Use of EM Technology in Intensive Shrimp Aquaculture: An Effective Research-Based Tool to Enhance Sustainability

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Abstract— **As the demand for farmed shrimp continues to grow worldwide, the use of probiotics to address the sustainability of intensive shrimp farming has gained much attention. Emerging diseases, such as acute hepatopancreatic necrosis disease (AHPND), pose a threat to sustainable intensification of shrimp aquaculture due to its devastating economic impact. This paper extends the application of Effective Microorganisms (EM) from a controlled setting to a commercial scale. A previous scientific study conducted on live shrimp evaluated the use of EM to mitigate the effects of the pathogenic *V. parahaemolyticus* strain that causes AHPND. In laboratory conditions, the analysis of shrimp survival and bacterial community composition in the gastrointestinal tract of shrimp showed 73.3% survival and higher weight gains (31.2%) versus the negative control (11.2%). In the present study, comparable results were obtained in a commercial shrimp farm located in Thailand, in a region where the shrimp industry had been decimated by AHPND. Survival rate increased from 58% to 91% and Food Conversion Rates decreased from 1.36 to 1.22. These results validate the efficacy of EM in inhibiting bacterial diseases and increasing the sustainability of intensive shrimp production systems.**

Index Terms— **AHPND, bacterial community composition, Effective Microorganisms, probiotics, sustainability**

I. Introduction

In the last decade, the aquaculture industry has become a significant productive activity, especially in developing countries that depend on it for food security and economy [1]. Even though the percentage of marine products obtained from aquaculture industry is smaller than those obtained from fishery and wild catch, the representation of the former has increased from 13% in 1990 to 40% in 2010 with a total of 148 million metric tons [2]. Furthermore, 87% of the total amount of shrimp exported from Asia is produced in shrimp farms [3]. According to the Food and Agriculture Organization (FAO) [2], approximately 220 million jobs around the world were generated by shrimp farming in 2012. This figure alone illustrates the detrimental effects of diseases in aquaculture, not only in terms of reduction in productivity, but also the social burden due to the loss of income. Therefore, ensuring the sustainability of shrimp farming is of upmost importance.

One characteristic of shrimp and other marine animals is that their gastrointestinal tract (GIT) hosts a vast diversity of microbial species, among which, bacteria are found in greater relative abundance [4], [5]. These intrinsic microbial communities are responsible for many vital functions such as nutrient digestion and immune response, [4], [6]–[8]. Despite the significance of microorganisms in the health of aquatic environments [9], there is limited literature regarding the microbial diversity and its impact on aquaculture systems [10].

The potential benefits of using probiotics in aquaculture include improvement of growth performance, immune system, survival, and reproduction of aquatic species such as fish, crustaceans and mollusks [11]. Previous studies demonstrated the importance of probiotics in improving gut health through stabilizing gut microflora. As a result, the overall health status, welfare, and performance of animals improved significantly [12], [13]. Furthermore, a comprehensive study investigated the effects of probiotics including EM™ on shrimp survival and bacterial community composition after an induced AHPND infection. EM is a registered trademark of EM Research Organization and/or its affiliated companies in the United States and many other countries. The findings revealed the effectiveness of probiotics against this devasting disease [14]. The *in vitro* studies demonstrated the antagonistic properties of probiotics solutions consisting of multiple microbial groups as opposed to solutions that include only one type of microorganism [8]. The *in vivo* study was conducted in a control setting following the typical conditions of intensive shrimp production systems. The scientists concluded that EM was effective at mitigating AHPND reaching survival rates of above 73.3%±4.71% SD ($P<0.05$) in comparison to other probiotic solutions tested that reached survival rates between 11.7% and 36.7%. In addition, the next generation sequencing analysis revealed that the bacterial community composition in the GIT of shrimp treated with EM maintained the diversity, which in turn impeded the proliferation of the pathogen. Moreover, analysis of dissimilarities based on relative abundance and diversity indices of microbial composition showed that treatments with higher survival had lower dissimilarity with the negative control, where shrimp were not exposed to the pathogen. On the contrary, treatments with lower survival presented lower dissimilarity with the positive control, where untreated shrimp were exposed to the pathogen [14].

The present study was designed to validate the findings of the research previously described by evaluating shrimp survival and productivity parameters in a commercial shrimp farm treated with EM located in a region of Thailand impacted by AHPND.

II. Methods

A. *Probiotics preparation*

EM was prepared following the conditions of the laboratory study [8]. The ready-to-use EM solution known as EMA (Activated EM) was anaerobically fermented in 3 m^3 plastic tanks for a period of 7 days or until the pH of the solution reached below 3.5. After the fermentation period, the microbial population was approximately 8 log CFU/ml consistent with the study conducted in a controlled environment [8].

B. *Ponds preparation and water conditions*

The shrimp farm was located in Phetchaburi, Thailand. Ponds lined with plastic geomembranes were used for this study. The approximate area of a pond was 4,800 m^2 (3 rai) with an average depth of 1.8 m. Prior to being filled with water, ponds and aeration paddles were cleaned manually with brush and a solution of 1-2 ppm of ClO_2, then rinsed thoroughly with water. Subsequently, a solid organic substrate called bokashi was applied at a rate of 1 kg per m^2, which served as a starter inoculum of effective microorganisms at the bottom of the ponds. Then, the substrate was irrigated with approximately 500 L of EMA. Next, ponds were filled up to 1.1 m before setting the aeration system. A total of 10 series of paddles were installed, 4 in the center and 6 in the outer area of the ponds. Aeration was maintained constant using a 3-HP motor for the paddles and salinity levels were kept at 13±2 ppt.

C. *Live specimens and density*

The species used was Pacific white shrimp *L. vannamei*. Shrimp were purchased at PL12 from the Charoen Pakphand Group hatchery located in Chumphon province, Thailand. Shrimp were raised for 30 days under semi-controlled conditions in an *in situ* hatchery prior to being transferred to the production ponds. Each pond was stocked with approximately 600,000 shrimp.

Fig 1. Application of bokashi prior to introducing water in the system.

Fig 2. Application of EMA onto the bokashi substrate.

D. *Probiotic treatment*

Probiotics were applied via feed and into the water. A 40% protein formulation was offered at a rate of 10% of body weight divided into 5 feeding sessions per day. Feed was inoculated with EMA at a rate of 1 L per 30 kg and applied manually or automatically using an auto feeder located in the center of the pond. For water applications, 500 L of EMA divided into 3 applications per week were applied directly into the ponds. The probiotic was poured in front of a series of paddles to ensure an even distribution by following the water flow.

Fig 3. Probiotic application (A) via feed and (B) into the water.

E. *Sludge removal*

The sludge of the pond was removed every day or when sign of high organic content was evident. Ponds were designed with a funnel-like structure in the middle, where most organic matter would accumulate due to the paddle configuration. A 3-HP pump removed the sludge from the middle of the pond for 30 minutes into the water channel for treatment and recirculation.

F. *Monitoring*

Shrimp were visually monitored after every feeding session for signs of erratic behavior, stress or diseases. In addition, shrimp hepatopancreas, guts, chelipeds, antenna, feed intake and excretion were monitored. Water quality parameters, such as acidity, NH_4, temperature, and minerals were recorded on a daily basis. Dissolved oxygen and turbidity were monitored weekly. At the drain, hepatopancreas, GIT and shells were visually inspected after sludge removal. In addition, shrimp survival and FCR were recorded after the harvest to evaluate the sustainability of the production cycle.

G. *Harvesting*

After approximately 65 days, ponds were harvested manually by a specialized local harvesting team. In brief, a large fishing net was placed at the perimeter of the pond to catch shrimp while the water level was been lowered to 1 meter in depth. Then, with the use of additional fishing nets, the team proceeded to harvest the entire pond. Immediately after harvested, shrimp were stored on icy water while been sorted by size and weight. Finally, shrimp were frozen for transportation to the local market.

Fig 4. Manual harvest by a specialized local shrimp harvesting team.

III. Results

Regarding the water quality parameters, a consistent fluctuating pattern in acidity levels in the morning and evening for the majority of the production cycle was evident (Fig 5). In the mornings, pH value started at 8.17±0.075 (SEM) on day 1 and reduced to 7.68±0.075 (SEM) on day 63. In the afternoons, the pH values reduced from 8.47±0.049 (SEM) to 8.05±0.076 (SEM). Turbidity values showed a significant difference during the first 4 days of production with an average of 82.8 cm±5.72 (SEM) in comparison to the average turbidity during the remaining of the production cycle (42.9cm ± 5.41 SEM). In addition, the patterns seen in acidity levels and turbidity measures were not affected by the increasing amount of feed used to meet the demand (Fig 5).

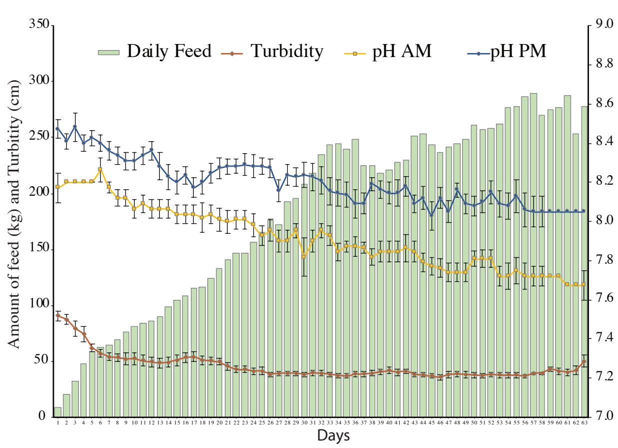

Fig 5. Water quality parameters and daily feed amounts throughout production cycle. Daily feed= total amount of 5 feeding sessions per day. Data represent means ± SEM of 5 production cycles conducted in 2017.

Shrimp survival was calculated based on 5 production cycles from January to December of 2017. Survival increased from 57.9% in March 2017 to 90.9% in December 2017 (Fig 6). In addition, food conversion rates (FCR) improved from 1.36 to 1.22 in the same time period.

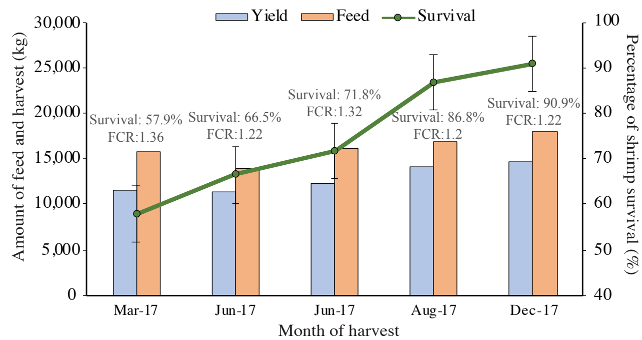

Fig 6. Productivity parameters observed after harvest of each production cycle. FCR= total amount of feed/total biomass gained. Data represents means ± SEM of 5 production cycles conducted in 2017.

IV. Discussion

Findings from the present study support the beneficial effects of EM in shrimp productivity parameters. Increasing shrimp survival and feed conversion rates are evident after each production cycle. These results are similar to those found in studies where probiotics were effective at inhibiting the growth of bacteria in white shrimp [15]–[17]. Moreover, productivity in relation to the use of probiotics is often associated with the health benefits. Healthier animals will yield higher levels of production. Nevertheless, probiotics affect productivity in a more direct way. Probiotics break down complex hydrocarbon molecules to basic elements and simpler molecules. These smaller compounds are easier to assimilate by the digestive system, which can be translated into more efficient food conversion rates [18]. This is evident in the consistent food conversion rates (FCR) obtained in 5 production cycles. FCRs are measures of efficiency since it represents how much of the feed used it transformed into biomass. Both survival rates and FCRs are directly related to profitability of shrimp production systems, which in turn has a positive impact on the economic aspect of the sustainability of the activity.

According to de Azevedo and Braga [19], bacterial diseases are the cause of most mortality occurring in post-larvae stages of shrimp. Additional studies recognize that administering a probiotic mix as opposed to solutions containing a single microbial type, have a positive effect on growth and survival of the larvae, and provide protection against the bacterial and viral diseases. Researchers attributed these results to the stimulation of the immune system, causing an increase in phagocytosis and antibacterial activity [14], [20]. The probiotics used in this study, EM, is a consortium of microorganisms from three microbial types: lactic acid bacteria, photosynthetic bacteria and fungi. The results of this study may be attributed to the formulation of the probiotic solution containing multiple species.

Maeda and Liao [21] reported some aspects of biological control in aquaculture that had a direct effect on the growth of prawn and crab larvae. The application of probiotics in aquaculture ponds create a biological equilibrium between competing beneficial and deleterious microorganisms. This phenomenon results in a bacteriostatic effect of opportunistic pathogens such as *Vibrio spp*. Therefore, incidences of diseases caused by members of the genus *Vibrio* are also reduced. In this case, the survival rates obtained after each production cycle increased, which can be attributed to a greater stability of the bacterial community composition in the water that impedes the proliferation of pathogenic species.

Finally, findings of this study validate the results obtained under controlled conditions in previous studies [14]. Furthermore, the increase in productivity parameter have a direct effect on the profitability of shrimp farming. Nonetheless, the implications go beyond the economic benefit. According to Godfray et al [22], the aquaculture industry plays a pivotal role in facing the challenge of feeding 9 billion people by 2050. The use of Effective Microorganisms is a research-based approach that can be used to maintain sustainability of intensive aquaculture production systems.

V. Conclusion

This study demonstrates that the results obtained in previous research using EM in aquaculture under controlled conditions can be extrapolated to commercial scale. The effectiveness of EM at improving survival and feed efficiency in intensive shrimp farms is directly related to enhancing the profitability and sustainability of the industry. By increasing productivity, improving efficiency of resources, and providing an effective biocontrol against newly emerged diseases, EM Technology may help close the gap of food security in the near future.

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