Findings on the Practical Models of Whiteleg Shrimp Farming Using Indigenous Microorganisms in Quang Tri Province, Vietnam

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ABSTRACT

This study evaluated the effectiveness of indigenous microorganisms in a two-phase whiteleg shrimp farming system in Quang Tri province. Four bacterial strains and two algal species were integrated into the shrimp pond. The first phase culture with a 21-day nursery in cement tanks (1000 PL12/m³), survival rate, growth rate, and feed conversion ratio (FCR) averaged 99%, 0.04 g/shrimp/day, and 0.99, respectively. The second phase culture investigated two models: a linning pond (LP) (150 shrimp/m², biofloc technology), a Earthen pond with lined around the banks (ELPB) (70 shrimp/m², semi-biofloc technology), and control ponds without indigenous microorganism supplied. Compared to controls, LP exhibited 96% survival (vs. 80%), 0.24 g/shrimp/day growth (vs. 0.22 g/shrimp/day), and an FCR of 1.29 (vs. 1.5). Whereas ELPB showed survival of 95.5% (vs. 89%), growth rates of 0.25 g/shrimp/day (vs. 0.23 g/shrimp/day), and an FCR of 1.26 (vs. 1.30). Linning ponds demonstrated double the yield of ELPB. Importantly, the system avoided water exchange, not use antibiotics, maintaining optimal water quality and disease prevention throughout both cropping cycles. This result support the development and nationwide implementation of indigenous microbial products and biofloc technology for sustainable whiteleg shrimp farming.

Keywords: FCR, biofloc technology, semi-biofloc technology, linning pond, *Litopenaeus vannamei*

INTRODUCTION

Shrimp farming using microbial technology is the addition of bacterial strains in conditions of increased oxygen to promote the conversion of waste into biomass of bacteria - algae - fungi (biofloc), increasing the ability to resist without having to change water. The current high level of development of microbial technology is biofloc technology and was first applied to some aquatic species that can be raised at high density such as tilapia and whiteleg shrimp (*Litopenaeus vannamei*) [1]–[3]. Some countries have successfully applied this model such as the US, India, Malaysia, Indonesia [4], Brazil [5], China [6], [7] and Korea [8]. With biofloc technology, shrimp farming productivity can be increased two to three times in the same farming area [9]. One of the requirements for biofloc technology development is the ability to apply local microbial sources to minimize negative impacts on the environment [10], [11]. Okaiyeto studied Halomonas strains capable of creating biofloc [12]. Kasan isolated microbial sources from whiteleg shrimp ponds to find strains with high biofloc creation ability [2], [10], [11]. Phan Cong Hoang (2013) provided additional useful information for the use of *P. pseudoalcaligenes* and *P. stutzeri* as probiotics in shrimp farming systems using biofloc technology and evaluated their floc-producing and ammonia-treating abilities. This group of authors also isolated heterotrophic bacteria including *Acinetobacter schindleri*, *Exiguobacterium aestuarii* and *P. pseudoalcaligenes* capable of producing exopolysaccharide and removing ammonia from shrimp pond water using biofloc technology [13]. Tang Minh Khoa (2015) applied semi-biofloc technology in intensive whiteleg shrimp farming in Dam Doi district, Ca Mau province, using wheat flour with a C/N ratio of 10/1, showing high survival and growth rates of shrimp, ensuring economic and environmental efficiency [14].

The study of applying indigenous microorganisms isolated from the Quang Tri aquatic environment in whiteleg shrimp farming using biofloc technology on a laboratory scale was successfully tested by the authors of the project in 2024, which is a premise for implementing this research on a farm scale [15]. In fact, in Vietnam, there have been a number of farms and companies applying the method of shrimp farming using microorganisms and biofloc, such as in Ninh Thuan province (2012), Ba Ria-Vung Tau (2013), Thua Thien Hue (2013) [16], [17]. These models are only at the semi-biofloc level and are mainly applied on a small outdoor scale, focusing on the nursery stage.

The results of the farming show that the pond environment when applying semi-biofloc technology is still highly variable, farmers still have to periodically change the water, and the risk of disease is still high. Practical research on the farm scale applying bifloc technology from indigenous microorganisms in whiteleg shrimp farming was conducted to evaluate the success of the model compared to traditional farming methods, thereby developing Vietnamese-branded microbial products and perfecting the technical process to expand nationwide.

MATERIALS AND RESEARCH METHODS

Materials

The indigenous microorganisms used include 04 bacterial strains including NQ1 (*Nitratireductor kimnyeogensis*), NQ3 (*Hyphomonas polymorpha*), BQ1 (*Bacillus sp*.), BQ2 (*B. velezensis*) and 02 algae species *Chaetoceros sp*. and *Thalassiosira pseudonana* which are products of Quang Tri provincial Science and Technology project [15]. *Litopenaeus vannamei* with age PL12, initial length 8-10 mm and weight 4-6 mg/individual.

Pond model: shrimp farming according to a 2-phases culture and experiment applied throughout the entire cycle from the nursery stage to the commercial farming stage. Mr. Nguyen Huu M's model (characteristic of a low-density farming model in the estuary area, the first phase pond is made of cement with an area of 100m^2, 1.5m deep; the second phase pond is ELPB with an area of 3000m^2, 1.5m deep) and Mr. Ho Ngoc C's model (characteristic of an high density farming model, the first phase pond is made of cement with an area of 100m^2, 1.5m deep, the second phase pond is a LP with an area of 1000m^2, 1.5m deep), repeated experiment for 2 crops, 1 farming crop includes 2 ponds (1 pond for phase 1 and 1 pond for phase 2), compared with the control unit as the result of 2 simmilar ponds of 02 experimental farms which were carried out in the traditional techniques without applied indigenous microorganisms.

Methods

Experiment management

- Phase 1: the water of the experimental ponds and control ponds were treated with 30 ppm chlorine for using. During the experiment, maintain biofloc vollume at 10-20 mg/L, arrange a bottom surface oxygenation system to prevent floc from settling at the bottom. The stocking density in the nursery stage was 1000 PL12/m^3. Check environmental parameters daily and use materials to maintain a stable pH in the pond at 7-8, alkalinity >100 mg/L.

- Phase 2: the water of the experimental ponds and control ponds were treated with 30 ppm chlorine for using, stocking density was 70 shrimp/m^2 (ELPB, maintain biofloc vollume at 5-10 mg/L) and 150 shrimp/m^2 (LP, maintain biofloc vollume at 10-20 mg/L) in the growthing.

Feed and indiginous microorganism suppied:

All the experimental ponds, shrimp were fed by Grobest's feed and follow the guidle of Grobest company. The experimental ponds used microbial preparations according to the project's process:

+ Add *Chaetoceros sp*. and *T. pseudonana* with a dosage of 50ml/species/100m^2 for phase 1 pond; 1 liter/species/3000m^2 for ELPB and 0.5 liter/species/1000m^2 for LB (multiplied to 50 L - 200 L in 03 days) applied 3-5 days before farming and periodically every 7 days during the farming period.

+ Add 04 bacterial strains NQ1, NQ3, BQ1, BQ2 with a dosage of 50ml/species/100m^2 for phase 1 pond; 1 liter/strain/3000m^2 for ELPB and 0.5 liter/species/1000m^2 for LB, release directly into the pond, apply 7 days

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after stocking and periodically every 7 days during the culture period. During the experiment, based on the N content measured in the pond, add molasses at 10 am to maintain a minimum C:N ratio of 15:1.

+ Microbiological products are produced from 02 production units: (1) University of Science, Hue University and (2) Center for Research, Technology Transfer and Innovation, Department of Science and Technology of Quang Tri province.

Methods of monitoring water quality, biofloc, and shrimp

- Water quality: Periodically checked water parameters every 10 days and analyzed at the laboratory of University of Sciences, Hue University (Table 1).

- Floc vollume: Every 10 days measured the floc vollume at the laboratory of University of Sciences, Hue University (Table 1).

- Shrimp: Every 10 days and the end of experimental crop, randomly measured 30 shrimps to evaluate FCR, weight gain, and survival rate. Calculation formulars as:

+ Feed conversion ratio (FCR) = Amount of feed used/(Weight of harvested shrimp – initial weight of stocked shrimp) (1)

+ Survival rate of experimental shrimp (SR) = (Number of harvested shrimp/Number of stocked shrimp)*100% (%) (2)

+ Absolute weight growth rate (DGR) = (W2 – W1)/N (g/day) (3)

W1, W2: initial and post-sampling shrimp weights (g), respectively; N: Experimental time.

Table 1. Methods of analyzing water parameters, floc and shrimp

Parameter	Sampling time	Sampling cycle	Analysis method/equipment
Water quality			
Temperature (^oC)	8-10 am	1 time/day	HANNA – HI 9142
pH	8-10 am	2 times/day	EcoSense
DO (mg/L)	8-10 am	1 time/day	HANNA – HI 9142
Salinity (ppt)	8-10 am	1 time/day	HACH – sension156
NH_4-N (mg/L)	8-10 am	1 time/week	OPP method
NO_2-N (mg/L)	8-10 am	1 time/week	SMEWW 4500 NO^-_3 E: 2005
Floc vollume			
Vollume (mg/L)	8-10 am	10 days/time	Imholf
Shrimp sampling			
Weight gain (g/day)	Weigh of shrimp at begin and the end of the experiment		Use electronic scale 0 decimal places
Survival rate (%)	Number of alive shrimp at the end of experiment		Count the number of shrimp remaining in each tank.

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Data processing

Data were calculated and processed using Microsoft Excel 2016 and IBM Statistics SPSS 20 (Paired-Samples T test) software.

Implementation time

The test period was conducted in 2 farming crops from May 13, 2024 to December 10, 2024

RESULTS AND DISCUSSION

Growth performance of shrimp

In phase 1, shrimp were cultured using biofloc technology in cement tanks for 21 days. In phase 2, shrimp were cultured for 69 days in ELPB and 61-65 days in LP. The results of shrimp growth performance are presented in Table 2.

Table 2. Shrimp performance in the experiment models

Phase	Criteria	ELPB model	LP model
I	Production (kg)	132 ± 0,27	141 ± 10,59
	Average shrimp harvest weight (g/shrimp)	0,88 ± 0,01	0,94 ± 0,30
	Survival rate (%)	99 ± 1,41	100 ± 0
	FCR	1,00 ± 0,07	0,99 ± 0,02
	Growth rate (g/shrimp/day)	0,04 ± 0,001	0,04 ± 0,014
II	Production (kg)	3086,2 ± 296,19	2190,7 ± 121,06
	Average weight of harvested shrimp (g/shrimp)	16,25 ± 0,91	16,15 ± 0,92
	Survival rate (%)	95,5 ± 3,54	96,0 ± 1,41
	FCR	1,26 ± 0,14	1,29 ± 0,29
	Growth rate (g/shrimp/day)	0,25 ± 0,003	0,24 ± 0,001
	Productivity (tons/ha/crop)	10,18 ± 0,98	21,91 ± 1,21

Note: Growth and yield data in phase 2 were recorded identically in terms of harvest time according to the LP model (61-65 days). Water quality data of control ponds were not available.

Phase 1 (cement tanks) with a density of 1000 PL12/m^3, higher than the previous nursing density applied by the experimental farm of 500-700 shrimp/m^3 and within the recommended density range for appropriate shrimp rearing using biofloc technology [17]. After 21 days, both farming models gave good results, the survival rate was 99-100% (the amount of shrimp added in the sample averaged 10%), FCR reached 0.99-1.00, the harvested shrimp weight was from 0.88-0.94 g/shrimp, these values were higher than the nursing results of the farming household, with an average survival rate of only 90%, shrimp weight reaching 0.5-0.7 g/shrimp in the same farming period.

Similar data between the two experimental models on shrimp growth indicators in phase 1 are completely consistent due to the uniformity of pond conditions and stocking density. The addition of 10-20 mg/L floc

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vollume provides additional sources of protein, essential amino acids, vitamins and trace minerals. The absolute weight growth rate of shrimp is high, 0.04 g/day in both farming models.

Phase 2 (culture in ELPB): apply low stocking density as the estuary farmers are still farming 50-70 shrimp/m^2. Growth results of shrimp in the ponds following the project model compared with the ponds following the current farming techniques: survival rate 95.5% (vs. 89%), FCR 1.26 (vs. 1.3), growth 0.25 g/individual/day (vs. 0.23 g/individual/day), harvested shrimp weight 16.25 g/individual (vs. 15.25 g/individual), productivity 10.18 tons/ha/crop (vs. 8.83 tons/ha/crop).

Phase 2 (culture in LP): apply a stocking density of 150 shrimp/m^2. Growth indicators of shrimp compared between ponds following the project model and ponds following the curent farming techniques: survival rate 96% (vs. 80%), FCR 1.29 (vs. 1.5), growth 0.24 g/shrimp/day (vs. 0.22 g/shrimp/day), harvested shrimp weight 16.15 g/shrimp (vs. 14.50 g/shrimp), productivity 21.91 tons/ha/crop (vs. 16.22 tons/ha/crop).

It can be seen that shrimp farming using biofloc and semi-bifloc technology recorded significantly higher growth efficiency than current farming techniques ponds without added microorganisms, with productivity 35.1% and 15.3% higher than the control pond, respectively. The FCR coefficient in the ELPB and LP models was lower because the shrimp used biofloc particles created in the pond, the lack of water exchange helped accumulate a large amount of bacterial biomass in the floc particles, reducing FCR [14], [18]. According to Emerencinao et al. (2011), shrimp farming using biofloc technology was provided with a source of amino acids, fatty acids and vitamins from the community of bacteria, microalgae, protozoa, rotifers and copepods in the system [19].

Comparison of the results of the second phase of the two experimental models: The survival rate remained high in both phases (>95%). The weight of harvested shrimp was different between the two models ($p<0.05$). The growth rate and weight of commercial shrimp were higher in the ELPB model, due to the lower stocking density. This result is consistent with the findings of the study by Le Quoc Viet and Tran Ngoc Hai (2018) [20], the growth rate in terms of volume gradually decreased with the stocking density. However, in terms of productivity, the model of lining pond with a density of 150 shrimp/m^2 was twice as effective as the model of pond with a density of 70 shrimp/m^2 as the advantage of a small area, making it easier to control shrimp health and reducing the risk of disease compared to ELPB.

Water quality fluctuations in the two-phase shrimp farming model

The changes of water quality during the experiment of 2 phases and 2 experiment models (Table 3).

Table 3. Water quality in two experimental models

Phase	Criteria	ELPB model	LP model
I	pH morning	7,6 ± 0,13	7,5 ± 0,13
	pH afternoon	7,9 ± 0,21	7,7 ± 0,21
	Temperature (°C)	31,0 ± 0,67	31,6 ± 0,47
	Salinity (ppt)	15,5 ± 0	17,0 ± 0
	DO (mg/L)	6,4 ± 0,31	6,5 ± 0,31
	Alkalinity (mg $CaCO_3$/L)	109,6 ± 6,58	122,1 ±6,58
	NH_4-N (mg/L)	0,75 ± 0,36	0,76 ± 0,36
	NO_2-N (mg/L)	0,47 ± 0,26	0,44 ± 0,26

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	Floc vollume (mL/L)	1 - 11	1 - 10
II	pH morning	7,9 ± 0,12	7,6 ± 0,07
	pH afternoon	8,1 ± 0,18	7,9 ± 0,17
	Temperature (°C)	31,2 ± 0,36	30,1± 0,66
	Salinity (ppt)	16,0 ± 0	15,0 ± 0,78
	DO (mg/L)	6,1 ± 0,80	6,2 ± 0,72
	Alkalinity (mg $CaCO_3$/L)	117,6 ± 15,11	123,8 ±15,15
	NH_4-N (mg/L)	1,8 ± 0, 75	2,3 ± 0,92
	NO_2-N (mg/L)	1,0 ± 0,50	1,4 ± 0,54
	Floc vollume (mL/L)	1 - 6,5	1 – 13,5

- pH: The difference in pH between morning and afternoon affects the physiological and biochemical processes of shrimp, affecting the growth and resistance of shrimp. The daily fluctuation range should not be greater than 0.5 [21]. The measured pH value increased slightly in the afternoon (7.7-8.1), compared to the morning (7.5 - 7.9) and was higher in the ELPB. The pH value did not differ between the two models and was always maintained within the growth range favorable for the growth of farmed shrimp and biological processes in the pond, pH 7.5-8.5 [22], [23], [24].

- Temperature: Whiteleg shrimp can adapt to a wide temperature range of 15-33°C [25]. In phase 1, the temperature is stable at 31-31.6°C. In phase 2, the average temperature of the two models of ELPB and LP is 31.2 ± 0.36°C and 30.1 ± 0.66°C respectively, the temperature fluctuation is within the favorable range for shrimp growth [26],[27].

- Salinity: The average salinity in the two farming stages ranges from 15.5-16 ppt for ELPB and 12.5-17 ppt for LP. The salinity fluctuation during the experimental shrimp farming process is within the optimal range (10-30 ppt) for whiteleg shrimp farming [28],[29].

- DO: Dissolved oxygen concentrations in experimental pond water were 6.4 ± 0.31 mg/L (phase 1) and 6.4 ± 0.80 mg/L (phase 2) for ELPB; 6.5 ± 0.31 mg/L (phase 1) and 6.2 ± 0.72 mg/L (phase 2) for LP. Low DO concentrations are a limiting factor in intensive shrimp farming systems [30]. The minimum DO concentration of whiteleg shrimp in the larval stage is 4.1 mg/L [31]. In the shrimp farming model using biofloc technology, it is necessary to maintain optimal DO from 7 to 8 mg/L to limit competition for oxygen demand between microorganisms in biofloc and farmed shrimp [23].

- Alkalinity: Alkalinity is directly related to the pH stability of the environment and the molting activity of shrimp. The average alkalinity in water between the two experimental models in the two stages was 109.6 ± 6.58 and 117.6 ± 15.11 mg $CaCO_3$/L in ELPB; 122.1 ± 6.58 and 123.8 ± 15.15 mg $CaCO_3$/L in LP. The appropriate alkalinity for the growth of whiteleg shrimp must be above 100 mg $CaCO_3$/L and in the condition of a pond system with limited water exchange, alkalinity should be controlled in the range of 100 - 150 mg $CaCO_3$/L [32]. Through the survey, the alkalinity in the two experimental models was maintained at an ideal level for shrimp growth.

- Ammonium content: NH_4-N concentration in water fluctuated significantly over time and was different between the two models ($p<0.05$), ranging from 0-3.10 mg/L in ELPB and 0-3.62 mg/L in LP. Ammonium concentration during the nursery phase ranged from 0 - 1.23 mg/L. According to Whestone [21], ammonium parameters less than 2 mg/L do not affect aquatic organisms. In phase 2, the highest measured ammonium concentration was in the middle of the cycle with 3.1 mg/L (ELPB) and 3.63 mg/L (LP).

- Nitrite content: The NO_2-N index had a statistically significant difference between the two treatments ($p<0.05$). The shrimp farming model on ELPB, LP recorded NO_2-N concentrations ranging from 0-2.08 mg/L and 0-2.15 mg/L, within the safe level (<4.5 mg/L) for shrimp growth according to Chen and Chin [33]. Boyd said that the best nitrite content for aquaculture is less than 2 mg/L, with a concentration of 4-5 mg/L being detrimental to shrimp life [20].

- Floc volume: In the nursery stage, floc volume increased from 1-11 mL/L in ELPB and 1-10 mL/L in LP, with no statistical difference between the two models ($p>0.05$). In stage 2, floc volume in ELPB was recorded lower at 1-6.5 mL/L ($p<0.05$), while in LP floc volume increased from 1-13.5 mL/L. Thong (2014) cited that the maximum floc volume used in biofloc and semi-biofloc systems was 15 mL/L and 5 mL/L, respectively, which helped reduce the sudden increase of ammonia and hydrogen sulfide and partially removed nitrate [9].

General discussion of the test results

The addition of indigenious microorganisms in the application of shrimp farming using biofloc technology has increased the natural feed, the microflora has helped clean the environment, increased the stability of the pond environment, helping shrimp grow well. The effects on the environment are reducing the need for frequent water changes, reducing the amount of waste discharged into the environment, only adding more water to compensate for the water lost due to evaporation. During the farming process, no drugs and antibiotics are used in shrimp farming, improving the quality and value of farmed shrimp products. This result correctly reflects the nature of biofloc technology, which plays an important role in stabilizing the water environment, biosafety, preventing pathogens, providing direct food for shrimp, enhancing natural nutrients, and reducing environmental pollution [34].

To apply and replicate the results of the model, the farm needs to invest in infrastructure that meets standards, invest in aeration systems and water fans to ensure water agitation and dissolved oxygen, and ensure power supply. Water must be disinfected, larvae shrimp must be tested for disease-free by PCR, and the farm must have a biosafety system to reduce the risk of infection in the pond. Microbiological products in liquid form should be stored in the refrigerator to ensure hygiene conditions, maintain product quality and extend shelf life.

CONCLUSIONS

The two-phase shrimp farming trial at farm scale using indigenous microorganisms in biofloc technology recorded good results. The trial results in ELPB showed that the productivity was 12.8 tons/ha/crop; FCR was 1.19; survival rate was 95.5%. The trial results in LP showed that the productivity was 21.5 tons/ha/crop; FCR was 1.32; survival rate was 96%. Both experimental models provided the good shrimp peformance result compared with current farming techniques ponds without added microorganisms of the farmer.

Shrimp pond waste is controlled by microorganisms used, organic matter is converted into biofloc biomass, and the Ammonium and nitrite indicators are controlled at safe levels for the growth of farmed shrimp in both models under conditions of no water change during the farming process.

The results show that the model has had a positive impact on the environment by limiting waste discharge due to water change and improving product quality due to not using chemicals and antibiotics during the farming process.

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