ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(2): 07-14

www.fisheriesjournal.com
Received: 15-01-2015
Accepted: 17-02-2015

Pratap Reddy C
Department of Zoology, Sri Venkateswara University, Tirupati - 517 502, India.

Hareesh K
Department of Zoology, V.S.U.P.G. Centre, Kavali - 524 201, India.

Suneetha Y
Department of Zoology, Sri Venkateswara University, Tirupati - 517 502, India.

Srinivasulu Reddy M
Department of Zoology, Sri Venkateswara University, Tirupati - 517 502, India.

Correspondence
Srinivasulu Reddy M
Department of Zoology, Sri Venkateswara University, Tirupati - 517 502, India.

Efficacy of Animal and Plant Feed Ingredients in the Formulation of feeds for induction of growth potentials in penaeid prawn *Litopenaeus vannamei*

Pratap Reddy C, Hareesh K, Suneetha Y, Srinivasulu Reddy M

Abstract
Probiotic use in aquaculture is increasingly as producers are attempting to improve pond, soil, water quality, enhance survival and improve the growth of candidate species. In the present investigation an attempt has been made to know the effect of different types of probiotics including soil, water and feed probiotics during the culture operation of pacific white shrimp *Litopenaeus vannamei*. In the present investigation soil, water and feed probiotics selected, super PS, super Biotic and UB Probizyme, respectively used to monitor the growth potentials in prawns. Super PS was broadcasted at the rate of 5 lit/ 0.5 ha, Super biotic i.e. 5kg/ 0.5 ha and UB Probizyme at the rate of 10 g/ 1 kg feed were applied by following the manufactures instructions. The results showed that probiotics plays an important role in maintaining water quality parameters, soil quality and health management as well as increase the growth and survival of shrimp. Probiotic treatment offers a promising alternative to the use of antibiotics in prawn culture activity.

Keywords: *Litopenaeus vannamei*, Probiotics, Feed ingredients.

1. Introduction
Shrimp aquaculture sector occupies very important role in the socio-economic development of the country and also provide proteinaceous food for the poor people. The issue of malnutrition has become important for the growing populations in India as well as in the world. Soon after the collapse of penaeid shrimp industry due to disease outbreak, the alternative prawn candidate species for culture operation were explored including freshwater prawn *Macrobrachium rosenbergii* and pacific white shrimp *Litopenaeus vannamei*. At present along with *M. rosenbergii*, the pacific shrimp *L. vannamei* has become the main crustacean species produced through culture, with production exceeding that of tiger shrimp *Penaeus monodon* since 2003 [11]. At present *L. vannamei* provides a significant contribution to the total value of Aquaculture production in India and also several parts of the world [33, 60]. The momentum of growth in Aquaculture acquired in India during late eighties and early nineties started declining due to various bio-physical and socio-economic factors including occurrence of white spot syndrome virus (WSSV), stipulations of supreme court and lack of Institutional credit support. It is possible to solve most of the production constituents through well-developed management practices. Hence, there is a strong need to facilitate the adoption of Better Management Practices (BMPs) to achieve the goal of sustainable shrimp farming. One of the principles of BMPs includes the usage of probiotics of different types during the culture operation of candidate species in order to increase productivity in the hygienic environment. Probiotics are live non-pathogenic microorganisms that provide colonization resistance to the pathogenic microbes and thus are effective in prevention and treatment of some diseases. Fuller [13] defined probiotics as live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance. Probiotics, including Lactic acid bacteria and *Bacillus* sp. can be introduced into the culture environment as 'Bio-friendly agents' to control the pathogenic organisms. The use of preventive and environmentally friendly approaches, namely Antibacterial peptides, probiotics and prebiotics are becoming increasingly important in Aquaculture, particularly in light of new trends toward organic production systems [2, 3, 8, 47]. The original definition of probiotics is organisms and substances contributing to intestinal microbial balance [32]. The probiotics are a live microbial

feed supplement, which beneficially affects the host by improving its intestinal balance. Moriarty *et al.* [29] proposed the definition of probiotics in Aquaculture to microbial "Water additives". Probiotics can also be defined as microbial cells administered through the gastrointestinal tract with the aim of improving health of the hosts [15]. The present investigation is aimed to study the impact of different types of probiotics in combination i.e. Soil Probiotics, Water Probiotics and Feed Probiotics on the growth patterns of pacific white shrimp *Litopenaeus vannamei.*

2. Materials and Methods

The present study was carried out in commercial shrimp ponds located near Ramayapatnam (Latitude 150 021 5511 N, Longitude 800 021 5011 E), Prakasam Dist., Andhra Pradesh, South India. Buckingham canal is passing near the ponds providing continuous water supply throughout the year. Ponds of Average size of 05-06 ha and rectangular in shape with clay loamy soil suitable for semi intensive type of culture was selected for present investigation. The entire farm has a covered area of 12 ha and six ponds were selected for this operation. The culture is semi intensive type with stocking densities of 20 PLs/m2. The pond water depth was maintained at 1.5 mts regularly. Initially all the selected ponds were allowed to dry and splinter to increase the capacity of oxidation of Hydrogen sulphide and to eliminate the fish eggs, crab larvae and other unwanted predators. The pond bottom was scrapped 3 to 5 cm by using a tractor blade to avoid top soil. Then the pond bottom was ploughed horizontally and vertically a depth of 30 cm to remove the obnoxious gases, oxygenate the bottom soil and remove the H_2S odour and to increase fertility. The soil pH was recorded in the ponds with the help of cone type pH meter. For increasing the availability of nutrients, required amount of lime was applied to neutralize the acid soil, condition of the soil based on the average pH level of the pond. During the course of Experimentation, the pond water Physico-chemical parameters were maintained at Temperature (26-30 °C), Salinity (12-24 ppt), pH (7.6-8.2), Dissolved oxygen (3.6-6.5 ppm), Transparency (35-55 cm) and Ammonia (0.1-0.3 ppm).

2.1. Feeding trails

Litopenaeus vannamei of 0.65±0.05 g were selected for present investigation and were stocked at the rate of 20 pcs/m2 in the culture ponds. In the present investigation three experimental feeds were selected/formulated i.e. one commercial feed (CP Brand feed), one experimental feed formulated with Animal ingredient source (EF-A) and one experimental feed formulated with plant ingredients source (EF-P) and the composition of formulations were presented in Tables. 1 & 2. The ponds of almost equal size were selected in the farm for broadcasting of selected experimental feeds during culture operation. Similarly another set of three equal sized ponds were also selected for usage of probiotics along with the above mentioned experimental feeds. In the present investigation soil, water and feed probiotics selected, Super PS, Super Biotic and UB Probiozyme, respectively. Super PS was broadcasted at the rate of 5 lit/ 0.5 ha, Super biotic 5 kg/ 0.5 ha and UB Probizyme at the rate of 10 g/ 0.5 ha feed were applied by following the manufacturer's instructions. Out of 6 ponds i.e. three ponds cultured without probiotics and the other were cultured with probiotics. The culture operation was continued for a period of 120 days.

Growth performance studies were conducted continuously with every 15 days break and observations were recorded. Growth parameters including Average weights, Average daily growth rates (ADG), Specific growth rates (SGR), Feed conversion ratio (FCR), Percent survival were monitored and tabulated.

$$\text{Survival Rate} = \frac{\text{Total number of live animals}}{\text{Total number of Animals Stocked}} \times 100$$

$$\text{Weight gain} = \text{Weight of the Animal (G) at the end of culture period} - \text{Weight of the animal (G) at the time of stocking}$$

$$\text{FCR} = \frac{\text{Total amount of feed broadcasted (kgs)}}{\text{Total Biomass (weight) of prawns (kgs)}}$$

$$\text{SGR} = \frac{\text{Log weight of the Animal at the end of the Experiment} - \text{Log weight at the stocking point of Experiment}}{\text{Total days of culture period of operation}} \times 100$$

$$\text{SGR} = \frac{(\text{Log W2} - \text{Log W1})}{\text{T Where}} \times 100$$

Where

W1= Weight of the Animal at the starting of the culture period

W2= Weight of the Animal at the end of the culture period

T= Total number of culture period

2.2. Analysis of Gut Micro flora

The Bacteriological analysis of the gut flora of the prawn was carried out after the completion of the experiment. The wet weight of each prawn was determined before the dissection. The prawns were dissected to remove the gut, after washing dorsal surface area of prawn with sterile distilled water. The entire gut was homogenised in 9 ml of diluents (Phosphate Buffer 0.01 M) and mixed with a vortex mixture and used as 10-1 dilution. Each sample was serially diluted, using pour plate method, poured in to four different media. The Total Plate count was determined using Tryptone Soya Agar (TSA) media. The *Lactobacilli* count was determined using MRS Agar media. The inoculated plates were incubated at 35±20 C for 3 days. The Anaerobic count was determined using Anaerobic Agar media. The inoculated plates were incubated at 35±20 C for 3 days in anaerobic condition. The total coliform count was determined using the most probable number method (MPN). Bacterial cultures were isolated and identified through standard methods [18]. The entire procedure adopted for gut Micro flora analysis was described by Bhavani [5] and Bhavani *et al.* [6].

Moisture content was determined by oven-drying at 105 °C for 24 h. Crude protein content was analysed by Kjeldahl method after acid digestion. Crude lipid content was analysed by the Ether extraction method by Soxhlet system.

2.3. Analysis of Profiles of Amino acids

The individual amino acids were determined in Muscle tissue using LKB Automatic Amino acid analyser. Amino acids were extracted into Ethanol medium and were subsequently discovered in Citrate buffer (0.1 M) and 0.5 ml was loaded for quantification suitable standards also run simultaneously. All the conditions pertaining to the quantification were standardized.

2.4. Biochemical parameters

Biochemical parameters including assaying of Catalase and Peroxidase activity [31], Super oxide dismutase (SOD) [27] and protein content [21] using Bovine serum albumin as standard.

The data obtained in the present investigation was subjected to Statistical Analysis by using SPSS 14version.

3. Results and Discussion

In the present investigation, Three experimental feeds were selected, i.e. one CP Brand experimental feed, the other two experimental feeds were formulated, one with Animal based ingredients (EF-A) and the other with Plant based ingredients (EF-P). The composition of the experimental feeds formulated was presented in Tables. 1 & 2. The proximate composition of experimental feeds was analysed and parameters like crude protein, total lipid, fibre content, and moisture and ash contents were determined and presented in Table. 3. The growth rates of pacific white shrimp *L. vannamei* was monitored after a culture period of 120 days. In the present investigation six ponds were stocked with PLs of *L. vannamei* and were fed with one commercial feed (CP Brand) and two formulated feeds i.e. one feed formulated with animal based ingredients. The other three ponds were broadcasted with above mentioned feeds along with probiotic treatments. The probiotics selected in the present investigation i.e super PS, super Biotic and UB probiozyme represents, Soil, Water and Feed probiotics, respectively. Shrimp were harvested after completion of 120 days of culture operation. One third of water from each pond was drained through outlet just before catching. While two third of water let out, the prawns were collected and handpicked. Shrimps were immediately transferred to changing tubes provided near the pond. They were rinsed and chilled before packing. Random samples were collected during the weighing process for determination of individual weight. After quantifying the biomass, mean final yield, feed conversion ratio, and survival were calculated. Specific growth rates, average daily growth and final weight % were determined by using the standard formulae.

Table 1: Composition of Experimental feed formulated with Animal based Ingredients (EF-A)

Ingredients	Percent inclusion
Prawn meal	26
Squilla meal	25
Chicken liver	20
Snail meal	12
Fish meal	12
Cholesterol	2.4
Vitamin and Mineral mixture	2
Choline chloride	0.5
Ascorbic Acid	0.1

Table 2: Composition of Experimental feed formulated with Plant based ingredients (EF-P)

Ingredients	Percent inclusion
Soybean meal	25
Ground nut cake	25
Corn gluten	15
Rice bran	10
Pea meal	10
Wheat Flour	5
Corn starch	2
Cholesterol	2.4
Soy oil	3
Choline chloride	0.5
Ascorbic Acid	0.1

Table 3: Proximate composition of Experimental Feeds (on Percent basis).

Parameter	CF	EP-A	EP-P
Crude protein	38.12±1.79	36.35±1.24	36.42±1.28
Total lipid	5.18±0.23	6.59±0.28	6.41±0.29
Fibre	4.13±0.18	4.24± 0.17	4.22±0.19
Moisture	11.15±0.63	16.18±0.63	17.14±0.72
Ash	15.62±0.73	17.71±0.79	16.89±0.82

All values are Mean ± SD of six individual observations.

Table 4: Average values of weighs of prawns recorded at different periods of culture operation with and without Probiotic treatments.

DOC	Commercial feed		EF-A		EF-P	
	Control (g)	With Probiotics (g)	Control (g)	With Probiotics (g)	Control (g)	With Probiotics (g)
0	0.65	0.65	0.65	0.65	0.65	0.65
15	2.02	2.16	2.17	2.89	1.98	2.18
30	4.15	4.74	4.76	5.46	3.96	4.19
45	7.11	8.38	7.88	8.96	6.98	7.38
60	12.19	13.94	13.04	15.12	11.75	13.42
75	17.25	19.85	18.19	20.46	16.49	17.85
90	21.19	23.49	24.13	26.45	19.76	21.62
105	24.88	26.92	26.76	28.18	22.12	24.13
120	26.15	29.04	28.03	30.39	24.19	26.46

Commercial feed: CP Aqua Feed, EF-A: Experimental Feed formulated with Animal based ingredients, EF-P: Experimental Feed formulated with Plant based ingredients.

Table 5: Growth Parameters of Prawns recorded after fed with three experimental feeds with and without Probiotic treatments.

Parameter	Commercial feed		EF-A		EF-P	
	C	WP	C	WP	C	WP
Initial weight (g)	0.65	0.65	0.65	0.65	0.65	0.65
Final weight (g)	26.15	29.04	28.03	30.39	24.19	26.46
Weight (g)	25.50	28.39	27.38	29.74	23.54	25.81
% weight gain	3923	4368	4212	4575	36.21	3971
Average daily growth (ADG)	0.2181	0.242	0.234	0.253	0.202	0.221
Specific growth rate (SGR)	1.33	1.38	1.36	1.39	1.31	1.34
Feed conversion ratio (FCR)	2.13	1.82	2.34	2.02	2.49	2.16
% Survival	83	86	85	87	80	83
Total feed consumed (Kgs)	13302	12545	12760	11801	12992	11649
Total Production(Kgs)	6245	6893	5453	5842	5218	5393

Growth rates of *L. vannamei* fed with three experimental feeds with and without probiotics during 120 day culture operation were monitored and presented in Tables 4 & 5. Among the three experimental feeds selected in the present study, maximum growth rates were recorded with prawns fed with EF-A i.e. experimental feed formulated with animal based ingredients as 28.03 g after 120 day culture operation, whereas 26.15 g for prawns fed with commercial feed CP brand and least of 24.19 g for prawns fed with EF-P i.e. experimental feed formulated with plant based ingredients. Similarly when the probiotic treatment were adopted during culture operation along with the broadcasting of the above mentioned three feeds, maximum growth rates observed with EF-A (30.39 g) followed by commercial brand of feed (29.04 g) and finally EF-P (26.46 g). The results obtained for growth parameters including average daily growth rate (ADG), specific growth rate (SGR), feed conversion ratio (FCR), production yields and total feed consumed in the present investigation clearly demonstrate that, the three experimental feeds selected are fairing relatively better. The FCR values obtained are in and around 2, therefore the three feeds selected in the present investigation are considered to be of superior quality feeds. Several authors demonstrated that, the inclusion of both plant and animal based ingredients in the formulation of feeds for penaeid shrimps and freshwater prawns induced the best and highest growth potentials during culture operation. [4, 7, 22, 28, 37, 40, 54].

Proximate composition of feed ingredients of animal and plant origin selected was estimated and presented in Table. 6. Amino acid composition of feed ingredients of animal and plant origin selected were analysed and presented in Tables. 7 & 8.

Table 6: Proximate Composition of feed ingredients selected in the present investigation.

Ingredients	Moisture (%)	Protein (% of dry matter)	Crude fat (% of dry matter)	Ash (% of dry matter)
Prawn meal	7.8	75.2	8.2	8.3
Squilla meal	7.9	71.3	7.7	7.4
Chicken liver meal	7.5	65.3	7.9	7.5
Snail meal	7.7	68.4	8.1	7.6
Fish meal	8.1	70.3	7.8	8.1
Soy bean meal	8.3	54.2	2.3	10.3
Ground nut cake	8.2	18.4	7.2	8.5
Corn gluten	7.4	16.5	0.9	8.2
Rice bran	10.4	13.4	1.2	6.5
Pea meal	9.3	18.2	1.3	6.8
Wheat flour	8.1	10.4	1.2	7.2
Corn Starch	7.3	13.2	1.1	7.1

Table 7: Amino acid composition of the ingredients of selected in the present investigation of Animal Origin

Amino acid	Ingredients of Animal origin				
	Prawn meal	Squilla meal	Chicken liver	Snail meal	Fish meal
Aspartic acid	80.95±3.28	83.45±3.76	82.34±3.75	85.49±3.85	79.38±3.72
Glutamate	12.84±0.25	19.42±0.28	13.25±0.34	12.14±0.54	15.16±0.65
Lysine	13.5±0.74	14.17±0.82	15.18±0.72	14.95±0.68	17.43±0.79
Arginine	18.73±0.65	22.14±0.72	26.15±0.76	22.49±0.78	23.45±0.72
Histidine	8.43±0.24	10.24±0.22	9.34±0.21	10.05±0.24	12.42±0.23
Glycine	28.22±0.79	29.32±0.84	31.44±0.83	30.24±0.82	28.24±0.85
Alanine	12.33±0.25	12.49±0.29	13.85±0.34	14.84±0.38	20.33±0.39
Valine	16.34±0.24	18.22±0.25	21.31±0.26	14.14±0.28	22.45±0.28
Leucine	26.74±0.75	28.24±0.74	30.13±0.68	32.13±0.65	29.42±0.62
Isoleucine	13.46±0.26	13.76±0.28	18.72±0.34	22.42±0.35	18.44±0.36
Cysteine	13.36±0.29	16.72±0.34	13.42±0.32	18.45±0.46	16.43±0.29
Methionine	6.74±0.13	8.12±0.22	6.76±0.21	10.24±0.25	11.45±0.27
Phenyl alanine	12.43±0.23	14.74±0.25	18.22±0.28	20.14±0.29	23.24±0.34
Tyrosine	45.46±2.79	65.34±2.72	54.49±2.05	52.46±2.14	62.73±2.42
Proline	16.74±0.85	18.35±1.05	22.45±1.12	21.42±1.10	29.44±1.15
Serine	64.75±3.14	82.18±3.15	70.15±3.46	69.74±3.42	88.13±3.15
Threonine	17.39±1.08	22.18±1.16	24.19±1.15	25.12±1.22	20.15±1.24
Tryptophan	12.45±0.18	16.46±0.25	19.74±0.29	25.42±0.31	26.15±0.28

The values are Mean ± SD of three individual observations.
The values are expressed as µmoles of Tyrosine equivalents/ gram weight

As India is mainly agro based country, a large variety of agricultural crops wastages, and by products are being used as aquaculture feeds including fish and prawn. Although most are available throughout the year and all over the country, some are much localized. In the present study both locally available feed ingredients of animal and plant origin were selected for feed formulation studies for *L. vannamei.* As far as prawn culture is concerned there are many factors relates the growth and feeding activity [30], which include a functional digestive system to efficiently utilize the nutrients present in the food offered [1] and the physiological conditions, and the rearing environment [19]. Growth of prawn is normally very fast during the early life and shows down during adult; the survival rates also very high during the early life and fell subsequently [26]. In the present investigation the feed ingredients of animal and plant origin selected for feed formulation are having relatively very good amounts of nutritional requirements in terms of protein, carbohydrate, lipid and amino acid contents. The

content of protein, carbohydrate, lipid are expression of an animal's adaptive characteristics. Many biotic factors including maturation, reproduction, food availability and food quality, and abiotic factors including photoperiod, temperature, pH and oxygen, are known to influence the physiology and biochemistry of crustaceans [23, 41, 55]. Protein is essential for growth and development. It is essential to provide the body with energy and is needed for the production of hormones, antibodies, enzymes etc. [16]. The protein requirement is dependent on many nutritional factors, such as lipid, carbohydrate contents or energy levels. In Juveniles of *Penaeus japonicus* and *P. monodon*, the optimal level of protein requirement is between 40-52% for good growth and survival and the level of protein exceeding 60% lead to a clear depressing effect on growth [9, 10, 42, 50, 51]. Dietary protein is the source of nitrogenous waste products. Consequently, optimization of dietary protein levels along with increasingly other nutrients retention could reduce nitrogen loading and positively influence production cost [53]. The gross dietary protein requirement is not influenced directly by the amino acid composition as the diet [52]. But the growth is strongly influenced by the digestibility and essential amino acid composition of protein sources [17, 20, 59]. In the present investigation the protein and amino acid contents are relatively higher quantities in all the feed ingredients of both animal and plant origin and also produced appreciable growth. Further, the higher carbohydrate contents in the some of the feed ingredients of plant origin also may aid to increase the protein sparing effect on growth patterns of prawns.

Carbohydrates play in important role in balancing the utilization of protein and lipid for energy production. They are the first to be exhausted when energy is required, followed by lipid and then protein. Most crustaceans do not have a specific requirement for dietary carbohydrate [12]. It has been reported that carbohydrates are used for short-term energy requirements in prawns [48]. According to Shiau and Peng [49], the protein sparing effect was more obvious in *P. monodon* when the dietary protein level was reduced from 40 to 30% by increasing the dietary corn starch level from 20 to 30%. Dietary lipids are known to play a vital role in nutrition as they provide energy, maintain the structural integrity of biological membranes and function as precursors for important steroids. The optimal level of dietary lipid required for crustacean generally ranged from 2 to 10% [44, 45]. The diet deficient in lipid affects molting frequency and weigh t gain due to insufficient lipid utilization. Similarly, diet with excessive lipid affects the growth due to inefficient lipid utilization and results in lipid accumulation and lowers meat quality [34]. This is particularly common when other energy source are available in right proportion. However, diets with higher lipid level have a protein- sparing effect on growth [1]. In the present study, the proportion of crude lipid content of ingredients of animal origin and feeds falls between 5–10%, and therefore the lipid content was ideal and capable of inducing highest and best growth potentials.

Table 8: Amino acid composition of the ingredients of selected in the present investigation of Plant Origin

Amino acid	Ingredients of Plant origin						
	Soybean meal	Groundnut Oil cake	Corn gluten	Rice bran	Pea meal	Wheat flour	Corn starch
Aspartic acid	25.75±0.82	30.42±0.75	26.76±1.12	33.34±0.95	45.15±1.12	28.12±0.85	21.35±0.83
Glutamate	75.33±2.18	85.42±2.13	85.41±2.18	88.74±2.18	90.12±2.35	82.18±2.34	70.15±2.13
Lysine	115.13±2.79	109.74±2.83	82.93±2.14	104.75±3.49	104.42±3.15	70.18±2.12	83.19±2.42
Arginine	28.42± 0.27	14.15±0.22	26.34±0.26	19.12±0.25	22.18±0.27	26.74±0.28	34.75±0.29
Histidine	18.75±0.28	28.42±0.29	30.12±0.32	29.44±0.31	34.75±0.35	30.14±0.29	35.75±0.31
Glycine	25.74±0.29	30.18±0.35	35.42±0.36	40.14±0.85	28.19±0.56	33.42±0.49	30.14±0.45
Alanine	23.15±0.28	28.74±0.29	30.13±0.34	42.14±0.35	20.16±0.42	27.45±0.44	29.15±0.42
Valine	12.18±0.12	9.14±0.15	8.85±016	12.14±0.17	10.75±0.25	17.19±0.22	25.12±0.24
Leucine	34.85±0.75	25.74±0.74	45.15± 0.85	35.14±0.89	45.19±0.93	36.75±0.94	25.13±0.95
Isoleucine	12.75±0.24	16.42±0.25	12.05 ±0.22	18.74±0.25	19.72±0.25	22.10±0.29	23.74±0.29
Cysteine	19.42±0.85	25.12±0.89	34.14 ±1.12	45.14±1.35	18.72±0.74	21.14±0.42	20.73±0.45
Methionine	98.13±2.14	145.18±2.74	132.74±3.39	105.43±3.75	114.45±3.18	115.44±3.24	143.72±3.42
Phenyl alanine	18.74±0.18	29.49±0.23	35.14±0.25	24.18±0.19	20.14±0.15	23.13±0.17	21.15±0.19
Tyrosine	22.18±0.42	39.49±0.46	28.13±0.84	29.74±0.72	33.18±0.56	30.04±0.64	29.74±0.62
Proline	10.12±0.22	20.14±0.24	16.74±0.24	12.18±0.20	20.12±0.22	13.15±0.24	12.49±0.25
serine	30.34±0.35	25.12±0.42	34.15±0.39	29.13±0.45	44.19±0.47	28.15±0.49	29.15±0.52
Threonine	25.13±0.45	20.13±0.57	26.74±0.59	21.39±0.45	20.45±0.40	29.18±0.45	32.75±0.49
Tryptophan	21.39±0.25	24.18±0.32	30.13±0.35	25.74±0.42	24.75±0.45	25.14±0.45	23.75±0.54

The values are Mean ± SD of three individual observations.
The values are expressed as µmoles of Tyrosine equivalents/ gram weight

Table 9: Gut microbial load of prawn *L. vannamei* after fed with three Experimental feeds with and without probiotic treatments.

Parameter	Commercial feed		EF-A		EF-P	
	C	WP	C	WP	C	WP
Total plate count	5.3×10^8	3.9×10^8	6.4×10^8	4.2×10^8	5.7×10^8	3.7×10^8
Total coli forms	2045	1236	2246	1342	2385	1305
Total fecal coli forms	503	175	640	218	638	242
Facultative Anaerobes	7.3×10^6	3.4×10^6	8.2×10^6	3.9×10^6	7.8×10^6	4.1×10^6
Lactobacillus sp.	2.1×10^6	5.2×10^6	2.5×10^6	4.3×10^7	2.7×10^6	3.9×10^7

All values are Mean ± SD of six individual observations
Values are expressed as CFU/g

Table 10: Antioxidant enzyme activity in the midgut gland of prawn *L. vannamei* after fed with three Experimental feeds with and without probiotic treatments.

Parameter	Commercial Feed		EF-A		EF-P	
	C	WP	C	WP	C	WP
SOD	23.48±1.32 PDC	36.72±2.08 (+56)	24.39±1.49 PDC	34.46±1.56 (+41)	24.72±1.84 PDC	32.89±1.56 (+33)
Catalase	43.12±2.43 PDC	58.74±2.49 (+36)	43.18±2.34 PDC	58.38±2.36 (+35)	43.42±2.38 PDC	57.94±2.33 (+33)
GPx	7.34±0.42 PDC	10.13±0.46 (+38)	7.36±0.39 PDC	9.72±0.43 (+32)	7.35±0.38 PDC	9.54±0.42 (+30)

All values are Mean ± SD of six individual observations
PDC: Percent Deviation over Control
Values are expressed as Units/g wet weight of tissue/hr
all values are statistically significant at $p< 0.05$

The Gut Microbial load of pacific white shrimp *L. vannamei* were estimated and presented in Table. 9. Total plate count, Total coliforms, Total faecal coliforms and Facultative anaerobes were found to be significantly reduced with the treatment with different types of probiotics compared to control ponds, maintained without probiotics. But, *Lactobacillus* sp. were significantly increased in the gut of prawns in ponds treated with probiotics compared to control ponds without use of probiotics. The Antioxidant enzyme activities in midgut gland tissue of *L. vannamei* were assayed and presented in Table 10. The Super oxide dismutase (SOD), Catalase and Glutathione Peroxidase (GPx) activities of m id gut gland showed a significant elevation in probiotics treated pond animals compared to control pond animals, maintained without probiotics. In the last decade, the consumption of aquatic products has been increased substantially, but the world fishery production was decreased and hence the productions of aquatic products through controlled conditions have been come in to lime light. The probiotics are known to play an important role in carrying out a wide variety of functions including modulation of mucosal and systemic immunity, improving microbial balance by preventing colonization of undesirable bacteria in the intestinal tract [15, 39, 43]. The common probiotics used in pond management are live Bacterial inocula (Non-Pathogenic organisms) rich in extracellular enzymes claims about the potential benefits of probiotics in Aquaculture ponds enhances decomposition of organic matter, reduction of Nitrogen and Phosphorous concentration s, enhances the availability of Oxygen, reducing the Blue-Green Algae, controls Ammonia, controls Nitrate and Hydrogen sulfide, enhances the production rates. The sustainability and the success of aquaculture depend on the quality of soil, water, seed selected and feed used. A good quality of soil, water and seed and feed plays an important role in the successful yield under skilful management practices. The ponds often accumulate with uneaten feed materials excreta, molted shells, dead algae and surface run of organic matter carried by win d and water. When all the above mentioned materials remain un-degraded or partially degraded in reduced oxygen condition and toxic gases such as H_2S and NH_3 will be produced. These gases give rise to stress to the cultured organ isms resulting in the loss of appetite sluggishness, gulping for oxygen etc. and ultimately results in the reduction of growth patterns. Due to usage of probiotics in the present investigation, the primary principle for acceleration of organic matter decomposition by probiotics and the function of C:N ration management by heterotrophic bacteria was carried out successfully [15]. In the present investigation, ammonia, H_2S and CO_2 contents were decreased consequent upon the usage of water probiotics in the culture ponds, whereas the dissolved oxygen content of the water was increased [38]. The probiotics used in the present investigation known to enhance the quantity of heterotrophic bacteria in the culture environment, which in turn maintains the C:N ratio in the ponds. The results obtained in present investigation also gains support from the earlier reports in the culture *Penaeus monodon* and *Litopenaeus vannamei* [35, 36, 38], so it is very clear that probiotics and feed probiotics are capable of maintaining good quality and inducing best growth potentials. In the present investigation prawns showed similar kinds of response to the probiotic treatments. Among the three different types of treatments adopted in the present investigation, the treatment with probiotics yielded good results in terms of productivity and growth potentials. The average body weight of the harvested prawns of probiotics treated and control prawns (Tab les. 4 & 5) showing the difference was statistically significant. The Average daily growth rates recorded in the present investigation also emphasizing that probiotic treatment clearly increasing the growth potentials. Results showed that all probiotic-supplemented ponds and diets resulted in higher growth in prawns than prawns fed with no supplementation of probiotics. This result was very inspiring in prawn culture with probiotics as the size of the prawn was directly related to better foreign exchange earnings. Several authors reported that probiotic treatments improved growth rates in prawn and crab culture activity [14, 24, 25, 46, 56, 57, 58, 61]. The average survival rates recorded are also appears to be relatively more in probiotic treated ponds compared to control pond prawns without treatment with probiotics. Here in this study with the application of probiotics, survival rate of prawns has been found to be more compared to the control ponds, which is similar to the report of Maeda and Liao [24] have also found higher survival and molt rates of prawn larvae of *P. monodon* by treating the pond with soil probiotics.

Organisms life depends upon oxygen as the final acceptor of electrons in mitochondrial electron transport, but the process also generates toxic metabolites, Reactive oxygen species (ROS), and leak from mitochondria in to the cytoplasm where they cause cellular damage by oxidizing a variety of biologically important molecules, including DNA, Protein s, Lipids and Carbohydrates. Aerobic organ isms possess a baseline status of antioxidant system, involved in a variety of detoxification reactions, to assure the maintenance of a balance between production and removal of reactive oxygen species (ROS) and other pro-oxidants. These ROS include Super oxide an ion radical (O_2), hydrogen peroxide (H_2O_2) and highly reactive hydroxyl radical (OH). As a con sequence of the reactivity of ROS and their potential to damage cells and tissues, majority of organisms balance the production of these radical with a wide variety of cellular antioxidant defences. Prominent among these antioxidants are the enzymes Superoxide dismutase (SOD), Catalase, Glutathione Peroxidase (GPx). SOD catalyses the conversion of superoxide an ion radical to H_2O_2, Catalase reduces H_2O_2 to water. Glutathione Peroxidase acts on con junction with other enzymes on H_2O_2 and to terminate Lipid Peroxidation. Under normal condition s i.e., without the influence of stress condition s, a balance exists between the generation of ROS

and other pro-oxidants and their detoxification and removal by Antioxidant defence mechanisms. A number of studies have demonstrated the potential for ROS generation, antioxidant enzyme and the radical scavenging responses and oxidative damage in species of in vertebrates including Molluscs. However, few studies have been undertaken on Crustacean s and little is known about such mechanisms. In the present investigation CAT, SOD and GPx activities, the key enzymes were found to indicate the involvement of detoxification of ROS species. In the present investigation, all the antioxidant enzymes are playing key role in the detoxification of ROS species and inducing the best growth potentials.

4. Conclusion

The present investigation may be concluded that the probiotics play a vital role inducing growth, survival and disease resistance of the aquatic animals by maintain in good water quality parameters throughout the culture period. Probiotic treatment offers a promising alternative to the antibiotics for prawn culture activity in Andhra Pradesh. By using water, soil and feed probiotics, the growth potentials of prawn *L. vannamei* was considerably elevated and which in turn results in good yields. In Andhra Pradesh sustainable prawn culture activity with a wide variety of probiotics is increasing and unemployment can be minimizes through this sector.

5. Acknowledgements

Prof. MSR thanks UGC, New Delhi for financial support.

6. References

1. Anderson T, De Silva Nutrition S. In: *Aquaculture; Farming Aquatic Animals and Plants* (Ed. by J.S. Lucas & P.C. Southgate), Blackwell Publishing, Victoria, Australia, 2003, 146-171.
2. Balcazar JL, De Blas I, Ruiz-zarzuela I, Cunningham DP, Vendrell D, Muzquiz JL. The role of probiotics in aquaculture. Vet. Microbiol. 2006; 114:173-186.
3. Balcazar JL, Luna TR, Cunningham DP. Effect of the addition of four potential probiotic strains on the survival of pacific white shrimp *Litopenaeus vannamei* following immersion challenge with *vibrio ralanaemolyticus.* J. Invertebrate. Pathol. 2007; 96:147-150.
4. Bautista-Teruel MN, Eusebio PS, Welsh TP. Utilization of feed pea *Pisum sativum* meal as a protein source in practical diets for juvenile tiger shrimp *Penaeus monodon*. Aquaculture. 2003; 225:121-131.
5. Bavani M. Studies on the determination of nutritional requirements for freshwater prawn *Macrobrachium rosenbergii*. Ph.D Thesis, S.V University, Tirupathi, 2015.
6. Bhavani M, Suneetha Y, Naidu CK, Srinivasulu Reddy M. Monitoring the effect of water and feed probiotics on the growth potentials in freshwater prawn *Macrobrachium rosenbergii*. Int. J. Fish. Aqua. Sci. 2015; 5(1):59-70.
7. Bhavan PS, Radhakrishnan S. Nutritional indices and biochemical constituents in the prawn *Macrobrachium malcomsoni* fed with formulated feeds. Int. J. Pharma. Bio. Sci. 2012; 3(2):327-336.
8. Decamp O, Moriarty DJW, Lavens P. Probiotics in shrimp culture: Review of field data from Asia and Latin America. Aquacult. Res. 2008; 39:334-338.
9. Deshimaru O, Kuroki K. Studies on a purified diet for prawn I. Basal composition of diet. Bull. Japanese Soc. Sci. Fish. 1974; 40:413-419.
10. Deshimaru O, Yone Y. Optimum level of dietary protein for prawn. Bull. Japanese Soc. Sci. Fish., 1978; 44:1395-1397.
11. FAO. Food and Agriculture organization of United Nations (FAO): The States of the world fisheries and aquaculture. Rome, 2014.
12. Fegan, D. Larval Shrimp Nutrition. Global Aquaculture Advocate, 2004, 64-66.
13. Fuller R. Probiotics: The Scientific Basics. Chapmen and Hall, London, UK, 1992, 162.
14. Garriques D, Arevalo G. An Evolution of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of *Penaeus vannamei* post Larvae in Equador. In: Swimming through troubled water. Proc. Special. Session on Shrimp Farming Aquaculture. (Eds. C.L.Browdy and J.S.Hopkins) World Aquaculture Society Blaton Rouse, LC 1995, 53-59.
15. Gate soupe FJ. The use of probiotics in aqua culture. Aquaculture. 1999; 180:147-165
16. Gimenez AVF, Dia AC, Velurtas SM, Fenucci JL. Partial substitution of fish meal by meat and bone meal, soybean meal, and squid concentrate in feeds for the prawn, *Artemesia longinaris*: Effect on digestive proteinases. The Israeli J. Aquaculture – Bamidgeh. 2009; 61:48-56.
17. Glencross BD. The nutritional management of barramundi, *Lates calcarifer* – a review. Aquacult. Nut., 2006; 12:291-309.
18. Holt JG, Krie NR, Sneath PHA, Stanley JT, Williams ST. Bergeys Manual of determinative Bacteriology. Williams and Wilkins. Baltmore, 1996, 787.
19. Lee PG, Lawrence AL. Digestibility. In: Crustacean Nutrition (Ed. LR. D'Abramo, DE Conklin, DM Akyama. Adv. World Aqua, 1997; 6:194-260.
20. Le Vay LA, Rodriguez A, Kamaludin MS, Jones DA. Influence of live and artificial diets on tissue composition and trypsin activity in *Penaeus japonicus* larvae. Aquaculture. 1993; 118:287-297.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Foln phenol Reagent. J. Biol. Chem. 1954; 193:265-276.
22. Mahmud NA, Hasan MDR, Hossain MB, Minar MH. Proximate composition of fish fed ingredients available in Lakshipur region, Bangladesh. 2012; 12(5):556-560.
23. Manivannan K, Sudhakar M, Murugesan R, Soundarapandian P. Effect of feed on the biochemical composition of commercially important mud crab *Scylla tranquebarica* (Fabricius 1798). Intl. J. Anim. Vet. Advan. 2010; 2:16-20.
24. Maeda M, Nogami K. Some aspects of the biocontrolling method in aquaculture. Current topics in marine biotechnology, Japan. Soc. Mar. Biotechnol. Tokyo, 1989; 395-397.
25. Maeda M, Liao IC. Effect of bacterial population on the growth of a shrimp larva *Penaeus monodon*. Bull. Natl. Res. Iust. Aqua. 1992; 21:25-29.
26. Miller GC. Commercial Fishery and biology of the freshwater shrimp *Macrobrachium* in the Lower St. Paul River. Liberia. 1952-53. P. 13. US. Department of commerce. Natl. Mar. Fish. Serv. Spec. Sci. Rep. Fish. 1971; 626.
27. Misra HP, Fridorch I. The role of superoxide anion in the autooxidation of epinephrine and a sample assay for superoxide dismutase. The. J. Biol. Chem. 1972; 247:3170-3175.
28. Mohanta KN, Subramanian S, Sidweerayya VK.

Evaluation of different Animal protein sources in formulating the diets for Blue Gourami *Trichogaster trichopterus* fingerlings. J. Aquac. Res. Dev. 2013; 4:164-171.
29. Moriarty DJW, Decamp O, Lavens P. Probiotics in Aquaculture. Aquaculture. Asia. Pacific. Mag., 2005, 14-16.
30. New MB, Valenti WC. Freshwater prawn culture: The Farming of *Macrobrachium rosenbergii.* Oxford, England. Blackwell Science. 2000.
31. Ohkawa H, Ohishi N, Yagi K. Assay for lipid Peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochem. 1979; 95:351-358.
32. Parker RB. Probiotics. The other half of the antibiotic story. Anim. Nutri. Health. 1974; 29:4-8.
33. Perez Farfante I, Kensley B. Penaeid and sergestoid shrimps and prawns of the world key and diagnosis for the families and genera. Memoires du Museum national d' Histoire naturelle, Paris. 1997; 175:1-233.
34. Ponat A, Adelung D. Studies to establish an optimal diet for *Carcinus maenas*: II. Protein and lipid requirements. Marine Biol., 1980; 60:115-122.
35. Porubcan RS. Reduction in chemical oxygen demand and improvement in *Penaeus monodon* yield in Ponds inoculated with aerobic Bacillus bacteria. Program and Abstracts of its 22nd Annual conference and exposition. World Aquaculture society. Sam. Juan Puerto Rico.
36. Prabu NM, Nazar AR, Rajagopal S, Khan SA. Use of probiotics in water quality management during shrimp culture. J. Aqua. Trop. 1999; 14(3):227-236.
37. Rajaram V, Dayal JS, Ambasankar K, Ali SA. Silk cotton cake as an alternative protein source in the diet of tiger shrimp *Penaeus monodon* and its effects on growth, nitrogen utilization and metabolism. Ind. Fish. 2012; 59(4):101-110.
38. Rangappa A. Studies on the monitoring of growth patterns of giant fresh water prawn *Macrobrachium rosenbergii*. Ph.D. Thesis. S.V University, Tirupathi. 2011.
39. Ravi VS, Ajmalkhan S, Rajagopal S. Influence of probiotics on growth of Indian white prawn *Penaeus indicus*. J. Sci. Rudi Res. 1998; 57(10):752-756.
40. Rebecca AA, Bhavan PS. Growth performance of *Macrobrachium rosenbergii* post larvae fed with vegetable wastes and palmolein supplemented formulated feeds. Rec. Res. Sci. Tech. 2011: 3(10):69-76.
41. Rosa R, Nunes ML. Biochemical composition of deep sea decapods crustaceans with two different benthic like strategies of the Portuguese South Coast. *Deep Sea Res.*, 2003*;* 50: 119-130.
42. Sailaja M. Studies on the determination of nutritional requirements through formulation of diets for the tropical penaeid prawn *Penaeus monodon* (Fabricius). Ph.D. Thesis. S.V. University. Tirupati. India, 2000.
43. Saha SB, Khan YS, Hakim MA, Anwar MN. Anote on the Bacterial flora of freshly caught prawn *Macrobrachium rosenbergii* from Bangladesh. Mahasapar 1988; 21:253-256.
44. Sheen SS. Lipid supplementation of semi-purified diets for *Penaeus chinensis* juveniles. J. Fish. Soc. Taiwan. 1997; 24:235-242.
45. Sheen SS, D'Abramo LR. Response of juvenile freshwater prawn, *Macrobrachium rosenbergii* to different levels of a cod liver oil/ corn oil mixture in a semi purified diet. Aquaculture, 1991; 93:121-134.
46. Saad SA, Habashy MM, Sharshar KK. Growth response of the fresh water prawn *Macrobrachium rosenbergii* to diets having different levels of biogen. World. App. Sci. J. 2009; 6:550-556.
47. Soltanian S, Francois JM, Dhont S, Arnouts S, Sorgelos P, Bossier P. Enhanced disease resistance in Artemia by application of commercial (beta) glucans sources and chitin in a biotic Artenie cla Lange test. Fish Shellfish Immunol, 2007; 23:1304-1314.
48. Stuck KC, Watts SA, Wang SY. Biochemical responses during starvation and subsequent recovery in post larval white shrimp *Penaeus vannamei*. Marine Biol., 1996; 125:33-45.
49. Shiau SY, Peng CY. Utilization of different carbohydrates at different dietary Protein levels in grass prawn *Penaeus monodon*, reared in seawater. Aquaculture, 1992; 101:241- 250.
50. Sujay Kumar G. Studies on the formulation of feeds for demonstration of nutritional requirements for penaeid prawn *Penaeus monodon.* Ph.D. Thesis. S.V. University. Tirupati. India, 2006.
51. Teshima S, Kanazawa A. Effect of protein, lipid and carbohydrate levels in purified diets on growth and survival rates of the prawn larvae. Bull. Japanese Soc. Sci. Fish., 1984; 50:1709-1715.
52. Tibbetts SM, Lall SP, Anderson DM. Dietary protein requirement of juvenile American eel *Anguilla rostrata* fed practical diets. Aquaculture, 2000; 186:145-155.
53. Thomas EJ, Davis DV, Arnold CR. Evaluation of grow out diets with varying protein and energy levels for red drum *Sciaenops Ocellatus.* Aquaculture, 1999; 176:343-353.
54. Udo IU, Umoren UE. Nutritional evaluation of locally available ingredients use for Least-cost ration formation for African catfish *Clarias garipinus* in Nigeria. Asia. J. Agri. Res. 2011; 5(3):164-175.
55. Vinagre AS, do APN, Amaral FP, Ribarcki EF, da Silveira, Perico E. Seasonal variation of energy metabolism in ghost crab *Ocypode quadrata* at Siriu Beach (Brazil). *Comp. Biochem.* Physiol., Part A: Mole. Integ. Physiol., 2007; 146:514-519.
56. Wang YB. Effect of probiotic on the growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture, 2007; 269:259-264
57. Wang YB, Xu ZR. Effect of Probiotic for common carp *Cyprinus carpio* based on growth performance and digestive enzyme activities. Ani. Feed Sci. Tech. 2006; 127:283-292.
58. Wang YB, Xu IR, Xia MS. The effectiveness of Commercial Probiotics in northern white shrimp *Penaeus vannamei* ponds. Fishery Science. 2005; 71:1034-1039.
59. Wilson RP. Amino acids and Protein. In: *Fish Nutrition* (ed. by J.E. Halver & R.W. Hardy), Academic Press, San Diego. CA, USA. 2002, 43-179.
60. Wurmann C, Madrid RM, Brugger AM. Shrimp farming in Latin America: Current status, opportunities, challenges and strategies for sustainable development. Aquactic. Econ. Manag. 2004; 8:117-141.
61. Yu MC, Li ZJ, Lin H, Wen GL, Ma S. Effects of dietary medicinal herbs and bacillus on survival, growth, body composition and digestive enzyme activity of the white shrimp *Litopenaeus vannamei*. Aquaculture International. 2009; 17:377-384.