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Enhancement of *Penaeus vannamei* shrimp growth using nanobubble in indoor raceway pond

Asri Ifani Rahmawati [a,b,*], Rizki Nugraha Saputra [a,b], Arief Hidayatullah [a,b], Agus Dwiarto [a,b], Hardi Junaedi [a,c], Dedi Cahyadi [a,b], Henry Kasman Hadi Saputra [a,d], Wendy Tri Prabowo [e], Ujang Komarudin Asdani Kartamiharja [e], Hanny Shafira [b], Alfian Noviyanto [b,f], Nurul Taufiqu Rochman [g]

[a] *Nanobubble Karya Indonesia, Ltd., Jl. Raya Serpong, South Tangerang, Banten, 15314, Indonesia*
[b] *Nano Center Indonesia, Jl. Raya Serpong, South Tangerang, Banten, 15314, Indonesia*
[c] *Department of Agricultural Industry, IPB University, Jl. Raya Dramaga, Bogor, West Java, 16680, Indonesia*
[d] *Department of Aquaculture, Vocational School, IPB University, Jl. Kumbang 14, Bogor, West Java, 16680, Indonesia*
[e] *Aquaculture Center for Brackish Water Situbondo, Jl. Raya Pecaron, East Java, 68351, Indonesia*
[f] *Department of Mechanical Engineering, Faculty of Engineering, Mercu Buana University, Jl. Meruya Selatan, Kebun Jeruk, Jakarta, 11650, Indonesia*
[g] *Research Center for Metallurgy and Materials, Indonesian Institute of Sciences, PUSPIPTEK, South Tangerang, Banten, 15314, Indonesia*

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ABSTRACT

The effects of nanobubble on the growth environment, such as dissolved oxygen (DO) and total virus-bacteria, and growth performance, such as growth, feed conversion ratio (FCR), survival rate (SR), total harvest, and productivity, were investigated in *Penaeus vannamei* shrimp ponds. The present study was carried out in 50 m^2 indoor raceway ponds for 81 days with stocking density being set to 680 shrimp m^{-3} in two types of treatment: nanobubble and diffuser aerator. A paddlewheel was used to distribute the oxygen saturation levels throughout the ponds. The presence of nanoparticles was detected using a dynamic light scattering method and was reported to be 82.38 nm in size and −26 mV for zeta potential value. DO was significantly higher under nanobubble treatment. Total virus-bacteria and FCR decreased, whereas SR, growth (weight and length), total harvest, and productivity increased with increasing DO. The SR was reported to reach 95%. The average weight and length of *P. vannamei* were 15.1 ± 1.8 g and 13.1 ± 1.1 cm, respectively. The total harvest and productivity have doubled to 436 kg and 8.7 kg/m^3. The results revealed that nanobubble has managed to maintain DO at the optimal range and affected the shrimp growth significantly ($P < 0.05$).

1. Introduction

Penaeus vannamei or whiteleg shrimp is one of the major shrimp species farmed in Indonesia (Ma, 2015). The farming practices of *P. vannamei*, both extensive and intensive, have grown to a very great extent in recent years due to increased potential market (Li, Li, & Wang, 2006; Suriya, Shanmugasundaram, & Mayavu, 2016). Successful shrimp production requires the best practices in technology and management of water quality (Lazur & Britt, 1997).

Some studies investigated that dissolved oxygen (DO) played an important role in improving water quality (Boyd, 2017; Musa, 2013). Aquatic animals showed the best growth when DO concentrations reached near saturation (Boyd, 2017). Therefore, the enhancement of DO has become a primary concern for shrimp farming practices.

The present study proposed nanobubble generator, a novel technology in producing nano-sized bubbles to increase DO levels throughout the ponds. Nanobubble generator that was being used combined the gas-liquid flow with honeycomb structures for high-efficiency nanobubble generation (Ren et al., 2018).

Nanobubbles have high specific surface areas and high stagnation

* Corresponding author. Nanobubble Karya Indonesia, Ltd., Jl. Raya Serpong, South Tangerang, Banten, 15314, Indonesia.
E-mail addresses: fani@nano.or.id (A.I. Rahmawati), rizki@nano.or.id (R.N. Saputra), ariefh@nano.or.id (A. Hidayatullah), agus.dwiarto@nano.or.id (A. Dwiarto), hardi_j@apps.ipb.ac.id (H. Junaedi), dedi@nano.or.id (D. Cahyadi), henry_khs@apps.ipb.ac.id (H.K.H. Saputra), wen_aquaculture@yahoo.com (W.T. Prabowo), ujang_k66@yahoo.com (U.K.A. Kartamiharja), hannyshafira@nano.or.id (H. Shafira), a.noviyanto@nano.or.id (A. Noviyanto), nurul@nano.or.id (N.T. Rochman).

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times because of its nano-sized, which leads to mass transport efficiencies at the gas-liquid interfaces (Meegoda, Hewage, & Batagoda, 2018). Nanobubbles have an electrically charged surface which prevents bubbles from coalescence, and because of its high densities, nanobubbles allow to create a high rate of gas dissolution in water (Ren et al., 2018).

Due to its properties and behavior, nanobubbles could be best uses for applications in a fishery. By adding nano-oxygen bubbles, oxygen levels in water are maintained, and hence, have a positive effect on the growth performance of fish (Meegoda et al., 2018).

The purposes of this study were to investigate the effects of nanobubble on pond carrying capacity, mainly DO levels, total virus-bacteria, shrimp growth, feed conversion ratio, survival rate, total harvest, and productivity in indoor raceway ponds.

2. Experimental

2.1. Study site

The experiment was carried out from September to December 2018 in Aquaculture Center for Brackish Water (BPBAP) Situbondo, East Java, Indonesia.

2.2. Experimental shrimp

A total of 34,000 juvenile *P. vannamei* shrimp (post-larval stage 10) from BPBAP Situbondo were used in each pond with mean body weight and length of 0.09 g and 0.5–0.7 cm, respectively. The shrimp underwent 1 h acclimation period prior to the experiment.

2.3. Experimental design

The study was carried out with two treatments of aeration (nanobubble generator combined with paddlewheel and paddlewheel only) and conducted for 81 days (Budhiman, Paryanti, & Sunaryanto, 2005; FAO, 2009; Jaspe, Caipang, & Elle, 2011). Treatments are termed as nanobubbles and diffuser aerator. Two ponds were set for each treatment for replication. Indoor raceway ponds sized 50 m^2 in the area were used with a stocking density of 680 shrimp m^{-3}. The water depth of the raceway at the deep end was 1 m. Nanobubble generator (1 HP) was used at a flow rate of 6 L/min and an oxygen flow rate of 0.2 L/min. Pond water was pumped and flowed inside the machine input. Pressurized oxygen gas was going from the oxygen source through the oxygen input in the machine pump into the liquid flow. The bubbles were cut off in the machine and flowed to the output with the liquid flow in form of nanobubbles. A paddlewheel (0.5 HP) was positioned on the center partition for each pond. The schematic diagram of the experiment was illustrated in Fig. 1. For each treatment, DO concentration, total virus-bacteria, growth (weight and length) of shrimp, feed conversion ratio (FCR), and survival rate (SR) were quantified.

2.4. Experimental procedure and sample collection

Commercial diet (36% protein, 7% fat, 3% fiber, 13% ash) which meets the shrimp nutrient requirements (Davis, 2005; FAO, 2020; Lee & Lee, 2018) were fed every 3 h (*ad libitum*). Water temperature (27–29 °C) and pH (7.5–7.8) were maintained to the optimal cultivation levels. DO concentration was measured three times a day by a YSI instrument (Pro20, YSI, Ohio, USA). The isolation of bacteria from water samples was carried out to determine total *Vibrio* bacteria (TBV). NaCl 2.5%, GSP 2.5%, and TCBS were added to TSA medium. Incubation of samples was conducted for 24 h at room temperature. The bacteria were separated by its morphologies, including colony shape and color. The dilution was performed until it reached 10^5 and poured into a petri dish. The petri dish was incubated for 24–48 h under 35 °C. The colony was measured using a colony counter. The presence of TSV (taura syndrome virus), WSSV (white spot syndrome virus), IHHNV (infectious hypodermal hematopoietic necrosis virus), IMNV (infectious myonecrosis virus) were analyzed using Polymerase Chain Reaction (PCR) method. Five percent of the shrimp population in the pond was taken as group samples and the pleopods were soaked by alcohol 95% before being extracted. DNA/RNA implication process of the extract was conducted

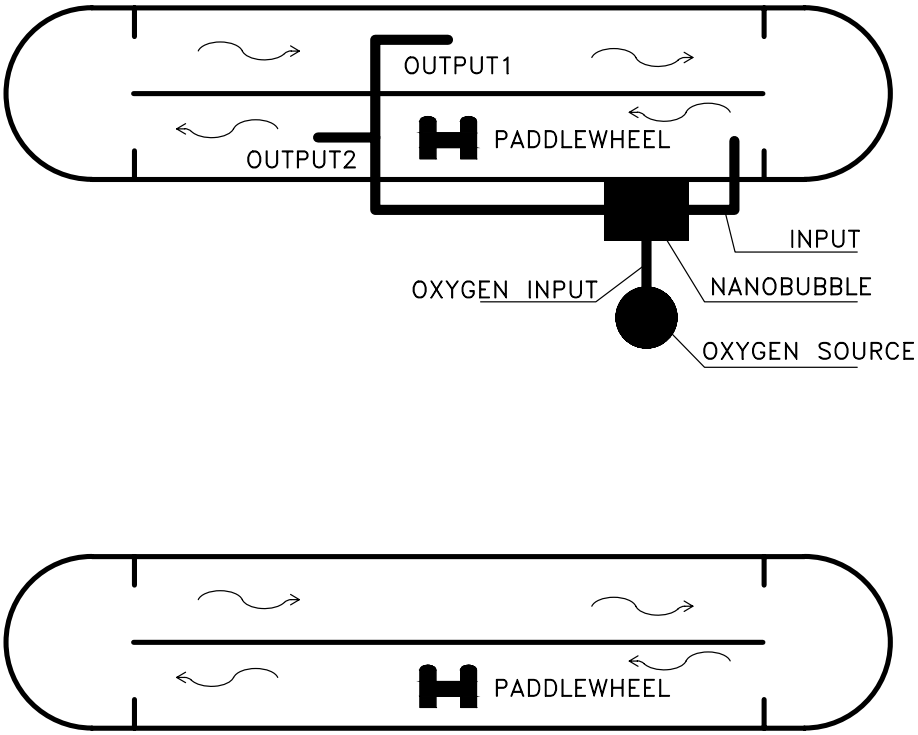

Fig. 1. Schematic diagram of *P.vannamei* shrimp pond experiment using nanobubble system (upper) and diffuser aerator (lower).

using PCR machine, which was divided into two steps: Reverse Transcriptase (RT)-PCR and Nested PCR. After the primers were propagated, PCR results reading, wherein occur as dot blot in WIT Chip Chamber, were managed.

FCR and SR were calculated by the given formula:

$$FCR = \frac{F}{W_t - W_o} \quad (1)$$

where F is the total weight of feed supplied, Wt and Wo are the final and initial weight of the shrimp during the experiment, respectively (Apun-Molina et al., 2015).

$$SR = \frac{N_t}{N_o} \quad (2)$$

Where Nt is the total number of surviving shrimp at the end of the experiment and No is the initial number of shrimp (Joseph, 2007). Shrimp length and weight were measured by ruler and electronic balance, respectively. Measurement of shrimp growth was determined by group samples (each with 20 shrimps) collected every seven days during total days of culture.

2.5. Measurement of size, zeta potential, and stability of nanobubble

Nanobubble size distribution, concentration, and zeta potential in water were analyzed using a Malvern instrument (Zetasizer Nano ZS, Malvern, Worcestershire, UK) which applied the principle of dynamic light scattering method. The Zetasizer is able to measure bubble sizes in the range of 0.3 nm–10 μm. Oxygen-nanobubble water was kept at room temperature. DO concentration was measured sequentially to identify the stability of the bubbles.

2.6. Statistical analysis

A *t*-test assuming equal variances was applied to examine significant differences between treatments. Differences were considered significant at the level of 0.05.

3. Results and discussion

3.1. Nanobubble characteristics

Nanobubble generator produced nano-sized bubbles by utilizing the multiphase gas-liquid flow contact principle and honeycomb structure. Water flow is pumped into the system, while the pressurized gas is being injected and form a multiphase flow (Levitsky, Gitis, & Tavor, 2015). Nano-sized bubbles occur due to the breakdown of multiphase flow when passing through the honeycomb structure (Ren et al., 2018).

The bubble size results, as reported in Fig. 2, were based on Number-Distribution Data. The peak value of the distribution curves approximately showed that 95.8% of the generated oxygen-bubbles were 82.38 nm in size. Nanobubble are defined to have radii <200 nm in aqueous solution (Meegoda et al., 2018). The zeta potential was reported to be −26 mV. Ushikubo et al. (2010) mentioned that oxygen nanobubble in water values 137 nm in size and 34–45 mV (negative values) for zeta potential. The disparity results of zeta potential between literature and the present study were expected to occur due to differences of water purification method (Ushikubo et al., 2010), however, the results were both showed negative values.

The negative value is assigned to the OH^- ion concentration at the bubble interface which leads to bubble stability. The electrically charged surface enables particles to repulse each other to avoid the coalescence of bubbles (Meegoda et al., 2018). Fig. 3 showed the stability of high DO levels even after a week since the generation of nanobubble. Water with the initial DO levels at 29 mg/L still remained to be 5 mg/L after being stored for a week. This is due to the very small size of nanobubble particles which resulted the characteristics of high mass transfer efficiency, high gas dissolution rate, and high stability in water (Ulatowski, Sobieszuk, Mroz, & Ciach, 2019; Yu & Felicia, 2015).

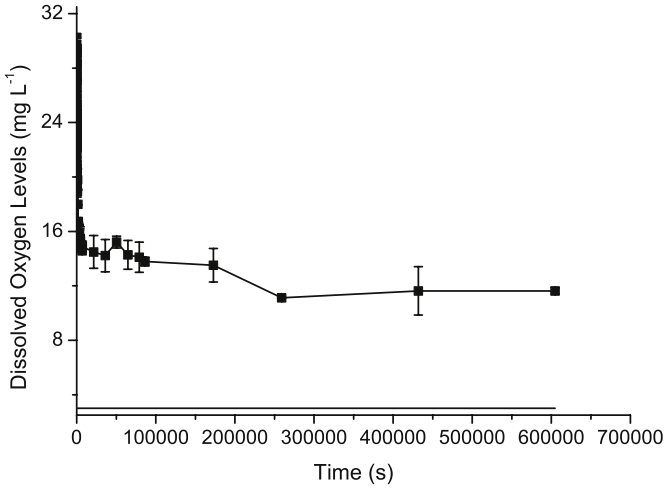

Fig. 3. Sequential changes of dissolved oxygen level of nanobubble water from day 0–7 (604,800 s), solid line (−) shows the initial dissolved oxygen level of non-nanobubble water.

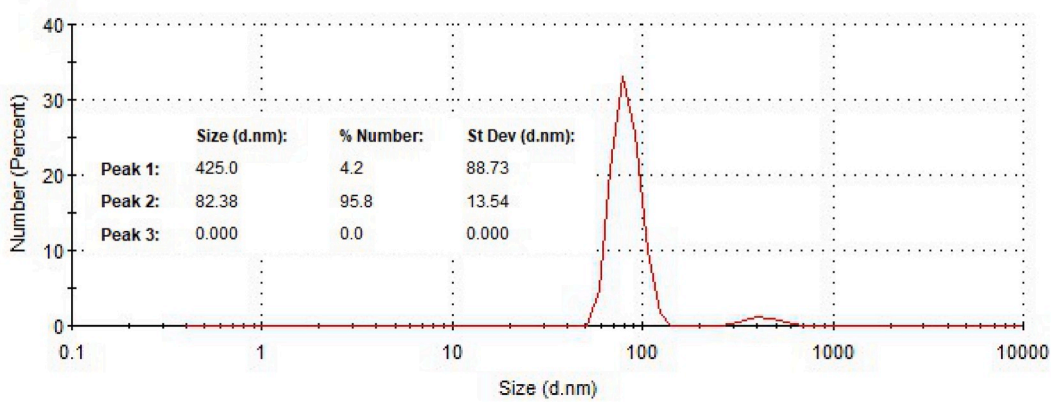

Fig. 2. Size distribution curve of nanobubble water.

3.2. Dissolved oxygen

Nanobubble generator being installed in *P. vannamei* shrimp ponds for 81-day cultivation period has shown a stable dissolved oxygen level. Examination of dissolved oxygen in the ponds for the first 12 h (pre-treatment; without shrimp) showed excellent performance of nanobubble generator in reaching optimal dissolved oxygen levels for shrimp growth (Fig. 4). The suitable aquatic environment for *P. vannamei* best growth should experience dissolved oxygen in the range of 4.5–7 mg/L (Li et al., 2006). This study reported that the nanobubble generator could maintain dissolved oxygen under these optimal conditions during cultivation days (Fig. 5).

Because of its unique characteristics, nanobubble has managed to improve the dissolved oxygen levels in the ponds. Several studies showed a similar result in the escalation of dissolved oxygen. The use of a nanobubble generator was reported to give rise in dissolved oxygen levels up to 9.0 mg/L (Ebina et al., 2013) and 10.8 mg/L (Galang et al., 2019).

Dissolved oxygen levels during cultivation showed a decrease after days of culture (DOC) 18 (Fig. 5). This phenomenon indicates an enhancement in oxygen consumption rate due to an increase in *P. vannamei* biomass (Kureshy & Davis, 2000). Bett and Vinatea (2009) reported evidence that there was a positive correlation between shrimp biomass and oxygen consumption rate. The oxygen consumption rate reported for *P. vannamei* juvenile sized 2 g was 0.66 mg/h, while the consumption rate for sized 12 g increased to 3.25 mg/h (Bett & Vinatea, 2009). The high oxygen level of the rearing water is proven to affect the rate of *P. vannamei* oxygen consumption rate (Li et al., 2006).

3.3. Growth of P. vannamei

The *P. vannamei* final weight and length (DOC 81) were reported in Table 1. There was a significant difference ($P < 0.05$) in the mean weight and length of the shrimp exposed to nanobubble and non-nanobubble treatments during the experiment. The shrimp cultivated in nanobubble pond shown a more significant growth (Fig. 6). The growth rate was measured per week. The highest rate was obtained for the shrimp maintained at the pond using nanobubble generator which were 15.10 g and 13.10 cm for weight and length average, respectively, whereas the shrimp which were maintained at the pond using diffuser aerator only obtained the growth at rate 12.70 g and 11.55 cm. The enhancement of growth proved that the energy absorbed by shrimp in a nanobubble pond is higher because of the oxygen levels available in the pond.

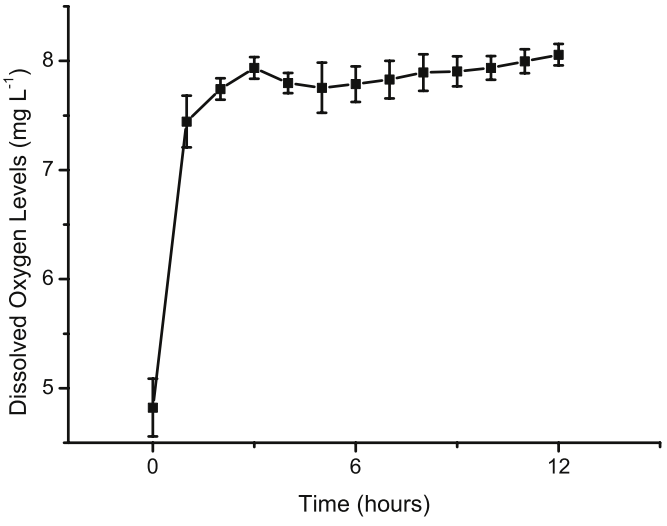

Fig. 4. Nanobubble generator performance to reach optimum dissolved oxygen levels in the ponds (pre-treatment; without shrimp).

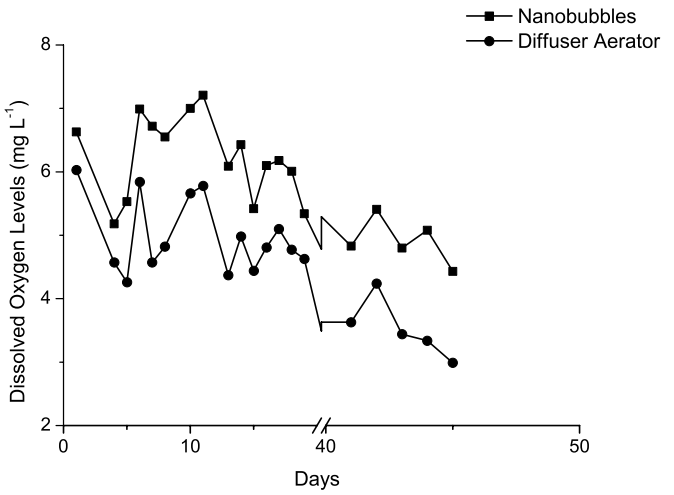

Fig. 5. Dissolved oxygen levels in *P. vannamei* shrimp ponds using nanobubble generator and diffuser aerator during cultivation period.

Table 1
Mean weight and length (±standard deviation) of *P. vannamei* reared at raceway ponds using nanobubble and diffuser aerator.

	t-test for equality of means		
	Mean	SD	t
Weight (g)			
Nanobubble	15.10	1.79	2.96*
Diffuser aerator	12.70	1.83	
Length (cm)			
Nanobubble	13.10	1.10	2.72*
Diffuser aerator	11.55	1.42	

N = 10.
*Significant at the 0.05 level.

According to Re and Diaz (2011), the ability of penaeids to acquire energy and distribute them effectively for growth depends on the effect of environmental factors. Oxygen level is one of these factors that influence the metabolism of the organism. If the cost associated with the consumption and processing of ingested food are not enough to be met by oxygen levels, shrimp stops eating, sacrificing the possibility of obtaining energy from food to be dedicated to growth. This was obviously proven by comparing oxygen levels and shrimp growth graphs in Figs. 5 and 7.

Shrimp weight and length increments during the entire growth period were shown in Fig. 7. Aquatic animal growth is strongly influenced by oxygen saturation levels (Carter, 2005; Li et al., 2006; Mallya, 2007). The dissolved oxygen levels specifically gave positive effect to survival rate and growth, as well as streamlined the feed conversion ratio.

High oxygen level induces bacterial autolysis, increases biological lysis reactions, and reduces sludge production (Ahmadi, Bidhendi, Torabian, & Mehrdadi, 2018). These conditions resulted in a decrease in the total *vibrio* and infectious virus in shrimp (Table 2). It can be seen from Table 2 that the total *Vibrio* bacteria (TBV) in nanobubble pond was much lower as compared to the use of diffuser aerator. Notably, the TBV number in nanobubble pond was afar from danger threshold (closely at a safe range) to cause infected shrimp, i.e 2.0×10^3 CFU mL^{-1}. There was no disease-causing virus detected in the nanobubble pond, whereas the other treatment pond showed a contradiction result which indicated the

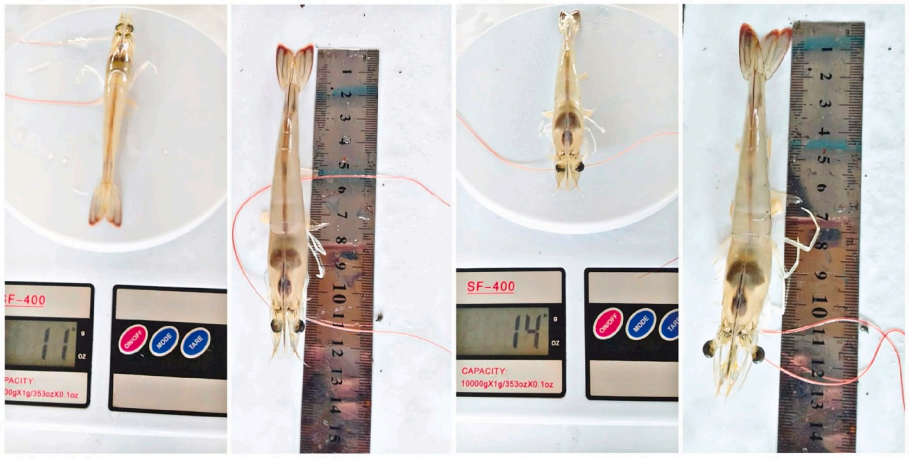

Fig. 6. Comparison between shrimp in nanobubble pond (right) and non-nanobubble pond (left). The shrimp in nanobubble pond shown a higher measurement in weight and length.

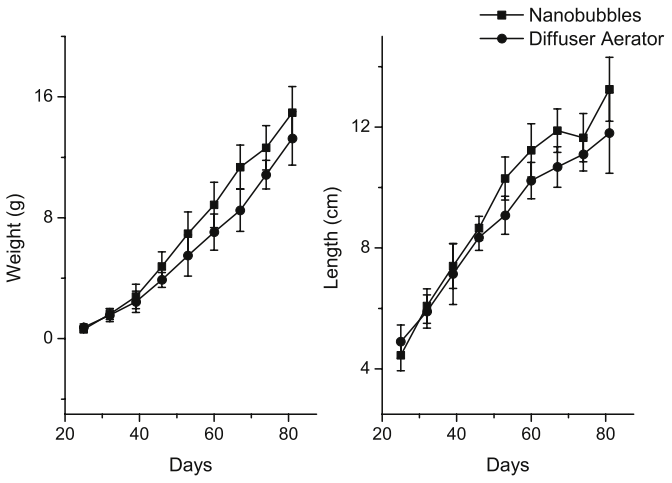

Fig. 7. Growth of *P. vannamei* in indoor raceway pond during cultivation period using nanobubble generator and diffuser aerator (a) average weight; (b) average length.

Table 2
Total virus and bacteria in *P. vannamei* pond using nanobubble and diffuser aerator.

Parameter	Nanobubble	Diffuser aerator	SNI
TBV (CFU/mL)	2.0×10^3	1.9×10^4	$<4.4 \times 10^4$
TSV	Negative	Negative	Negative
WSSV	Negative	Negative	Negative
IHHNV	Negative	Negative	Negative
IMNV	Negative	Positive	Negative

Remarks: SNI (Indonesian National Standards).

presence of IMNV.

This free-disease water exposed to nanobubble resulted in a high survival rate. The survival rate of *P. vannamei* in nanobubble pond reached 95% as compared to non-nanobubble which only reached 78%. Suriya et al. (2016) reported that oxygen levels at 7.5–7.9 mg/L is able to increase the survival rate to a range of 80%–90%.

High oxygen levels also improve feed conversion ratio and was proportional to feed availability (Musa, 2013). This study reported a lower value of feed conversion ratio in the pond exposed to nanobubble, which indicates efficiency in feed conversion (Table 3). These conditions promote an increase in feed intake (Thorarensen et al., 2017). Several studies have indicated that species in aquaculture are unable to digest food in low oxygen level since oxygen plays a decisive role in metabolism (Dmitry, 2013).

Therefore, the exposure of high oxygen levels in the pond has succeeded in improving the growth environment by specifically reducing total bacteria, virus, and diseases, and increasing feed conversion efficiency that promotes to maximum growth of *P. vannamei*. The study of Nonwachai, Purivirojku, Chuchird, and Limsuwan (2011) also proven that high oxygen levels help increased immune parameter levels measured as total hemocyte count, percentage phagocytosis, bactericidal activity, phenoloxidase activity, and superoxide dismutase activity. Shrimp were able to perform good physiological responses and have high resistance to the pathogen when exposed to constant oxygen levels at optimal range.

These suitable environments in nanobubble pond have doubled the total harvest to 436 kg as compared to the diffuser aerator pond. Remarkably, the productivity of vannamei shrimp in nanobubble pond reached 8.7 kg/m^3 (Table 4).

4. Conclusions

The present finding shows that nanobubble could increase DO concentration in *P. vannamei* shrimp pond, and hence, improve the growth environment. Nanobubble size was measured to be 80 nm with zeta potential value at −26 mV. The stability of nanobubble was remained on water after being stored for a week, therefore oxygen levels in nanobubble pond could be maintained at range 4–6 mg/L[1]. Total *Vibrio* bacteria and FCR were reduced to 2.0×10^3 CFU/mL and 1.1, respectively, while SR was increased to 95%. These good environments due to

Table 3
Survival rate and feed conversion rate in *P. vannamei* pond using nanobubble and diffuser aerator.

Parameter	Nanobubble	Diffuser aerator
Survival rate (%)	95	78
Feed conversion rate	1.1	1.5

Table 4
Total harvest and productivity in *P. vannamei* pond using nanobubble and diffuser aerator.

Parameter	Nanobubble	Diffuser aerator
Total harvest (kg)	436	222
Productivity (kg/m^3)	8.7	4.4

nanobubble existences also gave a significant effect on the shrimp growth to be 15.10 g for average weight and 13.10 cm for average length and had proven to double the total harvest and productivity to 436 kg and 8.7 kg/m^3, respectively.

CRediT authorship contribution statement

Asri Ifani Rahmawati: Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Rizki Nugraha Saputra:** Formal analysis, Funding acquisition. **Arief Hidayatullah:** Writing - original draft. **Agus Dwiarto:** Formal analysis, Resources. **Hardi Junaedi:** Methodology, Investigation. **Dedi Cahyadi:** Conceptualization, Validation. **Henry Kasman Hadi Saputra:** Validation, Data curation. **Wendy Tri Prabowo:** Investigation, Data curation, Project administration. **Ujang Komarudin Asdani Kartamiharja:** Project administration. **Hanny Shafira:** Validation, Data curation. **Alfian Noviyanto:** Validation, Data curation, Supervision. **Nurul Taufiqu Rochman:** Validation, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare no conflicts of interest.

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