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Identification, Impact of White Faecal Disease and Improving Disease Resistance for *Litopenaeus vannamei* Shrimp Forming Ponds through Application of Probiotics as Feed in Machilipatnam, Krishna District

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Abstract

This study firmly establishes the remarkable benefits of Aqua Fortuna probiotic (AFPB), which features a blend of bacterial strains sourced from fish gut microbiota, for enhancing the physiological performance of white-leg shrimp (*Litopenaeus vannamei*). As the most extensively farmed crustacean worldwide, this species is crucial to India's shrimp exports, contributing over 50% of the total. However, shrimp aquaculture faces significant challenges from various microbial diseases, predominantly viral and bacterial, which lead to severe mortality rates. The frequent use of antibiotics and chemical treatments in this sector poses serious risks to both environmental health and human safety, and is often ineffective against many pathogens, particularly viruses. Therefore, adopting effective, chemical-free solutions for managing infectious diseases in aquatic farming is essential. Recent findings unequivocally demonstrate that incorporating probiotics into aquaculture practices significantly reduces the incidence of white faecal syndrome, thereby optimizing shrimp production. In fact, the use of these probiotics resulted in a marked decrease in disease intensity after just 14 days.

***Key words*:** *Litopenaeus vannamei*, Probiotics, White faecal syndrome, Gut microbiota

Shrimp farming has emerged as a valuable aquaculture practice focused on cultivating marine shrimp in seawater or brackish water, ultimately providing a significant source of human food. This industry has experienced impressive growth on a global scale, and with strategic advancements, it holds great potential for further economic development. However, challenges such as outbreaks of viral diseases have been identified, highlighting the need for a concerted effort to mitigate risks and promote sustainable practices within the sector. In India, Andhra Pradesh stands out due to its extensive brackish water area, which is the second largest in the country. Covering approximately 37,560 hectares and boasting a 972 km coastline across nine districts, the region has become a hub for coastal aquaculture. The growth of shrimp farming, particularly of *Litopenaeus vannamei*, has been remarkable, with a significant increase in culture area from 264 hectares in 2009-10 to 37,560 hectares in 2014-15 [1].

As a leader in shrimp production, Andhra Pradesh accounted for 78% of the nation's output by 2015, producing 276,077 MT. Despite these achievements, the industry has faced setbacks due to disease outbreaks, leading to significant economic losses estimated at over Rs. 1,000 crores from 2006-2008. Continuously improving biosecurity measures and addressing the introduction of pathogens are crucial for sustaining growth. For instance, the implementation of best practices in pond management and sourcing shrimp from registered, specific pathogen-free hatcheries can greatly reduce disease risks. The successful introduction of specific pathogen-free strains, such as *Litopenaeus vannamei* in 2009, represents a positive step toward revitalizing the shrimp culture industry, although it is essential to ensure these strains are deployed in areas with minimum infection risk [2]. Overcoming challenges such as inadequate farm management, lack of infrastructure and varying local practices requires a collaborative approach that emphasizes technical training and support.

To address the complexities of aquaculture, it is important to enhance monitoring of health and disease risks, as these often stem from interactions among the farmed organisms, pathogens, and environmental conditions. A proactive strategy that focuses on disease prevention can significantly improve outcomes. The use of probiotics has shown promise in supporting shrimp health and enhancing immune responses.

Since their introduction in the 1970s as supplements in animal feeds, probiotics have proven effective in boosting growth and resilience against diseases. Additionally, antibiotics remain a common practice in aquaculture for both preventive and therapeutic purposes [3]. There are many commercially available probiotics tailored for shrimp farming, such as Bio-green and Aqualact, along with specific strains like *Lactobacillus rhamnosus* sourced from reputable suppliers in Andhra Pradesh. By leveraging these advancements and

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reinforcing best practices, the shrimp farming industry can not only recover but thrive sustainably, ensuring its vital role in food security and economic growth.

MATERIALS AND METHODS

Collection of shrimp larvae

Litopenaeus vannamei (6 lakhs of young shrimps) post larvae were collected from the hatchery at CP Acquaculturee Ltd. Chennai, Tamil Nadu. The post larvae of *Litopenaeus vannamei* with an average weight 1:8 ± 02 g.

Location of the and experimental design

The feeding evaluation was done in the Sreenu aquaculture, Machilipatnam, Andhra Pradesh, India. The shrimps were habituated in provided ponds comprising natural seawater (salinity from 02 to 05 ppt, pH 7.5 to 8.5, temperature 27±2 °C) for 7 days before and throughout the experiment. The post larvae of *Litopenaeus vannamei* with an average weight 1:8 ± 02 g were procured from Progutgen Aquaculture Gut Probiotic commercial laboratory.

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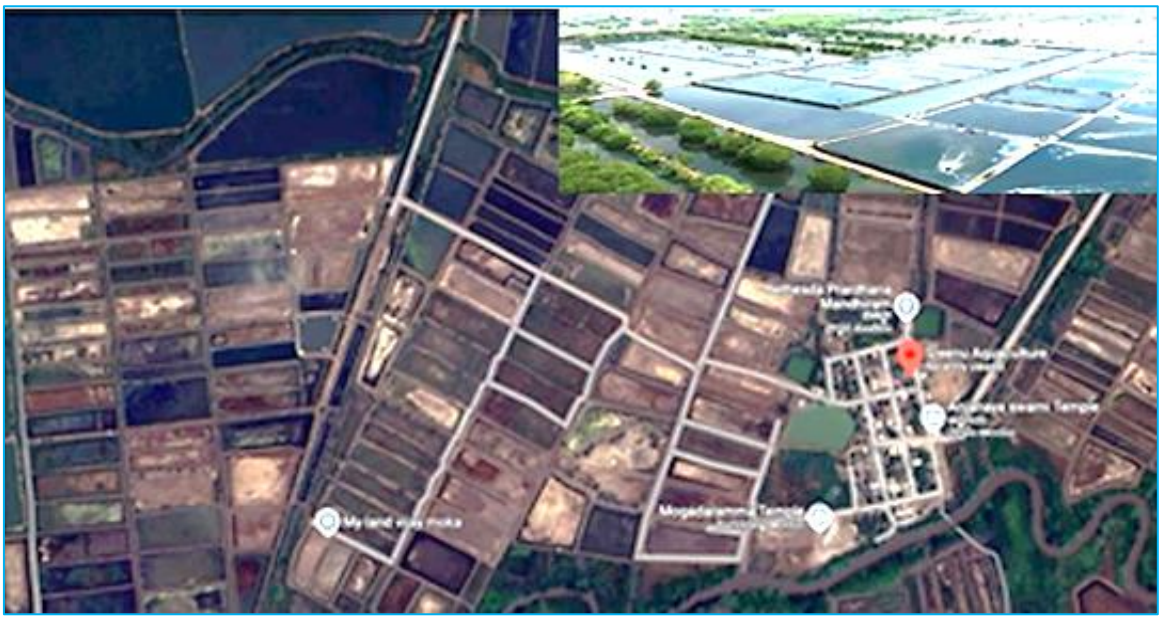

Fig 1 Location and preparation of the pond

Preparation of the Pond

In any earthen pond culture system, the bottom soil plays a crucial role in pond yield. High Organic matter content in neutral soil often promotes higher primary productivity and hence higher fish/ shrimp yield. Natural food organisms are one of the most important food sources in ponds. It contains rich in protein, vitamins, mineral and other essential growth elements that simple supplementary feed cannot complete. Aquatic organisms (Fish or Shrimp) yield in pond can also be affected by the presence of predators, deteriorating water quality and improper pond management. Hence, pond preparation is an initial step towards ensuring a better pond production. In any earthen pond culture system, the bottom soil is critical to pond productivity. High organic matter concentration in neutral soil frequently promotes increased primary production and thus higher fish/shrimp yield. Natural food organisms are a major food sources in ponds. It is high in protein, vitamin, minerals, and other vital growth nutrients that plain extra feed cannot provide. Aquatic creatures (shrimp or fish) yield in ponds can also be influenced by predators, worsening water quality, and poor pond management. As a result, pond preparation is an important first step in improving pond productivity.

With above sources our research work was going on white faecal disease symptoms and how the previous studies overcome from this disease in shrimp culture were applied optimum probiotics to get positive results.

RESULTS AND DISCUSSION

Clinical signs of WFS

According to the ICAR-CIBA Technical Advisory Series No. 6 revealed about the White Feces Syndrome (WFS) signs during the cultivation, in affected shrimp farms, WFS is evident with whitish faecal strings floating on the pond surface. White faecal threads could also be found in the feeding trays. Shrimp affected with WFS excrete white faecal strings and show white/golden brown intestine. Feed consumption in the affected ponds is significantly decreased. WFS usually become evident after 30 to 40 days of culture. Pond affected with WFS show white faecal strings, these strings floating on the surface of pond surface for a period of 10 days to 45 days or more, elevated FCR, growth reduction, size variation, loose shell and daily mortalities. Loose shell affected shrimps are less active and found sluggishly swimming at the surface of pond water.

White Feces Syndrome (WFS) is found in growing ponds of *Litopenaeus vannamei* (Fig 2a-d). Incidences of White Feces Syndrome were observed after 25-35 days of stocking of the PLs (Post larvae's) [4]. The occurrence of WFS in shrimp

is attributed to the transformation, sloughing, and aggregation of hepato-pancreatic microvilli into vermiform bodies, which bears a resemblance to protozoan gregarines. This white fecal matter floats on the water surface in the culture ponds. The presence of protozoan gregarines, along with a substantial amount of pathogenic *Vibrio* bacteria, is believed to be responsible for WFS. *Vibrio* bacteria have been identified in the fecal analysis from infected shrimps [5-6]. White Feces Syndrome (WFS) is one of the most serious problems in shrimp culture. According to feedback from farmers and technicians White Feces Syndrome (WFS) poses a significant challenge in shrimp culture across all four states. WFS typically emerge after 20 days of culture (DoC) and have been observed to reduce shrimp productivity and cause high mortality.

The White Faces Syndrome (WFS) is characterized by the presence of floating white faecal strings in ponds and can be observed in both farm-reared Black tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*). This syndrome causes several issues, including high mortality, growth retardation, size heterogeneity, and an elevated feed conversion ratio. Research has shown that survival is generally reduced by 20-30% [7] and cumulative mortalities can be as high as 50% in the summer period [8]. Early disease indications, such as floating faecal strings, appear in feed trays and at the water surface (Fig 2a-d), accompanied by a sudden reduction in feed consumption. Typically, it occurs in grow-out ponds around the second month after stocking.

Fig 2a-d Symptoms of white fecal disease. (e) Decreased white fecal disease by the usage of probiotics

White fecal disease in shrimp culture

White Feces Syndrome (WFS) has recently been recognized as a serious concern for shrimp aquaculture in major shrimp farming countries. The syndrome is named as WFS, since the affected farmed shrimps excrete white fecal strings. WFS are a multifactorial disease that is associated with acute hepato-pancreatic necrosis disease (AHPND) in Asian shrimp. According to the research work carried out at ICAR-CIBA, the WFS has been found to be significantly associated with the microsporidian *Enterocytozoon hepatopenaei* (EHP) in *P. vannamei* grow-out farms. It has been reported that the Thai production loss due to WFS was estimated to be about 10-15% in 2010. In India since 2015, 17% of shrimp farms in the east coast were affected with WFS. The disease can cause moderate to severe economic loss due to reduced productivity compared to the normal ponds. White Feces Syndrome (WFS) was first detected in Indonesia in 2014. This type of disease causes the death up to 40% of the total name shrimp intensive pond population. The presence of WFS shrimp is undoubtedly linked with seven species of Vibrio, including *V. vulnificus*, *V. fluvialis*, *V. parahaemolyticus*, *V. alginolyticus*, *V. damselae* (*Photobacterium damselae*), *V. mimicus*, and *V. cholerae*. The Total Vibrio Count (TVC) in the shrimp intestine and hepato-pancreas unmistakably increased by 90% when affected by White Faeces Disease (WFD) compared to non-WFD infected shrimp [9]. In current years, the white faecal syndrome (WFS) previous reported through South Asian Countries have become an important concern in India. Although findings indicate a strong association between EHP (*Enterocytozoon hepatopenaei*) infection and WFS (White faecal syndrome), the actual cause of WFS still remains to be explained [10]. However, the loss of brackish water aquaculture in India due to IMN (Infectious myonecrosis) has not been significant. The India Brackish water aquaculture until the year 2020 was free from the other OIE listed diseases such as Taura syndrome (TS), yellow head disease (YHD), AHPND and *Necrotizing hepatopancreatitis* (NHP). Other syndromes due to poor farm management such as stunted growth, WFS, white muscle syndrome (WMS) and running mortality syndrome (RMS) are important causes responsible for significant morbidity and productivity. High stocking densities and poor pond management are attributed to such syndromes [11].

Identify and treatment with gut probiotics

During the culture *Penaeus vannamei*, after 86th day of culture (DOC) there was a poor growth observed in both ponds due to due to white gut and white faecal matter (Fig 2e). Forthwith feed gut probiotic (Progutgen) mix with the fed for 14 days. The problem was steadily rectified. The white faecal disease was inhibited with in short span of time by the helping of probiotics and proper water quality management for the successful shrimp farming. It was completely inhibited by the biological methods indicated by the WHO. Previous studies accordance with the probiotics was helped in WFS reduced in shrimp culture [12].

White faecal disease in *L. vannamei* is a direct result of bacterial film formation in the pond and infections within the shrimp's gut and body. Irianto and Austin [13] also stated that the maintenance of proper good water quality parameters is essential for promoting the optimum growth and survival of shrimp. Excess feed, fecal matter and metabolites will exhibit continuous influence on water quality of the shrimp. For the getting of good shrimp we were maintained optimum salinity (between 15 to 25 ppt), in selected farming ponds. However, the farming of white leg shrimp, *L. vannamei*, is widely distributed and it can tolerate the changes of the salinity between 2 to 45 ppt [14] previous studies also recommended 10-35ppt was ideal for shrimp farming. Good water quality is defined by adequate levels of dissolved oxygen, optimal temperature, balanced pH, and appropriate salinity. During white faecal disease (WFS) period gut probiotics and immune stimulants were added as feed supplements to increase immunity [15]. Changes in plankton bloom is observed during DOC and 34 fluctuating quality parameters like enhance in pH 8.1 to 8.4 with Sacchi disc reading reduced from 21cms to 12cms during the WFS period. All these were overcome by using probiotics. Probiotics have generated significant interest in aquaculture due to their remarkable ability to control pathogens, stimulate the immune response, improve growth performance, and enhance feed utilization [16].

Another study revealed on the probiotics were used in a series of experiments, *L. vannamei* were fed a probiotic supplemented diet at various concentrations for a period of 60 days, and then challenged with *Vibrio harveyi* every 15 days. The results from in-vivo studies unequivocally demonstrated that supplementation with *L. lactis* (S_1 and S_2) and *E. faecalis* (F_3 and F_7) significantly enhanced growth performance and feed utilization in the probiotic-treated groups compared to the control group. The findings strongly indicated that the administration of F_7 and S_2 strains at 106 CFU g^{-1} for 30-45 days was exceptionally effective at suppressing *V. harveyi* infection compared to the other feeding regimens [17].

A study conducted in India conclusively demonstrated that feeding shrimp with commercial probiotics containing lactic acid-producing Bacillus, digestive enzymes, and organic acids (Gut Probiotics, V Sthiraa Bioscience, India) at a dosage of 5 kg^{-1} effectively facilitated the recovery from WFS [18].

CONCLUSION

White Feces Syndrome (WFS) has emerged as a major threat to shrimp aquaculture, particularly in *Litopenaeus vannamei* and *Penaeus monodon* grow-out systems. The syndrome is clinically characterized by floating white fecal strings, reduced feed intake, sluggish behavior, loose shells, growth retardation, and elevated mortality, leading to significant economic losses across shrimp-farming regions. Although WFS has been strongly associated with pathogens such as *Vibrio* spp. and the microsporidian *Enterocytozoon hepatopenaei* (EHP), its multifactorial nature arising from microbial infections, poor pond management, and environmental stressors makes its control challenging. The disease can reduce survival rates by 20-30% and cause cumulative mortalities up to 50%, severely affecting productivity and profitability. Research and field evidence indicate that probiotics, immune stimulants, and stringent water quality management play a crucial role in mitigating WFS, restoring gut health, and enhancing shrimp resilience against pathogens. Therefore, integrated disease management strategies combining biosecurity, farm management, and probiotic supplementation are essential for sustainable shrimp farming and minimizing WFS-associated losses.

Author contribution

Practical design and manuscript preparation was carried out by our respected research supervisor Prof. Sumanth Kumar Kunda, Department of Zoology and Aquaculture ANU College of Sciences University, Guntur. The first author Kalyani Atukuri has done field work and practical performance and assisted to our research supervisor Prof. Sumanth Kumar Kunda.

Declarations

Conflict of interest statement: *The authors declared that they have no interest of conflicts*

Ethical approval: *Not applicable.*

Consent to participate: *Not applicable.*

Consent to publish: *Not applicable.*

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