



Effect of Probiotics and feed supplements on Running Mortality Syndrome (RMS) in Pacific whiteleg shrimp (*Litopenaeus Vannamei*, Boone, 1931) culture in Andhra Pradesh

Abdul Malik Jainulapudheen^{1*}, Perumalla Papaiaha², Ashok Prabhu.V³, Inayathullah.N⁴, Srilaxmi.k⁵

*Annamalai University, Faculty of Marine sciences, Centre of Advanced study in Marine biology, Parangipettai, Chidambaram, Tamil Nadu Email: aquainayath76neayas@gmail.com

Abstract:

This study evaluated the performance of shrimp culture in four ponds with a stocking density of 50 m², harvested on the 116th day of culture. Water quality parameters, including salinity (20–22 ppt), alkalinity (220–260), pH (7.9–8.5), ammonia (0.1–0.2 ppm), nitrite (0.1 ppm), and dissolved oxygen (5.4–6 ppm), were maintained within optimal ranges. Microbial colony counts varied, with yellow colonies ranging from 600 to 660 and green colonies from 70 to 180. Pond 1 achieved the highest survival rate (97%) and growth (33.5 g), followed by Pond A (95% survival, 10,374 kg production). In contrast, Pond 2 and Pond B exhibited lower survival rates (73% and 70%, respectively), reduced growth (21.8 g in Pond B), and higher feed conversion ratios (1.5 in Pond B, 1.48 in Pond 2). Pond 2 and Pond B also showed signs of Running Mortality Syndrome (RMS), including antennae cuts, reddish uropods, reddish-yellow hepatopancreas, and pale white bodies, with continuous daily mortalities. Pond 1 and Pond A, however, recorded no mortalities, attributed to the consistent use of specific probiotics. This study highlights the critical role of probiotics in enhancing survival, growth, and production while mitigating RMS in shrimp farming.

Key words: RMS, shrimp culture, disease, *L. Vannamei*, Andhra Pradesh, probiotic

Introduction

Shrimp farming has evolved from a small-scale business in Southeast Asia into a global industry, surpassing other aquaculture farming systems in significance (Joseph Selvin and Ninawe Lipton, 2009). The remarkable growth of shrimp farming has established it as a major player in the global shrimp industry, playing a vital role in providing quality nutrition, generating employment, and fostering rural development.

In India, Andhra Pradesh boasts the second-largest brackish water area, covering approximately 37,560 hectares, second only to West Bengal. The state's extensive coastline spans 972 kilometers across nine districts: Srikakulam, Vijayanagaram, Visakhapatnam, East Godavari, West Godavari, Krishna, Guntur, Prakasam, and Sri Potti Sri Ramulu Nellore. Despite its potential, shrimp farming in the region faces challenges, including Running Mortality Syndrome (RMS). This syndrome causes low-level mortalities during the culture period, leading to reduced survival rates and production. Since 2011, RMS has been widely prevalent in vannamei shrimp farms across Andhra Pradesh and Tamil Nadu, posing a significant threat to the industry.

The affected shrimp show patches of whitish musculature in the junctions of 2nd and 4th abdominal segments as a clinical sign with continuous low-level mortalities. This condition results in a small percent shrimp mortality in the affected pond on a daily basis. As the mortality continues on a daily basis till the rest of the culture period, it is called as "Running Mortality Syndrome (RMS)". The infected shrimp pond exhibits perpetuating mortality throughout the culture period and is not interdependent on water quality parameters. This condition is termed Running Mortality Syndrome (RMS) in shrimp (Alavandi et al., 2019). It is correlated with both mortality and morbidity, which eventually leads to lower productivity (Rao and Satyanarayana, 2020).

Usually the RMS started after 35–40 days of culture (DOC) with low mortalities and as the culture progressed, the mortality rate also increased and the problem becoming acute at around 90 DOC and the farmers were forced to prematurely harvest the crops. Infectious diseases are a common factor in shrimp culture, particularly in Andhra Pradesh and Tamil Nadu (Rao and Satyanarayana, 2020). Environmental factors trigger the expeditious multiplication of pathogens that are already in lower concentrations in the habitat (Johnson et al., 2010). Most of the outbreaks in culture waters are reported to be associated with *Vibrio* populations and have led to a loss of one billion USD \$ in the Asia-Pacific region (Sung et al., 1999; FAO, 2013).

The shrimp aquaculture in India suffered significantly due to disease infections. In India, the gross economic losses due to shrimp diseases were estimated at more than Rs.1,000 crores in 2006–2008 and loss continues even now (kalaimani et al., 2013). The threat of the diseases continued due to poor practicing of biosecurity measures like Pond preparations, water filtration and disinfection before and after pumping water in to the pond, not arranging bird and crab fencing, procuring the seed from unregistered hatcheries which supply non SPF seed. The diseases may be caused by various etiological agents such as viruses, bacteria, fungi, parasites, algal toxins, nutritional deficiency or the adverse environment. The present study reports the incidence of RMS in Andhra Pradesh also find solution to overcome the RMS problem.

Materials and Methods

Description of the study area:

The first site, SCS Aqua Farms, is located in Mypadu, Nellore District, while the second site is situated in Beeramgunta, Ongole District. At both locations, two ponds were selected for the study.

At SCS Aqua Farms, ponds were managed across two sites: Site 1 (Pond 1 and Pond 2) and Site 2 (Pond A and Pond B). Each pond had a water spread area of 0.7 hectares. Comprehensive pond preparation and biosecurity measures were carried out in accordance with established protocols (Gunalan et al., 2011).

In Pond 1 and Pond A, following pond preparation, a disinfection process was carried out using a Trooper solution. A total of 7 kg of Trooper disinfectant per pond was evenly sprayed across the soil using an agri-auto spray machine. The solution was prepared by first mixing 1 kg of Trooper (A) with 25 liters of water, allowing it to sit for 10 minutes, and then slowly adding Trooper (B). After mixing, the solution was left undisturbed for 4 hours, ensuring no exposure to sunlight. Once ready, the solution was sprayed across the ponds. Additionally, all dykes, outlets, and inlets were thoroughly sprayed with the Trooper disinfectant to prevent EHP (*Enterocytozoon hepatopenaei*) contamination.

After the initial pumping of water, the water was treated with 10 kg of Trooper (used as an alternative to chlorine) for one meter of water depth. The application of Trooper was carefully managed to ensure it did not negatively impact the existing algae blooms (The product it won't disturb the algal boom). Following 36 to 48 hours of treatment, the pond was ready for stocking. To maintain optimal water quality, a maintenance dose of 3 kg of Trooper was applied every 10 days after stocking, prior to probiotic applications, and this practice was consistently followed until harvest.

From 3 DOC (Days of Culture) onwards until harvest, water probiotics (Bio Treat) were applied every 10 days. Starting from 30 DOC until harvest, soil probiotics (Bottom Care) were also applied every 10 days. Additionally, from 30 DOC onwards, GUT probiotics (GUT Rider) were mixed with feed at a rate of 10g/kg and administered during two meals every alternate day. From 40 DOC onwards, chelated minerals (VIGOURMIN) were added as a feed supplement at a rate of 10g/kg, mixed with feed and given during two meals every alternate day, continuing until harvest.

In Pond 2 and Pond B, after pumping, a chlorination process was carried out. Commercially available probiotics were used for both water and soil, while gut probiotics and mineral supplements were administered along with the feed. The microbial population in the ponds was monitored every 10 days. Based on the levels of beneficial bacteria, the appropriate dosage of probiotics was applied to the pond to maintain optimal water and soil conditions.

The *Litopenaeus vannamei* seeds (Post-Larval Stage 10), acclimated to a salinity level of 25 ppt and confirmed negative for White Spot Syndrome Virus (WSSV) through Polymerase Chain Reaction (PCR) assay, were sourced from Winner Hatchery, Marakannam for Site 1 and from Sai Druga Hatcheries, Ongole for Site 2.

For seed packing and transportation, the methods described by Gunalan et al., 2011 were followed. The seeds were transported to the farm site, and the bags were floated in the pond water for some time to allow the temperature to adjust. Gradually, pond water was added to the bags to acclimatize the seeds to the salinity and pH levels of the pond. After this adjustment, the seeds were slowly released into the ponds (Gunalan et al., 2011, 2013). The stocking density was maintained at 50 shrimp per square meter (50/m²) for Ponds 1, 2, A, and B.

Skreting feed was used until the shrimp reached 3 grams, after which Gargil feed was used until harvest. Feeding was done four times daily at 7 am, 10 am, 1 pm, and 4 pm. No water exchange was carried out during the entire culture period; however, water from a bore well was added at regular intervals to compensate for losses due to evaporation or soil seepage. Starting from the 30th day of culture (DOC), cast net sampling was conducted every 10 days to monitor the health and weight of the shrimp.

The water salinity, pH, temperature, and dissolved oxygen levels were measured using a hand refractometer, pH pen, thermometer, and dissolved oxygen meter, respectively. The ammonia and nutrient levels in the water samples were estimated by following the standard procedures outlined by Strickland & Parsons (1972).

Aeration was provided throughout the entire culture period for all ponds. A total of 8 aerators (2 HP each) were installed for each culture pond. The aerators were strategically positioned to maximize the dissolution of dissolved oxygen (DO) into the pond water, ensuring a shrimp-friendly culture environment. The Feed Conversion Ratio (FCR) and Average Daily Growth (ADG) were calculated using the following formulas:

$FCR = \text{Total feed given (kg)} / \text{Total biomass gain (kg)}$

$ADG = (\text{Final weight} - \text{Initial weight}) / \text{Culture period (days)}$

Results

The stocking density for all ponds was set at 50 m², and harvesting was conducted on the 116th day of culture (Table 1). The average salinity was maintained between a maximum of 22 ppt and a minimum of 20 ppt. Alkalinity levels ranged from a maximum of 260 in Pond A to a minimum of 220 in Pond 1. pH variations across all ponds were recorded between 0.3 and 0.4, with the highest pH of 8.5 observed in Pond A. Ammonia levels were recorded at a minimum of 0.1 ppm in Pond 1, while the other ponds showed 0.2 ppm. Nitrite levels were consistently noted at 0.1 ppm in all ponds. Dissolved oxygen levels reached a maximum of 6 ppm in Pond A, with a minimum of 5.4 ppm recorded in Pond B. Microbial colony counts showed a maximum yellow colony count of 660 in Pond B and a minimum of 600 in Pond 1. For green colonies, the maximum count of 180 was recorded in Pond 2, while the minimum count of 70 was observed in Pond 1 (Table :2).

Table: 1 shows the culture pond details

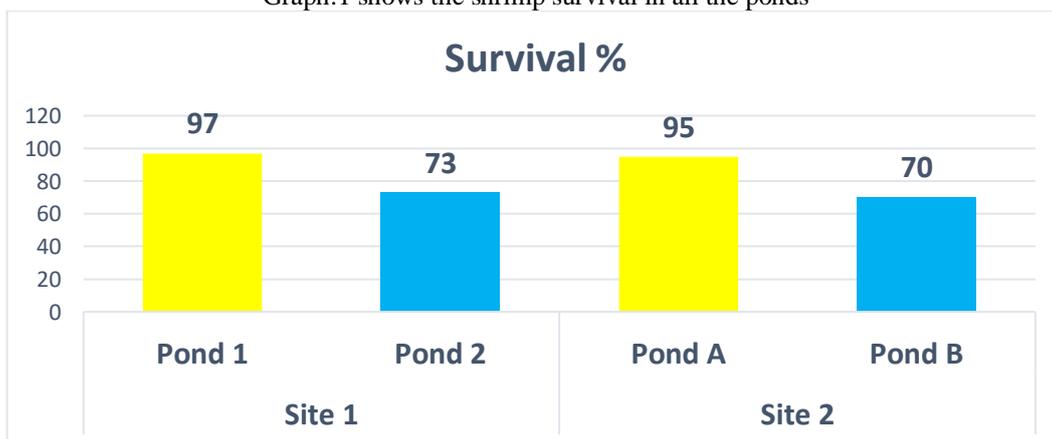
Details	Site 1		Site 2	
	Pond 1	Pond 2	Pond A	Pond B
Pond size	0.7	0.7	0.7	0.7
Initial stocking (L)	350000	350000	350000	350000
Density m ²	50	50	50	50
Stocking date	2.8.24	2.8.24	2.8.24	2.8.24
Harvest date	26.11.24	26.11.24	26.11.24	26.11.24
DOC	116	116	116	116

Table: 2 shows average water quality parameter in all the ponds

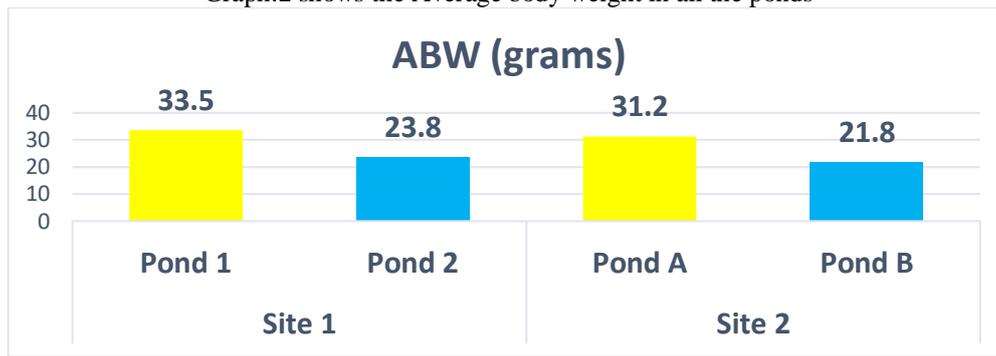
	Site 1		Site 2	
	Pond 1	Pond 2	Pond A	Pond B
Salinity (ppt)	20 - 22	20 - 22	20 - 22	20 - 22
pH	7.9 - 8.2	8.0 - 8.4	8.0 - 8.5	7.9 - 8.3
Alkalinity	220	250	260	240
Ammonia	0.1	0.2	0.2	0.2
Nitrite	0.1	0.1	0.1	0.1
Dissolved oxygen	5.6	5.8	6	5.4
Yellow colony	600	650	630	660
Green colony	70	180	90	160

In the present study, the highest survival rate was recorded in Pond 1 at 97%, followed by Pond A at 95%. In contrast, Pond 2 and Pond B showed lower survival rates of 73% and 70%, respectively (Graph:1). The maximum growth, recorded at 33.5 g, was observed in Pond 1, while the minimum growth of 21.8 g was noted in Pond B (Graph:2). The highest feed conversion ratio (FCR) of 1.5 was recorded in Pond B, followed by 1.48 in Pond 2 (Graph :3). The best yield and production were achieved in Pond 1 (11,373 kg), with Pond A following closely at 10,374 kg. The lowest production was recorded in Pond B at 5,341 kg (Graph:4).

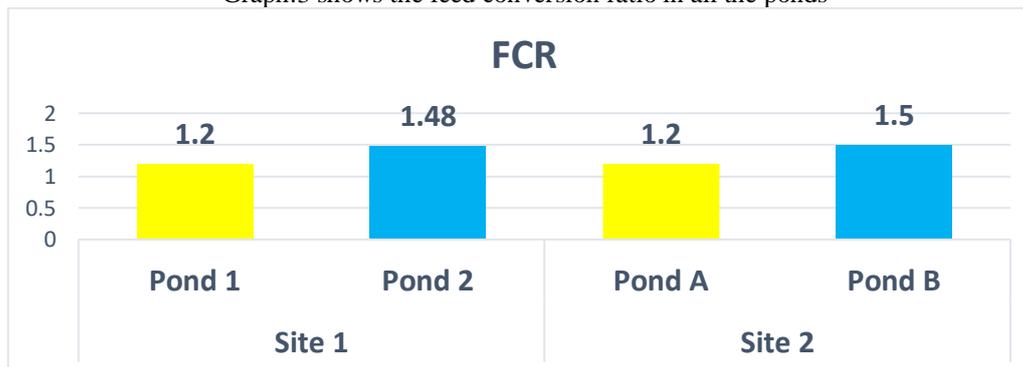
Graph:1 shows the shrimp survival in all the ponds



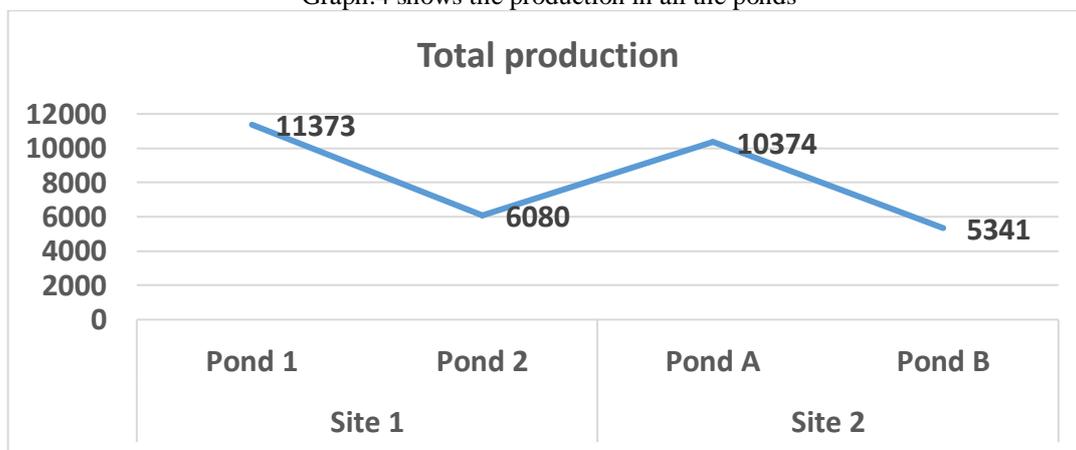
Graph:2 shows the Average body weight in all the ponds



Graph:3 shows the feed conversion ratio in all the ponds



Graph:4 shows the production in all the ponds



In the Pond 2 and Pond B certain percentage of shrimps noticed with antennae cut and Uropods turned normal to red in color, Hepatopancreas turns reddish yellow and finally entire body turns pale white in color. Continuous internal mortality is noticed daily in the check tray. But in the Pond 1 and Pond A no mortality was recorded because the continues usage of specific probiotic.

Discussion

In the present study, the water quality parameters in the grow-out ponds, including salinity, dissolved oxygen, and pH, consistently remained within the optimal ranges for Pacific white shrimp culture, as established by Gunalan et al. (2011, 2013) and Cohen et al. (2005). The average pH values, ranging from 7.9 to 8.5, align with the recommended range for optimal performance in the penaeid family, as reported by Soundarapandian and Gunalan (2008) and Cohen et al. (2005). Several studies have demonstrated successful ammonia reduction in limited or zero-exchange culture systems through the manipulation of the carbon-to-nitrogen ratio. This process facilitates the assimilation of inorganic nitrogen compounds and promotes the production of microbial biomass, as highlighted by Gao et al. (2012) and Samocha et al. (2007). In the present study, the lowest ammonia concentrations were consistently observed across all grow-out ponds. This outcome can be attributed to the maintenance of proper water quality and rigorous feed management practices, a finding consistent with the work of Gaona et al. (2011).

In the present study, Pond 2 and Pond B exhibited lower survival and growth rates compared to the other two ponds. Slow mortality was observed in these ponds starting from the 45th day of culture (DOC) onward. Initially, 2–3 cannibalized shrimp were found in the check trays, followed by an increase in the number of dead shrimp observed under aerators and in the center of the ponds. This indicated the onset of Running Mortality Syndrome (RMS), which gradually affected the shrimp population. As a result, Pond 2 and Pond B recorded significantly poorer survival rates compared to the other two ponds.

This issue has been widely reported in various studies. Venkateswarlu et al. (2019) documented RMS outbreaks in the Guntur, Krishna, West Godavari, and East Godavari districts of Andhra Pradesh. Similarly, Alavandi et al. (2019) recorded RMS problems in over 25 farms across the Nellore and West Godavari districts of Andhra Pradesh. Akshay Mandal and Malay Duyari (2024) studied RMS outbreaks in West Bengal and Tamil Nadu, while Anisha Shafni John et al. (2024) reported RMS infections in Tamil Nadu. Srinivas et al. (2016) identified RMS as a new syndrome in the Nellore district, and Srinivas Rao et al. (2021) reported RMS in 185 shrimp ponds in the Visakhapatnam district, located in the North Coastal Andhra region.

The use of probiotics in aquaculture is a well-established method to enhance the health status of aquatic animals, and their application for disease control has been widely discussed. Rammohan Rao et al. (2017) utilized herbal feed supplements to address the Running Mortality Syndrome (RMS) problem. However, in the present study, a novel technique was implemented to effectively overcome RMS.

In this study, Pond 1 and Pond A demonstrated exceptional results, with high survival rates ranging from 95% to 97%, a feed conversion ratio (FCR) of 1.2, and total production yields of 10.3 to 11.3 tons. These ponds were cultured alongside Pond 2 and Pond B, but Pond 1 and Pond A were managed with specific probiotic and feed supplement protocols to maintain optimal water quality and prevent RMS.

The management strategy included the application of 3 kg of Trooper every 10 days after stocking, starting prior to probiotic applications and continuing until harvest. From the 3rd day of culture (DOC) onward, water probiotics (Bio Treat) were applied every 10 days. Starting from 30 DOC, soil probiotics (Bottom Care) were also applied every 10 days. Additionally, from 30 DOC onward, GUT probiotics (GUT Rider) were mixed with feed at a rate of 10g/kg and administered during two meals every alternate day. From 40 DOC onward, chelated minerals (VIGOURMIN) were added as a feed supplement at a rate of 10g/kg, mixed with feed, and given during two meals every alternate day until harvest. This targeted use of probiotics and feed supplements played a crucial role in preventing RMS in Pond 1 and Pond A. Based on these findings, this study strongly recommends that shrimp farmers adopt specific probiotic and supplement protocols to effectively overcome Running Mortality Syndrome.

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