Growth Performance of G6 Transgenic Mutiara Catfish At Different Stocking Density

ABSTRACT

The optimum fish stocking density effected on fish growth performance as a response to growth and survival. Previous research showed that the G5 transgenic mutiara catfish produced 100% (twice) higher growth than non-transgenic catfish in indoor rearing. Further research on G6 at different stocking densities aims to evaluate the growth performance of transgenic mutiara catfish. The study was conducted using a Completely Randomized Design (CRD) with four treatments and three replications on the bucket media. The density treatments used: A (40 fish/60 L), B (50 fish/60 L), C (60 fish/60 L) and D (70 fish/60 L) in the *budikdamber* (fish farming in buckets) system. The results showed that a density of 70 fish/60 L (treatment D) increased the average biomass weight gain (ABWG) by 2,152 g for 56 days of rearing. In addition, the feed utilization efficiency (FCR) in all treatments of G6 transgenic mutiara catfish did not show any significant differences as compensation for the presence of exogenous GH inserts.

Keywords: Transgenic Fish, Stocking Density, ABWG, FCR, SR

1. INTRODUCTION

Problems often encountered in catfish fingerlings enlargement (non-transgenic) are related to the genetic quality of catfish broodstock growth. Growth quality is inseparable from the use of catfish broodstock strains that are often used in fish fingerlings production. Genetic improvement of catfish growth can be done with transgenesis technology (exogenous GH insertion, *CgGH*) The results of the growth increase shown by transgenic mutiara catfish G1-G5 around 2-3 times higher than the growth of non-transgenic fish [1]. However, the growth performance of G6 between transgenic fish is not yet known and needs to be evaluated through different stocking density tests in fish farming in *budikdamber*.

Differences in density levels can affect fish growth as one of the inhibiting factors for fish growth. Increasing density (270 fish/m3) in biofloc rearing media can cause slow growth due to competition for space, oxygen and feed [2, 3]. In addition, the accumulation of leftover feed and feces in the maintenance media will cause a decrease in air quality, thereby reducing fish appetite [4].

The use of transgenesis technology is one alternative to overcome the slow growth of catfish fingerlings. In principle, this technology transfers certain superior genes in related fish into the host genome which causes excessive growth (over expression).

The assembly of transgenic mutiara catfish has been successfully carried out using sperm electroporation as a vehicle for inserting the growth hormone gene by inserting the growth hormone gene or *CgGH* into the fish sperm. mutiara catfish inserted with the gene have a growth rate of 2.75-3.53 times greater than non-transgenic mutiara catfish [5] . The rate of exogenous GH inheritance in G1-G4 transgenic mutiara catfish ranges from 42.85 - 89%. [6,7] . Research with different stocking densities using transgenic mutiara catfish has been carried out on the fourth generation and resulted in 100% higher growth indoors and 87% higher outdoors compared to non-transgenic mutiara catfish. The results of the study showed that rapid growth in transgenic mutiara catfish was not affected by a certain stocking density level, while in non-transgenic mutiara catfish the growth rate tended to slow down [1].

Currently, transgenic mutiara catfish have been successfully developed up to the sixth generation in 2023. Further research needs to be carried out to evaluate the optimum stocking density for the growth and stability of the inheritance of superior traits of transgenic mutiara catfish fingerlings in the sixth generation.

2. MATERIALS AND METHODS

The research was conducted at the hatchery building 4 of Faculty of Fisheries and Marine Sciences, Padjadjaran University in July–September 2023 which included the preparation, implemantion and observation stages of the study. The materials used in this research were G6 transgenic mutiara catfish fingerlings measuring 5-7 cm (35 days old), commercial feed Prima Feed-1000 with a protein content of 39%. The tools used in this research were: bucket with a water capacity of 80 L, aerator installation, heater (Atman), DO meter (Lutron), pH meter (Xinweiqiang), digital scale (Joil), therometer (Lutron) and fish net. The overall study perod was 56 days

Completely Randomized Design (CRD) were used with four different stocking densities as many as 40 fish (treatment A), 50 fish (treatment B), 60 fish (treatment C) and 70 fish (treatment D) with volume 60 liters of water and each treatment was repeated three times. Twelve bucket with a top diameter of 51 cm, a bottom diameter of 39 cm, and a height of 57.5 cm) with a water capacity of 80 L were filled with water until it reached 60 L, each treatment bucket was equipped with an outlet pipe to facilitate water drainage. Then an aeration and heater installation were set at a temperature of 27±1°C. The distribution of fish according to each treatment density was carried out after measuring the total weight of the fish according to the number of fish used in the density treatment. Then covered with a hapa net to prevent fish from getting out of the maintenance bucket

Each treatment was repeated three times and fed as much as 3-5% of body weight. The frequency of feeding was twice a day at 08.00 and 16.00 WIB. To monitor and calculate growth parameters, fish were measured using a digital scale every week. Water quality parameters were measured every week using a DO meter, pH meter and thermometer. Water changes were carried out every two days with 100% new water replacement to maintain water quality. The growth test was carried out for 56 days to evaluate the growth performance of G6 transgenic mutiara catfish.

Test parameters carried out include average biomass weight gain, feed conversion ratio, survival rate of G6 transgenic mutiara catfish fingerlings.

Average biomass weight gain (ABWG) were calculated by reduce the biomass weight of the fish at the end of maintenance with initial biomass weight [8].

$$ABWG = W_t - W_O$$

Description :
ABWG = Average Biomass Weight Gain
W_t = Final Biomass Weight (g)
W_O = Initial Biomass Weight (g)

The value of the feed conversion ratio can be calculated by divided the total feed given by the difference between the total weight of the fish at the end of the period and the total weight of the fish at the beginning of the period. If there were fish that died, the weight of the dead fish was added to the difference between the initial and final weight values [9].

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

Desciption:
FCR = Feed Conversion Ratio
F = Amount feed eaten by fish
Wt = Final Weight (g)
W_0 = Initial Weight (g)
D = Dead Weight (g)

The survival rate (SR) value is obtained by comparing the number of live fish at the end of maintenance with the number of live fish at the beginning of maintenance. SR calculated by dividing number of survival fish with amount initial stocked fish [10].

$$\mathrm{SR} = \frac{\mathrm{N_t}}{\mathrm{N_O}} \text{ x } 100\%$$

Descirīption:
SR = Survival Rate
Nt = Number of Survival
No = Initial Stocking

The water quality parameters tested include temperature, acidity (pH) and dissolved oxygen (DO). Parameter measurements were carried out at the beginning of the study and repeated every 7 days. The method for measuring water quality parameters used in the study is shown in Table 1 and the procedure used is shown in Figure 1

Table 1. Water Quality Measuring Method

Parameters	Units	Tools	References
Fisika:			
Temperature	°C	Thermometer	[11]
Kimia:			
pH	-	pH-meter	[12]
Dissolved Oxygen	mg/L	DO-meter	[13]

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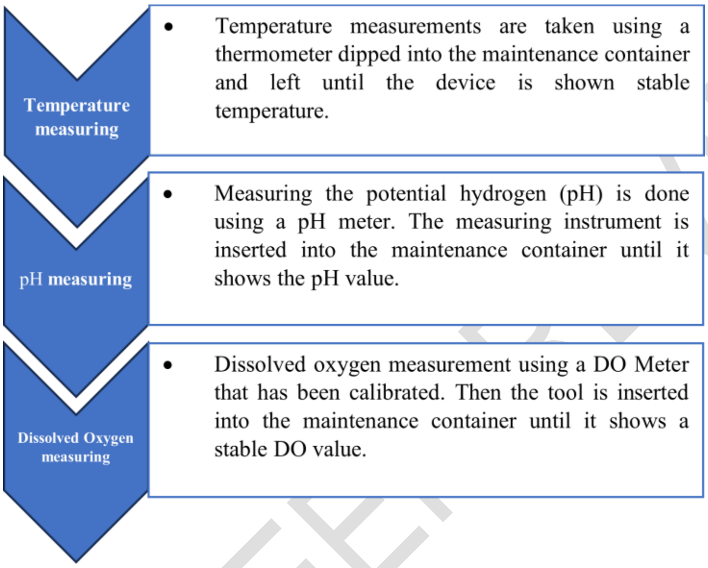

Fig. 1: Water Quality Measuring Procedure

The result of the feed conversion ratio (FCR), survival rate (SR) and Average Biomass Weight Gain (ABWG) were analyzed using Analysis of Variance (ANOVA) quantitatively. If there was an effect between treatments, it was continued with the Duncan's Multiple Range Test (DMRT) with a 95% confidence level using Sigmaplot 15 software. Data analysis of water quality observed as a supporting parameter was analyzed descriptively.

3. RESULT AND DISCUSSION

3.1 Growth Performance

3.1.1 Average Biomass Weight Gain

The average biomass weight gain value of fish obtained from each treatment during 56 days of maintenance was analyzed statistically using ANOVA. The results showed that there were significant differences between test treatments. The Value of average biomass weight gain shown by Figure 2.

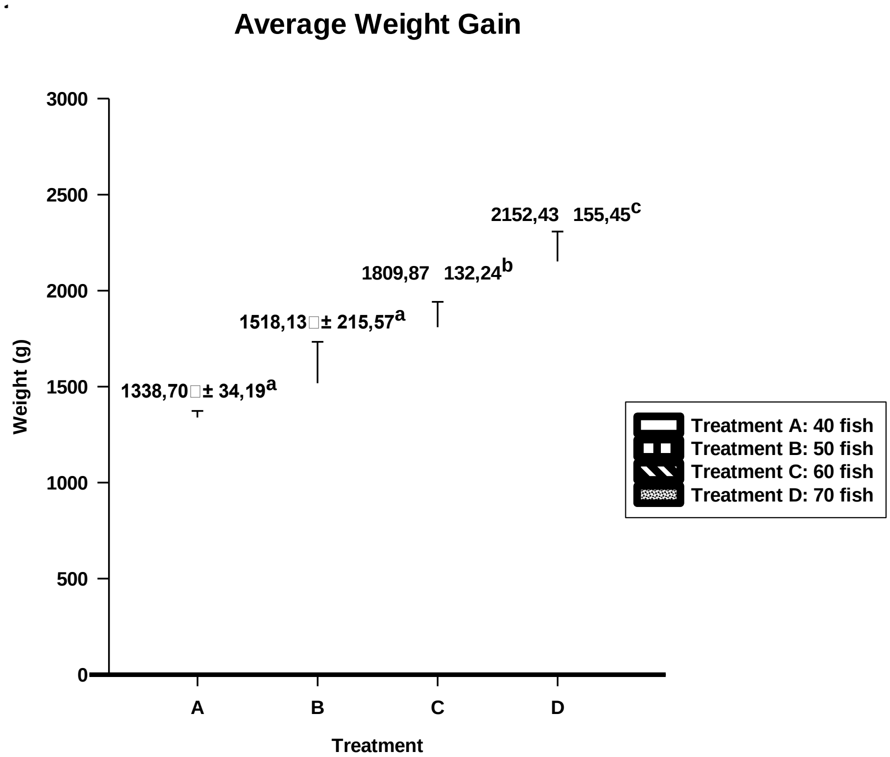

Fig. 2. Average Biomass Weight Gain A (40 fish), B (50 fish), C (60 fish) and D (70 fish). The mean value followed by SD with different letter notation, shows significance (p<0.05)

The results of observations during maintenance showed the average biomass weight gain of G6 transgenic mutiara catfish fingerlings with different stocking densities in the bucket rearing media. The highest average biomass weight gain was in treatment D which produced a weight growth of 2152.43 ± 155.45 g while the lowest length growth was obtained in treatment A, which was 1,338.70 ± 34.19 g. Treatments B and C each had average biomass weight gain values of 1,518.13 ± 215.57 g and 1,809.87 ± 132.24 g.

The highest average biomass weight gain was shown by treatment D (high density level). This statement is in accordance with the results of research shown in G4 transgenic mutiara catfish that high density (15 and 12 fish / 100 L) increases protein retention value and protein efficiency value higher than the density of 10 fish / 100 L [6] . This consequence shows that there is a positive relationship between increasing density and growth performance. Increasing density can stimulate appetite which can induce IGF-1 expression and overexpression of exogenous GH in transgenic mutiara catfish, thereby increasing the conversion of feed protein into body protein used for fish growth. In addition, increasing density can affect protein sparing effect so that biomass weight at relatively high density levels has a higher value compared to low density levels. The protein sparing effect is a protein savings that is utilized for growth due to the influence of GH transgenesis which can regulate energy metabolism in fish which increases the use of carbohydrates to meet energy needs [14].

The positive relationship between increasing density and growth performance was also shown in studies on grouper fish using the RAS system and raceway system [15], and japanese flounder [16]. The positive relationship between increasing density and growth is due to energy savings due to space limitations, which allows more efficient utilization of nutrients for growth [15], as well as overexpression of exogenous GH in transgenic fish [6].

3.1.2 Feed Conversion Ratio

Based on the results of the ANOVA during the study, it can be seen that feeding using commercial PF-1000 feed with differences in stocking density for G6 transgenic mutiara catfish fingerlings resulted in an average feed conversion value that was not significantly different (Figure 3).

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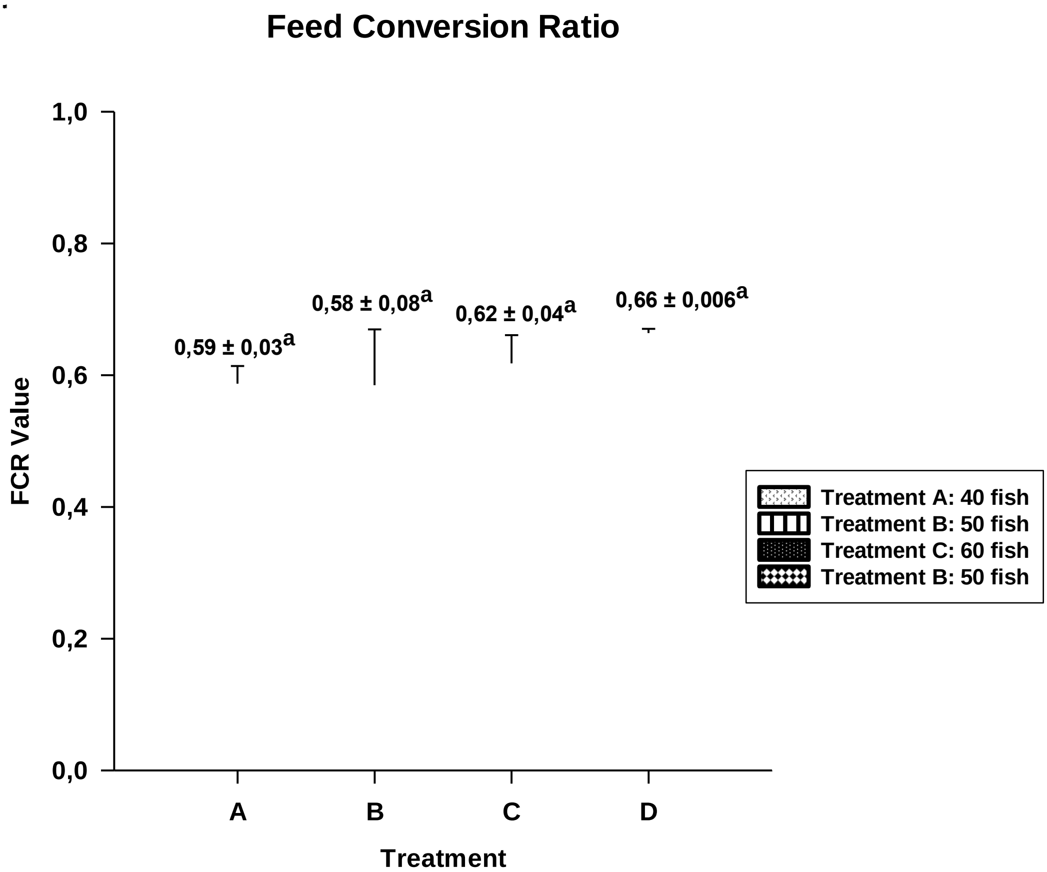

Fig. 3. Feed Conversion Ratio Value

The mean value followed by SD with different letter notation, shows significance ($p<0.05$)

Based on the results of observations during the study, it can be seen that feeding using commercial feed PF-1000 with different stocking densities on G6 transgenic mutiara catfish fingerlings produced different average feed conversion ratio values. The highest value was obtained in treatment D with a feed conversion value of 0.66 ± 0.006, while the lowest value was obtained in treatment B with a feed conversion value of 0.58 ± 0.08. Treatment A and Treatment C each had a feed conversion ratio value of 0.59 ± 0.03 and 0.62 ± 0.04.

The feed conversion ratio (FCR) in fish typically does not exceed 1.5. However, in this study, the observed FCR of less than 1 (Figure 3) suggests that transgenic catfish exhibit a significantly higher feed utilization efficiency for growth. This enhancement in FCR for transgenic catfish and atlantic salmon (*Salmo salar*) is attributed to the overexpression of exogenous growth hormone (GH) genes [17, 18]. The expression of the exogenous GH (*CgGH*) in transgenic fish is known to result in an FCR of less than 1.0, leading to reduced feed consumption. Furthermore, the exogenous GH facilitates the conversion of fats and carbohydrates into metabolic energy, thereby contributing to the improved feed efficiency observed in transgenic fish [19].

Increasing the density of G6 transgenic mutiara catfish seeds (70 fish/60 L) in the maintenance medium triggers a protein sparing effect due to the influence of feed consumption competition and leads to changes in carbohydrates or fat feed into protein. This effect greatly affects the efficiency of feed use and as a saving on feed savings and improving FCR to 0.66 [6, 20]

3.1.3 Survival Rate

The percentage of survival rate value of each treatment during 56 days of maintenance was statistically described using ANOVA. The results of the analysis showed the survival rate presented in Figure 4.

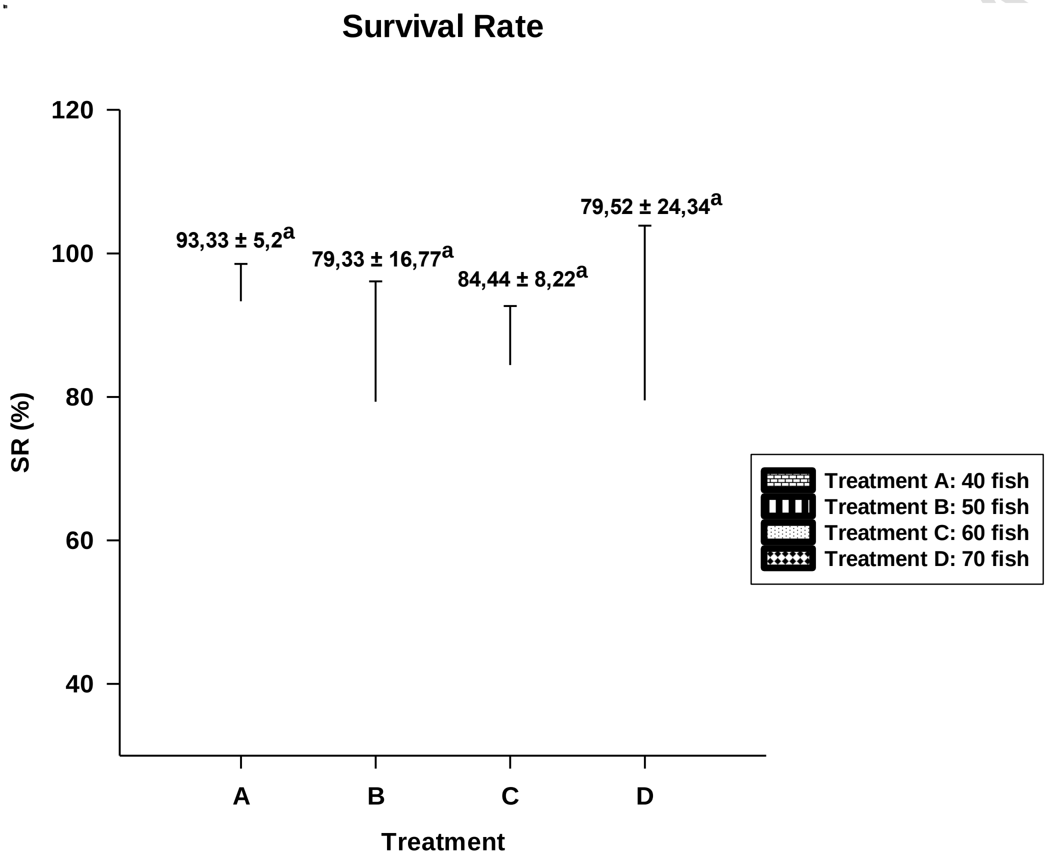

Fig. 4. Survival Rate Value.
The mean value followed by SD with different letter notation, shows significance ($p<0.05$)

The results of observations for 56 days on transgenic mutiara catfish G6 maintained in budikdamber media with different density levels resulted in the highest percentage value obtained in treatment A with a value of 93.33% while the lowest value was obtained in treatment B, which was 79.33%. Treatments C and D each had percentage values of 84.44% and 79.52%.

Based on the results of observations in treatments B and D, the survival rate was lower than treatments A and C. The number of mortalities in the study was caused by cannibalism. In treatments B and D, there was one replication that was suspected that not all fish received the same amount of feed, resulting in differences in size, the limited feed given triggered predatory behavior in fish with larger sizes because they needed more food. Several studies have shown that increasing density can cause cannibalism in fish, including Seatrout (*Cynoscion nebulosus*) [21], catfish larvae [22], European catfish [23] and african catfish fry [24].

3.2 Water Quality

Water quality measurements were carried out by measuring temperature, pH and dissolved oxygen (DO) parameters as supporting data in the maintenance and sustainability of G6 transgenic mutiara catfish fingerling. The average value of water quality can be seen in Table 2.

Table 2. Observation Water Quality Result and Standard for Mutiara Catfish water quality

Water Quality	Result	Standard	
Temperature (°C)	27-30	15-35	
pH	6,3-7,4	5-10	[25]
DO (mg/L)	3,4-6,6	> 0	

Water Quality has an important integral role in aquaculture [26]. At high density levels, survival rates will be affected, this is due to a decrease in water quality due to increased biomass and stress, which has a negative impact on fish survival [27]. The results of water quality observations during the study (Table 2) shown good conditions for the growth of transgenic mutiara catfish. Catfish has remarkable adaptability to environmental conditions that would be lethal for other fish species [28]. However, it's important to pay attention to ammonia levels. Elevated ammonia in the cultivation environment can be harmful to fish. The amount of ammonia is influenced by the quantity and protein content of the feed provided [29. Water exchange can reduce the ammonia content in cultivication media [30].

4. CONCLUSION

The stocking density of 70 fish/60 L produced average biomass weight gain (2152.43 g), FCR (0.66) and survival rate (79.52%) to support the growth performance of G6 transgenic mutiara catfish seeds.

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