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Assembling the superior biofloc tilapia strains for industry

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Abstract

The decreasing productivity and increasing environmental pressure on intensive tilapia culture in open waters, especially in floating net cages, have forced the development of a biofloc system for tilapia farming. This study aimed to develop the superior tilapia strain suitable for the biofloc system and to observe their productivity and economic profitability under commercial scale in the biofloc system. The selective breeding activity was carried out on 100 families reared and mated in hapa (a double-walled contrivance in which an inner hapa, made of mosquito netting material, is installed within an outer one made of whole cloth) in the biofloc pond for three generations. The individual selection was conducted at the age of 6 months on the characters of standard length and body weight with the best 10% of the top part of the population as a reference for the formation of the next generation. After three generations, the offspring obtained from mass spawning were grown out in round-limen ponds and square-concrete ponds for productivity and profitability observations. The results showed that heritability values of the biofloc tilapia strain were 0.4196 (standard length, SL) and 0.4898 (body weight, BW). The average response selection of BW and SL was 13.04% and 6.11%, respectively. Biomass gain was obtained between 19.29–28.83 kg/m^3. The production multiply value was 8.07-13.59x. The average daily weight growth was between 2.8g-3.0g. The Benefit-Cost ratio is valued from 1.81 to 2.71. The superior tilapia biofloc strain has a moderate heritability value. When it is raised in a biofloc ponds system at different volumes, it significantly affects the productivity on biomass, body weight, and multiplication value. Also, it is advantageous when applied on an industrial scale.

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Introduction

The decreasing productivity level and the increasingly heavier environmental pressure on the intensive tilapia culture, especially the floating net cages system (KJA) tilapia cultivation in open waters, in Cirata reservoir-Indonesia has happened (Nugroho et al*.* 2013). This has made one of the driving forces for the development of more controlled tilapia aquaculture technology such as the biofloc system (BFT) (Nugroho et al*.* 2020).

BFT has several advantages, including maintaining or cultivating fish with minimal water and land requirements, sometimes without changing water and a high level of feed efficiency (Avnimelech, 2012). As a new technology in Bangladesh, BFT is believed to have technical and economic advantages (Shamsuddin et al, 2022). The balance that occurs between beneficial bacteria, feed, and carbon supply and supported by strong aeration keeps water quality conditions good, and a flock composed of several organic materials, plankton, and bacteria can be used by fish as feed (Emerenciano at al*,* 2013).

Several research activities related to biofloc technology include Kumari et al (2021); Das and Mondal (2018); Haridas et al. (2017); Emerenciano et al. (2013); Crab et al. (2012); and Avnimelech (2009). Meanwhile, in Indonesia, research on biofloc has also been carried out including catfish (Hermawan et al. 2014; Puspita & Sari, 2018), tilapia (Widanarni et al. 2012), and shrimp (Nurhatijah et al. 2016). The productivity level of BFT in tilapia is relatively high. Rackocy et al*.* (2004) obtained the yield of tilapia between 13.7-14.4 kg/m^3. Meanwhile, Luo et al. (2017) found that tilapia productivity levels ranged from 32.60-35.83 kg/m^3. Nugroho et al. (2020) found that tilapia cultivation productivity with the BFT system was around 10.8-14.42 kg/m^3. However, the productivity of tilapia culture in the KJA system in the Jatiluhur reservoir ranges from 1.09-1.62 kg/m^3 (Nugroho et al*.* 2013).

Never less the success of cultivating tilapia with the biofloc system in Indonesia with existing productivity levels, this condition can be improved by using superior seeds specifically produced for aquaculture environments with a biofloc system. Several studies on the use of superior seeds that have been carried out in increasing cultivation productivity in Indonesia include tilapia (Nugroho et al. 2013) and catfish (Nugroho and Haryadi, 2017). While in Europe, the use of superior seeds in fish farming has successfully contributed to salmon farming implemented in 2012 (Jannsen et al. 2017).

Up to now, superior varieties of tilapia specifically for biofloc have not been obtained and used in cultivation in Indonesia. In addition, the biofloc system of tilapia aquaculture is still under development and optimization on a real-scale business in the field (Nugroho et al. 2021). This condition causes important information related to selection activities, such as heritability and productivity performance, and economic profitability, which are rarely found. The objective of this study is to obtain information about the assembly of superior seeds and the effect of their use in the cultivation of tilapia with the biofloc system on productivity and economic profitability values at the real business-scale cultivation level.

Materials and Methods

Time and location

The breeding programs of biofloc tilapia varieties were carried out during 2018-2022 at the Indohatchery pond in Cianjur, Indonesia. Fish were placed in hapa for naturally spawning, while nursery and grow out were carried out in a pond with a biofloc system.

Selection populations

The fish used as a population for breeding activities were tilapia variety which was produced by individual selection method of a synthetic population which constructed from six superior varieties of tilapia in Indonesia, namely Nirwana, Best, Nilasa, Pandu, Chitralada, and Sultana (Nugroho, 2018). The formation of the population for selective activity was carried out by random mating of 100 males and 100 females fish in pairs in hapa sized of 2x1x1 m with the proportion of 1 male and 1 female per hapa. The harvesting of larvae was conducted per batch with an interval of 4 days. The number of larvae collected was 500 individuals (from the parent with the number of larvae more than 800 individuals). Each hapa was pooled from the same batch.

Larvae were reared in a concrete pond with a size of 100 m^3 with a density of 300 individuals/m^3 using a biofloc system. Larvae were fed commercial pellets with 42% protein content of 10-20% biomass per day for the first 2 weeks, then continued with commercial pellets with 32% protein content for up to 3 months. Furthermore, the seeds produced were maintained in a biofloc-pond with a capacity of 25 m^3 with a density of 100 individuals/m^3 for 3 months. Fish were fed commercial feed with a protein content of 28% as much as 5-10% of weight per day and a frequency of 3 times a day until they reached a body weight of > 200 g.

The morphometric characters used as the basis for selection were weight and standard length when the fish age was six months old. The selection of broodstock was carried out using the best 10% reference limit based on the fish morphometric characters. The selected parent fish were reared until matured gonads in the biofloc system. The parents were then spawned in pairs as previously described to form the next population. After three generations, the offspring was obtained by mass spawning with the ratio of male and female parents 1:3. The offspring was carried out at the enlargement stage using four types of ponds, namely a round-limen pond with a volume of 10 and 25 m^3 and a square-concrete pond with a volume of 50 and 100 m^3.

Biofloc system

The Biofloc system used to refer to the Avnimelech method (2009). A modification was done by making preparations in the form of adding bacteria. The bacteria used in the prepping process were a commercial probiotic containing of *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus polimyxa*, which each of bacteria has 5 x 10^9 colony forming unit (CFU). Keeping the water quality of the biofloc system in the best condition by adding the *Bacillu*s sp is very important.

The floc condition was maintained by adding a carbon source (C) in the form of molasses with a concentration of C-N ratio higher than 15 (Crab et al. 2012). The biofloc system uses the circular ponds with volume of 10 and 25 m^3 equipped with an aeration system which was designed to the volume of the needed pond. The pond arrangements are regulated based on the available area and supported by the use of an air pump machine to increase the dissolved oxygen content in the water. Water exchange for each pond was carried out 4 times during the cultivation period. The addition of water by 10% by volume in the growing up stage was carried out on the 20th day; 40th; 60th; and 80th. It was based on the contained amount of floc (solids) in each liter of water measured using an *Imhoff* cone. During the cultivation period, no other materials were added to the pond because the system was running as expected. Sunlight and rainfall are not considered as things that can influence this biofloc system, so that there is no requirement for barriers such as paranet or fiber and others.

Monitoring and evaluation

Monitoring and evaluation of selected products were carried out at the growing up stages using four types of ponds, namely a rounded-linen pond with a volume of 10 and 25 m^3 and a square-concrete pond with a volume of 50 and 100 m^3. The seeds were maintained at a density of 100 fish/m^3 for 3 months. Fish were given commercial feed containing 32% protein, as much as 5-10% with a frequency of 3 times per day. An evaluation was carried out on morphometric characters and productivity. The treatments were four different ponds each of treatment had 4 replicate. Morphometric characters consist of parameters of body weight (BW), and standard length (SL). Productivity characters consist of parameters that are considered to have an effect on economic value, namely feed efficiency (FE), harvest biomass (HB), production multiples (M), the survival rate (SR), and daily weight gain (ADG).

Statistical analysis

The difference of measured parameters such as feed efficiency, biomass, survival rate and weight gain was analyzed with analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) with $\alpha = 0.05$ was used to determine the significance by using The SPSS software (version 16.0). Economic profitability values were also calculated. Monitoring of water quality and the total suspended matter volume was carried out every week.

Data analysis

Heritability was calculated based on the three parameters of body weight, and standard length with the following formula (Kirpichnikov, 1981; Tave,1993; Falconer, 1989):

Heritability (h^2)

$h^2 = R_s / D_S$

h^2 = Heritability value

R_s = Respons selection

D_s = Differential selection

The following equations are used to calculate the performance, productivity of a tilapia strain in a different pond:

Feed Efficiency (FE)

FE = (HB – IB)/TF x 100%

FE = Feed Efficiency

HB = Harvest Biomass (kg)

IB = Initial Biomass (kg)

TF = Total Feed (kg)

Biomass gain (B)

B = HB/V

B = Biomass gain (kg/m^3)

HB = Harvest Biomass (kg)

V = Water Volume (m^3)

Multiply (M)

M = HB / IB

M = Multiply (X)

HB = Harvest Biomass (kg)

IB = Initial Biomass (kg)

Survival rate (SR)

SR = Nt/No X 100%

SR = Survival Rate (%)

Nt = Number of fish at the harvest

No = Initial number of fish

Bodyweight gain.

ADG = (BWt – BWo)/T

ADG = Average Daily Growth (g/day)

BWt = Body weight at harvest (g)

BWo = Initial body weight (g)

T = time of rearing (days)

Economic profitability values of tilapia culture using biofloc calculated using the following equation (Jolly and Clonts, 1993):

Total Cost (TC)
TC = TVC + FC
TC = Total Cost (US$)
TVC = Total Variable Cost (US$)
FC = Fix Cost (US$)

Revenue (R)
R = P x Q
R = Revenue (US$)
P = Price (US$/kg)
Q = Quantity (kg)

Total Profit (TP)
TP = Q x ((P-AVC)-FC)
TP = Total Profit (US$)
Q = Quantity (kg)
P = Price (US$)
AVC = Average Variable Cost (US$/kg)
FC = Fixed Cost (US$/kg)

B/C ratio.
B/C ratio = R/TC
B/C = Benefit/Cost ratio
R = Revenue (US$)
TC = Total Cost (US$)

Results

Heritability

The heritability values of tilapia variety with biofloc system were 0.413 (Standard Length, SL) and 0.259 (Body Weight, BW). The average response to selection or genetic gain obtained for body weight was 13.04%, while the standard length was 6.11% (**Table 1**). Furthermore, the selection response to body weight and standard length of female fish was greater than that of male fish. The average percentage improvement of bodyweight and standard length were as follows: 11.52% and 6.98% for male fish and 16.26% and 8.18% for female fish.

Productivity

The average performance of the biofloc variety tilapia seed productivity raised in ponds with the BFT system is shown in **Table 2**. Statistically, there was a significant difference in the effect of a volume-type pond on the value of biomass gain, body weight gain, and multiplication ($P < 0.05$), but not significantly different on the value of feed efficiency and survival rate ($P > 0.05$).

The highest average value of feed efficiency is 87.54%, which is obtained in a 10 m^3 pond. The largest average value of biomass is 25.30 kg/m^3 (pond 100 m^3), while the largest average value of production multiplies, survival rate, and best body weight gain was 13.59± 3.35X (pond 25 m^3), 93.95±8.77% (pond 10 m^3) and 3±0.26 g/day (pond 10 m^3), respectively.

Tabel 1 Heritability of tilapia reared in BFT

Generation	Parameter	Bodyweight (g)			Standard Length (cm)		
		Population	Male	Female	Population	Male	Female
F1-F2	R_s	8.7	21	5	0.12	0.04	1.66
	D_s	64.5	197.8	142.7	0.79	0.68	2.22
	Heritability (h^2)	0.135	0.106	0.035	0.152	0.059	0.748
	Improvement (%)	3.04	6.67	2.18	0.63	0.2	9.64
F2-F3	R_s	68	55	71	2.24	2.7	1.27
	D_s	177.4	218.8	164.8	3.32	3.6	3.15
	Heritability (h^2)	0.383	0.251	0.431	0.675	0.75	0.403
	Improvement (%)	23.04	16.37	30.34	11.59	13.76	6.73
Average	*Heritability (h^2)*	0.259	0.179	0.233	0.413	0.404	0.575
	Improvement (%)	13.04	11.52	16.26	6.11	6.98	8.18

Table 2 Performance of Productivity of the biofloc tilapia strain

Pond Volume (m^3)	Parameter Productivity				
	Feed Efficiency (%)	Biomas (kg/m^3)	Multiply (x)	Survival Rate (%)	Body Weight Gain (g/day)
10	87.54±2.14[a]	24.98±0.81[a]	11.41±0.34[a]	93.95±8.77[a]	3.00±0.26[a]
25	86.48±1.65[a]	19.71±1.66[ab]	13.59±3.35[a]	89.91±13.41[a]	2.82±0.11[a]
50	86.88±1.70[a]	16.92±1.71[b]	8.76±2.25[b]	92.12±9.09[a]	2.48±0.13[b]
100	87.38±1.76[a]	25.30±8.94[a]	8.07±1.18[b]	91.85±8.89[a]	2.83±0.23[a]

Note: The same letter indicates not significantly different

Economic profitability

The average total cost production of tilapia culture with biofloc technology and bio-variety tilapia in this study hansisted of seeds feed (86.25%), electricity (7.01%), seeds (4.86%), and miscellaneous (1.89%). The average total cost required to operate tilapia cultivation with a biofloc system is US$ 1,096.57 with variations from US$ 323.85 to US$ 2,511.66. The average total revenue is US$ 2,751.38 with variations between US$ 586.71 to US$ 6,794.57. Meanwhile, the average total profit value is US$ 1,654.81 with variations between US$ 262.86 to US$ 4,282.92. The profit margins obtained ranged from 81.17% - 170.52% with an average value of 128.33% (**Table 3**).

The Benefit - Cost ratio is a common indicator normally used in capital budgeting to determine the financial desirability of an investment (Kahraman, 2001). Calculating a B/C ratio helps investors in assessing the certainty of how promising or successful an aquaculture enterprise might be. Investment is therefore profitable if the B/C ratio is greater than 1. The average B/C ratio value of tilapia culture using biofloc variety seeds in the biofloc system was 2.28. The smallest B/C ratio value was obtained in a pond with a volume of 10 m^3, namely 1.81 and the highest was 2.71 in a pond with a volume of 100 m^3.

Table 3 Cost and profitability of tilapia culture using biofloc system in Indonesia

Parameter	PONDS				Average
	10 (m^3)	25 (m^3)	50 (m^3)	100 (m^3)	
Stocking density (ind./m^3)	91	82	85	120	94.5
Feed Efficiency	87.54±2.13	86.48±1.65	86.88±1.69	87.39±1.76	87.07
Harvest Size (g)	321.3±24.6	292.3±19.7	249.0±17.6	267.5±17.3	299
Harvest Biomas (kg/m3)	27.4±0.81	21.4±1.24	19.3±2.60	28.3±9.77	26.2
Production (kg)	273.8±8.12	534.2±31.12	1157.1±209.51	3170.8±1458.6	262
Price ($/kg)	2.14	2.14	2.14	2.14	2.14
Culture duration (days)	98	97	90	84	92
COST					
Seeds ($)	51.43	35.4	50.94	75.52	53.32
Feed ($)	208.11±4.05	415.47±32.84	853.94±155.03	2305.43±1036.93	945.74
Electricity ($)	51.21	51.21	102.43	102.43	76.82
Man power ($)	8.93	8.93	17.86	17.86	13.39
Pond depreciation ($)	4.17	4.17	10.42	10.42	7.29
Total Cost ($)	323.85	515.18	1035.59	2511.66	1096.57
Revenue ($)	586.71	1144.71	2479.5	6794.57	2751.38
Total profit ($)	262.86	629.53	1443.91	4282.92	1654.81
Profit margin (%)	81.17	122.2	139.43	170.52	128.33
Benefit-Cost Ratio	1.81	2.22	2.39	2.71	2.28

Water quality

The observed water quality parameter values ranged from 28.5º to 29.1 ºC for temperature, 6.5 – 8.0 (pH), 0.25 – 0.30 ppm (TAN), 0.3 – 1.6 ppm (Nitrite) and 2.7 - 3.0 ppm (Dissolved Oxygen). While the measured of total suspended matter was 3-25 ml/L. The bacterial floc was dominated by *Bacillus* sp.

Discussion

Heritability

According to Tave (1993), the heritability value range in this study was considered as moderate to a high category. It has a large potential for successful selection if it is carried out on these three parameters. Several ranges of heritability values resulting from selection activities in tilapia are 0.25 - 0.65 on body weight (Nugroho *et al.* 2016; Nugroho *et al.* 2017; and Robisalmi et al., 2019). Heritability values generated by the selection on the standard length of tilapia is 0.30 (Neto *et al.* 2014).

Referring to the Indonesian regulations for fish release obtained from the selection method, the minimum selection response value that has to be achieved as a release requirement is more than 30% after three generations (10% for one generation). Therefore, according to their selection response value on body weight, this tilapia strain is enough to be released as a genetically improved fish after the third generation. Meanwhile, the standard-length character can be used as secondary characters for the selection of biofloc tilapia varieties.

Furthermore, the higher value of response selection of both characters in the female fish than those of the male one indicated that a tightening of the size of the selected reference in the male population is still required, namely to be higher than 5% of the best populations. The application for selection activities is that there will be more visible improvements in performance if using the body length character reference instead of body weight for tilapia culture with biofloc technology as well as shown in their heritability values.

Productivity

The statistically significant effect of the volume-type pond was only on biomass, body weight and multiplication parameters. The value of the productivity parameter resulting from the use of the bio-variety of tilapia is equivalent or better to the results of other studies. Rackoci *et al.* (2014) obtained tilapia biomass between 13.7-14.4 kg/m^3 and feed efficiency values between 45-53% with a survival rate of 78-81%.

Meanwhile, Luo et al. (2017) obtained productivity levels of tilapia ranging from 32.60-35.83 kg/m^3 and feed efficiency values between 68-71% with a survival rate of 95%. This condition indicates that the performance of tilapia aquaculture with the biofloc system can be used as an alternative as a substitute for tilapia culture with the floating net cage system which has a productivity level of 1.09-1.62 kg/m^3 (Nugroho et al., 2013). This result also supports Nugroho et al. (2020) who obtained results that the use of superior seeds can increase the productivity of tilapia aquaculture using the biofloc system. The productivity of culture tilapia using the biofloc system at that time was 10.8-14.42 kg/m^3.

The body weight gain in this experiment ranged between 2.48±0.13 g/day in the 50 m^3 pond to 3.00±0.26 g/day in the 10 m^3 pond. These values were much higher than that reported by Day et al. (2016). In the 10 weeks trial of biofloc experiment, Day et al (2016) found that tilapia species of *O. niloticus* revealed the highest growth rate among three tilapia species with an average daily gain of 0.693±0.018 g/day, better than two other species, i.e. *O. mossambicus* and *O. andersonii*.

In terms of feed efficiency, the obtained values were still below 100%. It showed that the application of biofloc has not fully functioned double, in terms of water quality and fish feed management. Widarnani et al. (2012), Emerenciano et al. (2013), Das and Mondal (2018), and Kumari et al (2021) stated that the floc composed of some organic materials, plankton, and bacteria can be used by fish as feed. Based on our result, an alternative of the required improvement is to reduce the amount and protein level of feed given so that it allows the fish to utilize biofloc as a protein source.

Furthermore, in ponds with a volume of 10, 25 and 50 m^3, the result showed that the feed affected the amount of the harvested biomass. Meanwhile, the initial size and stocking density affected multiply. It indicated that improving the amount of biomass harvested can be done by streamlining the feed management system. The improvement of multiplying production can be done by adjusting the initial size and stocking density.

Economic profitability

The average B/C ratio value of tilapia culture in this study is greater than one with the profit margins obtained ranged from 81.17% - 170.52%, which indicates how promising or successful an aquaculture enterprise might be. It is equivalent or better to the results of other studies. Phiri & Yuan (2018) obtained information that the BC ratio of intensively cultivated tilapia in China and Malawi is 1.21 and 1.61, with profit margins of 20.16% and 61.36%, respectively. The average total cost production is dominated by feed i.e., up to 86%. It was still high. This indicates, again, that the application of biofloc has not yet been as fish feed as mentioned by Emerenciano et al. (2013). However, seeing the relatively big of the above values and the factors of good water quality management, showed the potential that the biofloc system of tilapia culture technology is an alternative solution in the future for sustainable tilapia aquaculture in Indonesia.

Water quality

According to Das and Mondal (2018), biofloc systems are more efficient when water temperature is between 28 to 30ºC. Sarker et al. (2019) mentioned that nitrifying bacteria can support a range from 28-30ºC. pH < 6.0 and > 8.5 usually affect the efficiency of the biofloc components. Further, the concentration of suspended matter in the range of 250 - 450 mg/L ensures efficient bacterial activity. Improvement of the type and volume of bacteria in the composition of the floc is thought to increase the value of feed efficiency in this research as obtained in several studies.

In conclusion, the superior tilapia biofloc strain has a moderate heritability value, when it is raised in biofloc ponds system at different volume, significant affected the productivity on biomass, body weight and multiplication value and also is advantageous when applied on an industrial scale.

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Author contribution

Estu Nugroho, Riza Zulkarnain, Anang Hari Kristanto, Raden Roro Sri Pudji Sinarni Dewi, Bambang Gunadi, Adam Robisalmi, Wahyu Pamungkas, Lies Setijaningsih, and M. Rifaldi conceived conceptualization, methodology, carried out data acquisition, prepared the manuscript. E. Nugroho, Riza Zulkarnain, B. Gunadi, W. Pamungkas, and A. Robisalmi reviewed, edited and revised the manuscript. All authors reviewed the results, provided critical feedback, and approved this final version of the published manuscript.

Declarations

Ethical approval. This research does not require ethical approval from any institution, and there are no applicable laws in Indonesia.

Conflict of interest. The authors declare no competing interests.

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