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APPLICATION OF BIOFLOC TECHNOLOGY IN REARING RED TILAPIA *(Oreochromis niloticus)* AT DIFFERENT STOCKING DENSITIES IN BRACKISH WATER

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ABSTRACT

Red tilapia is a fish with a relatively wide tolerance to salinity (euryhaline) changes, making it possible to cultivate red tilapia in brackish waters. Additionally, intensive farming environments can support increased stocking density. The application of biofloc technology can help address this issue. Biofloc technology is an alternative approach to addressing water quality issues in water bodies, adapted from conventional domestic wastewater treatment techniques, and can also serve as a source of natural food. This research aimed to determine the best stocking density of red tilapia reared in brackish water using biofloc technology. The method used in this research is the experimental method of Completely Randomized Design (CRD) with one factor, five levels of treatment, and three replicates. The treatments applied were 20 fish/80 L (P1), 30 fish/80 L (P2), 40 fish/80 L (P3), 50 fish/80 L (P4) and 60 fish/80 L (P5). The red tilapia used as test fish were 3-5 cm in size and adapted to 17 ppt salinity. Feed with commercial pellets 3 times a day in sufficient amounts. This research was conducted for 50 days. The results showed the best stocking density was obtained in P4 (50 fish/80 L) which produced absolute weight of 8,87 ± 0,12 g, absolute length of 3,57 ± 0,07 cm, specific growth rate of 3,55 ± 0,12%, feed conversion ratio of 0,83 ± 0,06 kg, feed efficiency of 120,32 ± 7,81%, survival rate of 86 ± 4% and blood glucose level of 71 ± 5,13 mg/dL.

Keywords: Biofloc Technology, Red Tilapia, Stocking Density, Brackish Water, Intensive

1. INTRODUCTION

The growth of aquaculture production is constantly increasing. This is evidenced by the information released by the Ministry of Marine Affairs and Fisheries in the fourth quarter of 2022, which revealed that the production value of aquaculture commodities outside seaweed in 2021 was 1.4 million tons, increasing to 3.3 million tons in 2022, representing a 39.64% increase in production growth. The increase in production value, especially for tilapia, was 335,564 tons in 2021 and rose to 482,249 tons in 2022, resulting in a production growth rate of 43.71%[1]. This indicates that tilapia is one of the leading fishery commodities, with increasing market demand, particularly for red tilapia.

According to Lumbanbatu et al.[2], red tilapia has several advantages, including high body resistance to various diseases, tolerance to low and high temperatures, and tolerance to significant salinity changes (euryhaline), which makes tilapia a fish that can live in both freshwater and brackish water. Brackish water has a salinity of more than 5 ppt, a mixture of freshwater and seawater[3]. According to Manik et al.[4], the salinity suitable for tilapia is 0-35 ppt, but the salinity that allows tilapia to grow optimally is 0-30 ppt. Tilapia can still live at 31-35 ppt salinity, but growth is slow.

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Tilapia's tolerance to brackish water allows it to be cultured in this environment.

Adapting to changes in salinity enables tilapia to be reared in brackish water, but this must be balanced with optimal stocking density. Stocking density is one of the key factors that can impact the growth and survival of fish, as the number of fish in a water body influences both the amount of feed consumed and the availability of dissolved oxygen. Additionally, ammonia derived from the metabolic products of fish and feed residues, resulting from uncontrolled stocking density, will impact the waters.

One innovation that can be applied to overcome these problems is biofloc technology. Biofloc (BFT/bioflock technology) is an alternative to conventional domestic sewage treatment techniques, providing a solution to address water quality issues in water bodies[5]. Water quality and natural feed that is always available can serve as an alternative to meeting fish feed needs, supporting fish growth through biofloc technology.

The application of biofloc technology in raising red tilapia can not only provide natural feed. However, it can also be maintained at a high stocking density, allowing red tilapia to be classified as an intensive aquaculture practice. There is a lack of research on the optimal stocking density for red tilapia reared in brackish water using biofloc technology. The study aims to determine the optimal stocking density for supporting the production of red tilapia. Therefore, it is necessary to investigate the application of biofloc technology in maintaining red tilapia with varying stocking densities in brackish water media.

2. RESEARCH METHOD

Time and Place

This research was conducted from October to December 2024 at the Integrated Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Method

This research used a 1-factor Completely Randomized Design (CRD) with five treatment levels and three replicates, namely: P1 (20 fish/80 L); P2 (30 fish /80 L); P3 (40 fish /80 L); P4 (50 fish /80 L); P5 (60 fish /80 L). This refers to the research of Situmorang et al.[6], which demonstrated that different stocking densities impacted the growth and survival of red tilapia reared in brackish water with a recirculation system, with the optimal treatment being 40 fish /80 L.

Procedures

Container Preparation

A 100 L bucket is equipped with aeration in each container. Container preparation begins with washing and drying the container in the sun. After the container is dry, it is filled with water, treated with a disinfectant, and then incubated for 24 hours. It is rinsed again afterwards. The containers were labelled and arranged randomly.

Media Preparation

Brackish water is used as a rearing medium, obtained by diluting sea salt. The sea salt used was SPS Sea Salt Blue Treasure. Brackish water with a salinity of 17 ppt was obtained by diluting sea salt to a concentration of 1.4 kg/80 L of water for each container. This study employed a salinity of 17 ppt, based on the research results by Dahril et al.[7], which investigated the effect of different salinities on the growth and survival of red tilapia seeds. The best results were obtained at a salinity of 17 ppt. After dilution in each container, the media is aerated until the starter solution is ready for insertion.

Giving Starter Solution

Multi-cell booster probiotics are used as a source of floc-forming bacteria, and molasses as a carbon source. Multi-cell booster probiotics contain *Bacillus* sp, *Nitrosomonas* sp and *Nitrobacter* sp, which are given as much as 10 mL/m^3 in the maintenance media, which is done every 7 days[8], while molasses is given as much as 200 mL/m^3[4] for each container. Flocs that have formed are characterised by a change in the colour of the water to a brownish, turbid appearance, and the formation of bubbles in the maintenance media. After 7 days of floc formation, red tilapia seeds were stocked in each rearing container according to the predetermined treatment.

Preparation of Test Fish

The red tilapia used in this study were 3-5 cm in size and obtained from Jaya Abadi Fish Farm hatchery in Pekanbaru. The red tilapia used as test fish were first adapted to brackish water with a salinity of 17 ppt. The acclimatization method applied is continuous for 6-24 hours, with a gradual increase in salinity each day, until it does not exceed 5 ppt[9]. The red tilapia adaptation was carried out for 8 days.

Maintenance of Test Fish

Maintenance of test fish was carried out for 50 days with feeding in the form of floating commercial fish feed Prima Feed (PF-800) with a protein content of 39%, which was given 3 times a day, namely in the morning at 08.00 WIB, at noon WIB and in the afternoon at 16.00 WIB. The feeding dose was 3% of the test fish's biomass weight[6].

Measurement of Test Parameters

Fish length and weight measurements were taken every 10 days. In the morning, measurement or sampling is performed by taking a sample of test fish, which constitutes at least 50% of the total fish population, in one maintenance container. Blood glucose levels were measured twice, namely at the beginning and end of maintenance.

Research Parameters

Absolute Weight Growth

Absolute weight growth was calculated using the Effendie[10] formula:

$$W = Wt - Wo$$

Description:

W = Absolute weight growth of test fish (g)
Wt = Average weight of test fish at the end of rearing (g)
Wo = Average weight of test fish at the beginning of rearing (g)

Absolute Length Growth

Absolute length growth was calculated using the formula of Zonneveld et al.[11]:

$$L = Lt - Lo$$

Description:

L = Absolute length growth (cm)
Lt = Average length of test fish at the end of rearing (cm)
Lo = Average length of test fish at the beginning of rearing (cm)

Specific Growth Rate

Specific growth rate is calculated using the formula from Zonneveld et al.[11]:

$$SGR = \frac{LnWt - LnWo}{t} \times 100\%$$

Description:

SGR = Specific growth rate (%/)
LnWt = Average fish weight at the end of rearing (g)
LnWo = Average fish weight at the beginning of rearing (g)
t = Length of rearing (days)

Feed Conversion Ratio

The calculation of the feed conversion ratio was carried out using the formula from Effendie[10]:

$$FCR = \frac{F}{(Wt+D)-Wo}$$

Description:

FCR = Feed conversion ratio
F = amount of feed given (g)
Wt = Biomass weight of test fish at the end of rearing (g)
D = Total weight of dead fish (g)
Wo = Biomass weight of test fish at the beginning of rearing (g)

Feed Efficiency

Feed efficiency was calculated using the formula from Zonneveld et al.[11]:

$$EP = \frac{(Wt+Wd)-Wo}{F} \times 100\%$$

Description:

EP	=	Feed efficiency value (%)
Wt	=	Biomass weight of test fish at the end of rearing (g)
Wd	=	Biomass weight of dead test fish (g)
Wo	=	Biomass weight of test fish at the beginning of rearing (g)
F	=	Amount of feed consumed by the test fish (g)

Survival Rate

Calculation of survival rate (SR) is done by using the formula of Effendie[10]:

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

Description:

SR	=	Survival rate (%)
Nt	=	Number of test fish at the end of maintenance (fish)
No.	=	Number of test fish at the beginning of rearing (Fish)

Blood Glucose Level

Measurement of blood glucose levels using digital blood glucose test kits, namely GlucoDr test kits and GlucoDr strips. This is achieved by inserting strip paper into the GlucoDr digital device, and then on the glucometer monitor screen, a sign appears indicating that the device is ready for blood drops. The trick is to touch one drop of blood that comes out to the test strip and is pulled by itself through capillary action. When the container is filled with blood, the device begins to measure blood glucose levels. Measurement results were obtained for 11 seconds

3. RESULT AND DISCUSSION

Growth of Red Tilapia

The results of research on the growth of red tilapia during maintenance showed a significant effect ($P < 0.05$) on absolute weight growth, absolute length growth and specific growth rate (Table 1).

Table 1. Growth of red tilapia

Treatment	Absolute weight (g)	Absolute length (cm)	SGR (%)
P_1	$6{,}47\pm0{,}56^{a}$	$2{,}38\pm0{,}21^{a}$	$2{,}98\pm0{,}15^{a}$
P_2	$7{,}37\pm0{,}30^{ab}$	$3{,}17\pm0{,}51^{b}$	$3{,}01\pm0{,}19^{ab}$
P_3	$7{,}78\pm0{,}69^{b}$	$3{,}52\pm0{,}16^{b}$	$3{,}39\pm0{,}24^{bc}$
P_4	$8{,}87\pm0{,}12^{c}$	$3{,}57\pm0{,}07^{b}$	$3{,}55\pm0{,}12^{c}$
P_5	$6{,}45\pm0{,}52^{a}$	$2{,}25\pm0{,}52^{a}$	$2{,}70\pm0{,}23^{a}$

Notes: Different *superscript* letters in the same column indicate significant differences between treatments ($P<0.05$)

Based on Table 1, the growth in absolute weight varies among the different treatments. The highest absolute weight was obtained in P4, with an absolute weight of 8.87 g. In treatment P1 and P2, the absolute weight was 8.87 g. The treatments P1 and P5 had almost equal absolute weights of 6.47 g and 6.45 g, which means that P5 was the treatment with the lowest absolute weight. The low absolute weight growth in both treatments was caused by stocking densities that were too high and too low. One of the impacts of dense stocking in P5 is the high competition for dissolved oxygen, which can hinder the maintenance of optimal body condition. Simangungsong et al.[12] stated that fish reared with stocking densities that are too high will cause competition for food, space and dissolved oxygen, and vice versa.

Absolute length growth reached 3.57 cm in treatment P4, followed by P3 at 3.52 cm and the lowest absolute length of 2.25 cm in treatment P5. The difference in length growth between treatments indicates that fish reared in optimal numbers can achieve better absolute length growth. Additionally, the effectiveness of bioflocs is influenced by stocking density, as the balance between organic waste production and the capacity of bacteria to decompose organic matter determines their effectiveness. This finding aligns with the opinion of Sallam et al.[13], who reported the results of their research indicating that red tilapia reared in biofloc technology under optimal conditions can improve growth performance due to enhanced feed consumption and increased salinity.

The highest specific growth rate was obtained in P4 at 3.55%. The decrease in specific growth rate coincided with a decrease in stocking density. The decrease in specific growth rate, which is not too significant, is likely due to similar growth between treatments that are not significantly different. This is supported by the statement of Delgado et al.[14], based on the results of their research, which indicate that a smaller number of fish can produce a growth rate similar to that of a larger number of fish. However, inversely proportional to this, a specific growth rate increased when the number of fish in the treatment increased. This was triggered by the presence of bioflocs in the maintenance media so that at the optimal stocking density, P4 can balance the mass of bacteria (which is routinely added from multi-cell boosters along with molasses carbon sources) with the production of organic waste, so that the capacity of bacteria to decompose organic matter in the treatment with stocking density can make biofloc work well. Additionally, the growth of the average weight of red tilapia resulted in increased growth during maintenance, as shown in Figure 1.

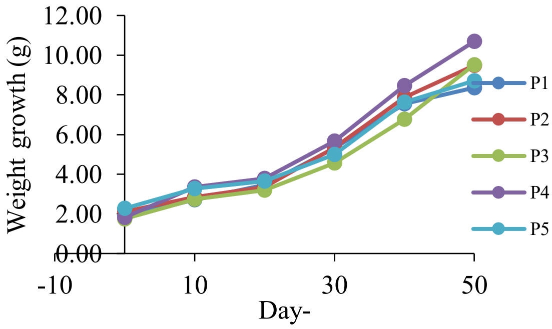

Figure 1. Average weight growth

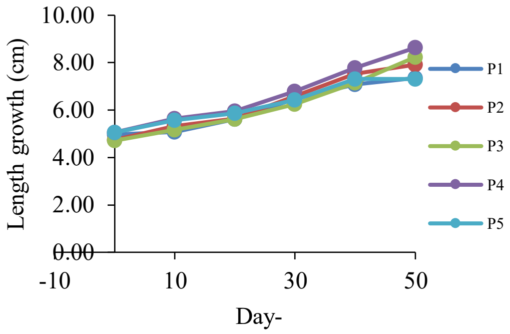

Figure 2. Average length of growth

Figure 1 shows an increase in average weight during maintenance. However, weight has not significantly increased from day 0 to day 20. This is because the maintenance time is still classified as the initial maintenance period. At that time, the fish are still adjusting to their new environment and body condition. Then, on day 20, the average weight had increased significantly. This increase is likely due to the fish having successfully adapted to their environment, and the feed provided can enhance the growth of fish weight. In line with the opinion of Simangungsong et al.[12], the availability of feed and the fish's ability to adjust to a new environment both affect fish growth.

Higher growth started on day 20, but from day 40 to day 50, P5 and P1 did not experience the same high growth as the other treatments (Figure 2). One factor that affects fish length growth is the availability of sufficient space for movement. According to Li et al.[15], fish reared with optimal stocking density in a biofloc system allow fish to swim and move naturally, thus supporting muscle development and length growth.

Feed Conversion Ratio and Feed Efficiency

Feed conversion ratio is the ratio of the amount of feed utilised by the fish to the Biomass of the fish. The feed conversion ratio value is inversely proportional to the feed efficiency value. The lower the feed conversion ratio value, the higher the feed efficiency value, which means the fish can utilise feed more effectively, resulting in better growth. Different stocking densities affect feed conversion ratio and feed efficiency (Table 2).

Table 2. FCR and feed efficiency

Treatment	Feed Conversion Ratio	Feed Efficiency (%)
P1	$1,05\pm0,05^{bc}$	$2,38\pm0,21^{a}$
P2	$0,98\pm0,12^{ab}$	$3,17\pm0,51^{b}$
P3	$0,88\pm0,08^{a}$	$3,52\pm0,16^{b}$
P4	$0,83\pm0,06^{a}$	$3,57\pm0,07^{b}$
P5	$1,15\pm0,04^{c}$	$2,25\pm0,52^{a}$

The lowest feed conversion ratio was obtained in P4 with a value of 0.83. Then, the highest feed conversion ratio value was obtained in P5 at 1.15. The range of feed conversion ratio is also influenced by the feeding rate (FR) applied. In this study, the feeding rate of the total body biomass was 3%. This dose is given because this study has applied biofloc technology, which can produce floc as an additional feed for fish. This is also supported by Magouz et al.[16], who reported the results of their research, which investigated the effect of different feeding rates on tilapia performance in a biofloc system. The low feed conversion ratio in all treatments is attributed to the utilisation of additional feed from the flocs formed. When there is an increase in floc biomass as a source of nutrients or extra food for aquaculture cultivars, the feed conversion ratio due to the utilization of these flocs is lower[17].

Then, the feed efficiency value of red tilapia ranged from 87.08 to 120.32%. P4 produced the highest feed efficiency value, which aligns with its low feed conversion ratio. Feed efficiency values exceeding 50% in all treatments indicate that biofloc technology is highly effective in enhancing the feed efficiency of red tilapia, demonstrating the superiority of the biofloc system.

Lumbanbatu et al.[2] stated that feed efficiency values above 50% are considered good. The high value of feed efficiency is attributed to the utilisation of biofloc as feed in maintaining red tilapia. This finding aligns with the results of Simanullang[18], which showed that adding molasses as a carbon source in the maintenance of red tilapia with biofloc technology resulted in the highest efficiency value of 134.59%. Meanwhile, red tilapia cultivated without biofloc technology has a feed utilization efficiency of 67-74%[2].

Survival Rate

Survival rate is the percentage of fish that can survive until the end of the study. Based on the results of the 50-day study, the survival rate of red tilapia is presented in Table 3.

Table 3. Survival rate of red tilapia

Treatment	Survival Rate (%)
P1	$63\pm7,64^{a}$
P2	$76\pm5,13^{bc}$
P3	$84\pm4,04^{c}$
P4	86 ± 4^{c}
P5	$72\pm1,73^{ab}$

The analysis of variance (ANOVA) test conducted on the parameter of survival rate of red tilapia showed $P<0.05$. The survival rate of red tilapia ranged from 63 - 86%. The results of this study indicate that stocking density P4 is the optimal stocking density for red tilapia when applying biofloc technology in brackish water rearing, achieving the best survival rate. However, the treatment with a higher fish density (P5) obtained a better stocking density than the low stocking density treatment (P1).

The higher increase in floc volume in P1 compared to other treatments, due to the amount of stocking density that is not proportional to the mass of biofloc produced, is thought to be one of the causes of fish mortality. Lyu et al.[19] state that the lower survival rate in rearing red tilapia with biofloc technology is related to the size and composition of bioflocs. Suppose the dispersion solids (biofloc particles consisting of agglomerates of organic compounds, substrates or microorganisms in the water) are lower. In that case, it can reduce damage to the gill lamellae caused by exposure to bioflocs and increase respiratory efficiency, thereby supporting survival.

The survival rate is influenced by several factors, including the availability of microalgae that form into flocs. According to Iba et al.[20], biofloc systems have been shown to increase growth and survival. However, the specific growth rate is not significantly affected; according to the research results by Brito & Silva[21], red tilapia can grow optimally in brackish conditions and achieve a survival rate of 85% when optimal water quality is maintained. However, the survival rate value during the study was still relatively good. This statement is supported by Simanullang's[18] opinion, which states that a survival rate of greater than 50% is considered good, a survival rate of 30-50% is moderate, and a survival rate of less than 30% is considered low.

Blood Glucose Levels

The results of the blood glucose level measurements of red tilapia during maintenance, carried out twice (at the beginning and end of the study), are presented in Table 4.

Table 4. Blood glucose levels of red tilapia

Treatment	Blood Glucose (mg/dL)
P_1	$50±6,11^a$
P_2	$54±10,41^a$
P_3	$61±4,51^{ab}$
P_4	$71±5,13^b$
P_5	$76±6,11^b$

Based on the results of the analysis of variance (ANOVA) test, the application of biofloc technology in maintaining red tilapia in brackish water with different stocking densities has a significant effect on the blood glucose levels of red tilapia ($P < 0.05$). The increase in glucose levels occurred along with the increase in stocking density. The best stocking density treatment, as indicated by the results of this study, P4, resulted in a blood glucose level of 71 mg/dL. These results indicate that the blood glucose range of red tilapia ranges from 50 to 76 mg/dL.

The increase in the average blood glucose level in the treatment is likely due to increased stocking density. According to Windarti et al.[22], blood glucose levels in fish can increase due to stress. Typically, stress in fish is caused by environmental changes resulting from various factors or treatments. Blood glucose levels will increase when fish experience stress due to these changes. At the same time, the thyroid gland is stimulated, and its thyroxin production increases, causing lymphocitemia and neutrophilia in the blood. Then, the sympathetic nervous system overreacts, which causes spleen contraction and increased breathing and blood pressure.

The highest blood glucose level in P5 was 76 mg/dL. That value is still within the optimal limit for fish, which is 40-90 mg/dL[23]. However, Aketch et al.[24] state that the optimum blood glucose level for tilapia species is 40.1-80.5 mg/dL. This suggests that excessively high stocking density leads to increased blood glucose levels, reflecting the higher stress levels in red tilapia. This is in line with the findings conducted by Aketch et al.[24], which showed that fish reared with high stocking density experienced a significant increase in blood glucose levels (136 mg/dL) compared to fish reared with low stocking density (70.2 mg/dL).

4. CONCLUSION

The application of biofloc technology in rearing red tilapia in brackish water with different stocking densities provided absolute weight growth of 8.87±0.12 g, absolute length of 3.57±0.07 cm, specific growth rate of 3.55±0.12%, feed conversion ratio of 0.83±0.06 kg, feed efficiency of 120.32±7.81%, survival rate of 86±4% and blood glucose level of 71±5.13 mg/dL.

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