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Effect of Biofloc on Feed Efficiency and Growth of Pacific White Shrimp, *Litopenaeus vannamei* (Boone, 1932)

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ABSTRACT: The use of antibiotics in shrimp culture in Indonesia is prohibited under new Fisheries Law. Biofloc Technology was introduced since 2003 and getting more interest as proved to provide positive impact on production. This study aimed to evaluate the effect of biofloc on shrimp growth and feed efficiency. A laboratory experiment was set up using biofloc media and control (without biofloc). Five treatments of feeding levels (10%, 9.5%, 9.0%, 8.5%, and 8.0% body weight day^{-1}) were applied, and compared with control at 10% feeding level but without biofloc. Nitrite concentration of the control was five times higher (± 0.51 mg l^{-1}) than in the biofloc system (± 0.1 mg l^{-1}). The similar pattern also occurred for Ammonia-Nitrogen. Shrimp survival rate was statistically comparable for both systems, ranging from 70.0% to 78.6%. Specific Growth Rate in the biofloc system was significantly higher (ranging from 8.1 – 9.2% body weight day^{-1}) than the control (7.5% body weight day^{-1}). This was due to higher feed efficiency attained in the biofloc system that finally resulted in higher shrimp biomass at harvest. It seems that organic material from the excess of feed and feces were degraded and converted into *in-situ* protein in form of bacterial cell (biofloc) and partly consumed by the shrimp. The recovery of intensive shrimp culture in Indonesia seems to be dependent on the ability of individual farm to maintain biofloc system during grow out period.

Key words: Ammonia, Feed Conversion Ratio, Nitrite, Shrimp biomass

INTRODUCTION

As the consequence of complete trawl ban in 1980 (through Presidential Decree No. 39, 1980), Government of Indonesia (GoI) has set aside subsidies to promote development of shrimp culture (tambak system). In five years, more than 40% of traditional tambaks have been converted into intensive shrimp culture and culture-based shrimp production increased more than threefold in ten years [1]. However, significant shrimp diseases were observed since 1990, and shrimp farmers experienced production collapses afterward [2]. Intensive system has caused accumulation of organic-waste due to excessive feed given during the culture period.

Key success in intensive shrimp farming in Indonesia is determined by keeping certain survival rate at higher stocking density [3]. As usually common in Southeast Asia [4, 5], shrimp farmers applied antibiotics to maintain survival rate. However, this was becoming an issue in meeting criteria of shrimp export to European Countries [5]. In 2004, GoI enacted Fisheries Law provides that the use of chemical or biological substances which may harm aquatic resources or the environment is forbidden in both fisheries and aquaculture. In addition to that, the Law also prohibits the use of additives that may endanger human health. Since then, antibiotics were no longer practiced within shrimp culture in Indonesia.

Shrimp aquaculture industry in Indonesia is now days under scrutiny due to its contribution to environmental degradation and pollution. Furthermore, the expansion will be limited by land (converting mangrove into shrimp pond) and water replacement system to remove organic waste from the pond. In order for shrimp aquaculture to be successful, it needs to be economically viable but environmentally benign. Biofloc Technology (BFT) is a technique of enhancing water quality in aquaculture through balancing carbon and nitrogen in the system [6]. The technology has recently gained attention as a sustainable method to control water quality, with the added value of producing *in-situ* proteinaceous feed. In addition to those advantages above, researches have recently shown [7] that BFT constitutes a possible alternative measure to fight pathogenic bacteria in shrimp culture.

BFT is being introduced for shrimp culture in Indonesia since early 2003. Many shrimp farms have shown the positive results of the new technology, with very limited water replacement. This study aimed to evaluate the ability of BFT in providing *in-situ* proteinaceous feed for target shrimp, *Litopenaeus vannamei*. Feed efficiency and growth rate are both used as main indicators of the success.

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MATERIAL AND METHODS

Bioflocs

Bioflocs were prepared in 180 l tank. It filled with 150 l of sea water (20 ppt), sterilized with chlorine at a concentration of 1 ml l^{-1} sea water. The solution was then neutralized with Na-Thiosulphate 1 ml l^{-1} sea water. A 25 l of sterilized sea water was taken out, prepared for control treatment. The N source was made available in the media (125 l remained in the tank) by adding Ammonium Sulphate from commercial ZA fertilizer source (21% N) at a concentration of 0.14 g l^{-1} sea water media. Starter microorganisms (probiotic-treated (PROBIO) fish) was added into sea water media at concentration of 3 ml l^{-1} water. The starter probiotic was aerated and kept at room temperature for three days [8]. At day-4, a carbon source, sago flour (49.8% C) was added to reach a C/N ratio of 12.5:1 [8,9]. The mixture was aerated and maintained for another 7-days prior to use as bioflocs.

Stock for White-Shrimp's Post Larvae (PL's)

PL's 12 of white shrimp were obtained from a commercial hatchery in Bali (± 350 km from experimental location). The PL's were carefully acclimatized for eight days in aquarium that filled with 25 l of sea water (20 ppt). During this period, shrimp PL's were fed with commercial diet (33.6% protein), at satiation. The culture media was fully aerated with air-blower.

Experimental design

All bioflocs were spread into 15 jars, each filled with 7 l of bioflocs. Another three jars were each filled with 7 l of sterilized sea water, prepared for control treatment. Each jar was stocked with white shrimp PL's at density of 10 PLs l^{-1} (70 PLs each jar). All bioflocs received five different treatments of feeding levels, i.e.: 10% body weight day^{-1}, 9.5%, 9.0%, 8.5%, and 8.0% body weight day^{-1}, respectively. All controls were fed at feeding level of 10% body weight day^{-1}. The experiment was completed for 35 days.

Sampling

Sampling on individual body weight was done every week. Total 20 shrimp were selected randomly in each jar and weighed using digital scale (0.01 mg). Gains in body weight were used in determination of feeding ration in the coming week. It also used as based in the calculation for the addition of carbon source for bioflocs. At the end, total individual and shrimp biomass were measured to calculate feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate, and specific growth rate (SGR). Crude protein content of feed was measured based on Kjeldahl [11]. Daily water quality parameters were monitored as follows: temperature (ºC) and Dissolved Oxygen (mg l^{-1}) using DO-meter, and pH using digital pH-meter. Water salinity was monitored every week, using refractometer, and used to rearrange water salinity at 20 ppt. Ammonium (NH_4-N), Nitrite (NO_2-N), and Nitrate (NO^{3-}N) were measured on bi-weekly basis [12].

Calculation

Survival rate (SR) was estimated based on equation [13]: $SR = (N_t/N_0)*100\%$, where N_t is total lived shrimp at the end of experiment, N_0 is total PL's stocked. Feed Conversion Ratio (FCR) was determined as follows, FCR = total dry weight of feed offered divided by total shrimp weight gain [14]. Specific growth rate was estimated based on: $SGR = [(\ln W_t - \ln W_0)/t]*100$, where: W_t = average individual body weight at the end of culture period; W_0 = average initial body weight, and t = experiment duration (days) [15]. Protein efficiency ratio (PER) was estimated based on: PER = Bt/P, where: Bt = total shrimp biomass at the end of experiment and P = total protein consumed [14].

Data analysis

Homogeneity and normality of all data were assessed based on Lavene's and Kolmogorov-Smirnov's test. Effect of reduced feeding levels (treatments) on feed efficiency and growth rate were based on one-way analysis of variance (ANOVA). Tukey's test was applied to compare differences within treatments and between treatments with control. All statistical analyses were supported by software of SPSS, version 20.

RESULTS AND DISCUSSION

Water quality parameters

All water quality parameters (Table 1) were in the range to support optimal growth for white shrimp culture. Temperature and salinity were similar in all treatments and control ($p>0.05$). pH of control was significantly higher than all treatments. This proved that bioflocs could maintain water quality optimal for culture target [6]. Biofloc composes varieties of hetero, and autotrophic microorganisms. This diversity could maintain the balance of carbonate system in the water. Shifting the system into more autotrophic will lead to higher pH that difficult to bounce back.

Dissolved oxygen at all treatments were significantly lower ($p<0.05$) than the control. Bioflocs need oxygen in order to degrade organic material as most of the works were completed by heterotrophic bacteria [16, 17]. Degradation of organic material, which is mainly protein, surely need carbon source to keep the balance of C/N ratio. Consequently, biofloc always need additional carbon source that added into the system. However, during degradation process, these microorganisms will consume most of the oxygen available in the water. In the contrary, as less bacterial work in the control, oxygen is maintained at higher level.

Table 1. Mean and standard deviation of water quality parameters during experimental period

Parameters	Treatments					
	Control	Biofloc and feeding levels				
		10%	9.5%	9.0%	8.5%	8.0%
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
Temperature (°C)	27.8±0.14^{a}	27.7±0.04^{a}	27.7±0.09^{a}	27.8±0.14^{a}	28.0±0.15^{a}	27.9±0.08^{a}
pH	8.07±0.03^{a}	7.89±0.04^{b}	7.99±0.02^{bc}	7.99±0.02^{bc}	7.95±0.05^{b}	7.98±0.01^{bc}
Salinity (ppt)	21±0.17^{a}	22±0.50^{a}	23±0.19^{a}	22±0.66^{a}	22±1.23^{a}	22±0.35^{a}
Dissolved oxygen (mg l^{-1})	5.75±0.04^{b}	5.62±0.10^{ab}	5.59±0.14^{ab}	5.46±0.05^{a}	5.43±0.04^{a}	5.49±0.04^{a}
Ammonia (mg l^{-1})	0.54±0.03^{b}	0.48±0.03^{a}	0.46±0.04^{a}	0.39±0.07^{a}	0.38±0.07^{a}	0.33±0.02^{a}
Nitrite (mg l^{-1})	0.51±0.02^{c}	0.08±0.00^{ab}	0.10±0.01^{ab}	0.11±0.01^{b}	0.08±0.00^{a}	0.09±0.01^{ab}
Nitrate (mg l^{-1})	4.04±0.07^{a}	7.22±0.16^{b}	7.49±0.38^{bc}	6.89±0.43^{b}	8.02±0.23^{c}	7.30±0.12^{bc}

Remark: The different superscript letter in common per factor, in the table above indicates significant difference ($p<0.05$) with Tukey Test. $\bar{X}$ = mean; SD = Standard Deviation

Ammonia-Nitrogen (NH_4-N) and Nitrite (NO_2-N) in control were significantly higher than that in all treatments. All heterotrophic and autotrophic organisms within bioflocs will transform Ammonia-Nitrogen and Nitrite into Nitrate and protein that finally accumulated in bacterial cell [18]. In the system without biofloc will maintain Ammonia-Nitrogen and Nitrite at higher level. The more feed given into the system, the more Ammonia-Nitrogen will accumulate in the system and this will lead to unbalanced environment. On the contrary, bioflocs could balance the water quality parameters and maintain it optimal to support shrimp growth.

Nitrate (NO_3-N) in the control was found to be significantly lower ($p<0.05$) than all treatments. This is in line with the reduction of Ammonia-Nitrogen and Nitrite in the biofloc system and was transformed partly into Nitrate (Table 1). In the system without bioflocs, organic material will be accumulated in the forms of Ammonia-Nitrogen and Nitrite which harm the culture target (shrimp). However, Ammonia-Nitrogen and Nitrite in the control were still in the range to support shrimp growth [19].

Shrimp growth and feed efficiency

Shrimp survival rate (SR) in all treatments and control were statistically similar ($p>0.05$) and maintained at ≥ 70% (Table 2). As mentioned above, Ammonia-Nitrogen and Nitrite in the control treatment seems to be lower than the concentration that can affect culture target [19]. Shrimp growth (SGR) in all biofloc systems were significantly higher than the control treatment. This indicates that biofloc system provides *in-situ* proteinaceous feed for shrimp [6, 7, 15].

Table 2. Feed Efficiency, growth, and total biomass of Pacific White Shrimp at harvest

Parameters	Treatments					
	Control	Biofloc and feeding level				
		10%	9.5%	9.0%	8.5%	8.0%
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
SR	72.4±9.5	78.6±13.6^{a}	72.9±17.5^{a}	65.2±2.2^{a}	71.9±15.7^{a}	70.0±4.3^{a}
SGR	7.54±0.40	8.11±0.54^{ab}	8.08±0.54^{ab}	8.96±0.36^{b}	8.78±0.12^{ab}	9.19±0.59^{b}
Shrimp Biomass	14.12±0.17	18.98±4.95^{ab}	17.06±3.30^{ab}	21.16±3.16^{ab}	21.61±3.73^{ab}	24.59±3.54^{b}
FE	66.82±6.03	101.8±27.7^{ab}	94.03±23.3^{ab}	120.2±31.1^{ab}	120.2±22.7^{ab}	140.4±9.95^{b}
FCR	1.50±0.13	1.03±0.28^{ab}	1.11±0.3^{ab}	0.87±0.2^{b}	0.85±0.15^{b}	0.71±0.05^{b}
PER	1.82±0.14	2.82±0.79^{ab}	2.60±0.64^{ab}	3.38±0.89^{ab}	3.37±0.63^{ab}	3.96±0.25^{b}

Note: The different superscript letter in common per factor indicates significant difference ($p<0.05$) with Tukey Test. $\bar{X}$ = mean; and SD = Standard Deviation; SR = Survival rate; SGR = Specific Growth Rate; FE = Feed Efficiency; FCR = Feed Conversion Ratio; PER = Protein Efficiency Ratio; SR = Survival Rate.

The highest total shrimp biomass at the end of culture period was reached at the lowest feeding level (8.0% body weight day^{-1}). Biofloc seems to reach certain capacity to degrade organic material from the excess of feed [8, 20]. The lowest feed conversion ratio, and hence, the highest Feed Efficiency (FE) was reached at the lowest feeding level. Generally, it showed that biofloc could efficiently use the feed available for shrimp. In addition, the excess of feed and other organic materials will be converted into protein that accumulated in bacterial cell and consumed by the shrimp. It seems that biofloc provides multi-positive functions for target shrimp, improve water quality, increase shrimp growth, and more efficiently use of feed.

CONCLUSION

This study proved the advantages of biofloc system in shrimp culture that function as improve water quality (nitrite reduction), increase shrimp growth (higher SGR), and more efficiently use of feed (higher FE). As feed contributes the highest cost in shrimp culture, this biofloc technology will gain more interest for shrimp culture in Indonesia. Additional cost needed to provide extra oxygen will be paid off. So, the recovery of intensive shrimp culture in Indonesia seems to be dependent on the ability of individual farm to maintain biofloc system during grow out period.

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