Effect of biofloc density and crude protein level in the diet on the growth performance, survival rate, and feed conversion ratio of Black Tiger Prawn (Penaeus monodon)

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Abstract. The objectives of the present study were to evaluate the best combination of biofloc density in culture media and percentage crude protein content on feed on growth performance, survival rate and feed utilization of the black tiger shrimp (Penaeus monodon) culture system. The Completely Factorial Random Design (CFRD) was used in this study. The first tested factor is the percentage of crude protein in the diet at three levels namely; 35%, 30%, 25%, and the second factor is biofloc density at in three levels namely; 5mlL⁻¹, 10mlL⁻¹, 15 ml L⁻¹. The results showed that the combination of 35% crude protein combined with 10mlL⁻¹ biofloc gave the best results for all measured parameters, with a survival rate of 100%, absolute growth rate was 17.207g, daily growth rate was 0.58g day⁻¹, specific growth rate was 3.528% day⁻¹, feed conversion ratio (FCR) was 0.974, and the feed efficiency was 102.7%. It is concluded that the best combination of crude protein and biofloc density that 10 ml L⁻¹ floc and 35% crude protein diet.

1. Introduction
Tiger prawn Penaeus monodon is one of the fishery export commodity for Indonesia. Tiger prawn businesses ever experienced heyday during period 1990 - 1995 since Indonesia is known as one of largest tiger prawn producers in the world [1], where one of the centers of tiger prawn production was in Aceh Province. Tiger prawn has several advantages, for example, rapid growth and adaptable in a wide range of salinity [2]; therefore, it is very profitable to cultivate. However, the tiger prawn business has been decline over the last two decades; this is because of the contagious of various diseases, for example, White Spots Syndrome (WSS) and Monodon Baculo Virus (MBV) which until now has not to be addressed effectively BPS. The tsunami disaster exacerbated this condition at the end of December 2004 which destroys most coastal ponds, hatchery and other utility facilities [3, 4].
Currently tiger shrimp farming in Aceh have started backstretch, this is because many aquaculture ponds have been revitalized post-tsunami disaster and the prawn price is increasing in the global market, even the second highest after the kuruma shrimp *Marsupenaeus japonicus* [5], but the prawn farmers still always worried there will be the diseases. Several approaches have been developed by scientists to prevent the diseases in cultured prawn, for example, closed water recirculation systems and best management of aquaculture practices, but these methods were not yet effective [6]. Therefore, it is very crucial to explore for other alternatives that are more effective and economical, one such alternative is by application of biofloc technology. Biofloc technology offers several advantages such as disease and water quality control [7] (Nurhatijah et al. 2016), and recycle the feces and leftover food into a microbial protein that can be utilized as a feed supplement by cultured prawns [8]. According to De Schryver and Verstraete [9], biofloc has 23-61% crude protein content, 13 % lipid and 12.6% fibre [10], fatty acids (0.04–0.07% Omega 3 and 0.7–2.0% Omega 6) [11]. Therefore, biofloc is very promising for tiger prawn culture.

The previous studies showed that penaeid shrimp could utilize biofloc as a complementary feed source [12-15]. Therefore, this technology has been successfully applied in several species of fish, for example in milkfish Chanos chanos culture [16], tilapia *Oreochomis niloticus* [17], and African catfish *Clarias gariepinus* [18], while in shrimp this technology has been applied in freshwater prawn *Macrobrachium rosenbergii* [19] and vaname shrimp *Litopenaeus vannamei* [20]. However, for tiger prawn *P. monodon* has never been evaluated.

In term of protein requirement, in general, the shrimps need the crude protein ranges from 18% to 60% [5] (Jory and Carbera), it depends on the species and their life stages. For tiger prawn *P. monodon* the protein requirement about 35-45% [21, 22], this needs will increase during gonadal maturation [23]. Hence, the objective of the present study was to determine the optimum biofloc density and crude protein levels in the diet for tiger prawn (*P. monodon*).

2. Material and Methods
2.1 Time and site
The experiment was conducted in the Center for Brackish water aquaculture, Ujung Batee, Aceh Besar District, Indonesia from June to August 2015. The shrimp postlarvae (PL-30) were purchased from this center produced by artificial breeding.

2.2 Experimental design
The completely randomized design method with two independent variables were used in this study. The first variable was crude protein (CP) level in the diet (three levels: 25%, 30%, 35%), and the second variable was floc density (FD) in the reared medium (three levels: 5 ml L⁻¹, 10 ml L⁻¹, 15 ml L⁻¹). Every treatment was performed at three replications. The tested experiment as follow: (A) 25% CP + 5 ml L⁻¹ FD, (B) 25% CP + 10 ml L⁻¹ FD, (C) = 25% CP + 15 ml L⁻¹ FD, (D) 30% CP + 5 ml L⁻¹ FD, (E) 30% CP + 10 ml L⁻¹ FD, (F) 30% CP + 15 ml L⁻¹ FD, (G) 35% CP + 5 ml L⁻¹ FD, (H) 35% CP + 10 ml L⁻¹ FD, (I) 35% CP + 15 ml L⁻¹ FD.

2.3 Feed preparation
The experimental diets were formulated using the raws materials of fishmeal, ebi shrimp meal, soybean meal, fine bran, corn bean meal, vitamins, and minerals. The composition of each diet was presented in Table 1.

Table 1. The raws materials composition of the experimental diet used in this study
| Raws materials          | Diet 1 (25% CP) | Diet 2 (30% CP) | Diet 3 (35% CP) |
|-------------------------|-----------------|-----------------|-----------------|
| Fishmeal                | 24              | 31              | 38              |
| Ebi shrimp meal         | 15              | 20              | 26              |
| Soybean meal            | 7               | 10              | 12              |
| Fine bran               | 31              | 23              | 15              |
| Corn bean meal          | 22              | 15              | 8               |
| Vitamin mix             | 1               | 1               | 1               |
| Mineral mix             | 1               | 1               | 1               |
| Total                   | 100             | 100             | 100             |

2.4 Biofloc preparation
The floc was growing in the two fiber tanks (vol. 2000 L) with water salinity ranges 20-23 ppt. Every tank was stocked with 2000 fingerling (3-5 cm) of tilapia fish *Oreochromis niloticus*. After one day, a total of 20 g or equal to 100 kg ha⁻¹ [12] of soil sediment from mangrove pond were put into the tank. The fish was fed on a commercial feed contains 38% crude protein at 5% feeding ration. The commercial feed was mixed molasses prior given to the fish. After 45 days, the floc was ready to use.

2.5 Biofloc density
The floc was added into culture media calculated based on Isnansetyo, and Kurniastuti. [31] as follow:

\[ v_1 = \frac{n_2v_2}{n_1} \]

where \( v_1 \) is floc volume which added into the culture media (ml), \( n_1 \) is floc density in the growing tank (cells L⁻¹), \( n_2 \) is floc density in cultures media (cells L⁻¹), \( v_2 \) is water volume in the culture media. The floc density in the reared media was controlled and maintained at certain level one week interval, if the floc was higher, a volume of water in the culture medium was siphoned, while when the floc density decreased, a volume of floc from the growing tank was taken and added into the culture media.

2.6 Stocking density and feeding of experiment shrimp
The postlarvae (PL-30 experimental shrimp was reared in the 27 units of the plastic basin (vol. 100 L) at a stocking density of 25 shrimp per liter. The experimental diet was fed at the feeding level of 0.03 g per day (equivalent to 100 g per 100,000 postlarvae of shrimp. The feeding level was increased by 10% every 15 days interval for 90 days of the experiment. The shrimp were fed three times a day (07.00 AM, 13.00 PM and 18.30 PM).

2.7 Measured parameters
The survival rate was calculated based on Muchlisin et al. [24, 25] as follow:

\[ SR(\%) = \frac{N_0 - N_t}{N_0} \times 100 \]

where SR is the survival rate (%), \( N_0 \) is total shrimp at the start of the experiment, \( N_t \) is total shrimp dead during the study. The weight gain was calculated based on Muchlisin et al. [26] (2017) as follow: \( W_g = \)
Wt - Wo, where Wg is weight gain (g), Wt is body weight at the end of the experiment (g), Wo is body weight the start of the experiment (g). The daily growth rate was calculated based on Muchlisin et al. [26] (2017) as follow: \( \text{DGR} = \frac{(Wt - Wo)}{t} \), where DGR is daily growth rate (g day\(^{-1}\)), and t is duration time of the experiment (day). The specific growth rate (SGR) and Feed conversion ration (FCR) were calculated based on De-Silva and Anderson [27] (1995) as follow: \( \text{SGR} = \frac{\ln(Wt) - \ln(Wo)}{t} \times 100 \), where SGR is specific growth rate (% day\(^{-1}\)). \( \text{SGR} = \frac{F}{Wt - Wo} \), where F is total feed during the experiment (g), while the feed efficiency (FE) was calculated as follow: \( \text{FE (\%) = } 100 / \text{FCR} \times 100 \). The water quality was measured and monitored daily and the data has been published by Nurhatijah et al. [7].

2.8 Data analysis

The data were subjected to two-way Analysis of Variant (two-way ANOVA) and followed by Duncan's multiple range test at 95% confidence limit.

3. Results and Discussion

The results showed that the weight gain ranges from 10.70 to 17.21 g, daily growth rate ranges from 0.36 to 0.58 d day\(^{-1}\), and specific growth rate ranges from 3.03 to 3.53 % day\(^{-1}\) (Table 2). The feed conversion ratio ranges from 0.97 to 1.23, and the survival rate was reached 100% in every treatment (Table 3).

The ANOVA test showed that floc density and protein level in the diet gave the significant effect on the growth performance (Wg, DGR and SGR) and feed utilization (FCR and FE) of tiger shrimp *P. monodon* (\( p<0.05 \)), but did not give a significant effect on the survival rate (\( p>0.05 \)). There was an interaction effect between this floc density in the cultured medium and crude protein level in the diet. The highest growth performance was recorded at shrimp feed 35% crude protein combined with 10 ml L\(^{-1}\) of biofloc; this value was significantly different from other treatments. Besides, there were significant differences in survival rate among the treatments.

| Treatment | Weigh gain (g) | Daily growth rate (g day\(^{-1}\)) | Specific growth rate (% day\(^{-1}\)) |
|-----------|---------------|-----------------------------------|-------------------------------------|
| 25 % CP + 5 ml/L BF | 10.690±0.026\(^{a}\) | 0.36±0.00\(^{a}\) | 3.028±0.003\(^{a}\) |
| 25 % CP + 10 ml/L BF | 11.067±0.012\(^{b}\) | 0.37±0.00\(^{b}\) | 3.064±0.001\(^{b}\) |
| 25 % CP + 15 ml/L BF | 11.033±0.012\(^{b}\) | 0.37±0.00\(^{b}\) | 3.060±0.001\(^{b}\) |
| 30 % CP + 5 ml/L BF | 11.527±0.035\(^{c}\) | 0.38±0.00\(^{c}\) | 3.106±0.003\(^{c}\) |
| 30 % CP + 10 ml/L BF | 13.473±0.064\(^{d}\) | 0.45±0.00\(^{d}\) | 3.270±0.005\(^{d}\) |
| 30 % CP + 15 ml/L BF | 13.427±0.012\(^{d}\) | 0.45±0.00\(^{d}\) | 3.266±0.001\(^{d}\) |
| 35 % CP + 10 ml/L BF | 17.207±0.129\(^{f}\) | 0.58±0.00\(^{f}\) | 3.528±0.008\(^{f}\) |
| 35 % CP + 15 ml/L BF | 16.017±0.015\(^{g}\) | 0.53±0.00\(^{g}\) | 3.452±0.001\(^{g}\) |

Different superscripts show different values between treatments

The study revealed that shrimp fed on the experimental diet contains 35% crude protein with the addition of 10 ml L\(^{-1}\) biofloc resulted in higher growth performance and best feed utilization. The interesting finding that at these doses, FCR can be reduced to 0.974, it means that only 0.97 kg of feed
was needed to produce 1 kg of shrimp. The floc needs for tiger shrimp is lower than that of vaname shrimp, where to produce the best growth, the density of floc was 15 ml L⁻¹ [28].

This study also showed that combination of feed contains crude protein 25% and 30% with any floc density resulted in lower growth performance and feed utilization, this is probably due to the protein requirement has not yet met the needs for tiger shrimp. According to Wickins and Lee [29] the tiger shrimp needs protein level ranges from 35% to 39%.

**Table 3.** Feed utilization and survival rate of the tiger shrimp *P. monodon* fed on experiment diet with three level of crude protein and three levels of biofloc density for 90days

| Treatment                  | Feed utilization | Survival rate (%) |
|----------------------------|------------------|-------------------|
|                            | Feed Conversion Ratio | Feed Efficiency (%) |                 |
| 25 % CP + 5 ml/L BF        | 1.229±0.005 b     | 81.36±0.30 a      | 100±0.00 a      |
| 25 % CP + 10 ml/L BF       | 1.222±0.002 h     | 81.85±0.12 a      | 100±0.00 a      |
| 25 % CP + 15 ml/L BF       | 1.193±0.000 c     | 83.81±0.01 b      | 100±0.00 a      |
| 30 % CP + 5 ml/L BF        | 1.155±0.004 d     | 86.61±0.27 d      | 100±0.00 a      |
| 30 % CP + 10 ml/L BF       | 1.097±0.005 e     | 91.12±0.44 c      | 100±0.00 a      |
| 30 % CP + 15 ml/L BF       | 1.164±0.003 f     | 85.87±0.22 c      | 100±0.00 a      |
| 35 % CP + 5 ml/L BF        | 1.040±0.010 g     | 96.12±0.96 e      | 100±0.00 a      |
| 35 % CP + 10 ml/L BF       | 0.974±0.005 h     | 102.7±0.55 b      | 100±0.00 a      |
| 35 % CP + 15 ml/L BF       | 1.019±0.001 i     | 98.17±0.09 f      | 100±0.00 a      |

Different superscripts show different values between treatments.

4. Conclusion

It is concluded that the flock density and protein level gave a significant effect on the growth performance and feed utilization, but did not give a significant effect on the survival rate. The growth performance and feed utilization were recorded at shrimp fed experimental diet contains 35% crude protein, and floc density of 10 ml L⁻¹, this treatment gave the best feed conversion ratio of 0.97.

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