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Enhancing Water Quality, Growth Performance and Profitability of Outdoor Earthen Pond Shrimp Aquaculture Using Biofloc Technology

Benedict Terkula Iber [1,2], *Shahadat Hossain* [1], *Mohd Nazli Mohd Nor* [1], *Suhairi Mazelan* [1], *Nurul Hayati Ismail* [1], *Mohamad Jalilah* [1], *Ahmad Shuhaimi Draman* [1], *Wahidah Wahab* [1], *Mohd Syafiq Mohd Razak* [1], *Hidayah Manan* [1], *Ahmad Ideris Abdulrahim* [1], *Amyra Suryatie Kamaruzzan* [1], *Azmie Ghazali* [1], *Mohd Ihwan Zakariah* [1], *Nor Azman Kasan* [1*]

[1] *Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries (AKUATROP), Universiti Malaysia Terengganu, Kuala Nerus, Kuala Terengganu 21030, Malaysia*

[2] *Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University, Makurdi P.M.B. 2373, Nigeria*

ABSTRACT

Global seafood demand has continued to rise amidst challenges to traditional aquaculture operations. Current shrimp aquaculture practice requires high water exchange and discharges toxic effluent to the environment. Biofloc technology (BFT) is self-sustaining and emphasizes nutrient cycling through microbial activity to maintain water quality. The effect of BFT on water quality and profitability of shrimp (*Litopenaeus vannamei*) culture was determined over a 70-day period. Ponds P1, P2, and P3 were treated with BFT and compared to a control group (P4) without BFT. *Bacillus infantis* cultured inoculum initiated biofloc development while molasses-maintained C:N ratio of 15:1. One-way ANOVA determined the mean differences in Temperature, pH, dissolved oxygen (DO), total dissolved solid, alkalinity, salinity, ammonia (NH_3), nitrates, nitrites (NO_2^-), calcium, magnesium, as well as shrimp body weight (BW) and total length (TL) across treatments. Profitability was determined by comparing the cost of production with sales and plotting it on a bar chart. BFT shrimp exhibited significantly higher BW (13.6 g) compared to 8.1 g in the control, and maintained a

*CORRESPONDING AUTHOR:
Nor Azman Kasan, Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries (AKUATROP), Universiti Malaysia Terengganu, Kuala Nerus, Kuala Terengganu 21030, Malaysia; Email: norazman@umt.edu.my

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higher survival rate (80–90%) by day 70. Water quality was better managed in BFT, with NH_3 consistently kept below 0.5 mg/L; transient peaks of NO_2^-, more stable pH (averaging at 7.5), and better DO management, maintained above 5 mg/L. BFT provided higher profitability of Ringgit Malaysia (RM) 11,019.67 (P1) and RM 8651.83 (P2) compared to financial losses in the non-biofloc system. Although operational challenges were reported, BFT showed superior resilience, suggesting that proper technical training and farm management are crucial for its optimization.

Keywords: Bacteria; *Bacillus Infantis*; Carbon Source; FCR; Microbial Activity; *Penaeus Vannamei*; Survival

1. Introduction

The ever-increasing demand for seafood due to rise in world population has further placed aquaculture as one of the major global food production industries. Increasing consumer preferences for high-quality protein foods have also contributed to this steady rise [1]. White-leg shrimp (*Litopenaeus vannamei*) is one of the most economically important species among others under culture in global aquaculture today due to its high growth rate and international market demand [2–4]. In spite of the promising prospects of white-leg shrimps, challenges such as water quality management, nutrient imbalances and incessant outbreaks of diseases have been reported to adversely impact shrimp health and overall productivity [5]. In order to keep culture shrimps in healthy condition, water quality related occurrences such as ammonia accumulation, adverse pH changes and DO level instability must be closely monitored. In addition, increased nitrogenous compounds like nitrates and nitrites must also be constantly kept in check [6,7]. In traditional shrimp culture systems, water quality challenges are often tackled by water exchange or external filtration system. However, this leads to high cost of operation and discharge of nutrient rich effluents to the environment, causing more pollution issues [2].

BFT is a unified aquaculture system that takes advantage of the relationship between heterotrophic bacteria and other microorganisms in the culture system to recycle organic matter and nitrogenous waste. The flocs produced by these microorganism form flocs that enhance waste management and serve as a food supplement for culture shrimps [4]. The application of BFT in shrimp aquaculture has significantly decreased the need for water exchange as it is the culture in traditional practice. This leads to water resource conservation and less environmental impact [8,9]. Additionally, BFT functions by stabilizing pH, DO, regulating ammonia and nitrate levels in the culture system thereby providing a good environment for shrimp growth and health [7].

The success stories of BFT have continued to spread, thanks to its ability to initiate a self-sustaining ecosystem that enables the bioavailability of nutrients that are important for growth and development of shrimp. Many studies have reported the ability of BFT to enhance growth rates, survival, and health conditions of shrimp by making available a continuous supply of microbial protein as well as water quality optimization [10,11]. Furthermore, other studies have highlighted the efficiency of BFT systems in maintaining salinity, temperature, and alkalinity thereby reducing stress on shrimp during culture [12]. Nevertheless, the full potential of BFT is still far from being explored despite its numerous achievements. This is especially true in the area of its impact on long-term shrimp culture in different environmental conditions and the comparative effectiveness across diverse pond systems.

Many recent studies have expressed the positive outcomes of BFT for shrimp culture especially in the area of water quality improvement and higher growth rates [10,13,14]. However, there is a need for a more comprehensive study to validate these findings and establish detailed insights into the operational advantages and disadvantages of implementing BFT in diverse farming systems. This should be important to farmers especially in Southeast Asia, a major hub for shrimp farming [15]. This study aims to assess the impact of BFT on water quality in relation to shrimp growth in natural outdoor earthen ponds. It also examines the profitability of BFT application in real shrimp farming scenarios. Determining the impact of BFT on these parameters will further expand its potential for improving sustainable shrimp farming practices. Most of the previous BFT studies were carried out in indoor close systems. Due to variability of environmental conditions, outdoor aquaculture systems are often not viewed as suitable for BFT implementation. In this study, the comprehensive analysis of long-term effects of the system in real-world shrimp farming environment in Southeast Asia will bridge the knowledge gap on the operational challenges and economic benefits of BFT under diverse pond systems.

2. Materials and Methods

2.1. Geographical Location of Study

The study was conducted in Bukit Keluang, Besut, Terengganu, Malaysia (**Figure 1**). The geographical coordinates of the research location are approximately 5.739º N latitude and 102.7656º E longitude.

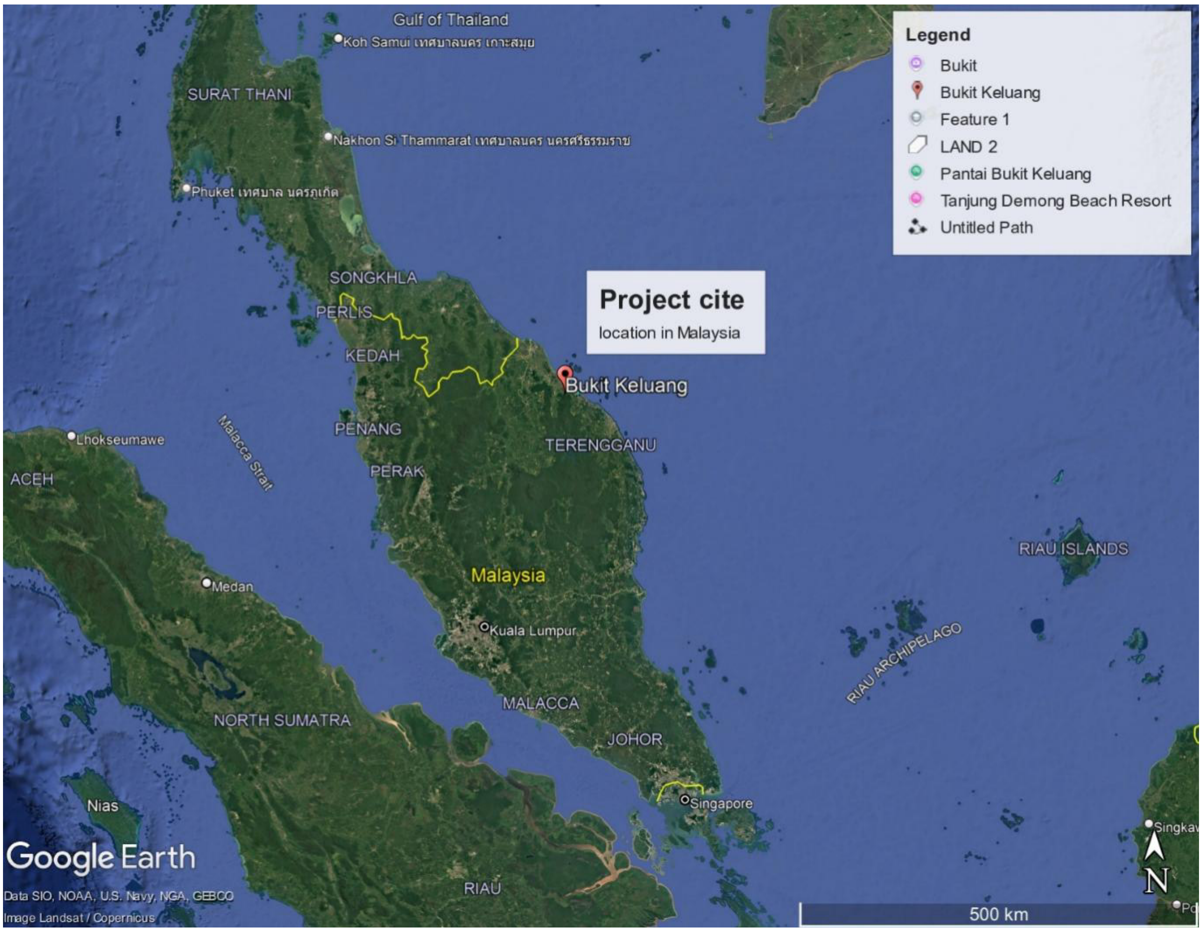

Figure 1. Map of Study Location.

2.2. Experimental Design

The study employed a comparative experimental design, where 12 natural outdoor earthen ponds from four different farmers (3 from each farmer) were selected as experimental units. These ponds were labelled P1, P2, P3, and P4 (control) (**Figure 2**) with average areas of 7,500 ± 1.22 m^2, 7,503 ± 0.12 m^2, 7,510 ± 2.05 m^2 and 7,508 ± 3.11 m^2, respectively. Control pond was a normal static earthen pond just like others with no water exchange and without biofloc application. All ponds contained 2 paddle wheels each for constant aeration throughout the culture period to ensure oxygenation and biofloc suspension. The ponds were located within an average of 1 km of each other to minimize environmental variability and ensure similar ecological conditions. The ponds were selected from four local farmers who practiced aquaculture. These ponds had similar characteristics in terms of size and management practices, allowing for a more accurate comparison of the applied treatments. Basically, the selection criteria include; average size (square meters), similar water depth and salinity, similar historical use for shrimp farming and in proximity.

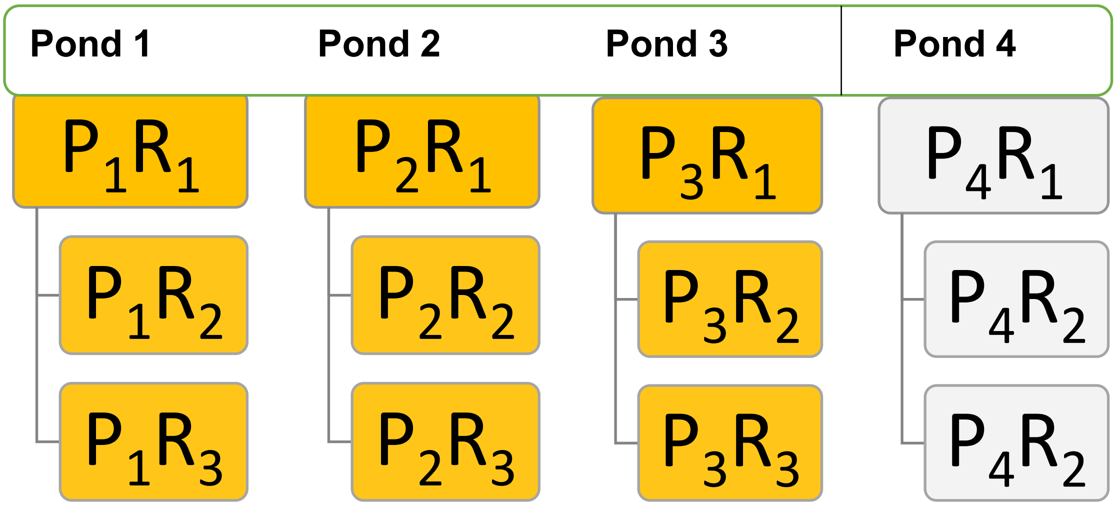

Figure 2. Experimental Design Showing Biofloc Ponds and Control Ponds with Replications.

2.3. Biofloc Preparation and Application

The *Bacillus infantis* strain was collected from the Rapid BFTTM® patent product [7]. The strain was cultured in the laboratory using nutrient agar broth. This single strain bacteria were chosen to ensure consistency in microbial activity and avoid variability that may be associated with multiple strains. In addition, the authors have a patent for the chosen bacteria whose performance in BFT was previously studied in indoor controlled systems [7]. In this study, 1 mL of inoculum of the pure bacterial culture was taken into 1 L of saline broth and incubated for a period of 24 hours. The biofloc inoculum was added following the procedure by Kasan at 1 litre per 100 tons of pond water [7]. Pond water volume was calculated by multiplying the area by the average depth. Bacterial inoculum was introduced at the beginning of the experiment and repeated every two days in the first week to ensure the establishment of beneficial bacteria. Molasses was used as a source of organic carbon to promote the growth of biofloc. The carbon to nitrogen (C:N) ratio was maintained at 15:1 by carefully adjusting the molasses quantity added to the ponds as reported by Kasan [7]. Throughout the study, biofloc formation was monitored by measuring floc density using Imhoff cone. Molasses was adjusted to keep floc volume within 10–15 mL/L [7]. Measurement was made using graduated imhoff cone.

2.4. Shrimp Stocking and Feeding

Post larvae of *Litopenaeus vannamei* were sourced from a local hatchery in Kuantan, Pahang, and Manjung, Perak, Malaysia. The 15-day-old PL were of uniform size and age at the time of stocking, with an average weight of 0.117 ± 1.02 g. At 15 days old, shrimp are considered to have passed the vulnerable early development stages; therefore, they have reduced risk of mortality due to stress [16,17]. Also, 15-day-old shrimp have more developed digestive and immune systems, making them more suitable for most experimental conditions [18]. The PL were stocked at a predetermined density of 200 shrimp/m^2 of earthen ponds and fed with commercial feed (Evergreen®) containing 35% crude protein for 70 days (21st January to 1st April, 2024). The feeding schedule was set at 5% of the shrimp's body weight per day [7], with rations divided into two sections of morning (7 am) and evening (6:30 pm).

2.5. Water Quality Parameters and Nutrient Analysis

Water quality parameters such as temperature, pH, DO, alkalinity, and TDS were monitored daily using the YSI Multiparameter Pro Plus (ProDSS 2, Yellow Springs, Ohio, USA). There was no water exchange during the culture period. The levels of ammonia, nitrate (NO_3^-), nitrite

(NO_2^-), Mg and Ca were determined using the UV-vis1800 Shimadzu spectrophotometer (Shimadzu Corporation, Japan), applying the phenate method and the cadmium reduction method. This was done following the protocols outlined by Pearson [19]. The water quality and nutrient data were recorded every 10 days during the culture period.

2.6. Determination of Profitability and Issues Faced by Farmers

Questionnaires were issued to farmers to obtain their demographic information such as names, age, marital status, educational level, level of knowledge in shrimp aquaculture and income levels. Farmers were closely monitored to obtain data on operational expenditure in order to calculate cost of production. In addition, their market experiences were recorded including shrimp selling price per kilogram and ease of finding buyer for their products. Other data collected through questionnaires included the challenges faced by the farmers from production to marketing of products.

2.7. Data Analysis

Data analysis was achieved through One-way ANOVA of post hoc and Tukey test using SPSS 25.0 package, which determined the mean differences between the Biofloc-treated ponds. Line graphs were plotted to show the trends in behaviour of measured parameters over the 70-days culture period. The mean was considered significantly different at $p < 0.05$ from the statistical analysis conducted. Bar charts were used to show profits and losses as well as the frequency of issues faced by different farmers during the cultivation period.

3. Results and Discussion

This study investigated the effects of BFT on water quality parameters and shrimp growth across four ponds (P1, P2, P3 and P4 (control) over 70 days. Generally, biofloc-treated ponds showed considerable improvements in water quality and shrimp performance compared to the control.

3.1. Effect of Biofloc on Parameter Behaviour and Trends in All Ponds

3.1.1. Growth Performance

In the present study, shrimp cultured in biofloc showed consistently higher growth rates compared to the control system. In all ponds, there was a steady increase in body weight as the culture period progressed, with faster body gains reported in biofloc ponds, thereby resulting in significant mean final figures at the end of culture (**Figure 3**). The better growth performance in biofloc ponds is attributed to supplemental nutrition and improved water quality conditions provided by suspended microbial aggregates [20,21]. Miao reported that shrimp reared in biofloc grew faster and demonstrated better overall health conditions due to stable pH, low NH_3 and NO_2^- levels than those in conventional systems [22]. Relatedly, Alam observed that biofloc-treated shrimp grew at a significantly faster rate compared to those in non-Biofloc systems [23]. In the biofloc ponds, unconsumed shrimp feed and wastes were not only recycled but converted into microbial biomass which was further utilized directly as feed source rich in proteins, lipids, vitamins and other relevant nutrients [24]. Nutritional boost by microbial biomass could translate to faster weight gain as corroborated by Kasan where shrimp in biofloc achieved higher weights and weekly growth rates than those reared in clear-water system [7]. In addition, Ramiro revealed that whiteleg shrimp reared in biofloc gained 13.6 g compared to 8.1 g in a recirculating clear-water system at the end of culture period [25]. The study added that approximately 1.35 g/week and 0.8 g/week respectively, were recorded in both systems. Such performances are attributed to the ability of shrimps to graze on biofloc thereby decreasing the reliance on conventional farm feed, effectively reducing FCR. Other previous studies have also noted that biofloc-derived nutrition accounts for 20–30% of growth in BFT reared shrimps, improving feed efficiency, growth performances and survival [26–28]. The variability in body weight experienced between biofloc-treated ponds could be due to slight differences in earthen pond conditions, which were part of the limitations experienced from the field experiment in natural environments.

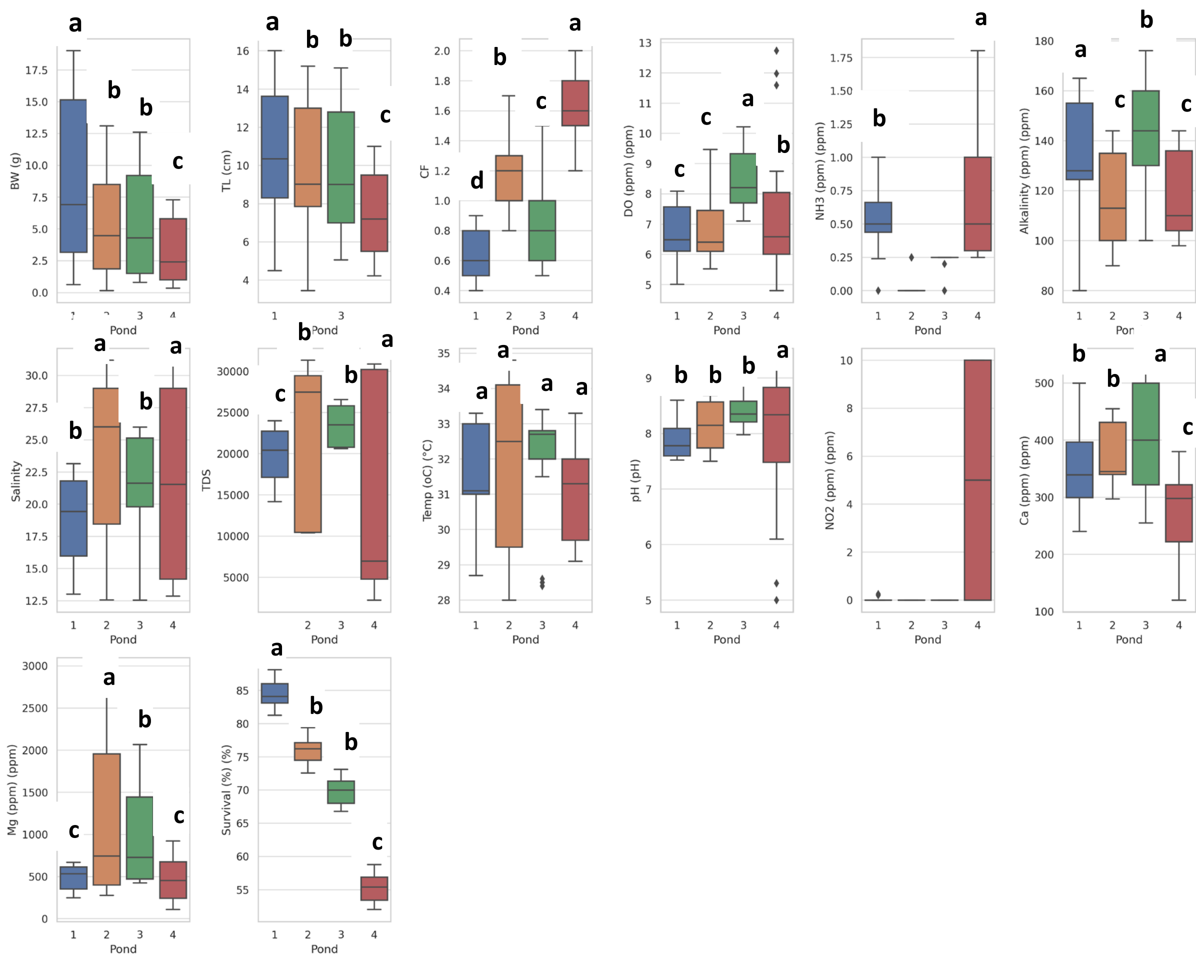

Figure 3. Average Parameter Behaviour During Culture Period.

Notes: Letters "a, b, c, and d" Are Used to Mark Significant Differences Between Parameters, with "a" as the Highest Value, Followed by Others in Decreasing Order. Shrimps in Pond 1 Recorded Highest Body Weight and Total Lengths Which Were Significantly Different from Other Ponds.

The condition factor, K, which relates weight to length maintained a healthy range for all shrimp, though slightly higher towards the end of culture period (**Figure 4**). In terms of total length, biofloc-raised shrimps showed average higher values, indicating better energy reserves and a well-fed condition. This performance could be connected to continuous availability of natural productivity in form of biofloc biota as feed supplement [29]. Therefore, it suffices to state that shrimp grew proportionally heavier without stunting during the period, an indication that there was a balanced diet and low stress environment in the systems. Furthermore, shrimp survival was also better in biofloc ponds, remaining in the upper 80–90% range at day 70 which was in agreement with other intensive shrimp trials in biofloc [30,31]. The survival level in the control pond remained relatively good, likely due to good water quality management and natural pond conditions. The higher survival in biofloc ponds depicts a generally enhanced health and resilience of shrimp in such conditions. It has been reported that biofloc improved immune responses and disease resistance and promoted better physiological status of shrimp [32]. In addition, microbial community in biofloc serve as probiotic reservoir with bacteria like *Bacillus spp*., in floc acting to suppress pathogens like *vibrio species* [33]. Borges reported that Vibrio counts were lower in biofloc system during early and mid-culture periods compared to clear water system [34]. This showed that biofloc has the ability to delay or reduce pathogenic outbreaks in culture systems. In the present study, the microbiome advantage as well as the more stable water quality may have been responsible for the higher survival recorded in the biofloc ponds. Lopez reported that, the numerous management challenges associated with shrimp culture in clear water system can significantly affect survival when compared to biofloc system [35].

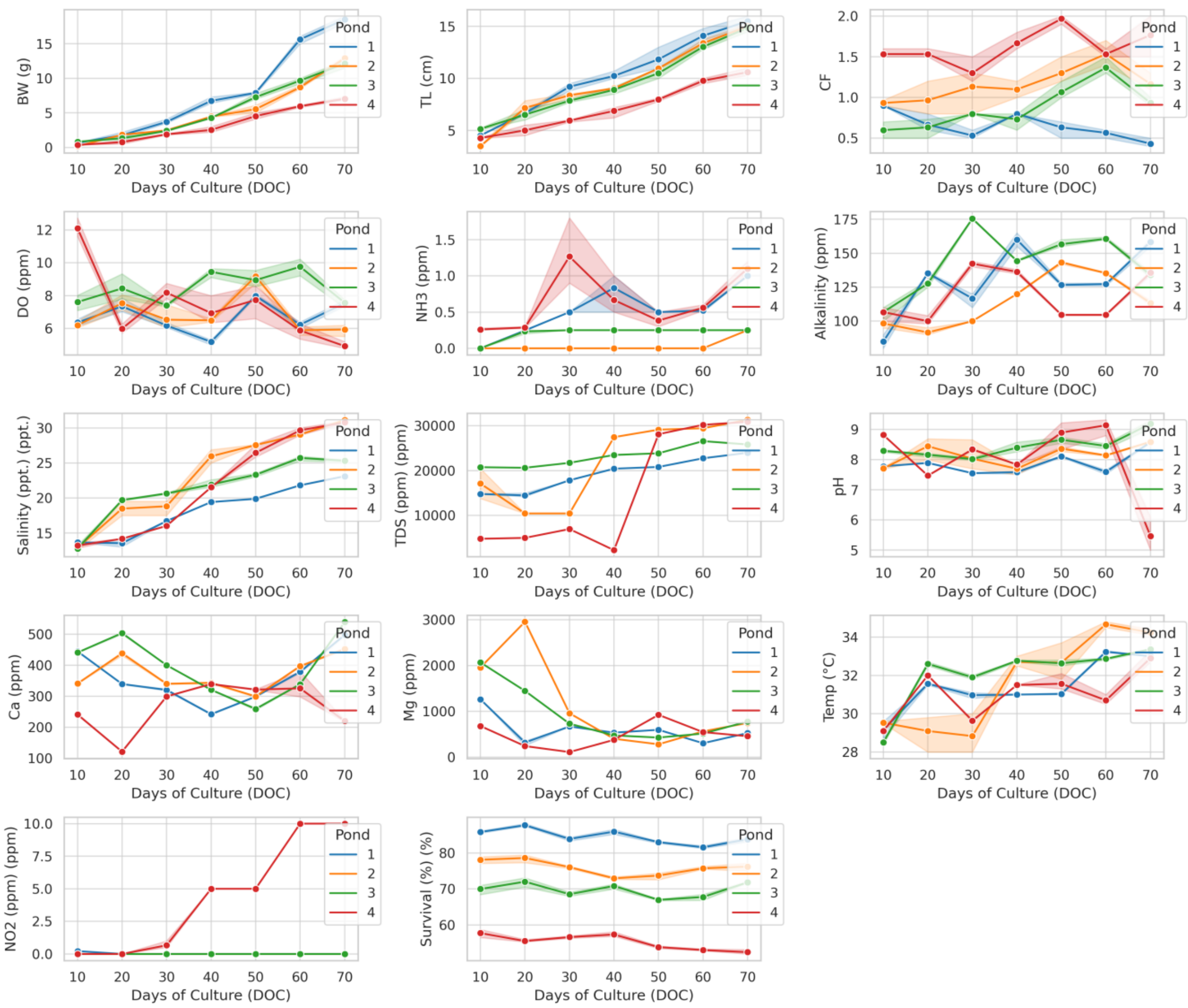

Figure 4. Trends of Measured Variables During the 70-Day Culture Period.

Notes: In Each Parameter, Ponds Are Depicted in Different Colours. The Line Graphs Show a Conspicuous Rise and Fall in the Average Values of All Parameters from the Beginning to the End of Culture.

3.1.2. Temperature and Dissolved Oxygen

Results of water temperature followed a similar trend influenced by ambient climate, maintaining an optimal range of 28–30°C throughout culture period, suitable for the culture of *P. vannamei* [25,36]. Although there were no significant differences ($p \leq 0.05$) in temperature between biofloc and control ponds (**Table 1**), the former tended to run slightly warmer (approx. 0.5–1 °C higher) during the latter half of the experimental period. This little difference could be due to the heat generated by microbial metabolic activity in heavy biofloc systems or higher absorption of solar radiation due to the darker colouration of biofloc water. This effect was also reported by Ramiro who recorded 29.9°C in biofloc and 28.2°C in clear water, with average difference of 1.6°C [25]. Shrimp growth and immunity can be compromised by extreme temperature fluctuations; however, the fairly stable results in all ponds without large swings were considered favourable. This study noted that both BFT system and control provided suitable thermal environment which implies that temperature was not a limiting factor for growth and healthy development. Furthermore, in all ponds and almost at all times, DO was actively managed through aeration and was kept above

5 mg/L (**Figure 3**). It was revealed that DO exhibited diurnal pattern, typical of aerated ponds but never dropped to hypoxic levels. There was elevated oxygen demand in biofloc ponds due to high microbial respiration in addition to oxygen consumption by shrimp [25,36]. The general DO level (5–6 mg/L or higher) throughout the culture period conforms with best practices for biofloc systems [33]. In a biofloc system, adequate oxygenation is critical for nitrifying and heterotrophic bacteria to avoid stress. The driving force behind DO improvement using biofloc is the enhancement of microbial community taking part in organic waste degradation thereby reducing the need for external aeration [21]. According to Dong [33], biofloc systems significantly decreased the need for mechanical aeration by over 08% while maintaining optimal DO levels for shrimp growth. Keeping DO near saturation (>5 mg/L) as it was the case in the present study optimized shrimp performance and prevented dense solids in biofloc water from impairing respiration. This was vital in sustaining high growth and survival and preventing stunted growth and shrimp mortalities that may have been precipitated due to low DO.

Table 1. Demographic Characteristics of Respondents (Farmers).

Category	Number
Respondents	4
Gender–Male	4 (100%)
Race–Malay	4 (100%)
Education	
Never attended school	0
Higher School	3
University	1
Marital Status	
Single	1
Married	3
Single Father/Mother	0
Age Group	
20–29 years	2
30–49 years	1
>50 years	1
Income	
<1000	0
1000–2000	3
2001–3000	1
3001–4000	0
>5000	0

3.1.3. pH and Alkalinity

This study revealed that BFT had a buffering effect on pH with 7.8–8.0 recorded in all ponds at the beginning of trial (**Figure 3**). Subsequently, pH experienced a slight downward trend in the control pond, steadying at approximately 7.3 while an average of 7.5 was reported in biofloc ponds (**Figure 4**). While variation in pH between biofloc and non-biofloc ponds was significant, the difference within the biofloc ponds was not. The pH fluctuations in biofloc ponds demonstrate the cyclical nature of microbial activity which indicates that a more stable phase was preceded by increased microbial degradation and subsequent equilibrium in microbial populations. Hasan-Nataj-Niazi reported stability in pH between 7.5 and 8.5 and considered it optimal for shrimp in biofloc [24]. The study maintained that pH below or above the recorded range can stress the organism and lead to poor growth and survival.

The variability in pH of the control pond has also been agreed upon by Amin and Chetpattananondh, who reported that greater variations in pH exist in traditional ponds due to by-products of organic degradation [37]. These studies reinforce the importance of Biofloc in water quality control especially in areas with variable water conditions. Also acting as both a nutrient sink and microbial habitat are the tightly linked nitrogen and carbon cycles in biofloc systems [38]. This cycle adds weight to the continuous overall stability and buffering ability of the water in the biofloc system [39]. This arrangement sheds more light on the consistent rise in alkalinity recorded in ponds 1 and 3. In addition, the water chemistry of the biofloc ponds may have also been affected by the release of organic acids and bases that altered the alkalinity. Acidic by-products such as lactic acid or acetic acid are produced during breakdown of organic materials. However, these acids can be readily consumed by bicarbonates where the buffering capacity is high [26]. The relative stability of pH in biofloc ponds can be attributed to the rapid uptake of nitrogen by heterotrophic bacteria that produce less acidic by-products than nitrification.

Alkalinity which is measured as calcium carbonate ($CaCO_3$) was observed to decrease gradually in all ponds due to consumption of bicarbonates by nitrification (**Figure 4**). Nevertheless, ponds treated with biofloc demonstrated better ability to conserve alkalinity at the onset because ammonia was assimilated into microbial biomass rather than fully oxidized to nitrite [32]. The variation in alkalinity of biofloc ponds has also been reported by Khanjani who noted that biofloc-treated ponds showed a significant increase in alkalinity over time which was due to enhanced microbial activity and nutrient cycling [38]. It could be observed that alkalinity began to fall as the trial progressed into the later days due to the establishment of a bacterial community within the biofloc ponds, maintaining an optimal level above 100 mg/L (**Figure 4**). Although there was no significant difference ($p \leq 0.05$) between biofloc pond 2 and control pond in terms of alkalinity (**Figure 3**), there was a faster drop in the latter at the onset of the trial as ammonia was nitrified, likely due to a lack of initial heterotrophic cushion. This was also evident in its slightly lower pH by the end of culture period. Nevertheless, pH remained within optimal range for shrimp growth across all ponds with minor differences, highlighting the importance of alkalinity management in long-term water stability. This finding agrees with recommended water quality regimes of keeping pH above 7.0 and alkalinity sufficient to buffer against nitrification acids [25]. Previous studies have also reported significant pH declines in unbuffered biofloc systems due to activity of nitrifiers, necessitating addition of bases like sodium bicarbonates to culture ponds [33,40]. Also, the unstable nature of alkalinity as a result of microbial activities in shrimp culture has also been investigated by many authors with reports in agreement with findings in this study [26,41].

3.1.4. Ammonia, Nitrite, and Nitrate

Similarly, considerable dynamics were observed in ammonia levels between biofloc and control ponds. There was steady rise in NH_3 levels when feeding commenced, peaked during the third week when the feeding rate increased faster than nitrifying bacteria could colonize (**Figure 4**). In biofloc ponds, especially in pond 1, NH_3 levels were increased but could not reach near or above safe limits that would necessitate either water exchange or reduced feeding to prevent toxicity [42]. The initial relatively high NH_3 levels in the biofloc ponds could be due to the fact that the microbial community was still developing. This can also be explained when the NH_3 level began to decrease and stabilize by day 15 to 20 even as feeding continued (**Figure 4**). It was observed that NH_3 levels in the three biofloc ponds remained consistently below 0.5 mg/L. On the other hand, control pond exhibited significantly higher NH_3 levels until its natural nitrification process caught up. The result of NH_3 had a similar trend to that reported by Nguyen [43], where lower NH3 levels were observed in biofloc systems compared to non-biofloc ponds. The control pond, which showed NH_3 spikes, agrees with the report of Li [44] that without proper filtration, NH_3 can build up rapidly in shrimp ponds. This study confirms the better NH_3 management ability of Biofloc systems. While other studies have also reported NH_3 reduction, this study provides comprehensive data showing consistent NH_3control across multiple Biofloc treatments. Furthermore, the active assimilation of NH_3 into cell protein by heterotrophic bacteria when supplied with an adequate carbon source, removes the toxicant from the water column [6,45]. In this study, the molasses utilized as additional carbon source in biofloc ponds served the purpose. In a recent study, it was reported that biofloc successfully maintained low NH_3 in shrimp culture system across various aeration regimes when molasses was added, thereby demonstrating the NH_3 assimilation capacity of the system [32,46]. Although both biofloc and control systems were able to keep NH_3 at low levels, the former was able to achieve this much sooner and with smaller peak amplitude. Chronic exposure of shrimp to elevated unionized NH_3 can damage their gills and reduce growth [22]. Therefore, keeping NH_3 accumulation low, as it was observed in the biofloc ponds, likely kept shrimp in a more comfortable environment, thereby resulting in their superior growth. non-Biofloc ponds such as the control in the present study, tend to accumulate NH_3 over time, leading to poor shrimp growth and health [37]. The effectiveness of the present trial is an indication that zero water exchange was required to control NH_3. The environmental implication of this result is that far less nitrogenous waste is released to the environment during and at the end of culture.

Nitrite remained near zero in the biofloc ponds for the first couple of weeks, likely due to minimal nitrification and heterotrophic activity. The slight increase in NO_2^- at the later period (**Figure 4**) indicated the onset of nitrifying bacteria activity converting NH_3^- to NO_2^-. This NO_2peak was modest and transient in biofloc ponds when NH_3 was put under control (**Figure 3**). Though some NO_2^- still appeared, they were quickly either oxidised to nitrate (NO_3^-) or assimilated. On the other hand, the peaks were more prominent in the control pond, likely due to initial surge in NH_3 [42]. In addition, NH_3-oxidizing bacteria in the control pond produced NO_2^- faster than it could be converted to NH_3, especially in the early to middle phase of the culture. This led to accumulation of NH_3 up to suboptimal levels in the water column. Studies have shown that high levels of NH_3 can be hazardous with significant effect on oxygen transport in shrimp by oxidizing hemocyanin, especially where salinity is low [27,47]. In shrimp culture, NO_2^- has been studied alongside the ability of biofloc to control it. NO_2^- toxicity reduction promotes better shrimp survival and growth [12,48]. In addition, NO_2^- and NO_3^- build up in shrimp ponds can result in oxidative stress and impaired growth because they interfere with oxygen uptake by shrimp [48]. Increased microbial denitrification by application of BFT has been shown to reduce both NO_2^- and NO_3^- [42]. This study demonstrated the importance of detailed tracking of these compounds over time in real shrimp farm situations. Furthermore, the moderate salinity level (15–20 ppt) of the control pond likely mitigated the NO_2^- toxicity since chloride ions must have competitively inhibited nitrite uptake by shrimp gills [49]. The NO_2^- level began to decline by week 6 to 7 as oxidizing bacteria populated and grew (**Figure 4**), converting NO_2^- to NO_3^-. At the end of the culture period, the magnitude and duration of shrimp exposure to NO_2^- were higher in the control pond. This observation has been supported by a previous study which reported that when the system nitrification is slow to establish, a temporary nitrite buildup can occur [50]. Biofloc pond kept NO_2^- levels consistently below the recommended threshold of less than 1–2 mg/L for marine shrimp [21]. This result conforms with the report by Zhu who noted a clear trend of reduced NH_3 and NO_2^- in BFT based system compared to the traditional aquaculture system [48]. The study added that BFT significantly lowered NO_3^- concentration and off-farm nutrient discharge thereby contributing to environmental sustainability.

3.1.5. Salinity and Total Dissolved Solids

In all ponds, the initial salinity was recorded as approximately 15 ppt. However, a slow upward creep was observed in both biofloc and control ponds, likely due to evaporation, and concentration of minerals from feed and metabolism [12]. Salinity fluctuations may have also been influenced by freshwater top-up from rainfall and surface water flow into the earthen ponds. According to Martínez-Montaño [51], more stable salinity and temperature are associated with culture systems treated with biofloc, leading to better shrimp growth. Although all ponds were located in the same area, the amount of surface flow into the control pond may have been less compared to biofloc ponds, resulting in less dilution and higher salinity in the former. Generally, the final salinity in both systems was considered stable for the culture shrimp. The consistent methodology and data collection every 10 days in the present study provide a detailed temporal analysis of the biofloc long-term impact on water quality and shrimp growth. This provides a deeper comprehension of the sustainability of biofloc systems in aquaculture. Similarly, TDS which is an important indicator of water quality, reflecting the overall concentration of dissolved ions, minerals, and organic compounds followed the same trend to rise alongside salinity with no large divergence between the two systems (**Figure 3**). In the present study, lower TDS levels were observed in the biofloc ponds. This was attributed to the low aggregation of dissolved organic matter due to microbial metabolism and recycling [52]. Such nutrient cycling where organic materials are degraded into forms that can be utilized by shrimp has also been reported [53]. Said recorded similar behaviour of TDS in biofloc systems during a 120-day shrimp culture [28]. The study observed that high TDS did not negatively impact health of shrimp growth. However, TDS levels can become harmful if allowed to grow excessively high. This is more so especially when dissolved solids arise from waste byproducts instead of beneficial minerals.

3.1.6. Calcium and Magnesium

In the present study, calcium and magnesium levels were also monitored due to their importance in molting and osmoregulation. Initial Ca and Mg levels were observed as 100–150 mg/L and 300–400 mg/L, respectively, within the range typical for brackish water culture, agreeing with the results of salinity (**Figure 3**). As the trial period approached the final stage, a downward trend was observed in Ca and Mg levels in all ponds (**Figure 4**), owing to their continuous utilization by shrimp to form exoskeleton and possibly taken up into biofloc biomass or better still precipitated [54,55]. Ca is essential in shrimp health especially during molting stage, formation of shell and for osmoregulation, while Mg is critical for better enzyme activity, energy metabolism, and osmoregulation [56]. Mg showed a more stable trend than Ca (**Figure 4**), considering its higher concentration and lower fraction needed in the shrimp carapace. At the last phase of culture, Ca/Mg in the biofloc and control ponds were marginally lower than at the start indicating some level of removal. The differences in Ca/Mg in biofloc and control ponds were not remarked (**Figure 3**), indicating that biofloc did not cause any extraordinary depletion of these ions beyond the normal dictates of shrimp growth. However, this finding differs from previous studies, which reported that biofloc systems supply a constant amount of minerals like Ca due to organic matter degradation and the growth of biofilm-forming microorganisms, which absorb and mineralize nutrients [22,57]. In addition, Miao stated that culture systems treated with biofloc showed significantly higher Ca levels in shrimp ponds compared to conventional system thereby disagreeing with the findings of this study [22]. Similarly, the importance of high Ca levels in shrimp growth and shell formation has been explained by Rodrigues [58]. It was revealed that all ponds remained above critical hardness levels, signifying no evident molting problems. This result also suggests that regular feeding and pond natural mineralization processes helped in sustaining Ca/Mg availability. In addition, Huang determined that boosting Mg relative to Ca in low-salinity biofloc water significantly enhanced shrimp growth and survival [27]. In the present study, the ambient salinity water had naturally appropriate Mg/Ca ratio of approximately 2–3:1, indicating that shrimp experienced normal growth without ionic stress. In addition, the stable survival and growth was an indication that Ca and Mg levels remained sufficient in all ponds throughout the culture period. The higher growth and survival in biofloc ponds led to higher biomass yield compared to control. Management of floc density as the floc volume increased over time was key in preventing excessive biofloc which can lead to gill clogging or off-flavour. This was achieved by reducing feed and relying on the shrimp grazing to consume floc [59].

3.2. Profitability and Financial Outcome

Table 1 shows the demographic features of shrimp farmers involved in the project. Farmers were all male with a greater percentage having higher education and evenly distributed income. The impact of BFT on shrimp farmers focused on knowledge improvement, profitability and challenges faced during operations. Results showed significant knowledge gains among all participants and mixed financial outcomes. Respondents 1, 2, and 3 with knowledge gains of 0–70%, 25–75% and 50–70% respectively, who operated biofloc ponds reported highest improvements which suggested that training and practical exposure to BFT improved technical competencies (**Figure 5**). Although respondent 4 managed pond without biofloc, shrimp farming knowledge improvement (0–50%) was recorded at the end of training and operation. These findings agree with previous studies which reported that BFT-based training programs significantly improved farmer technical knowledge and practical skills in aquaculture [10,60]. Similarly, Nguyen reported that BFT promotes a deeper comprehension of water quality management and microbial community control which is important for sustainable aquaculture operations [61]. Furthermore, the biofloc utilizing respondents achieved profitability exceptions of 3 and 4, while those without biofloc suffered financial losses. The profit recorded in Malaysian Ringgit (RM) was RM 11,019.67 and RM 8,651.83 for respondents 1 and 2, respectively (**Figure 6**). These dynamics suggest that though BFT provides economic advantages, successful financial returns also depend on effective farm management and operational skills. This conclusion has also been made in previous studies where BFT reduced FCR and improved yield but precise management measures were noted to avoid system crashes and financial losses [42,62,63]. In addition, Megahed reported that BFT system enhanced shrimp survival rates and yield in Egyptian aquaculture but further submitted that farmers who lack sufficient technical support would face operational failures [64]. Considering the low-level literacy of most small-scale shrimp farmers, benefits from biofloc are only achievable with continuous technical guidance [21].

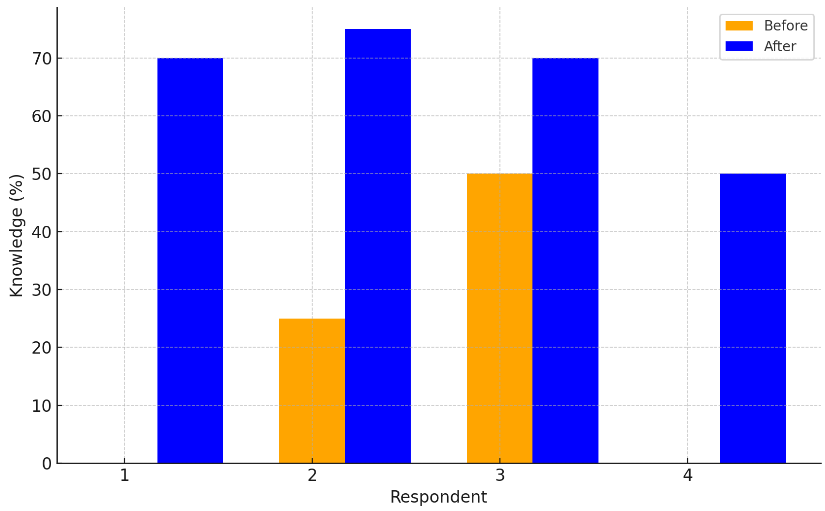

Figure 5. Knowledge of Respondents to Shrimp Farming Before and After Project Implementation.

Notes: Respondents 1 and 4 Had Hitherto Engaged in Finfish Culture and Had Zero Knowledge of Shrimp Farming. The Blue Bars Appear in All Respondents, Indicating That All Farmers Reported Significant Knowledge Gain at the End of Training and Culture Period.

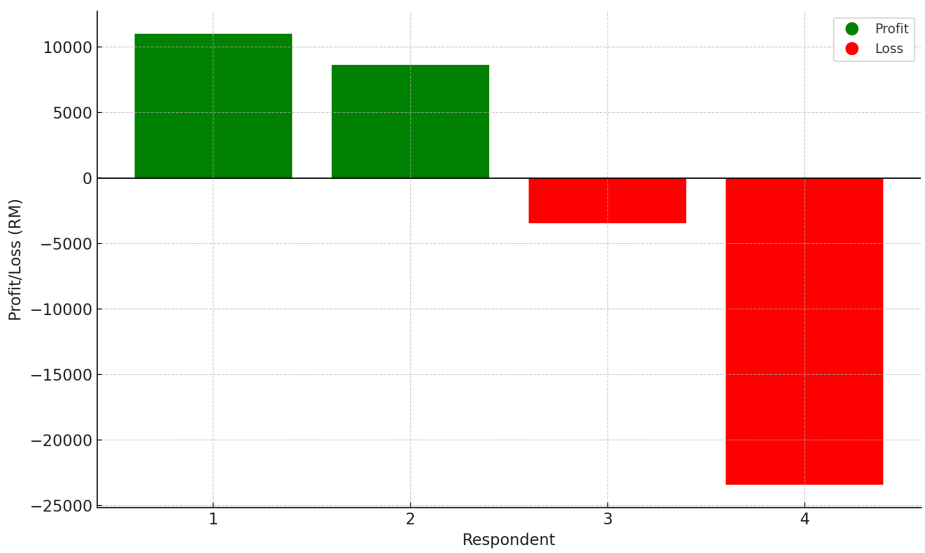

Figure 6. Total Profit/Loss of Farmers in Shrimp Farming.

Notes: Highest Profit in Respondent 1, Followed by Respondent 2. Havier Losses Were Suffered by Respondent 4 Who Cultured Shrimp in Pond Without Biofloc.

3.3. Challenges Faced by Farmers

Figure 7 is a bar chart of issues faced by farmers where operational challenges were the most frequent. This was followed by financial and lastly, marketing issues. The major operational challenges faced were disease control, water quality fluctuations and oxygenation in the culture water. This finding has also been reported in a previous study where operational management was reported as critical for biofloc systems, with disease outbreaks and extreme water quality changes being common problems [29]. In the case of financial challenges, prominent issues among farmers were high cost of feed and limited access to affordable inputs. According to Kuhn et al. [65], though biofloc has the ability to decrease FCR, high cost of inputs and pond maintenance remain a challenge for adoption by smallholder farmers. In recent years, many studies, especially in south-east Asia have further corroborated the fact that financial limitation, access to feed and technical support are key influential factors for biofloc system sustainability [5,7,66–68]. In this study, marketing issues were less reported compared to others. This suggests a readily available market for shrimps and shrimp product. It was an indication that once production is successful market access for shrimp was relatively less problematic for the farmers. This finding disagrees with previous studies that reported poor market infrastructure as a significant barrier for shrimp farmers in other regions [69,70].

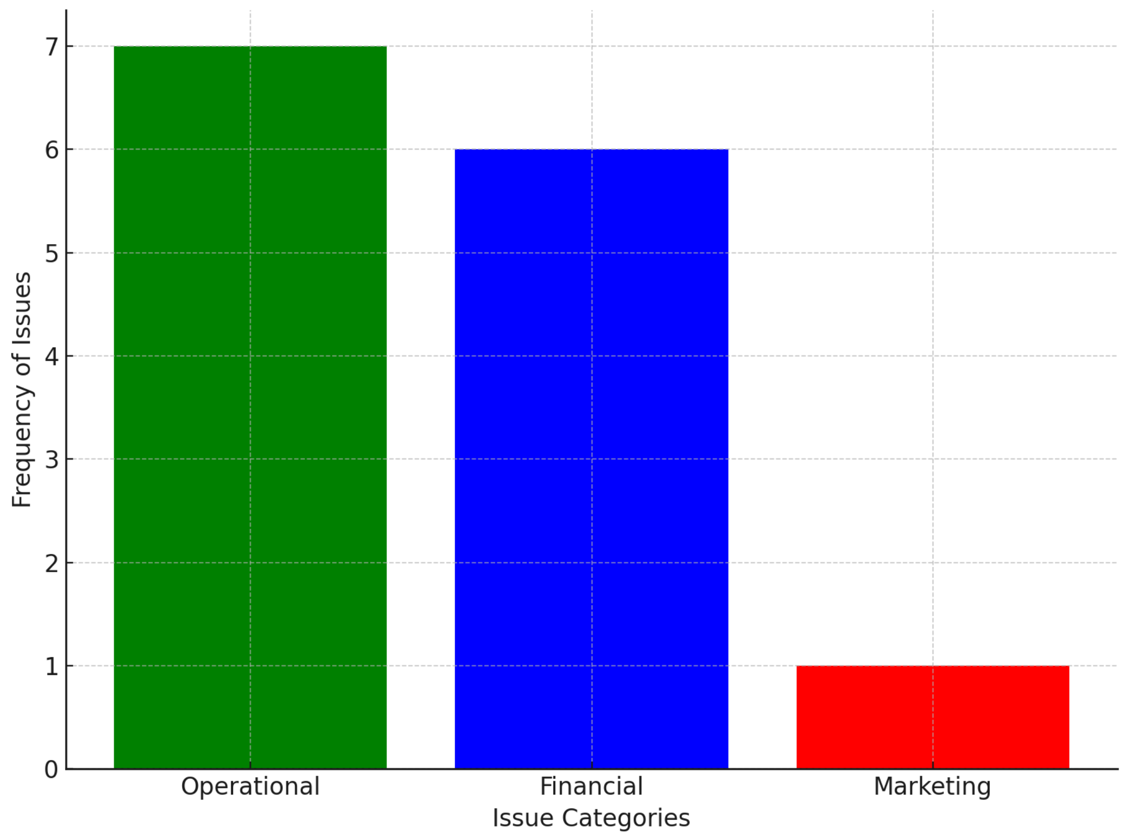

Figure 7. Major Issues Faced by Farmers in Shrimp Farming.

4. Conclusions

This study determined the significant benefits of BFT in shrimp aquaculture, focusing on shrimp growth, survival, water quality management and general farm profitability. BFT shrimp consistently outperformed those in control system, exhibiting higher body weight and survival (80–90%) at the end of the 70-day culture. This was attributed to supplementary microbial nutrition from biofloc, which contributed essential nutrients such as proteins, lipids and vitamins. The improved body weight and K in biofloc ponds indicated optimal feeding and minimal stress. In contrast, higher survival rates can be linked to improved immune response and disease resistance promoted by the microbial communities within the biofloc system. In addition, the system successfully kept NH_3 and NO_2^- within safe limits (below 0.5 mg/L) thereby preventing toxicity and promoting stable environment for shrimp growth.

Furthermore, higher profitability was recorded in biofloc ponds due to improved growth rates, lower FCR and reduced reliance on commercial feed. Nevertheless, some of the farmers faced financial challenges as a result of high costs of feed and operational expenses. These notwithstanding, the profitability from the biofloc system was generally positive, suggesting that with proper management and training, the economic potential of the technology can be fully realized. This study identified operational issues such as disease control, fluctuating water quality and maintaining adequate oxygenation. Although these challenges were recorded from both systems, biofloc provided a more stable environment up to the end of culture period. Financial barriers like high cost of feed and limited access to affordable inputs were also identified as affecting widespread adoption of BFT. However, market access for shrimp was never a major concern for the farmers, suggesting that once shrimp were produced, market availability was relatively easy. The results of this study are proof that the capacity of BFT for sustainable shrimp aquaculture goes beyond laboratory applications. It is a sustainable and effective approach for water quality enhancement and growth of shrimps. The consistent results across multiple biofloc-treated ponds are a proof of the reliability of the system in diverse shrimp culture environments.

Limitations of the Study

The general limitations of this study came from greater influence of environmental conditions due to outdoor nature of the earthen ponds which could not be controlled. This study could not control or measure the nature and pattern of rainfall as well as evaporation within and around the culture environment. In addition, the surface flow into the ponds as well as nature and composition of bottom sediments could not be determined. Further studies may be conducted taking into account all these limitations in order to design an optimized protocol for biofloc application in outdoor earthen pond.

Author Contributions

Conceptualization, B.T.I.; validation, N.A.K.; writing—original draft preparation, B.T.I.; writing—review and editing, B.T.I., A.G., M.I.Z., and N.A.K.; visualization, A.G. and M.I.Z.; Review and editing of final draft, S.H., M.N.M.N., S.M., and N.H.I.; Data cleaning and analysis, M.J., A.S.D., and W.W.; data collection, review of initial draft, proofreading, M.S.M.R., H.M., A.I.A., and A.S.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data will be made available upon request.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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