Production of Pacific whiteleg shrimp, *Litopenaeus vannamei* through implementation of rapid biofloc technology

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Abstract. A green technology known as rapid biofloc was implemented to reduce environmental damage while improving production of Pacific whiteleg shrimp, *Litopenaeus vannamei* to meet global market demand. Since there was no intensive application of biofloc technology being implemented at an industrial scale, this study aimed to assess the effect of biofloc in order to optimize water quality and maximize overall shrimp production. To speed-up the development of biofloc in *L. vannamei* cultures, an isolated biofloc boost-up bacteria inoculum was added during a new shrimp post-larvae (PL) stocking program. Samples of water, shrimp and biofloc were collected at ten day intervals from the new stocking of shrimp PL until harvest time (±100 days). Biofloc was observed starting to form as early as 10 days after shrimp the cultivation period began, and biofloc formation was speeded up by the addition of the inoculum. The biofloc effectively enhanced good water quality which resulted in an increase in shrimp biomass. The rapid biofloc formation and aggregation of beneficial microbes in the biofloc were responsible for maintaining good water quality and optimizing shrimp survival and production. Therefore, knowledge on the microbial composition in biofloc is deemed important for the successful design and application of biofloc technology to support a sustainable shrimp aquaculture industry.

1. Introduction

Aquaculture is an increasingly important source of food, nutrition, income and livelihoods, supporting many millions of people around the world. Recent reports by experts have highlighted the great potential of the oceans and inland waters to contribute significantly to meeting the demand for seafood and nutritional needs of the global human population, which is expected to reach 9.7 billion by 2050 [1]. The production of aquatic animals from aquaculture reached 73.8 million tons in 2014, with an estimated first-sale value of US$160.2 billion [1]. This total comprised 49.8 million tons of finfish (US$99.2 billion), 16.1 million tons of molluscs (US$19 billion), 6.9 million tons of crustaceans (US$36.2 billion) and 7.3 million tons of other aquatic animals including amphibians (US$3.7 billion). China accounted for 45.5 million tons in 2014, or more than 60 % of global fish production from aquaculture.

The prime goal of aquaculture expansion is to produce more aquaculture products without significantly increasing the usage of the basic natural resources of water and land [2]. The second goal is to develop sustainable aquaculture systems that will not harm the environment. The third goal is to
accumulate systems providing an equitable cost or benefit ratio to maintain the economy and social sustainability. It has been proposed that all these three requirements for sustainable aquaculture development can be achieved with the implementation of biofloc technology (BFT) [3]. BFT is considered as an innovative aquaculture system, and is based on limited water exchange (also known as ‘zero’ water exchange), that has been developed as a good alternative solution to solve the problems of biosecurity and value added nutrition in domesticated breeders.

BFT can enhance water quality through the addition of extra carbon to the aquaculture system, through an external carbon source or raising the carbon content of the feed [2]. Active heterotrophic microorganisms are manipulated to control the water quality by the immobilization of ammonium into microbial biomass. Moreover, as the microbial community develops, biofloc is formed containing a heterogeneous mixture of microorganisms and organic particles [4].

The major market for shrimp in the United States of America was expected to import approximately 477,000 tons worth USD 3.1 billion in 2005, 1.8 times more than the 264,000 tons imported in 2000. Productivity of Pacific whiteleg shrimp, Litopenaeus vannamei can reach 1.71 to 6.73 tons/ha/crop (or 3.42 to 13.459 metric tons/ha/year). This shows that productivity of L. vannamei production has not even reached the minimum productivity of intensive L. vannamei culture which was expected to produce 7 to 20 metric tons/ha/year [5].

Unexpectedly, Malaysian shrimp farmers found that the productivity of L. vannamei was unable to compete with other ASEAN countries. For instance, productivity in Indonesia, Vietnam and Thailand ranged between 10 to 15 metric tons/ha/year, 10 to 20 metric tons/ha/crop and 24 metric tons/ha/crop, respectively. Hence the low productivity of L. vannamei in Malaysia indicated that this industry is facing inefficiency in overall shrimp production. Moreover, intensive shrimp farming also has a number of environmental problems such as low feed utilization and water quality [6]. As an alternative approach, biofloc could enhance the growth performance and could be used as a supplementary food source for cultured shrimps, thus reducing the costs of feeding [7,8]. Therefore, this study was conducted to investigate the effectiveness of implementation of rapid biofloc, using commercial probiotics as a control, in terms of shrimp growth performance and water quality in L. vannamei culture ponds.

2. Methodology

2.1. Study site
Two treatments using rapid biofloc (GO-UMT) and probiotic (GO-HANNAN) were set-up at Pacific whiteleg shrimp Litopenaeus vannamei culture ponds in Kuala Gula, Perak, Malaysia (4°92'93" N, 100°49'32" E). The procedures for developing rapid biofloc were implemented as described by [8]. In each treatment pond, 400,000 shrimp post-larvae (PL) were stocked, giving a stocking density of 200 ind/m²). The climate can be considered as tropical with warm and humid conditions throughout the year. In total, the aquaculture complex comprises 150 L. vannamei culture ponds of 0.5 hectare each and is expected to produce around 3,000 tons of shrimp per year.

2.2. Data collection
This experiment was conducted for up to 100 days from PL to harvestable size. In-situ water quality parameters were checked weekly using a YSI multiprobe model 556 for temperature, dissolved oxygen (DO), pH and salinity. Ammonia, nitrite and phosphate were evaluated using a phenate method, diazotization method and ascorbic acid method [9], respectively. Biological parameters including shrimp growth rate, feed conversion ratio and survival rate were calculated as follows:
Specific Growth Rate, \( SGR = \frac{(\ln w_2 - \ln w_1)}{(t_2 - t_1)} \times 100 \) \((Equation 1)\)

where: \(w_2\) and \(w_1\) are the final and initial weight, respectively. \(t_2 - t_1\) is the duration of the experimental period.

Feed Conversion Ratio, \( FCR = \frac{\text{Feed consumed by the shrimp}}{\text{Weight gain by the shrimp}} \) \((Equation 2)\)

Survival Rate, \( SR = \frac{\text{No. of shrimp alive at the end of the time period}}{\text{No. of shrimp alive at the start of the time period}} \times 100 \) \((Equation 3)\)

2.3. Data collection
Statistical analyses were performed using SPSS Software version 2.0. Analyses were conducted for each treatment pond, and graphs were produced showing the mean values and standard error. The t-Test for Two-Samples Assuming Unequal Variances was used to determine the significance level of differences in water quality parameters.

3. Results

3.1. In-situ water quality parameters
There was no significant different between GO-UMT and GO-HANNAN grow-out pond water temperature \((P = 0.692, P>0.05)\) (Figure 1). The highest and the lowest of water temperature for the GO-UMT were 34.67 ± 0.01°C and 28.00 ± 0.00°C, respectively. On the other hand, the highest water temperature for the GO-HANNAN was 32.54 ± 0.01°C and 27.0 ± 0.00°C was the lowest. The water temperature in the GO-UMT was decreased on the day of culture 32 until 75 and was slightly increased on the day of culture 92 and 100.

Dissolved oxygen (DO) levels in the GO-UMT and GO-HANNAN treatment ponds followed a similar pattern. DO decreased on culture days 32 and 65, increased on day 75, decreased slightly on 92 and increased slightly on day 100. The highest DO for the GO-UMT biofloc treatment was 6.18 ± 0.13 mg/L and the lowest was 5.40 ± 0.13 mg/L. Meanwhile, the highest DO for the Hannan (probiotic) treatment grow-out pond was 5.68 ± 0.09 mg/L and the lowest was 5.28 ± 0.09 mg/L (Figure 2). The difference in DO concentration between GO-UMT and GO-HANNAN grow-out ponds was not significant \((P = 0.115, P>0.05)\).
Similarly, the difference in pH between the GO-UMT and GO-HANNAN grow out ponds was not significant (P = 0.765, P>0.05). The highest and the lowest pH for the GO-UMT were 8.70 ± 0.00 and 6.87 ± 0.03, respectively, while the highest and the lowest pH in the GO-HANNAN grow-out ponds were 8.60 ± 0.05 and 7.23 ± 0.10, respectively (Figure 3).

There was also no significant difference in salinity between the GO-UMT and GO-HANNAN treatment grow out ponds (P = 0.318, P>0.05). The highest and the lowest salinity for the GO-UMT ponds were 36.00 ± 0.00 ppt and 31.65 ± 0.02, respectively (Figure 4). The salinity level in the GO-UMT (biofloc) treatment grow-out ponds increased from day 32 to 65, decreased day 75 to 92 and slightly increased on day 100. Meanwhile the salinity level in the GO-HANNAN (probiotic) treatment grow-out ponds increased on day 32 to 65, decreased and then remained stable on day 75 to 92 and slightly increased on day 100.
3.2. *Ex-situ* water quality parameter measurements

The concentration of ammonia in both GO-UMT and GO-HANNAN grow-out ponds ranged between 0.12 ± 0.01 ppm and 2.00 ± 0.00 ppm, however the patterns of ammonia level variation differed (Figure 5). The ammonia level in the GO-UMT treatment ponds increased on day 32 to 45, decreased from day 65 to 75 and increased from day 92 until 100. In contrast, the ammonia level in the GO-HANNAN treatment ponds increased from day 32 to 42, decreased on day 65 to 75, remaining stable to day 92, and then increasing to day 100.

![Figure 4. Salinity levels in the grow-out ponds with GO-UMT (biofloc) and GO-HANNAN (probiotic) treatments](image)

![Figure 5. Ammonia concentrations in the grow-out ponds with GO-UMT (biofloc) and GO-HANNAN (probiotic) treatments](image)

There was a significant difference in nitrite concentrations between GO-UMT and GO-HANNAN grow out ponds (P = 0.009, P<0.05). The highest level of nitrite for the GO-UMT ponds was 2.00 ± 0.00 ppm and the lowest was 0.00 ppm, while, the highest level for the GO-HANNAN was 2.71 ± 0.04 ppm and the lowest was 0.25 ± 0.00 ppm (Figure 6). The nitrite level in the GO-UMT (biofloc) treatment ponds increased on the day 65 to 92 and decreased by day 100. In the GO-HANNAN (probiotic) treatment ponds, the nitrite level decreased on day 32 to 65, then increased and remained stable from day 75 to 92 and decreased by the last day of culture.
Figure 6. Nitrite concentrations in the grow-out ponds with GO-UMT (biofloc) and GO-HANNAN (probiotic) treatments.

The highest phosphate concentration in the GO-UMT treatment ponds was 2.00 ± 0.00 and the lowest was 0.00 ppm (Figure 7), while the highest level of phosphate in the GO-HANNAN treatment ponds was 2.01 ± 0.01 and the lowest was 1.00 ± 0.00 ppm. The phosphate level in the GO-UMT ponds increased on day 32 to 45, then decreased and remained stable from day 65 to 75 and was absent from day 92 to 100. Meanwhile, in the GO-HANNAN ponds, the phosphate level decreased on day 32 to 45 and then remained stable from day 45 to 100.

Figure 7. Phosphate concentrations in the grow-out ponds with GO-UMT (biofloc) and GO-HANNAN (probiotic) treatments.

3.3. Pond production performance
Table 1 shows details regarding the growth performance of Pacific whiteleg shrimp in both rapid biofloc (GO-UMT) and probiotic (GO-HANNAN) ponds. The survival rate in the GO-UMT ponds (75%) was higher than in the GO-HANNAN ponds (69%). The feed conversion ratio (FCR) was lower in the GO-UMT ponds (1.66) compared to the GO-HANNAN ponds (1.85). The growth performance in the GO-UMT ponds (86%) was higher than in the GO-HANNAN ponds (74%).
Table 1: Growth performance of Pacific whiteleg shrimp *Litopenaeus vannamei* in GO-UMT (biofloc) and GO-HANNAN (probiotic) ponds.

| Parameter                                | GO-UMT (biofloc) | GO-HANNAN (probiotic) |
|------------------------------------------|------------------|-----------------------|
| Pond area (m²)                           | 1500 m²          | 1500 m²               |
| Stocking density (ind/m²)                | ± 133 pcs/m²     | ± 133 pcs/m²          |
| Initial stock-take (number of shrimp)    | 346,000          | 288,000               |
| Harvest stock-take (number of shrimp)    | 301,550          | 276,831               |
| Initial weight (g)                       | 4.00             | 5.00                  |
| Final weight (g)                         | 10.00            | 10.20                 |
| Final Biomass (kg)                       | 2645.92          | 2567.88               |
| Total feed used (kg)                     | 4385             | 4744                  |
| Feed conversion ratio (FCR)              | 1.66             | 1.85                  |
| Average daily gain (gram/day)            | 0.074            | 0.086                 |
| Survival rate (%)                        | 75               | 69                    |
| Specific growth rate (%)                 | 86               | 74                    |

4. Discussion

This experiment was conducted to investigate the effect of rapid biofloc on the growth performance of Pacific whiteleg shrimp, *L. vannamei* culture. In addition, the experiment was also carried out to determine the optimized condition of water quality in *L. vannamei* farming operations. Throughout the culture period, water temperature remained within suitable limits for *L. vannamei* culture [10]. Water temperature in the shrimp aquaculture ponds changes diurnally and seasonally as it depends on water depth, air temperature, pond management and pond design [11]. The microorganisms present in the biofloc can help maintain water quality because they can decrease the concentration of nitrogen compounds [12]. Generally, the water quality parameters measured during this experiment were considered as within suitable ranges for the culture of *L. vannamei*.

The growth performance and survival rate were higher in the GO-UMT ponds which contained biofloc compared to the GO-HANNAN ponds which were treated with a probiotic. The percentage survival rate in the biofloc ponds was 87% as compared to 72% in probiotic ponds. In addition, the stock-take at harvest on day 100 was also higher for the GO-UMT pond (322,521 shrimp) compared to the GO-HANNAN pond (221,908 shrimp). This shows that biofloc not only maintained water quality, but also supplied nutrition to supplement the diet of the cultured shrimp.

Biofloc can enhance growth performance and help maintain good water quality in shrimp culture [13]. This is because biofloc consists of a medium rich in organic matter composed of friendly protozoa, phytoplankton, bacteria, filamentous bacteria, nematodes, ciliates, flagellates and also rotifers [14]. All the beneficial microorganisms found in the biofloc can serve as natural food for Pacific whiteleg shrimp and thus improve shrimp growth rate and survival rate. Pacific whiteleg shrimp has the ability to collect and use the biofloc suspended in ponds as extra feed [15]. Furthermore, some of microalgae aggregating in the biofloc are able to assimilate ammonia and nitrate in the water and utilize these compounds to build cellular protein which can provide nutritious feed for the shrimp [12]. The lower the Feed Conversion Ratio (FCR), the higher the weight gain obtained from the feed [16], and in this study the FCR for the GO-UMT ponds was lower than for the GO-HANNAN ponds. Therefore, biofloc can provide additional high quality feed with the protein necessary to boost shrimp growth with a low FCR [16].

5. Conclusion

The study has demonstrated that, compared to a probiotic treatment, Pacific whiteleg shrimp performed well under biofloc treatment in terms of survival rate, growth rate and FCR.
water quality under the biofloc treatment remained within the optimum range for Pacific whiteleg shrimp culture. An additional advantage of the biofloc system was that no water exchange was needed during the culture period. Avoiding water exchange could increase biosecurity as water is often a source of pathogens. Biofloc also supplies additional nutrition to the diet of the cultured shrimp, thus improving growth rate performance under this treatment. In conclusion, rapid biofloc can help to decrease the environmental impact of aquaculture, reducing the use of water, decreasing FCR, and reducing feed costs as well as supporting efficient energy use.

References
[1] Kungvankij P, Chua T E, Pudadera J B J, Tiro L B, Corre G, Potestas I O, Borlongan E, Taleon G A, Alava and Paw J N 2017 Shrimp Culture: Pond Design, Operation and Management (Rome: Food and Agriculture Organization of the United Nations)
[2] Crab R, Defoirdt T, Bossier P and Verstraete W 2012 Biofloc technology in aquaculture: Beneficial effects and future challenges Aquaculture 356–357 351–6
[3] Emerenciano M G, Martinez-Córdova L R, Martinez-Porchas M and Miranda-Baeza A 2017 Biofloc Technology (BFT): A Tool for Water Quality Management in Aquaculture Water Quality ed H Tutu
[4] Xu W and Pan L 2012 Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile Litopenaeus vannamei in zero-water exchange tanks manipulating C/N ratio in feed Aquaculture 356–357 147–52
[5] Ghee-Thean L, Ismail M M and Harron M 2016 Malaysia white shrimp (P. vannamei) aquaculture: An application of stochastic frontier analysis on technical efficiency Int. Food Res. J. 23 638–45
[6] Khatoon H, Banerjee S, Yuan G T, Haris N, Ikhwuanuddin M, Ambak M A and Endut A 2016 Biofloc as a potential natural feed for shrimp postlarvae Int. Biodeterior. Biodegrad. 113 304–9
[7] Arnold S J, Coman F E, Jackson C J and Groves S A 2009 High-intensity, zero water exchange production of juvenile tiger shrimp, Penaeus monodon: an evaluation of artificial substrates and stocking density. Aquaculture 293 42–4
[8] Kasan N A, Ghazali N A, Jauhari I, Jusoh A and Ikhwuanuddin M 2018 18s rDNA sequence analysis of microfungi from biofloc-based system in Pacific whiteleg shrimp, Litopenaeus vannamei culture Biotechnology 17 135–41
[9] APHA 2012 Standard Methods for the Examination of Water and Waste Water ed L S Eugene WR, Rodger BB, Andrew DE (Washington DC: American Public Health Association/American Water Works Association/Water Environment Federation)
[10] Hargreaves J A 2006 Photosynthetic suspended-growth systems in aquaculture Aquac. Eng. 34 344–63
[11] Rahman M M, Corteel C M, Dantas-Lima J J, Wille M, Alday-Sanz V, Pensaert M B, Sorgeloos P and Nauwynck H J 2007 Impact of daily fluctuations of optimum (27 °C) and high water temperature (33 °C) on Penaeus vannamei juveniles infected with white spot syndrome virus (WSSV) Aquaculture 269 107–13
[12] Manan H, Moh JHZ, Kasan NA I M 2016 Biofloc application in closed hatchery culture system of Pacific white shrimp, Penaeus vannamei in sustaining the good water quality management J. Fish. Aquat. Sci. 11 278–86
[13] Avnimelech Y 2007 Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds Aquaculture 264 140–7
[14] Rivera D A, Davo A P, Escalante K, Chavez C, Cuzon G and Gaxiola G 2014 Probiotic effect of FLOC on Vibrios in the pacific white shrimp, Litopenaeus vannamei Aquaculture 424–425 215–9
[15] Kasan N A, Ghazali N A, Ikhwuanuddin M and Ibrahim Z 2017 Isolation of potential bacteria as inoculum for biofloc formation in Pacific Whiteleg shrimp, Litopenaeus vannamei culture
ponds *Pakistan J. Biol. Sci.* 20 306–13

[16] Jamabo N A, Fubara R I and Dienye H E 2015 Feeding frequency on growth and feed conversion of *Clarias gariepinus* (Burchell, 1822) fingerlings *Int. J. Fish. Aquat. Stud.* 3 353–6