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Review Article

Probiotics in Water Quality Management and Disease Resistance in *P. Vannamei* Farms of Razole, East Godavari, Andhra Pradesh

Saif Pilli*

Department of Industrial Fish and Fisheries, University of Calcutta, West Bengal, India

ABSTRACT

Incidences of white feces syndrome was reported in reared farms of Penaeus vannamei during January 2022 in many shrimp farms of Razole, East Godavari, and Andhra Pradesh, India. This was commonly observed in 30-50 DOC (days of culture). A total nine types of water quality parameters were taken to observe how this parameter enhances the White feces syndrome. The early indication of white feces syndrome is identified by white fecal strings is floating on the surface of the water. In severely affected shrimp hepatopancreas and gut becomes white and pale yellow in color. In this study recommendations were made on how to control White feces syndrome by using Probiotics and natural remedies.

Keywords: White feces syndrome; *Enterocytozoon hepatopenaei*; *Penaeus vannamei*; Probiotics; DOC (days of culture)

INTRODUCTION

The world population is increasing day by day, but the production of nutritious food is not increasing in the same pace. The production of capture fisheries is also not enough for the population, it is not sufficient to reach the needs of total population so that people are driving towards aquaculture. To increase the production of the aquatic organisms (Fishes, Crustaceans, Mollusca's, etc.,) humans adopted new methods collectively known as Aquaculture methods. Aquaculture is generally considered as a part of Fishery Science [1]. Aquaculture is divided into three types of Marine water Aquaculture, Fresh water Aquaculture, Brackish water Aquaculture based on the water source. Coming to the statistics of fisheries, the world capture fisheries production is 12.02 million tons, while aquaculture production is 82,095 thousand tons (FAO 2020). Among the aquaculture production Crustacean production is 9387 thousand tons. Among the crustaceans, shrimp production (*Penaeus monodon, Penaeus vannamei*) occupies the major portion (5716.8 thousand tons). In tropical and subtropical countries, the gradual increase of shrimp aquaculture is most prominent [2]. When compared to other culture systems, Shrimp farming has grown to a level of global industry from a traditional, small-scale business in Southeast Asia [3].

India is blessed with a long coastline of over 8000 km providing immense scope for brackish water and marine aquaculture. The land base aquaculture systems include vast potential for freshwater and cold-water ecosystems. Although the aquaculture activity in India is not very new, modern practices with intensification on scientific bases using hatchery produced seeds, formulated feeds and pond and water management methods have been initiated over the last 20 years, and species cultured include diverse aquatic fauna such as finfish, shrimps, crabs, lobsters, prawns, oysters and mussels. The culture of shrimp is the most successful industry among all aquatic species culture. Indian shrimp farming has been evolved as a leading shrimp culture industry in the international scenario, using locally available crustacean species such as *Fenneropenaeus indicus* and *Penaeus monodon* growing in both salt and brackish waters. Before the outbreak of white spot syndrome disease, the culture of tiger shrimp *P. monodon* was in a steady progress globally [4]. Due to difficulties in captive breeding, disease resistant, Specific Pathogen Free (SPF) and genetically improved strains of tiger shrimp could not be developed [5]. In this context SPF status of pacific white leg shrimp (*Litopenaeus vannamei*) was introduced in India in 2009 which revived the shrimp culture in India. The SPF status does not ensure that it is free from all the diseases and therefore, the threat of shrimp diseases continued to remain. The pacific white shrimp *Litopenaeus vannamei* is also known to be vulnerable to a wide range of viral and bacterial diseases and reports of mass mortalities and crop failures of culture systems have also been recorded. Unfortunately, the SPF shrimp was also introduced in those areas where infections already existing. The present scenario of shrimp culture in Andhra Pradesh is beset with all types of diseases. With

Correspondence to: Saif Pilli, Department of Industrial Fish and Fisheries, University of Calcutta, West Bengal, India, E-mail: philippilli143@gmail.com

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the expanding shrimp culture, disease incidence has become a major threat to the aquaculture industry and has a tremendous impact on both socio-economic development and rural livelihood of people. Deterioration of quality in the seed creating the stress leading to viral and bacterial infections is due to supply of post larvae developed from domesticated SPF brood stock by some unregistered hatcheries. Rapid expansion of shrimp culture leads to outbreak of some diseases like WSSV (White Spot Syndrome Virus), IHHNV (Infectious Hypodermal and Hematopoietic Necrosis Virus), YHV (Yellow Head Virus), TSV (Taura Syndrome Virus), WFS (White Faeces Syndrome). Among these, WFS (White Feces Syndrome) reporting huge losses to Aqua farmers. WFS were first reported in Venezuela-South America (FAO). WFS are commonly observed in pond after 30 DOC (Days of culture). WFS is identified by the appearance of white faecal strings, which are aggregated transformed microvilli floating in Figure 1 on the surface of water [4]. WFS are caused by *Enterocytozoon hepatopenaei* (EHP), a microsporidian parasite (fungi) in shrimp hepatopancreas [6]. EHP is a double stranded DNA fungus, which is transmitted both horizontal and vertical manner. Affected shrimp Hepatopancreas and gut becomes whitish in color (Figures 2 and 3). WFS shrimp is also associated with seven species of Vibrio, namely *V. vulnificus, V. fluvialis, V. parahaemolyticus, V.alginolyticus, V. damselae (Photobacterium damselae), V. mimicus* and *V. cholerae.* Total Vibrio Count (TVC) when hit by WFD (White Faeces Disease) was increased 900% in shrimp intestine and hepatopancreas compared to non WFD infected [7]. WFD also associated with EHP. High stocking densities, spurious seed, and use of large quantities of feed materials and chemicals etc. cause disease outbreaks in intensive system due to stress in the environment. Even though many disease-carrying pathogens are present, epidemic diseases occur only when the environment is favorable and the host is weak due to malnutrition or under stress.

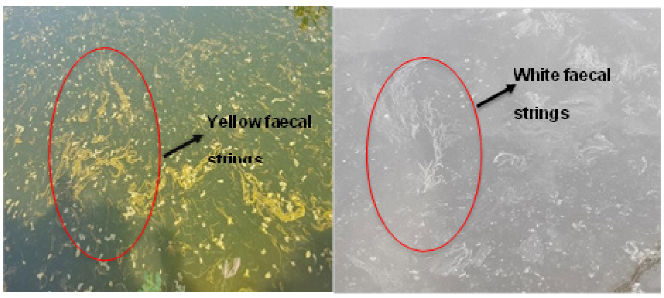

Figure 1: White and yellow faecal strings floating on water.

Figure 2: White faeces syndrome affected *P. vannamei*

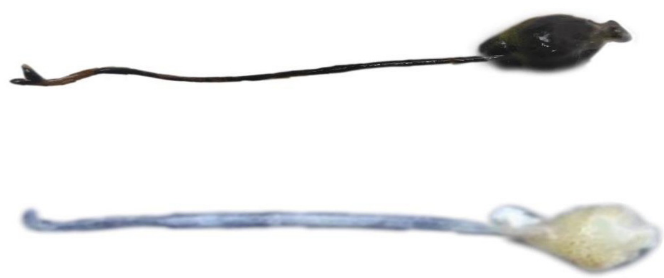

Figure 3: Hepatopancreas with gut of a) Healthy *P. vannamei* b) WFS affected P. vannamei

Pollutants are present in water in the form of solid or gaseous particles. Pollutants are frequently monitored to avoid the huge losses in production of the shrimp organisms [1]. Water quality is assessed based on the results of toxicity tests, which in turn indicate the response of aquatic organisms to certain quantities of pollutants [8]. Different aquatic species have different tolerances against specific toxic compounds. Fluctuation in water quality parameters like dissolved oxygen, temperature, pH, salinity, variety of toxins and inadequate nutrition cause stress on the prawns making them vulnerable to disease. When the water quality parameters exceed the permissible limits, then the water environment becomes unfavorable. It is generating high stress levels in shrimps; this stress levels are favorable to pathogens to enter freely into shrimp. Probably the most common way for the pathogen to enter an aquaculture pond is via introduction of water, and the other way is through brood stock introduction [9]. Solid waste is generated either directly or indirectly from supplementary feed added to the system that is present as uneaten feed residuals, metabolites, manures, and microorganisms growing in the system/pond [10]. Erosion of pond soil was one of the major sources for both solids and organic matter (40%-60%). Because water exchange in pond is mainly through the plastic-lined or PVC pipes, it stands to reason that most organic matter and solids are ultimately derived from feed.

Probiotics, defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' [11]. To promote growth and prevent disease in shrimp aquaculture probiotics are becoming increasingly popular antibiotic alternatives. Though the exact mechanisms of action of probiotics is limited, there is strong evidence showing their probiotic effects through the competitive exclusion of pathogenic bacteria, nutrient and enzymatic contribution to shrimp digestion, enhancement of the shrimp immune response and antiviral effects [12-14]. To date, approximately 20 genera of bacteria have been shown to have a probiotic effect in shrimp. Due to more prevalence and successful application of *Bacillus* and Lactic Acid Bacteria (LAB), such as *Lactobacillus* as probiotics in mammals and poultry, research has focused mainly on *Bacillus* and *Lactobacillus*. Probiotics can be administered orally with the feed (including bio encapsulation with live food vectors such as artemia, directly into the water as purified cultures or spores, or within a fermented growth media, for example *Bacillus subtilis* E20-fermented soybean meal [14-19]. Similarly, probiotics may be administered in combination with a 'non-digestible food ingredient', a complementary prebiotic, that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, to form treatments known as 'symbiotics' [20,21].

Exponential growth of pathogens with the increasing population of stressed prawns in the pond leading to diseases. Hence, addressing the aquatic animal health issues has become an urgent requirement for sustainable growth of aquaculture. Numerous strategies have been used to try to control pathogens through preventive measures. The implementation of Better Management Practices (BMP) at every stage helped to contain the spread of the disease.

Objectives

The objective of this study was to investigate

• How water quality parameters influence EHP (*Enterocytozoon hepatopenaei*) transmission.

• To hypothesize that usage of probiotics will improve the quality of water which in turn can decreases the number of vibrio species in pond and resulting resentence to vibrio infection and WFS outbreak

• To provide a new strategy using Probiotic supplementation to avoid antibiotic use and prevent shrimp production losses from infectious diseases, especially WFS.

MATERIALS AND METHODS

Study area

East Godavari district of Andhra Pradesh has an area of 12805 Sq. Kms with population of 52.86 lakhs (2011 census) (Figure 4). This district ranks 2nd in shrimp farming with 5160 ha spread area. *P. vannamei* is the major cultured shrimp species in East Godavari. Coastal part of East Godavari district practice vannamei farming since its introduction to 2009 (CAA). The present study made use of primary data. The primary data was mainly collected directly from farmers who are doing shrimp farming. In this study, water samples were collected from five farms located at different areas in East Godavari, Razole (Andhra Pradesh). Samples were collected from both WFS affected and unaffected ponds to compare the water quality parameters like salinity, pH, temperature, dissolved oxygen etc. shrimp farms of winter crop located at Karavaka (16.416779,81.946228), Turpupalem (16.399483,81.916317), Sankaraguptam (16.400890,81.847592), Chintalamori (16.387488,81.815431), Gudimula (16.374735,81.736713), of Razole division from Figure 5, East Godavari district, Andhra Pradesh.

Figure 4: Survey location of East Godavari district.
Note: (■) Karavaka; (■) Turupupalem; (■) Sankaraguptnam; (■) Chinatalamori; (■) Gudimula

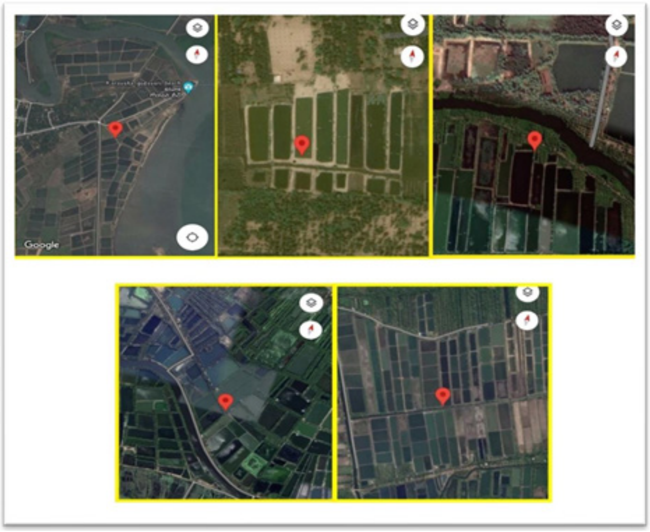

Figure 5: Map showing locations of sample collection.

Sample collection

Samples were collected from five different areas from 9th January 2022 to 26th January 2022 as shown in Figure 1. In each pond water is taken from four different areas of same pond and mixed thoroughly; 250 ml of sample is taken, and immediately transported to the laboratory.

Water analysis

Temperature: Water temperature was measured by using mercuric thermometer. Temperature readings were taken at regular intervals (Morning, Afternoon, Evening) during the study period and the average value was calculated, readings with mean ± standard deviation. Same protocol was repeated for all five different farms.

pH: pH was calculated by using mercuric electrode pH meter. The pH of all five farms was measured by using pH meter, and the values were with mean ± Standard deviation.

Salinity: Salinity was measured by using salinometer. The salinity of all farms was measured by using salinometer, and the values were with mean ± standard deviation.

Alkalinity: Alkalinity was calculated by using volumetric titration method. The alkalinity readings of all farms were calculated, resultant values were with mean ± standard deviation.

Hardness: Hardness of water is due to the presence of dissolved calcium and magnesium particles. Hardness of water in 5 farms was checked by using hardness testing kit, resultant values were with mean ± standard deviation.

Ammonium: Ammonium levels in water are due to faecal pollution of aquatic organisms. Ammonium of water in all five farms was calculated by using volumetric analysis, results were with mean ± standard deviation.

Nitrite: The nitrite value increases with the fluctuations in temperature and environment. Nitrite content of all five farms was calculated by using volumetric analysis and the resultant values with mean ± standard deviation.

Bacterial colonies: Bacterial colonies present in water contain Vibrio species. To test the presence of bacterial colonies we must prepare the TCBS (Thiosulfate-bile-salts- sucrose) agar.

Preparation of TCBS agar: 89.08 gm of dehydrated TCBS powder medium was taken in 1000 ml of distilled or deionized water and gently mixed the medium with distilled water. Then the solution was heated to dissolve the medium completely. It should be cooled at 45-50˚C. Mixed well and poured into sterile petri plates.

Formation bacterial colonies: The prepared TCBS agar was taken into petri plate. Few micro liters of sample water were taken on petri dish and streaked the water sample by using L shaped glass rod. Streaked well up to 2-3 minutes and a glass lid was placed on the Petri dish. The entire procedure was done in laminar air flow. After completion of streaking the samples were kept in incubator, at a temperature of 38˚C for 18-24 hours. After that the samples were taken out from incubator and the yellow and green colonies were counted on the petri plate (Table 1).

Preparation of natural probiotics: 250 liters plastic tub was taken and filled with pond water up to 60-80 liters. 50 kgs of rice bran taken into tub and stirred it gently using a rod. Then 10 kgs of ground nut oil cake was added and stirred it gently. To that mixture, 10 kgs of jaggery or molasses were added and mixed gently, if necessary, water added, mixed gently. After that 1-2 Kgs of yeast bacterial powder available in market was added. Finally mixed gently up to 10-15 minutes, and for fermentation the tub was left for 2-4 days (Figure 6).

Exchange of pond water: Water exchange is additionally a method of improving other water quality conditions. If dissolved oxygen concentration in a very pond is low and also the water within the supply canal includes a high dissolved oxygen concentration, high water exchange rates can improve dissolved oxygen concentrations within the pond. Likewise, excessive nutrients, plankton and toxic metabolites may be flushed from ponds through water exchange. Influent water should enter ponds at the surface and effluent pond water should be discharged from a drain located near the pond bottom and on the alternative side of the pond. The foremost beneficial means of exchanging water during a pond is to first drain out the quantity to be exchanged then pump or let in an equal volume of prime quality water [5]. As the pond got affected by white feces syndrome there were some fluctuations in water quality, so the water got exchanged immediately (Figure 7).

Applying natural probiotics and market available probiotics: Application of probiotics was done in different ways. There are two types of Probiotics i.e., naturally prepared probiotics and Probiotics available in market, like Avant pro W, Vib-go, Pro-B-Aqua, GEO-PROB. The probiotics were applied in this manner i.e., natural probiotics, already in the form of liquid were sprayed into pond.

Avant pro W (artificially available probiotic) contains Bacillus subtulis, Pediococcus acidilactici. Vib-go contains Bacillus subtilis-2.5 billion/g, Bacillus megaterium-2.5 billion/g, Bacillus licheniformis-2.5 billion/g, Bacillus pumilus-2.5 billion/g. Pro-B-Aqua contains 14 different strains with consortium of lactic acid bacteria *Lactobacillus* Biofidobacterium Rhodospeudomonas. The probiotic powder was mixed in 20-30 liters of fresh water, and 1-2 kg of jaggery/molasses added and mixed thoroughly and brewed overnight. Then broadcasted all over the pond surface during morning hours with running aerators. Then 5-10 gram of probiotic liquid added to the feed of 1 kg.

Biosecurity: Care was taken to prevent the mixing of water from diseased pond to healthy pond. A proper fencing was constructed around the pond to prevent entry of predators and infected organisms. Entry of unknown persons into the pond was strictly prohibited. Nylon mesh was constructed over the pond for prohibition of infected shrimps from near ponds by predators.

Statistical analysis: To calculate the influence of water quality parameters in outbreak of WFS, we used the following statistical methods i.e., Simple mean, Median and Standard deviation in MS Excel (Tables 1 and 2).

Table 1: WFS affected pond water analysis report.

S. No	Water Parameter	P-1	P-2	P-3	P-4	P-5
1	Temperature (˚C)	29 ± 1.6	30 ± 1.3	30.5 ± 1.4	31 ± 1.5	29.5 ± 1.2
2	pH	7.8 ± 0.26	7.3 ± 0.78	7.2 ± 0.39	7.4 ± 0.65	7.1 ± 0.44
3	Salinity (PPT)	6 ± 2.6	15 ± 4.3	13 ± 2.9	10 ± 3.3	12 ± 3.7
4	Alkalinity (mg/l)	140 ± 12.6	160 ± 12.3	140 ± 13.3	110 ± 13.2	120 ± 11.8
5	Hardness (PPM)	5400 ± 211	4800 ± 236	4650 ± 320	5930 ± 295	5760 ± 275
6	Ammonium (NH4)	0.20 ± 0.012	0.25 ± 0.021	0.20 ± 0.043	0.20 ± 0.014	0.25 ± 0.022
7	Nitrite (No2)	0.05 ± 0.0010	0.07 ± 0.002	0.08 ± 0.006	0.06 ± 0.004	0.09 ± 0.007
8	Green colonies (CFU/ml)	40 ± 5.9	30 ± 6.3	45 ± 6.1	50 ± 7.0	35 ± 6.9
9	Yellow colonies (CFU/ml)	250 ± 7.8	240 ± 8.3	280 ± 7.5	300 ± 8.5	270 ± 8.1

Note: P-1 to P-5, P-Pond

Figure 6: Preparation of Natural Probiotics.

Figure 7: Exchange of water.

RESULTS

Water analysis

Physico-chemical factors: Results of all five farms were summarized before and after application of Probiotics in WFS affected ponds. After usage of natural remedies and probiotics, finally WFS was controlled. The results of WFS affected farms Table 1 before control and after control by Probiotics showed that Table 2 and Figure 8, the temperature was optimal in WFS controlled farms than the affected farms. Hydrogen potential (pH) in WFS affected farms resulted low pH Table 1 when compared with WFS controlled farms in which basic nature existed (Table 2 and Figure 9). Alkalinity was low in WFS affected farms from Table 1 as compared with WFS controlled farms (Table 2). Figure 10 showed variation in alkalinity of both affected and controlled farms. When hardness of water was taken into consideration, WFS affected farms resulted in high levels of hardness and Ammonium Table 1 as compared with that of controlled farms (Table 2). After application of probiotics hardness and ammonium levels decreased with control of WFS (Figures 11 and 12). High levels of nitrate content were observed in WFS affected farms from Table 1 than in WFS controlled farms (Table 2). Figure 13 showed the variation of nitrate values before and after the control of WFS (Figure 14).

Table 2: After WFS controlled water quality parameters.

S No	Water parameter	P-1	P-2	P-3	P-4	P-5
1	Temperature (°C)	27.9 ± 1.3	28.5 ± 1.1	28.6 ± 1.4	28.9 ± 1.0	28.8 ± 1.2
2	pH	7.9 ± 0.56	8.1 ± 0.23	8.3 ± 0.46	8.4 ± 0.21	8.7 ± 0.14
3	Salinity (PPT)	7 ± 2.1	14 ± 3.2	13 ± 2.7	12 ± 2.3	16 ± 3.0
4	Alkalinity (mg/l)	210 ± 9.2	230 ± 12.5	280 ± 13.5	260 ± 12.2	230 ± 11.9
5	Hardness (PPM)	3000 ± 150	3980 ± 199	3680 ± 160	3700 ± 156	3200 ± 140
6	Ammonium (NH4)	0.03 ± 0.002	0.02 ± 0.001	0.01 ± 0.001	Nil	0.03 ± 0.002
7	Nitrite (No2)	0	0.02 ± 0.002	0	0	0.03 ± 0.002
8	Green colonies (CFU/ml)	20 ± 2.2	10 ± 2.4	0	20 ± 3.1	0
9	Yellow colonies (CFU/ml)	280 ± 9.3	300 ± 8.6	320 ± 8.2	340 ± 8.5	380 ± 9.5

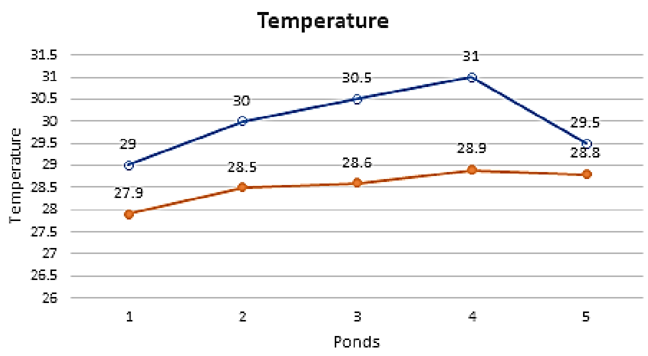

Figure 8: Comparison of Temperatures. **Note:** (—o—) WFS affected; (—●—) WFS controlled

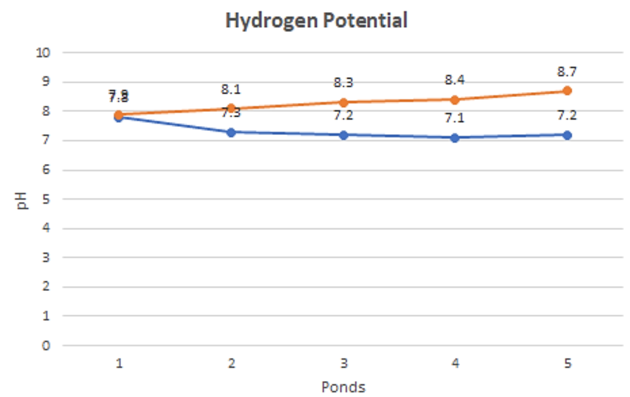

Figure 9: Comparison of hydrogen potential. **Note:** (—o—) WFS affected; (—●—) WFS controlled

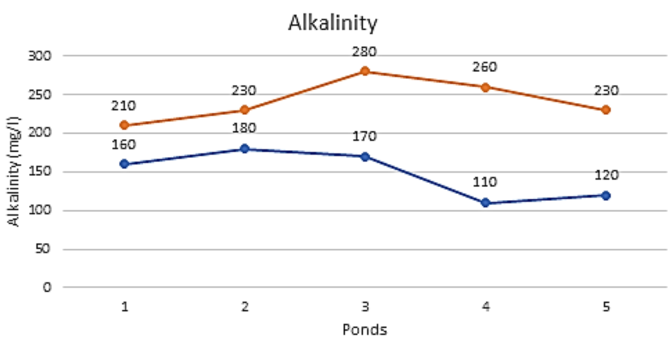

Figure 10: Comparison of alkalinity. **Note:** (—o—) WFS affected; (—●—) WFS controlled

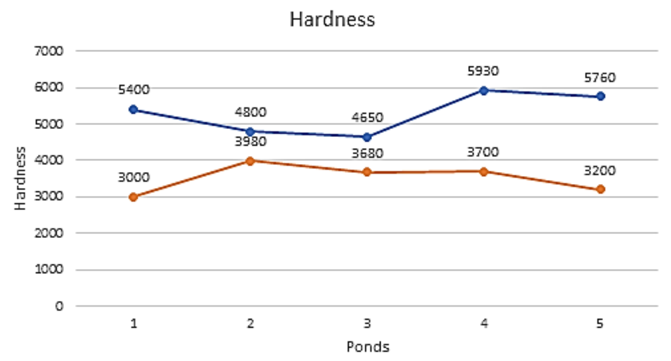

Figure 11: Comparison of hardness. **Note:** (—◆—) WFS affected; (—◆—) WFS controlled

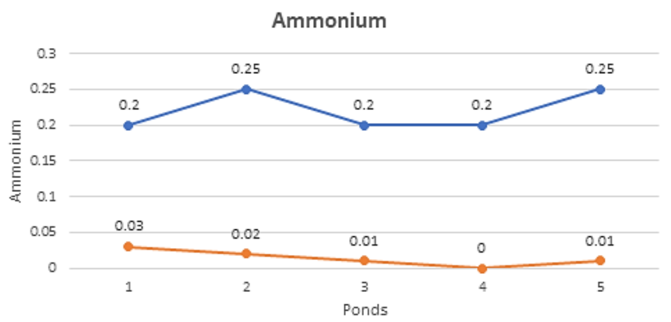

Figure 12: Comparison of ammonium. **Note:** (—◆—) WFS affected; (—◆—) WFS controlled

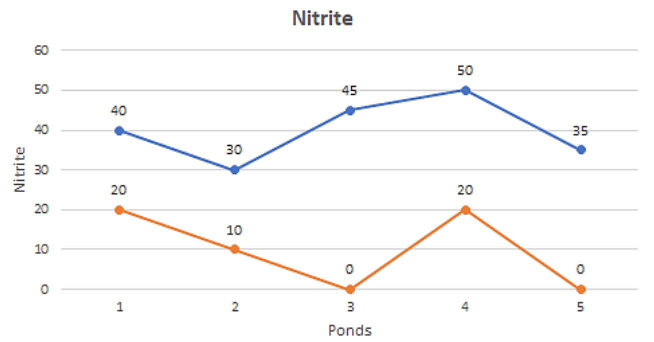

Figure 13: Comparison of nitrite. **Note:** (—◆—) WFS affected; (—◆—) WFS controlled

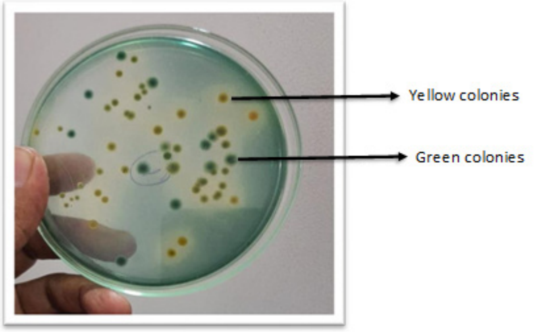

Figure 14: Formed bacterial colonies (CFU/ml) in WFS affected pond.

Bacterial colonies

Bacterial colonies were formed in the presence of TCBS agar. Bacterial colonies were counted, and results were shown in Table 1 and 2. Two different types of bacterial colonies were formed (Figure 14). When the bacterial colonies of the pond water were considered, yellow colonies of the water resulted in low count in WFS affected pond Table 3, when compared after treatment. So, the count of yellow conies increased after treatment with Probiotics and control of WFS (Table 4 and Figure 15). Green colonies are high in WFS affected in Table 3 when compared to count in controlled farms (Table 4 and Figure 16).

Table 3: WFS affected pond water analysis report.

S No	Pond	Green colonies (CFU/ml)	Yellow colonies (CFU/ml)
1	1	40 ± 5.9	250 ± 7.8
2	2	30 ± 6.3	240 ± 8.3
3	3	45 ± 6.1	280 ± 7.5
4	4	50 ± 7.0	300 ± 8.5
5	5	35 ± 6.9	270 ± 8.1

Table 4: After WFS affected pond water analysis report.

S No	Pond	Green colonies (CFU/ml)	Yellow colonies (CFU/ml)
1	1	20 ± 2.2	280 ± 9.3
2	2	10 ± 2.4	300 ± 8.6
3	3	0	320 ± 8.2
4	4	20 ± 3.1	340 ± 8.5
5	5	0	380 ± 9.5

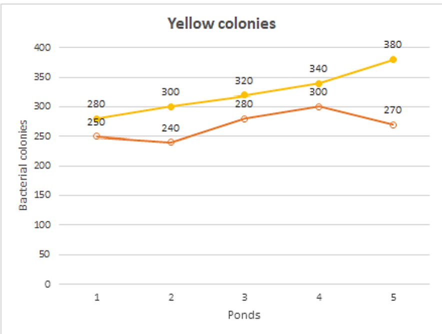

Figure 15: Comparison of yellow colonies.

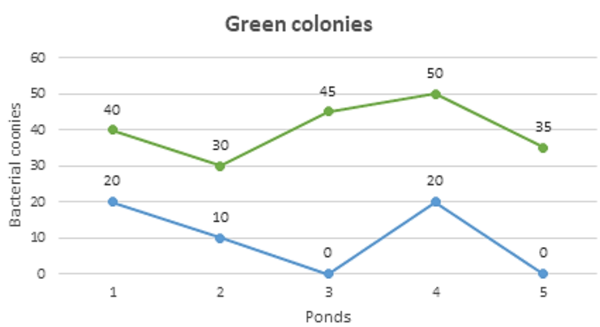

Figure 16: Comparison of green colonies. **Note:** (—●—) WFS affected; (—●—) WFS controlled

DISCUSSION

The shrimp culture industry has been confronted with serious disease problems, which have limited the success of the industry worldwide. Pathogenic Vibrio spp. has been implicated as one of the major causes of disease problems [22]. There has been a widespread use of antibiotics as a remedial measure. However, the excessive and inappropriate use of antibiotics has resulted in the presence of resistant strains of bacteria in shrimp culture, to avoid problems of drug resistance, alternative methods have been used to improve shrimp health and yields, such as the use of probiotic bacteria [23-25]. White feces syndrome has caused a huge economic loss to the shrimp farmers, because affected shrimp population showed elevated Feed Conversion Ratio (FCR), reduction in growth, and size variations of individual shrimps at the time harvesting. From last 5 years White Feces Syndrome (WFS) incidents were especially very high in **Penaeus vannamei** shrimp farms after 30-50 DOC (days of culture). I have performed a direct analysis of the samples of WFS affected farms, the physico-chemical factors influencing the incidence of white feces syndrome. Water quality is one of the major factors in the enhancement of white feces syndrome in *P. vannamei* culture ponds. Physico-chemical parameters of water were influenced by both environmental and management practices. The result analysis (Mean ± SD) of the water quality parameters in shrimp, *P. vannamei* culture farms of Razole, East Godavari district in Andhra Pradesh were shown in Tables 1 and 2. In shrimp farms water exchange was a good management tool, which intended to reduce organic and solid waste loadings in shrimp farms. If water exchange was not being practiced or is significantly reduced, it could cause the increase in concentrations of waste load which leads to chances of disease input.

Temperature: Temperature is one of the most important factors controlling the photosynthesis which in turn influence the growth and disease management (Bower CE, Bid well J P, 1978). Seasonal changes in temperature are common. The high temperature will cause the increases of bacterial load and other chemical loads in culture ponds or farms. During the study period, Table 1 the high temperatures noticed in the WFS affected ponds causes the physiological stress to the marine shrimps.

pH: pH is an important water quality parameter to control the growth and survival of the shrimp. When it is low the water becomes acidic (below-7) and high the water becomes basic (above-7). 7.5-8.5 is the optimum ranges for shrimp growth. The most common cause of high pH is in shrimp ponds were high rate of phytoplankton blooms, water alkalinity, and soil pH and lime applications. During the study period, optimal ranges were observed in affected and controlled farms.

Salinity: Salinity is one of the important parameters to increase the growth of marine shrimps in culture ponds. Optimum salinity is above 5 ppt in culture pond. This parameter does not show any impact on the WFS in culture ponds.

Alkalinity: Alkalinity plays a major role in marine shrimp culture because alkalinity involves in the shrimp moulting process. In the present study, in WFS affected ponds when in Table 1 alkalinity is low, after WFS is controlled in Table 2 the alkalinity increased to optimum level. Low alkalinity levels led to pH variations which resulted in reduced growth and increase in the bacterial loads. High alkalinity levels cases the process of moulting.

Total hardness: Total hardness (calcium and Magnesium) is essential for the shrimp growth. Hardness of water in WFS affected farms have shown in Table 1 high values compared with WFS controlled ponds Table 2, which in turn enhances the chances of WFS in Shrimp ponds.

Ammonium: Ammonia is the main product in protein catabolism in crustaceans and can account for 50-90% of nitrogen excretion. Large scale fluctuations in ammonium have been demonstrated in closed shrimp culture systems [26]. The present study coincides with the findings of Thakur and Lin. By having high ammonium levels in affected ponds (Table 1). Many farmers are very careful about their culture farms in maintaining good water quality using Probiotics. As temperature rises in the pond water, toxicity of ammonium increases, which enhances the white faeces syndrome.

Nitrite: Nitrites are mainly formed by the process of nitrification. Nitrite, intermediate product during the two steps of oxidation of ammonium. Nitrite is commonly found in intensive ponds because large amounts of nitrogen are added in the form of feed, fertilizer, or manure. The nitrite may also accumulate in the water after sudden increase of ammonium concentrations by phytoplankton decay. In the present study, Nitrite in WFS affected ponds Table 1 was high due to improper management practices. But after application of Probiotics, nitrite levels were reduced.

Bacterial colonies: Bacterial colonies or Bacterial load is due to the presence of useful and harmful bacteria present in culture ponds. In culture ponds bacterial colonies are mainly of two types i.e., yellow colonies and green colonies. Compared with yellow colonies, green colonies have a high load of vibrio species. WFS affected ponds in Table 1 having high count of green colonies and the normal range of green colonies was up to 20 CFU. Whereas yellow colonies in WFS affected in Table 1 ponds were low in count, and in the WFS controlled ponds normal counts of bacterial loads were observed.

Some of the shrimp farmers are adopting new methods on their own. For example, through discussion with local farmers in Razole, East Godavari, I have observed that farmers are adopting two main strategies:

1) By frequent addition of probiotics to the water they are attempting to reduce the vibrio species population in the culture ponds

2) Through use of feed additives and probiotics attempt to reduce the pathogens in shrimp digestive tract. For example: feed additive is garlic in the form of freshly crushed, and turmeric powder is also used by local farmers to reduce the white faeces in shrimp.

We recommended the use of probiotics to kill harmful bacteria and to improve the gut appetite. Use of natural remedies, Exchange of water and maintaining a good biosecurity measure will also reduce the white faeces in shrimp ponds [27-31].

CONCLUSION

Maintenance of good water quality parameters is essential to any shrimp culture ponds. Disease out breaks in culture ponds is mainly due to the improper maintenance of water quality. A combination of inorganic fertilizers, aeration, waste removal and water exchange and application of water probiotics once or twice a week should be must to maintain the efficient water quality. It is necessary to control the disease out breaks in shrimp culture. The present study helps the farmers to decreases the WFS out breaks and increases the shrimp production. The study concludes that the maintenances of optimal water quality parameters in all *P. vannamei* culture ponds helps the environmentally friendly practices of shrimp culture in India.

REFERENCES

1. Mercier V, Fox D, Khan H, Taylor D, Raymond B, Bond W, et al. Application and Testing of the Water Quality Index in Atlantic Canada: Report Summary. CCME. 2004.

2. Clesceri LS. Standard methods for examination of water and wastewater. Am J Public Health. 1998;9.

3. Bower CE, Bidwell JP. Ionization of ammonia in seawater: Effects of temperature, pH, and salinity. J Fish Res Board Can. 1978;35(7):1012-6.

4. Sriurairatana S, Boonyawiwat V, Gangnonngiw W, Laosutthipong C, Hiranchan J, Flegel TW. White feces syndrome of shrimp arises from transformation, sloughing and aggregation of hepatopancreatic microvilli into vermiform bodies superficially resembling gregarines. Plos one. 2014;9(6):e99170.

5. Boyd CE. Water quality in ponds for aquaculture. Alabama Agricultural Experiment Station. 1990

6. Tangprasittipap A, Srisala J, Chouwdee S, Somboon M, Chuchird N, Limsuwan C, et al. The microsporidian *Enterocytozoon hepatopenaei* is not the cause of white feces syndrome in whiteleg shrimp Penaeus *Litopenaeus vannamei*. BMC Vet Res. 2013;9(1):1-0.

7. Somboon M, Purivirojkul W, Limsuwan C, Chuchird N. Effect of Vibrio spp. in white feces infected shrimp in Chanthaburi, Thailand. J Fish Environ. 2012;36(1):7-15.

8. Carbajal-Hernández JJ, Sánchez-Fernández LP, Carrasco-Ochoa JA, Martínez-Trinidad JF. Immediate water quality assessment in shrimp culture using fuzzy inference systems. Expert Syst Appl. 2012;39(12):10571-82.

9. Lightner DV. Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. J World Aquac Soc. 2005;36(3):229-48.

10. Viadero Jr RC, Noblet JA. Membrane filtration for removal of fine solids from aquaculture process water. Aquac Eng. 2002;26(3):151-69.

11. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014.

12. Hoseinifar SH, Sun YZ, Wang A, Zhou Z. Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. Front Microbiol. 2018;9:2429.

13. Li E, Xu C, Wang X, Wang S, Zhao Q, Zhang M, et al. Gut microbiota and its modulation for healthy farming of Pacific white shrimp *Litopenaeus vannamei*. Rev Fish Sci Aquac. 2018;26(3):381-99.

14. Ringo E. Probiotics in shellfish aquaculture. Aquac Fish. 2020;5(1):1-27.

15. Immanuel G. Bioencapsulation of brine shrimp *Artemia nauplii* with probionts and their resistance against Vibrio pathogens. J Fish Aquat Sci. 2016;11(4):323-30.

16. Liu KF, Chiu CH, Shiu YL, Cheng W, Liu CH. Effects of the probiotic, Bacillus subtilis E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. Fish Shellfish Immunol. 2010;28(5-6):837-44.

17. Tseng DY, Ho PL, Huang SY, Cheng SC, Shiu YL, Chiu CS, et al. Enhancement of immunity and disease resistance in the white shrimp, *Litopenaeus vannamei*, by the probiotic, Bacillus subtilis E20. Fish Shellfish Immunol. 2009;26(2):339-44.

18. Tsai CY, Chi CC, Liu CH. The growth and apparent digestibility of white shrimp, *Litopenaeus vannamei*, are increased with the probiotic, Bacillus subtilis. Aquac Res. 2019;50(5):1475-81.

19. Wang YC, Hu SY, Chiu CS, Liu CH. Multiple-strain probiotics appear to be more effective in improving the growth performance and health status of white shrimp, *Litopenaeus vannamei*, than single probiotic strains. Fish Shellfish Immunol. 2019;84:1050-8.

20. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J Nutr. 1995;125(6):1401-12.

21. Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics-approaching a definition. Am J Clin Nutr. 2001;73(2):361s-4s.

22. Rengpipat S, Phianphak W, Piyatiratitivorakul S, Menasveta P. Effects of a probiotic bacterium on black tiger shrimp Penaeus monodon survival and growth. Aquaculture. 1998;167(3-4):301-13.

23. Riquelme C, Hayashida G, Toranzo AE, Vilches J, Chavez P. Pathogenicity studies on a Vibrio anguillarum-related strain causing an epizootic in Argopecten purpuratus larvae cultured in Chile. Dis Aquat Org. 1995;22(2):135-41.

24. Moriarty DJ. Control of luminous Vibrio species in penaeid aquaculture ponds. Aquaculture. 1998;164(1-4):351-8.

25. Itami T, Asano M, Tokushige K, Kubono K, Nakagawa A, Takeno N, et al. Enhancement of disease resistance of kuruma shrimp, *Penaeus japonicus*, after oral administration of peptidoglycan derived from *Bifidobacterium thermophilum*. Aquaculture. 1998;164(1-4):277-88.

26. Thakur DP, Lin CK. Water quality and nutrient budget in closed shrimp (Penaeus monodon) culture systems. Aquac Eng. 2003;27(3):159-76.

27. Rengpipat S, Rukpratanporn S, Piyatiratitivorakul S, Menasveta P. Probiotics in aquaculture: A case study of probiotics for larvae of the black tiger shrimp (Penaeus monodon). Biotechnol Adv. 1998:177-81.

28. Palanikumar P, Wahjuningrum D, Abinaya P, Babu MM, Citarasu T. Usage of plant natural products for prevention and control of White Feces Syndrome (WFS) in Pacific whiteleg shrimp *Litopenaeus vannamei* farming in India. Aquac Int. 2020;28(1):113-25.

29. Mastan SA. Incidences of White Feces Syndrome (WFS) in farm-reared shrimp, *Litopenaeus vannamei*, Andhra Pradesh. Indo Am J pharm. 2015;5(9):3044-7.

30. Phillips MJ, Lin CK, Beveridge MC. Shrimp culture and the environment: Lessons from the world's most rapidly expanding warmwater aquaculture sector. 1993:171-97.

31. Ringo E, Doan HV, Lee S, Song SK. Lactic acid bacteria in shellfish: Possibilities and challenges. Rev Fish. Sci Aquac. 2020;28(2):139-69.