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Determination of stocking density on vannamei (*Litopenaeus vannamei*) fingerling with biofloc technology for improving growth and feed efficiency

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APPROVAL OF RESEARCH PROPOSAL

1.	Tittle	:	Determination of stocking density on vannamei (*Litopenaeus vannamei*) fingerling with biofloc technology for improving growth and feed efficiency
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4.	Period	:	9 months
5.	Research cost proposed	:	

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1. INTRODUCTION

1.1 Background

Culture of vannamei shrimp *Litopenaeus vannamei* has received a great attention in terms of economic benefits, for which this requires superior quality and sufficient quantity of the juvenile for grow-out culture. Currently, marine resources are intensively researched for marine culture activities using floating net cages. This attempt also resolves the shrinkage of land space for the farming. Shrimp culture using floating net cages is applicable and requires high quality seeds, locally known as *benur*, which are resistant to stressors. To meet the seed demand, seedling stages need to be perfectly prepared, using intensive culture system. However, seedling phase with high stocking density is undesirable, requiring more feed. In fact, some of the feeds often remains uneaten, becoming debris; while some is consumed and converted into biomass and excreted as ammonia and feces. The excreta and debris are deposited in culture medium, which provokes increment of nitrogen concentration as represented by ammonia which is toxic to the shrimps (Avnimelech dan Ritvo, 2003). Significant attempts to reduce ammonia are inevitable, including water exchange. However, it requires enormous quantity of water and potentially pollute environment when the wastewater is untreated. Other attempt is biofloc culture system, enabling to manage water quality in intensive aquaculture.

To date, biofloc technology (BFT) has occurred as an outstanding ecofriendly technology capable of minimizing the sewages from culture activities (Avnimelech, 2006; Avnimelech, 2007). As an exceptionally ecofriendly technology, BFT is based on assimilation of inorganic nitrogen (ammonia, nitrite, and nitrate) promoted by microbes (heterotroph bacteria) in culture medium as their nutrition source (De Schryver *et al*., 2008). Biofloc is a suspension in water, which is present as phytoplankton, bacteria, viable aggregrate, organic materials and bacteria eater (Avnimelech, 2007). Biofloc technology is developed to improve and control water quality in culture unit, biosecurity, promote efficient use of water and feed (Avnimelech, 2012). This microbial floc contains protein (19,0-40,6%), fat (0,46-11,6%) and ash (7-38,5%), being a source of nutrients for cultured species (Tacon, 2000; Ekasari, 2008). Avnimelech (1999) stated that bioflock system contained heterotroph bacteria forming flocs that could be utilized by aquatic species; thus, this is proven to reduce inorganic nitrogen and replace feed protein. The shrimp feed often contains more protein than carbohydrate, for which carbon supply from feed is low, with C/N ratio of 9:1. On the other hand, bacteria need 20 carbon for 1 nitrogen assimilated (C/N = 20:1). The low C/N ratio in feed adversely affect the growth of heterotroph bacteria. Therefore, in

intensive fish farming, organic carbon is added to maintain C/N ratio of 20-30 (McIntosh, 2000; Brune et al., 2003). • The use of biofloc culture system undeniably needs to consider a stocking density in order to produce optimum result, since this novel aquaculture technology enables to control harmful nitrogenous substances produced in the culture pond. Principally, the intensive fish culture shall optimize C/N ratio (McIntosh, 2000; Brune et al., 2003). Therefore, stocking density and the quantity of discharged components in the culture system are crucial for performance of biofloc system. In this regard, additional carbon can be easily added through various sources. A aquaculture system with high density produces more discharged components, which forms more abundant floc capable ot acting as source of feed. This leads to a higher feed conversion ratio and a lower feed cost.

1.2 Research Purposes

This research aimed to determine a stocking density of vannamei shrimp in fingerling with biofloc system to optimize growth and feed supply.

1.3 Research Output

The research is designed to obtain following outputs:

1. The output of research is the optimum level of stocking density for shrimp culture under biofloc technology.
2. A research publication in either reputable national journal or Scopus-indexed journa.

2. BENEFIT AND IMPORTANCE OF RESEARCH

Performance of biofloc system relies on its ability to control water quality or provision of extra feed sources for microorganism. To ensure the technology works efficiently, it is important to manage the composition of nitrogen and carbon as source of main nutrients in the biofloc system. In this work, carbon source originates from shrimp disposal waste, which is cheap and abundant. Considering that production of waste linearly relates to density of shrimp, there is a need to find the best level of shrimp density in biofloc system.

3. METHODS

3.1 Preparation of Culture Unit and Biofloc

Shrimp rearing was carried out in aquarium (100 × 60 × 80 cm) sterilized using chlorine (100 ppm) and rinsed with clean water. It was then filled with water up to height of 40 cm (equal to 72 L) and aerated at 5 spots. Inoculated bacteria EM4 at dose of 0.16 ml/Aquaria and ammonium sulfat at dose of 4 gram / akuaria. To increase carbon concentration, molase was added directly to the aquarium (once per day, after 2 h of feeding) at 10.00 am. Quantification of carbon which was required for prompting floc formation by heterotroph bacteria followed previous equation by De Schryver *et al*. (2008).

3.2. Shrimp Rearing

Vannamei PL16 (average weight of 0,03±0,04 g/shrimp and size of 1,60±1,69 cm/shrimp) were reared at density of 458 shrimp/m^2 (equal to 110 shrimp/aquarium) for 28 days. They were fed 3 times per day (08.00, 12.00, and 16.00 WIB) at feeding rate of 25%. Experiment followed completely randomized design consisting of 4 treatment groups (A = control; B = C/N ratio 10; C = C/N ratio 20; D = C/N ratio 30) with 3 replications. Commercial feed containing protein of 40% was applied (Fengli – Matahari Sakti). Position of rearing containers was randomly distributed.

Treatement A = stocking density 56 shrimp/ aquarium

Treatement B = stocking density 68 shrimp/ aquarium

Treatement C = stocking density 80 shrimp/ aquarium

Treatment A is control with stocking density 56 shrimp/ aquarium. Treatment A referred to shrimp culture in absence of molase as external carbon source. Water was syphoned 3 times per day in control groups. In treated groups, shrimp were fed with feed containing 40% of protein and GE (Gross Energy) was defined as followsL 1 g protein = 5,6 kcal GE, 1 g fat = 9,4 kcal GE, 1 g carbohydrate/NFE = 4,1 kkal GE (Watanabe, 1988).

3.3. Research Parameters and Data Analysis

Growth of shrimp (weight and length) was observed 2 weeks (per 14 days), and viable shrimp was totally counted at the end of experiment. Weight and length of the shrimp was key indicator for determining amount of daily feeding, regarding the survival rate. Several research parameters were collected as follows: floc volume, absolute growth, feed

efficiency, survival rate, FCR. Chemical analysis was also carried out during 30-day experiment.

3.3.1 Floc Volume

Floc volume (FV) represented the density of floc particles in water as described by Avnimelech (2012). Water sample (50 mL) was filled to a 50 mL-conical cone and left for 30 min to allow floc accumulating on the bottom of the cone. FV (mL/L) was determined as follows:

$$FV = \frac{\text{floc volume}}{\text{water volume}} \times 1000$$

3.3.2. Relative Growth

Relative growth (RG) presented biomass gain of shrimp during experiment. The calculation followed the equation below (Acarli & Lok, 2008):

$$\text{PR} = \frac{\text{lnLt} - \text{lnL0}}{\text{t}}$$

where

RG : relative growth (%)

Lt : average length at the end of experiment (cm)

Lo : average length at the initial of experiment (cm)

T : period of experiment (day)

3.3.3. Feed Efficiency

Feed efficiency (FE) constituted a comparison between shrimp biomass and feed applied during experiment. FE was determined as follows (Takeuchi, 1988):

$$\text{E} = \frac{(\text{wt} + \text{wd}) - \text{w0}}{\text{F}} \text{x } 100$$

where:

EP : Feed efficiency (%)

F : total amount of feed (g)

Wt : final shrimp weight (g)

Wo : initial shrimp weight (g)

Wd : total weight of died shrimp (g)

3.3.4. Survival Rate

Survival rate (SR) indicated percentage of shrimp harvested compared with initial stock, determined using formula by Goddard (1996) as follows:

$$SR = \frac{N_t}{N_0} \times 100$$

where:

SR : survival rate (%)

N_t : number of shrimps harvested

N_o : number of shrimps stocked

3.3.5. Proximate Analysis

Proximate analysis included crude protein, crude fat, ash and water content, carried out at the initial and end of experiment. Shrimp (20 g) in each experimental unit was used for proximate analysis.

3.3.6. Water Quality Measurement

Water quality parameters were observed, i.e. dissolved oxygen (DO), pH, temperature, ammonia (NH_3), nitrate, nitrite, salinity and phosphate. Daily observed parameters included DO, pH, salinity, measured using DO meter, pH meter and refractometer, respectively. Meanwhile, other parameters (ammonia, nitrate, nitrite) were measured two times: initial and end of experiment

3.3.7. Data Analysis

Experimental design was arranged according to completely randomized design (3 treatments, 3 replications). Data were tabulated in Microsoft Excel 2013 and statistically evaluated in SPSS 22.0. To check the effect of treatment on each research parameter, independent sample t-test (p=0.05) was applied using SPSS 16.0.

4. RESULTS AND DISCUSSION

4.1 Results

Growth of shrimp reared for 30 day in the biofloc system is presented in Figure 1. Treatment A (56 shrimp / aquarium) resulted in better result compared with treatment B (68 shrimp / aquarium) and C (68 shrimp / aquarium). As depicted in Figure 1, fish growth differed greatly between treatments, i.e. 74.50%, 70.99%, 68.06%, respectively.

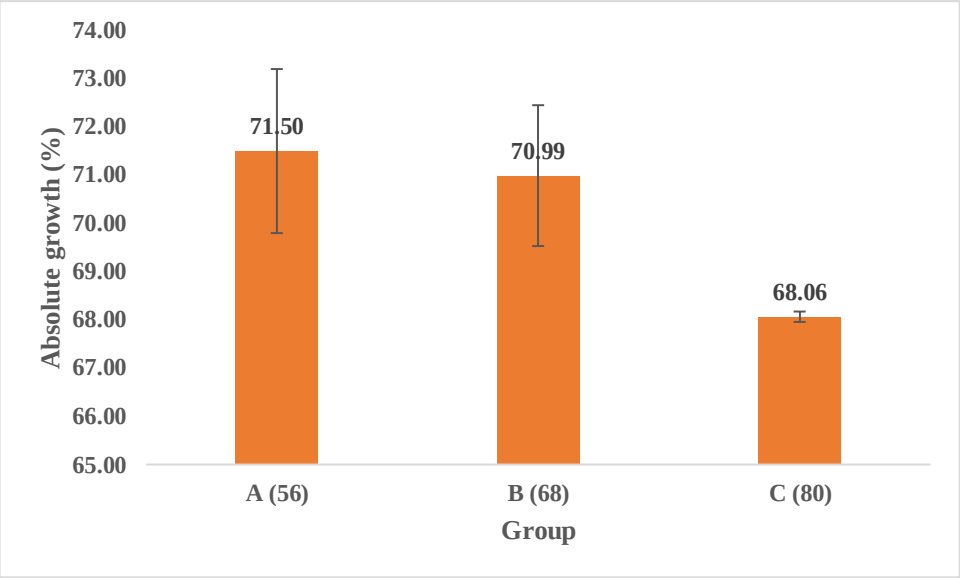

Figure 1. Absolute growth of vannamei between groups

Table 1. Feed consumption (Σ feed), FCR, EP, and SR of vannamei (*Litopennaeus vannamei*) for 30 days of rearing.

Group	Σ feed (g)	FCR	EP (%)	SR (%)
A	310.83±2.62	1.12±0.12	89.38±7.83	83.93±4.72
B	371.54±8.77	1.14±0.07	88.14±5.12	72.55±7.25
C	426.87±4.90	1.27±0.01	78.72±0.79	74.17±14.05

Note
1. A = 56 shrimp; B = 68 shrimp; C = 80 shrimp;
2. FCR: *feed convention ratio;* EP*: feed efficiency*; SR : *Survival Rate*;
3. Different superscripts in similar column represent significant difference at P<0.05.

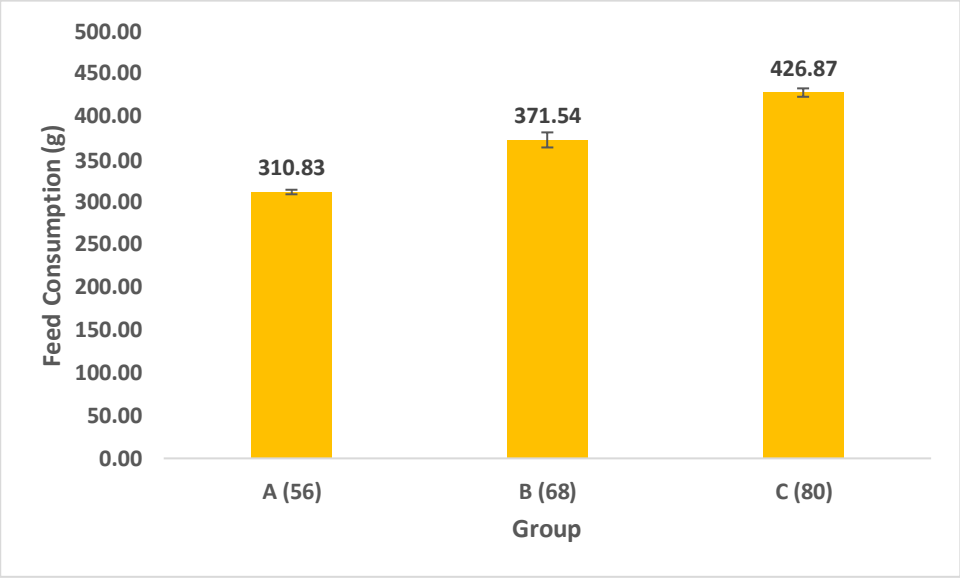

Figure 2. Feed consumption of shrimp between groups

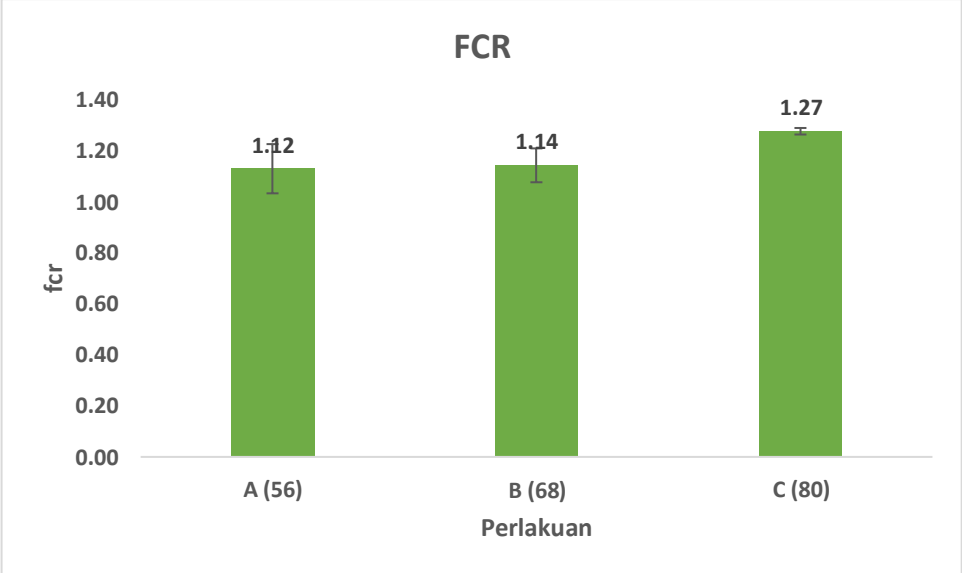

Figure 3. FCR of shrimp between groups

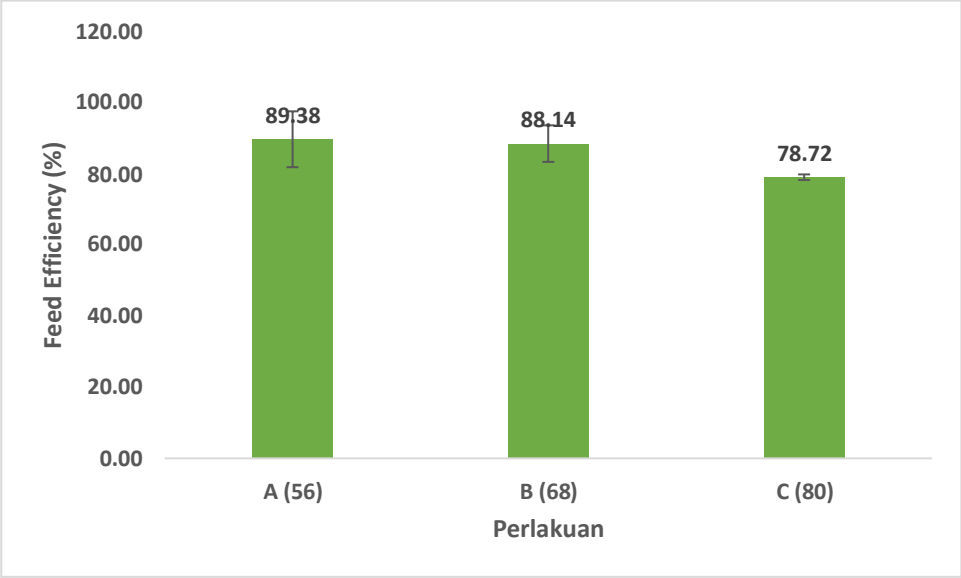

Figure 4. Feed efficiency of shrimp between groups

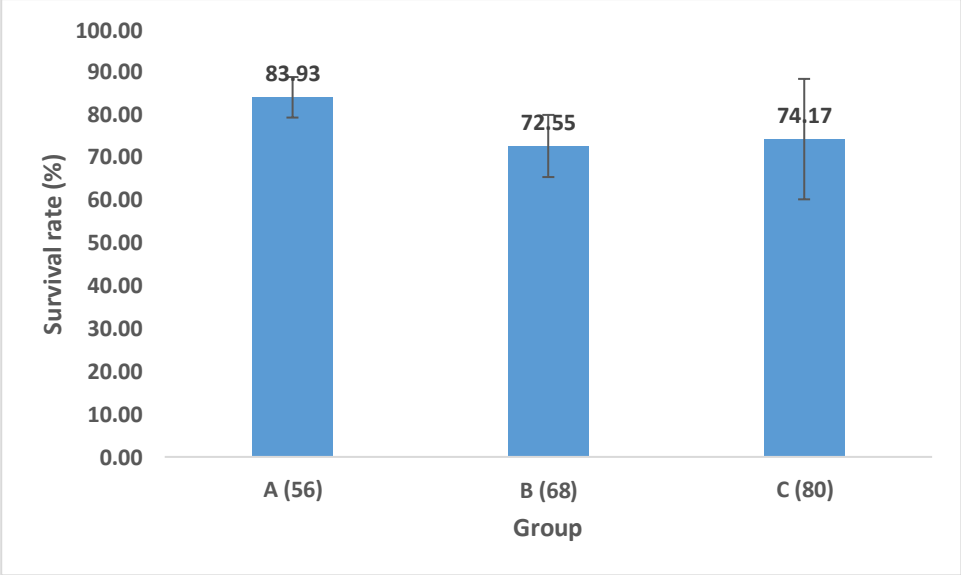

Figure 5. Survival Rate (SR) of shrimp between groups

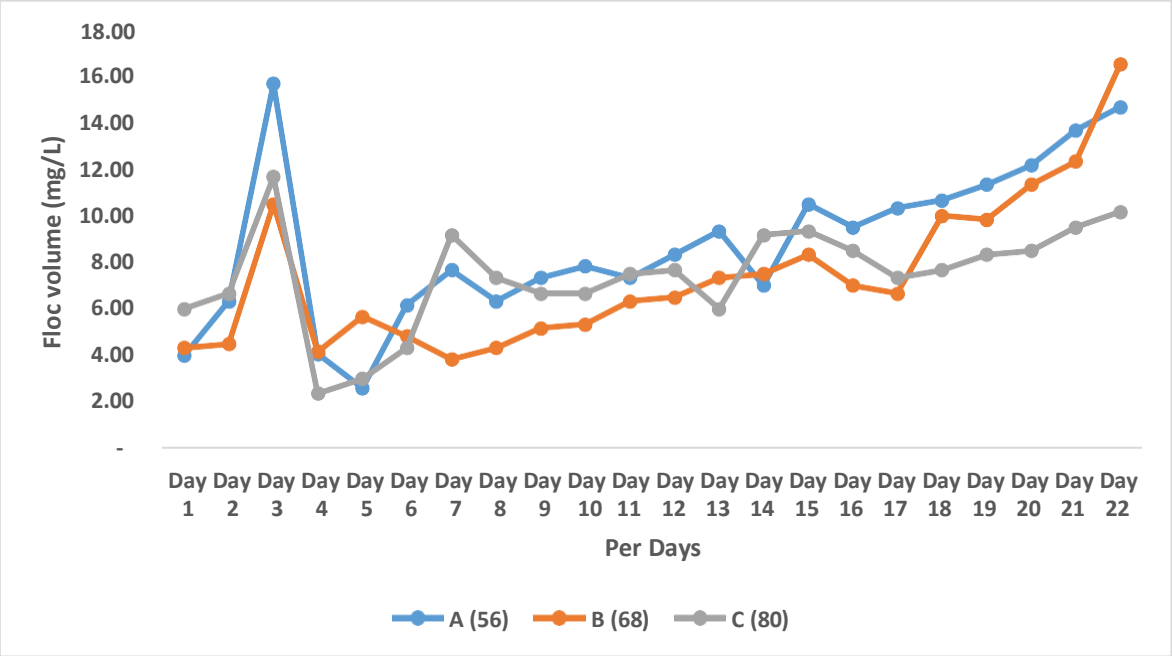

Figure 6. Floc volume between groups

The highest feed consumption occurs in group C, reaching 426.87 g, while the lowest is attributed to group A reaching 310.83 g. The greatest FCR is found in group C (1.27). The highest feed efficiency is found in group A (89.38%), meanwhile the lowest is found in group C (78.72%). In terms of survival rate, group A produces the highest value (83.93%), but group B produces the lowest (72.55%), as described in Figure 2-5. Figure 6 exhibits floc volume. The volume increases gradually, in which group A reaches the highest value. Table 2 presents water quality recorded during 30 days of experiment. The result suggests that water quality in the biofloc system complies with standard level for shrimp farming. Several parameters, e.g. TAN, nitrite, nitrate, are above the recommended level (Table 2).

Table 2. Water quality parameters

No	Variables	Group			Acceptable value
		A	B	C	
1	Temperature (℃)	25.4-26.7	24.7-25.3	23.2-24.5	25-32
2	DO (mg/L)	6.23-7.87	6.73-7.73	6.43-7.70	5-7
3	pH	5.70-7.23	5.70-7.23	5.10-7.17	7-9
4	TAN (mg/L)	1.5 -3	1.5-5	1.5-5	<1
5	Nitrate (mg/L)	0-100	12.5-100	0-100	<1
6	Nitrite (mg/L)	<0.3-33	0.3-33	<0.3-33	<1
7	Salinity (ppm)	30	30	30	25-35

4.2 Discussion

Despite no significant difference on growth parameter between treatments, the absolute value for this parameter showed a negative correlation between growth and stocking density. Liu *et al.* (2017) reported a negative correlation between growth and density, which resulted in 40% reduction of growth. In addition, Schveitzer *et al*. (2013) applied combination of density and substrate, showing that stocking dendity did not affect growth performance. Based on previous works, there is a possibility that growth performance in biofloc system depends on supporting factors of the system such as water quality paramters (e.g. ammonia, nitrite, and nitrate). Similarly, feed conversion did not differ significantly among groups Although there is no significant difference, the absolute value showed that higher level of stocking density would reduce feed efficiency.

In addition, survival rate was also not different significantly between treatments. However, we can observe that the highest survival rate corresponded to the lowest stocking density, with group B and C reaching up to 72-74%. This suggests that data are not sufficient to reveal the effects of stocking density on survival rate. In this case, the survival rate is in the acceptable range. The density used is at tolerable level for shrimp; and no mortality is found due to poor water quality.

Meanwhile, floc volume increases drastically at day 8 – 11. After this, it decreases and increases again till day 29. Such increase in day 8 to day 11 was affected by addition of ammonium sulfate which enables to accelerate formulation of floc. Despite decreased, the production of floc increases remarkably as more concentration of ammonia nitrite and nitrate. The data also revealed that formation of floc increases gradually as more nitrogen

was produced in culture system. However, difference in floc volume is not observed between treatments. This means that the system can still perform at higher density.

The experiment concludes that biofloc system can facilitate shrimp culture in aquarium with density up to 144 shrimp/m^2. Generally, most shrimp farmers applied density of 80-200 shrimp/m^2, which suggests that the current technique is at good range in Indonesia.

5. CONCLUSION AND SUGGESTION

5.1 Conclusion

The research revealed that biofloc technology in aquarium can be filled with shrimp at density up to 144 shrimp/m^2. This conforms with farmer's practice in Indonesia using density of 80-200 shrimp/m^2, suggesting that the biofloc system is applicable with shrimp farming in Indonesia.

5.2 Suggestion

The research future can determination ratio C/N for biofloc technology.

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APPENDIX

Gambar 7. Wadah pemeliharaan

Gambar 8. Proses pengisian air laut

Gambar 9. Tandon air laut

ANALISA STATISTIK

Biomasa Akhir

Table Analyzed	Final Biomass					
Data sets analyzed	A-C					
ANOVA summary						
F	0.7428					
P value	0.515					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					
R squared	0.1985					
Brown-Forsythe test						
F (DFn	DFd)	1.632 (2	6)			
P value	0.2717					
P value summary	ns					
Are SDs significantly different (P < 0.05)?	No					
Bartlett's test						
Bartlett's statistic (corrected)						
P value						
P value summary						
Are SDs significantly different (P < 0.05)?						
ANOVA table	SS	DF	MS	F (DFn	DFd)	P value
Treatment (between columns)	2752	2	1376	F (2	6) = 0.7428	P=0.5150
Residual (within columns)	11114	6	1852			
Total	13866	8				
Data summary						
Number of treatments (columns)	3					
Number of values (total)	9					

Pertumbuhan

Table Analyzed	Growth					
Data sets analyzed	A-C					
ANOVA summary						
F	5.051					
P value	0.0517					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					
R squared	0.6274					
Brown-Forsythe test						
F (DFn DFd)		1.232 (2	6)			
P value	0.3563					
P value summary	ns					
Are SDs significantly different (P < 0.05)?	No					
Bartlett's test						
Bartlett's statistic (corrected)						
P value						
P value summary						
Are SDs significantly different (P < 0.05)?						
ANOVA table	SS	DF	MS	F (DFn	DFd)	P value
Treatment (between columns)	2564	2	1282	F (2	6) = 5.051	P=0.0517
Residual (within columns)	1523	6	253.9			
Total	4088	8				
Data summary						
Number of treatments (columns)	3					
Number of values (total)	9					

FCR

Table Analyzed	FCR					
Data sets analyzed	A-C					
ANOVA summary						
F	0.02792					
P value	0.9726					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					
R squared	0.009222					
Brown-Forsythe test						
F (DFn DFd)		0.1174 (2 6)				
P value	0.8912					
P value summary	ns					
Are SDs significantly different (P < 0.05)?	No					
Bartlett's test						
Bartlett's statistic (corrected)						
P value						
P value summary						
Are SDs significantly different (P < 0.05)?						
ANOVA table	SS	DF	MS	F (DFn	DFd)	P value
Treatment (between columns)	0.000356	2	0.000178	F (2	6) = 0.02792	P=0.9726
Residual (within columns)	0.0382	6	0.006367			
Total	0.03856	8				
Data summary						
Number of treatments (columns)	3					
Number of values (total)	9					

SR

Table Analyzed	SR					
Data sets analyzed	A-C					
ANOVA summary						
F	1.253					
P value	0.3511					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					
R squared	0.2945					
Brown-Forsythe test						
F (DFn DFd)	0.6611 (2 6)					
P value	0.5502					
P value summary	ns					
Are SDs significantly different (P < 0.05)?	No					
Bartlett's test						
Bartlett's statistic (corrected)						
P value						
P value summary						
Are SDs significantly different (P < 0.05)?						
ANOVA table	SS	DF	MS	F (DFn	DFd)	P value
Treatment (between columns)	227.4	2	113.7	F (2	6) = 1.253	P=0.3511
Residual (within columns)	544.7	6	90.78			
Total	772.1	8				
Data summary						
Number of treatments (columns)	3					
Number of values (total)	9					

Volume Flok

Table Analyzed	Final Flock Volume					
Data sets analyzed	A-C					
ANOVA summary						
F	2.385					
P value	0.1729					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					
R squared	0.4429					
Brown-Forsythe test						
F (DFn	DFd)	0.7406 (2	6)			
P value	0.5159					
P value summary	ns					
Are SDs significantly different (P < 0.05)?	No					
Bartlett's test						
Bartlett's statistic (corrected)						
P value						
P value summary						
Are SDs significantly different (P < 0.05)?						
ANOVA table	SS	DF	MS	F (DFn	DFd)	P value
Treatment (between columns)	65.72	2	32.86	F (2	6) = 2.385	P=0.1729
Residual (within columns)	82.67	6	13.78			
Total	148.4	8				
Data summary						
Number of treatments (columns)	3					
Number of values (total)	9					