level 14.75±0.5 mg/dl was recorded in 10% treated group (fig-4).The treated shrimp groups does not showed significant ($p<0.01$) increase in DNA levels than control groups(fig-5). RNA levels increased in all the treated groups but significant increase ($p<0.01$) was observed in 10% group (fig-6). All probiotic treated shrimp groups showed significant increase ($p<0.05$) in percentage of protein levels when compared to control groups. The 10% probiotic treated group showed highest protein leves30.42 ± 0.74% than its control 23.1 ± 0.51% (fig-7).

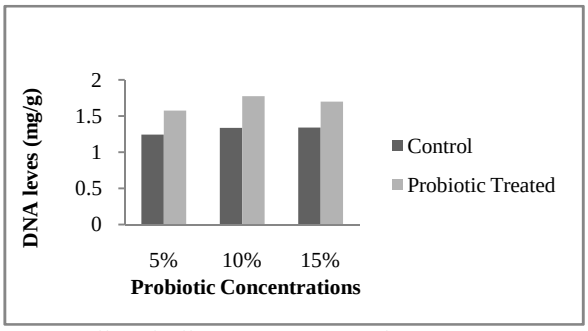

Fig-5 Effect of different concentrations of probiotic bacterium On protein levels (%)

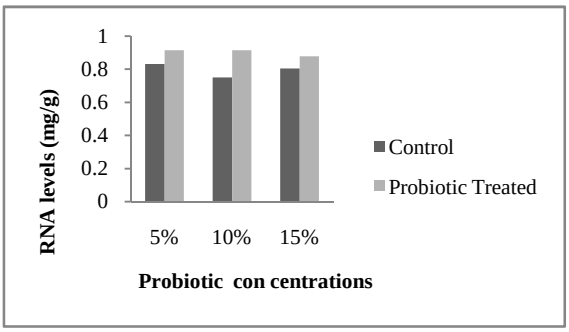

Fig. 6 Effect of different concentrations of probiotic RNA levels (mg/g)

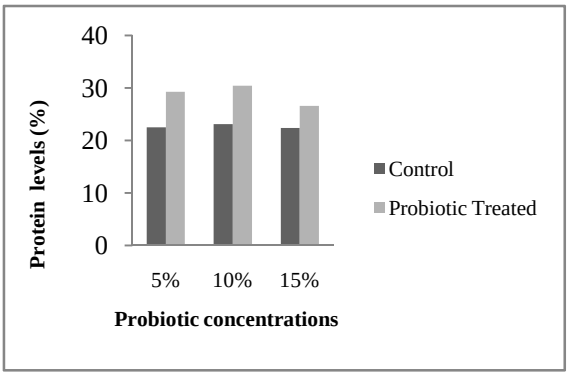

Fig-7 Effect of different concentrations of probioc bacterium on Levels DNA (mg/g)

DISCUSSION

In the present study dietary administration of lactic acid bacteria for 3 weeks significantly increased the weight gain in treated shrimp compared to the control group. Previous studies have showed that when probiotics were added as water additives, they can improve the growth performances of shrimp species (De Souza *et al*., 2012, Silva *et al*., 2012; WangY-B *et al*., 2005), the probiotic bacterium *Bacillus subtilus* had beneficial effects on the final weight and weight gain (Hadizakaeifar *et al*., 2014). This indicated that when probiotic bacteria such as *lactobacillus* were supplemented in diets

may also promote the weight gain which have resulted from secretion of digestive enzymes in the gastro intestinal tract. Some studies have shown that enzymes of genus *Bacillus* can easily and efficiently break down a variety of proteins, lipids and carbohydrates in to smaller units (Ochoa.S *et al*., 2006, Inatsu *et al*., 2006, Tari.C *et al*., 2006). All the probiotic supplemented diets resulted in an increase in weight gain, DWG and RGW which shows that probiotics increased the growth performance of shrimps (Y-B.Wang *et al*., 2007).

Glucose is the major sugar component in hemolymph (Honke and Scheer 1970). It has central metabolite importance in virtually all organisms from microbes to man. Glucose metabolism through the pentose phosphate pathway genereates NADPH and precursor for a many anabolic pathways. Lactate is the end product of glycolysis after reduction of pyruvate by NADH (Hochachka, 1970). Lipids are important one in constituting the cellular membrane and are good source of energy in the shrimp. In the present study better plasma glucose, lactate and tryglyceride levels were observed in *L.vannamei* maintained on the diet supplemented with probiotic bacteria showing significant differences ($P<0.01$) from the control and 10% probiotic supplemented shrimp showed better glucose and lactate levels than other groups. High glucose levels were found by Ming-Chaoyu *et al*. (2007) when they feed both *Bacillus* (0.2%) and medical herbs(0.3%) in shrimp *L. vannamei*. This indicates support for the suggestion that shrimp fed probiotic supplemented diets are healthier than control. In another study also better glucose, lactate and cholesterol levels were observed when shrimp *Litopeneus Vennamei* fed with probiotic bacteria *Bacillus* along with soyabean meal and carbohydrates. (Jorge olmos etal., 2011).

Plasma protein content in the present study was significantly ($p<0.01$) increased in all probiotic treated groups with the highest in 10% diet fed shrimps when compared to that of control diets fed shrimp The present study results are in agreement with the findings of Zhou *et al*. (2009) who applied the *Bacillus coagulans Sc8168* as a water additive enhanced the protein content of shrimp *P. vannamei.*, the protein content (PC) was significantly high in probiotic treated groups, the highest PPC was observed in $5X10^5$ CFU concentration of probiotic fed shrimp when compared to that of control and other $1x10^6$ and $1X10^6$ concentration diets fed shrimp.

In the present study we found that probiotics also have their effect on nucleic acids levels in shrimp tissue. High nucleic acid levels were found in probiotic treated groups. This observation probably indicates that shrimp fed probiotic supplemented diets were healthier than control.

CONCLUSION

In a conclusion, the probiotic bacterium belonging to *Lactobacillus* species isolated from shrimp culture ponds with 10% concentration was beneficial for shrimp *Litopenaeus vannamei* in terms of increasing growth performance and the concentrations of serum protein, other biochemicals like glucose, lactate, triglycerides and enhancing the health of the shrimp.

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