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THE EFFECTS OF PROBIOTICS ON THE GROWTH PERFORMANCES OF WHITE SHRIMP (*Penaeus vannamei*) REARED THROUGH BIOFLOC TECHNOLOGY

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Abstract: The use of probiotics may give beneficial effects on shrimp culture. Biofloc technology also improves water quality and feed efficiency. This study aimed to evaluate the effects of probiotics on shrimp growth reared through biofloc technology. This study consisted of six treatments and triplicates including Control (without probiotic and biofloc), F (Biofloc), BF (Bacillus and biofloc), IF (IW and biofloc), B (Bacillus), I (IW). Biofloc technology was applied using molasses as carbon source (C/N ratio 12). The experimental animals were white shrimp (Penaeus vannamei) (3.8 ± 0.8 g), stocked into glass tanks (60 x 40 x 50 cm3) with a stocking density of 40 shrimp per tank. The shrimp were fed experimental feed four times a day for 30 days. Experimental feed for control and F treatments was composed by commercial feed (32% protein) and 1% binder, while experimental feed for BF, IF, B, and I treatments was composed by commercial feed (32% protein), probiotics (0.2%), and 1% binder. The application of probiotics into the rearing media was conducted using Bacillus and IW probiotics (5 ppm) every week for BF and IF treatments. The parameters observed included final weight, growth rate, survival, feed conversion ratio (FCR), and water quality. The highest shrimp weight was achieved in treatment B (19.0 ± 0.8 g) followed by treatment F (18.4 ± 0.5 g). The lowest FCR was shown by treatment B (1.32 ± 0.12) followed by treatment IF (1.33 ± 0.01). The highest survival was indicated in treatment B (86.67 ± 3.81%) followed by treatment F (85.00 ± 2.5%). The best water quality was found in treatment F with the optimum results in nitrate, alkalinity, and floc volume.

Keywords: growth performances, biofloc, *Penaeus vannamei*, probiotics

Introduction

Pacific whiteleg shrimp (Penaeus vannamei) is the most popular species produced worldwide in 2020 with a total of 5.8 million tons (FAO, 2022). Indonesia is one of the largest suppliers of cultivated shrimp on the global market. The Ministry of Maritime Affairs and Fisheries is targeting shrimp production of up to 1.29 million tons by 2024. The increase in Pacific whiteleg shrimp production can be met by intensive cultivation. Intensive cultivation system is characterized by high stocking density

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and additional external feed. This can cause problems in the form of decreasing environmental quality caused by organic waste from leftover feed and feces. One of efforts that can be made is to provide probiotics in feed and rearing water.

This study uses two different probiotics including Bacillus spp. bacteria and IW. The Bacillus probiotic used is a commercial probiotic according to the study by Kusmiatun et al. (2022) that reported positive impacts on growth performance of Pacific whiteleg shrimp resulted from the administration of this probiotics type. According to Zokaeifar et al. (2014), the administration of Bacillus subtilis at a dose of 108 CFU/mL in the shrimp rearing water for 8 weeks provided beneficial effects for shrimp cultivation, including improving water quality, growth, digestive enzyme activity, immune response, and resistance to disease. Apart from Bacillus, IW contain Saccharomyces sp., Lactococcus rafinolactis, and Bacillus spp. This probiotics type has never been tested on Pacific whiteleg shrimp. Generally, probiotics contain three categories, including Lactobacillus, Bifidobacteria, and some gram-positive cocci, such as the effective microorganisms (EM), Bacillus (BA), Lactobacillus (LA), photosynthetic bacteria (PB), Saccharomyces (SA), etc. The beneficial effects of these probiotics include improving growth performance, enhancing the enzymatic contribution to nutrition, inhibiting of adherence and colonization of pathogenic bacteria in the digestive tract, and increasing hematological parameters and immune response (Ringo, 2020). Therefore, the differences in the influence of the use of multi-species probiotics, especially Bacillus and IW, on the cultivation of Pacific whiteleg shrimp through the biofloc system, need to be made. It is hoped that the administration of probiotics will be an effort to improve shrimp production performance by improving water quality and increasing the feed conversion ratio.

Materials and Methods

Experimental design

The experimental design used was completely randomized design. The treatments applied consisted of six treatments, triplicates, presented in Table 1.

Table 1. Experimental design of Bacillus and IW probiotics towards Pacific whiteleg shrimp

Treatments	Description
Control	Without probiotic and biofloc
F	Biofloc
BF	*Bacillus* and biofloc
IF	IW and biofloc
B	*Bacillus*
I	IW

Experimental preparation

This experiment used 18 aquariums sizing 60 x 40 x 50 cm^3. The aquarium was washed and sterilized using potassium permanganate solution. The aeration system in the experimental tanks sourced from 3 units of LP-20 aerator. The rearing media was prepared through several steps started by the deposition of organic matter in the water, followed by disinfection using cupric sulfate, TCCA (25 ppm), and CaCO3 (10-15 ppm). The rearing media that had been prepared was distributed to all experimental tanks with a working volume of 96 L/tank.

Shrimp preparation

The shrimp used in this study were Pacific whiteleg shrimp post larvae 12 (PL 12) obtained from PT. Suri Tani Pemuka, Penyaringan, Jembrana, Bali, Indonesia. The Pacific whiteleg shrimp were acclimatized in a round pond with a diameter of 3 m and filled with water to a height of 1 m. The shrimp fed commercial feed with adjusted feeding rates four times a day. The Pacific whiteleg shrimp with an average weight 2.7-5.0 g/ind were stocked into experimental tanks with a stocking density of 40 ind/tank.

Feed preparation

Experimental feed for control and F treatments was composed by commercial feed (32% protein) and 1% binder, while experimental feed for BF, IF, B, and I treatments was composed by commercial feed (32% protein), probiotics (0.2%), and 1% binder. Probiotics used in this experiment included *Bacillus* and IW probiotics activated using a mixture of 3 g/L molasse, 3 g/L fish meal (60% protein), 1 g/L monosodium glutamate, dan water, prior to use.

Shrimp rearing

Pacific whiteleg shrimp were reared for 30 days. The supplementation of *Bacillus* and IW into the shrimp feed was carried out every 3 days. The shrimp fed experimental feed with adjusted feeding rates four times a day (08:00, 12:00, 16:00, and 20:00). The application of probiotics into the rearing media was conducted using *Bacillus* and IW probiotics (5 ppm) every week for BF and IF treatments. Biofloc technology was applied using molasses as carbon source (C/N ratio 12).

Sample collection

Sampling for growth parameters was conducted every 10 days. Sampling was performed by measuring the shrimp weight and length. The shrimp survival was monitored through daily monitoring.

Experimental parameters

- *Absolute Weight Growth*

  Absolute weight growth during the rearing period was calculated using a formula according to Effendi (1997):

$$W = W_t - W_o$$

Notes:
W = Absolute weight growth (g)
W_t = Final weight (g)
W_0 = Initial weight (g)

- *Absolute Length Growth*

  Absolute length growth during the rearing period was calculated using a formula according to Effendi (1997):

$$L = L_t - L_o$$

Notes:
L = Absolute length growth (cm)
L_t = Final length (cm)
L_0 = Initial length (cm)

- *Survival Rate*

  Survival rate (SR) is the number of population at the end of the rearing period. Survival rate is calculated using a formula referred to Effendie (1997):

$$SR = \frac{Nt}{No} \times 100\%$$

Notes:
SR = Survival rate (%)
Nt = Final population (individual)
No = Initial population (individual)

- *Feed Conversion Ratio*

  Feed conversion ratio during the rearing period was calculated using a formula by Zonneveld *et al*., (1991):

$$FCR = \frac{F}{Bt - B0}$$

Notes:
FCR = Feed conversion ratio
F = Total feed amount (g)
Bt = Final biomass (g)
Bo = Initial biomass (g)

- Water Quality

  Several parameters including temperature, pH, dissolved oxygen (DO), and salinity were measured every day (08:00 and 16:00). Temperature and DO were measured using multi-tester, while pH was measured using pH meter, and salinity was measured using a hand refractometer. Other parameters consisting of nitrite, nitrate, ammonia, alkalinity, and phosphate were measured every 10 days using test kits. Floc volume was measured with an aid of Imhoff cone.

Statistical analysis

All data obtained were tabulated using Microsoft Excel 2010. Growth performances parameters were analysed through ANOVA, followed by Tukey's test with a confidence level of 95% (*a*=0.05). Water quality parameters were presented using descriptive statistics.

Result and Discussion

Weight growth

The average initial weight of the experimental shrimp was 2.7-5.0 g/ind. After the experiment was conducted, the final weight of the shrimp ranged 12.0-19.7 g/ind. The control had the lowest final weight (12.8 ± 0.69 g), followed by BF (15.6 ± 0.5 g), I (17.0 ± 0.8 g), IF (18.3 ± 0.6 g), F (18.4 ± 0.5 g), and B (19.0 ± 0.8 g), respectively. Treatments F, IF, and B showed significant differences from the control treatment ($P<0.05$) (Figure 1).

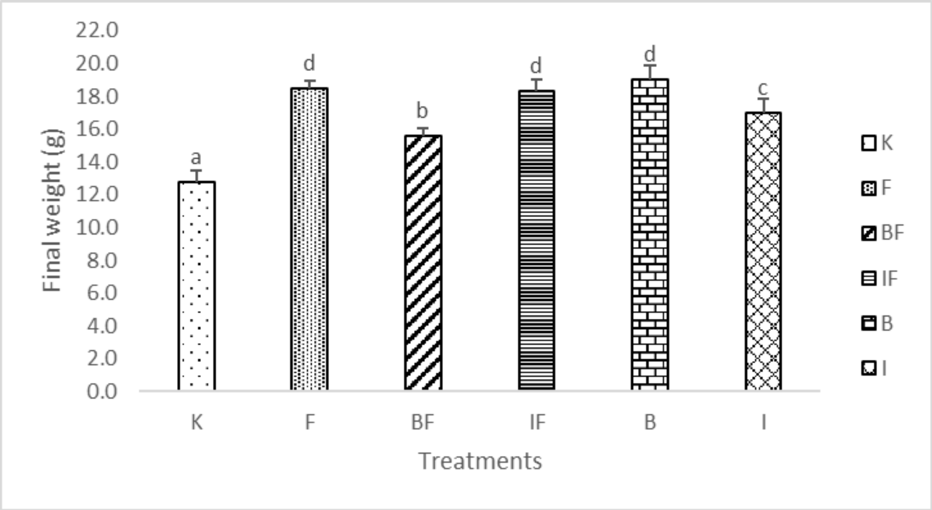

Figure 1. Final weight of Pacific whiteleg shrimp administered probiotics and reared through biofloc technology. Different letters on each bar indicated significant different results (P<0.05). Control (without probiotic and biofloc), F (Biofloc), BF (Bacillus and biofloc), IF (IW and biofloc), B (Bacillus), and I (IW).

The same results were found in the absolute weight growth of the shrimp. Treatments F, IF, and B showed higher daily growth rates than those of other treatments ($P<0.05$). Treatment F (15.7 ± 0.33 g) did not indicate a significant different result ($P>0.05$) to IF (15.1 ± 0.51 g) and B (15.4 ± 0.51 g). The lowest absolute weight growth was found in the control treatment (8.6 ± 0.69 g) (Figure 2).

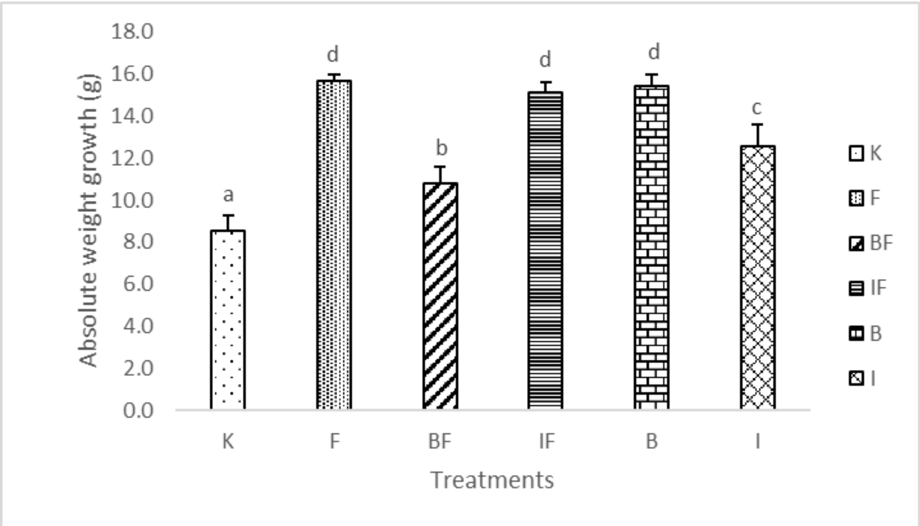

Figure 2. Absolute weight growth of Pacific whiteleg shrimp administered probiotics and reared through biofloc technology. Different letters on each bar indicated significant different results (P<0.05). Control (without probiotic and biofloc), F (Biofloc), BF (Bacillus and biofloc), IF (IW and biofloc), B (Bacillus), and I (IW).

Based on the weight growth of the experimental shrimp, the application of biofloc, the application of IW + biofloc, and the application of *Bacillus* probiotics to shrimp cultivation showed no differences among treatments. This indicated that shrimp cultivation can be carried out using the biofloc system alone or with the addition of Bacillus probiotics, or with the biofloc system + IW probiotics, because those treatments produced higher final shrimp weights compared to other treatments. However, if viewed from a cost efficiency perspective, Pacific whiteleg shrimp can be cultivated through the biofloc system alone, without the addition of *Bacillus* or IW probiotics.

The high growth in probiotic treatment is thought to be due to the presence of *Bacillus* probiotics tested in this present study. According to Gatlin & Peredo (2012), *B. subtilis*, *B. licheniformis*, *B. circulans*, and *B. clausii* are also often used as probiotics in aquaculture. *Bacillus coagulans* is an indigenous bacteria in Pacific whiteleg shrimp culture ponds. These bacteria are not pathogenic, grow well in the small intestine, can be used to increase growth rate, maintain the balance of intestinal flora, and produce several vitamins (Majeed *et al.*, 2019).

Length growth

The average initial length of the experimental shrimp was 9.0-10.3 cm/ind. After the treatments were applied, the final length of the shrimp ranged 10.8-13.5 cm/ind. The control treatment resulted in the lowest final length (11.0 ± 0.2 cm), followed by BF (12.4 ± 0.2 cm), F (12.4 ± 0.8 cm), I (12.6 ± 0.2 cm), IF (12.9 ± 0.4 cm), and B (13.3.0 ± 0.2 cm). Treatment Control showed a significant difference (P<0.05) from other treatments.

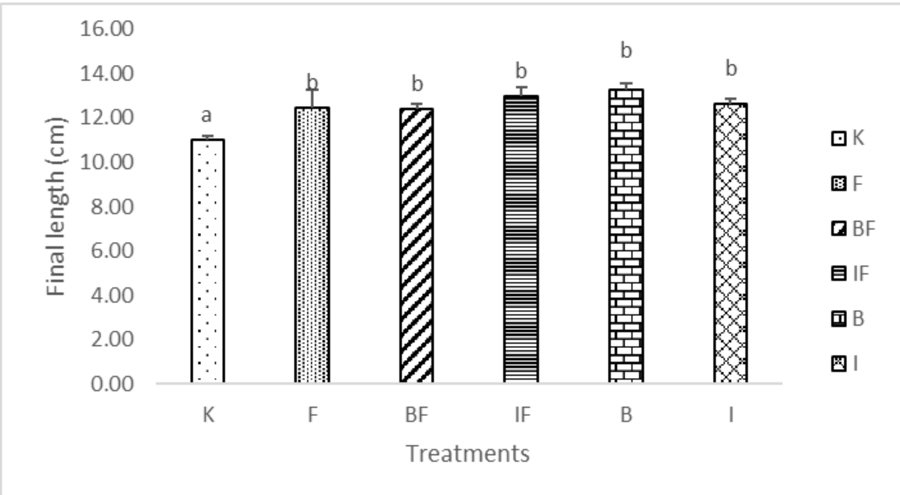

Figure 3. Final length of Pacific whiteleg shrimp administered probiotics and reared through biofloc technology. Different letters on each bar indicated significant different results (P<0.05). Control (without probiotic and biofloc), F (Biofloc), BF (Bacillus and biofloc), IF (IW and biofloc), B (Bacillus), and I (IW).

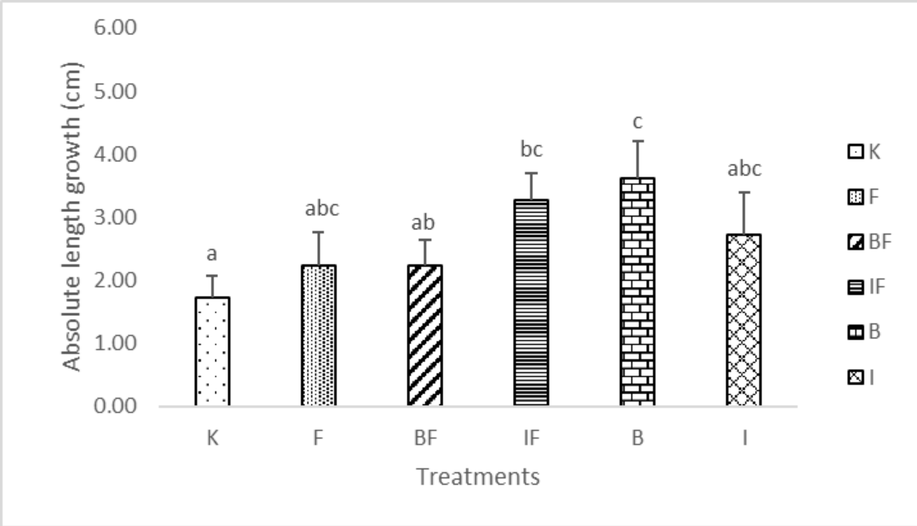

Figure 4. Absolute Length Growth of Pacific whiteleg shrimp administered probiotics and reared through biofloc technology. Different letters on each bar indicated significant different results (P<0.05). Control (without probiotic and biofloc), F (Biofloc), BF (Bacillus and biofloc), IF (IW and biofloc), B (Bacillus), I (IW).

The absolute length growth of vaname shrimp after administration of multi-species probiotics and biofloc is shown in Figure 4. Based on this figure, the absolute length growth in the control treatment gave significantly different results (P<0.05) to all other treatments. Absolute length growth in treatment B (Bacillus) was 3.61 ± 0.59 cm, indicating more significant results than other treatments. This shows that the administration of *Bacillus* probiotics through the shrimp feed has an effect on the length growth of Pacific whiteleg shrimp. *Bacillus* probiotics can increase the daily growth rate of Pacific whiteleg shrimp (Kewcharoen & Srisapoome, 2019). The addition of commercial probiotics containing *B. subtilis* and *B. licheniformis* to the feed can increase growth, feed efficiency, and immunity of Pacific whiteleg shrimp (Madani *et al.*, 2018).

Survival

One of factors in the success of shrimp farming is the high survival of the cultivated shrimp. Toledo *et al.* (2019) conducted a meta-analysis of Pacific whiteleg shrimp survival from 35 scientific articles examining the effect of probiotic treatment compared to control treatment. The results of the analysis showed that the addition of probiotics increased the survival of Pacific whiteleg shrimp. Treatments B (86.67 ± 3.8%), F (85 ± 2.5%), and IF (82.5 ± 2.5%) showed higher survival values that were significantly different (P<0.05) from control and BF (Figure 5). Based on these results, treatments with the *Bacillus* probiotics, biofloc, and a combination of IW and biofloc gave better results on the survival of Pacific whiteleg shrimp. Indariyanti & Aprilia (2022) reported that the addition of commercial probiotics containing *Lactobacillus casei* and yeast (*Saccharomyces cerevisiae*), *Bacillus* spp., and *Lactobacillus* spp. resulted in survival ranged 92-94% that was not significantly different from control (90%).

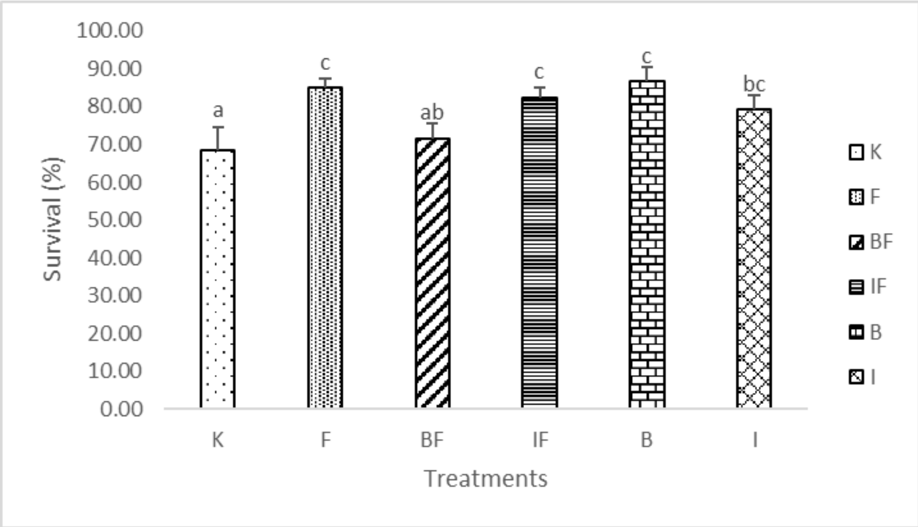

Figure 5. Survival of Pacific whiteleg shrimp administered probiotics and reared through biofloc technology. Different letters on each bar indicated significant different results (P<0.05). Control (without probiotic and biofloc), F (Biofloc), BF (Bacillus and biofloc), IF (IW and biofloc), B (Bacillus), and I (IW).

Feed conversion ratio

The feed conversion ratio is used to determine the effectiveness of feeding, shows the level of unconsumed feed, and also the profits of aquaculture business (Bachruddin *et al.*, 2018). Treatments B (1.32 ± 0.12), IF (1.33 ± 0.01), F (1.61 ± 0.08), and I (1.84 ± 0.01) showed higher feed conversion ratio values than those of control and BF The positive influence of probiotic application on the digestive health of aquatic organisms is demonstrated through increased of feed efficiency. This can be seen from the host feed conversion ratio. The smaller the feed conversion value produced indicates the more efficient use of the feed (Supono *et al.*, 2020). The study by Xie *et al.* (2019) demonstrated that the combination of *B. subtilis*, *B. licheniformis*, and *Lactobacillus* sp. probiotics improved the composition of the intestinal microbiota of Pacific whiteleg shrimp, so as to provide a low feed conversion ratio. According to Olmos *et al.* (2020), *B. subtilis* is able to increase the digestibility of the feed because this species can secrete protease, lipase, and amylase enzymes so that they play a role in feed digestion. Utilization of *B. subtilis* as probiotic bacteria in shrimp and fish culture can overcome several problems because this probiotic can increase growth and weight gain in animals by increasing feed conversion ratio as well as reduce feeding costs using high concentrations of cheaper nutrients.

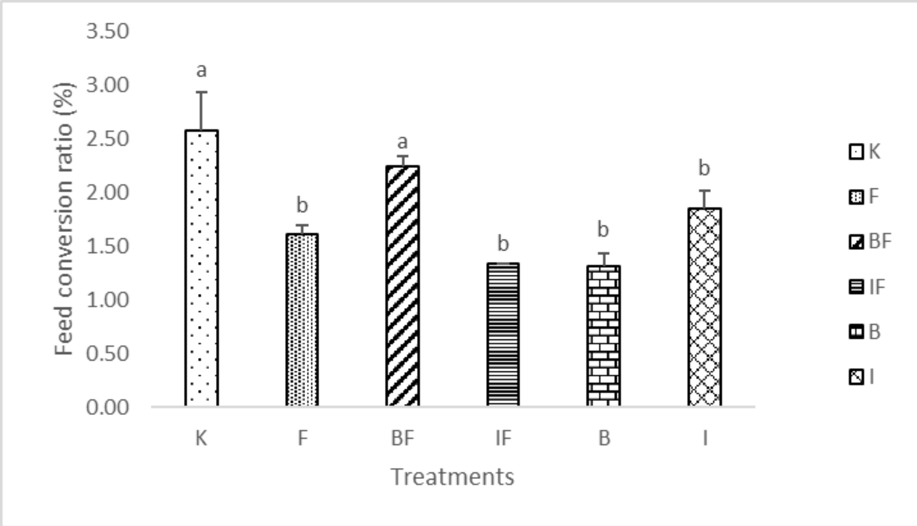

Figure 6. Feed conversion ratio of Pacific whiteleg shrimp administered probiotics and reared through biofloc technology. Different letters on each bar indicated significant different results (P<0.05). Control (without probiotic and biofloc), F (Biofloc), BF (Bacillus and biofloc), IF (IW and biofloc), B (Bacillus), and I (IW).

Water quality

There were no differences in temperature, pH, salinity, dissolved oxygen and ammonia among treatments. However, treatment I (Probiotic IW) resulted in the lowest nitrite range (0-0.2 mg/l) compared to those of other treatments that ranged 0-0.4 mg/l. Treatment F had the lowest alkalinity that ranged 80.1-210.04 mg/l. The addition of multi-species probiotics (*Bacillus* and IW) into the rearing media could not support the floc formation. This was shown by the floc volumes that were similar among treatments (Table 2).

Table 2. Water quality of the rearing media of Pacific whiteleg shrimp administered probiotics and reared through biofloc technology

Parameters	**Treatments**						**Optimum**
	K	**F**	**BF**	**IF**	**B**	**I**	
Temperature (°C)	26.1-29.2	26.2-28.9	24.4-28.4	26.2-28.2	26.1-27.9	25.9-28.3	29-32[a]
pH	7.58-7.82	7.6-8.02	7.8-8.11	7.8-7.91	7.9-7.94	7.6-8.07	7.5-8.5[a]
Salinity (ppt)	31-33	31-33	32-33	31-34	31-33	31-33	28-32[a]
Dissolved oxygen (mg/l)	6-7.2	6-7.3	6-7.8	6-7.3	6.1-7.5	6-7.5	min 4[a]
Amonia (mg/l)	0.075-0.125	0.075-0.125	0.075-0.125	0.075-0.125	0.075-0.125	0.075-0.125	Maks 0.1[b]
Nitrite (mg/l)	0-0.4	0-0.4	0-0.4	0-0.4	0-0.4	0-0.2	Maks 1[a]
Nitrate (mg/l)	0.2-1	0.2-1	0.2-1	0.2-1	0.2-1	0.2-1	Maks 0,5[c]
Phosphate (mg/l)	0-0.25	0-0.25	0-0.25	0-0.15	0-0.5	0.015-0.125	0.1-5[a]
Alkalinity (mg/l)	133.5-211.82	133.5-210.04	133.5-215.38	133.5-233.18	133.5-222.5	133.5-279.46	100-150[a]
Floc volume (ml/l)	0.2-6.5	0.1-8.0	0.1-7.0	0.2-8.0	0.2-8.5	0.2-8.5	Maks 50[d]

Sources: SNI 8118:2015[a], Anna (2013)[b], SNI 01-7246-2006[c], Avnimelech (2012)[d]

Treatment F showed the best results compared to other treatments. This is because the nitrogen cycle in treatment F run perfectly with the process of converting ammonia to nitrite which then continues to become nitrate which can be seen from the lower concentrations of nitrite and nitrate than other treatments. The ammonia concentration in the maintenance media was successfully converted into a non-toxic compound, namely nitrate. Apart from that, the success of biofloc formation could also be seen in treatment F with a floc volume of 0.1-8.5 ml/l that was higher than other treatments. The biofloc formed from organic materials left over from the feed and feces containing nitrogen, which was then converted by heterotrophic bacteria into biofloc. The floc can be eaten by shrimp and can increase shrimp growth. According to Avnimelech (2009), floc density could be distinguished by per liter of volume. Biofloc is classified as low if the volume ranges from 1-10 mg/L, classified as medium if the range is 10-20 mg/L, and high if the floc volume is > 20 mg/L, and is very low if the floc volume < 1 mg/L. In this present study, the floc volumes in all treatments were in the low categories.

Conclusion

Treatments F and B showed higher growth performances in the Pacific whiteleg shrimp, so the shrimp cultivation can be performed through biofloc system or the supplementation of Bacillus probiotics into the shrimp feed. However, if seen from cost efficiency, it is recommended to apply biofloc system in the shrimp culture. Treatment F also showed the best results in the water quality compared to other treatments. This was because the ammonia concentration in the rearing media has been successfully converted into a non-toxic compound, namely nitrate.

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