Original Research Article

Growth Performance in Transgenic Mutiara Catfish using Low Protein Feed

ABSTRACT

This research was conducted at the Hatchery Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The implementation starts from November to December 2022. The aim of this experiment is to test the growth performance of the G5 transgenic Mutiara catfish by providing low protein feed. The treatment design used the RAL method with 4 treatments (A:39% feed protein; B:31% feed protein; C:14% feed protein for transgenic catfish and D:39% feed protein for non transgenic fish) with 3 replications. . Data were analyzed using Sigmaplot 15.0 for test parameters Absolute weight gain (Wg), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein retention (PR) and using the Duncan Multiple Range Test (DMRT) with a 95% confidence level . The best treatment results for feed efficiency and protein efficiency were obtained in treatments A and B with values of 1.25 and 1.43 as well as 2.04 and 2.23. Transgenic Pearl catfish can grow normally on low protein diet is a new finding from this study.

Keywords: Transgenic Fish, Absolute Growth. FCR. RP, PER

1. INTRODUCTION

Growth in fish is influenced by external and internal factors. External factors that affect growth are feed quality (nutritional balance) and environment (dissolved oxygen, temperature, pH, and ammonia) [1], while internal factors are related to the condition of the fish such as genetic factors, age, sex and disease resistance This factor will later describe the growth rate of fish.

One of the factors that affect growth in fish is the quality of feed. This can affect the increase in growth performance as one of the important factors for the success of aquaculture business. Slow growth rates can reduce the productivity of aquaculture caused by the relatively long maintenance time and the large production costs that must be incurred [2]. Growth performance in fish is not only influenced by external factors such as the feed given, but there are internal factors derived from the fish's own genes, one of which is the growth gene. Each fish has a growth hormone (*growth hormone*) which is essential and serves to stimulate the process of protein synthesis [3]. Differences in protein levels in feed will certainly result in different fish growth performance. The minimum requirement of fish for protein to meet normal growth*catfish* by 20% [4].

The Mutiara Catfish is a fish resulting from fish breeding research carried out by the Sukamandi Fish Breeding Research Institute (BPPI) in 2014. This fish shows an increase in weight gain of up to 21% compared to other types of catfish [5]. However, this high growth rate cannot be maintained in the next generation, to increase the growth performance again by utilizing the transgenesis process.

The use of transgenesis technology has been widely carried out to improve fish growth performance, one of the studies is to insert a growth hormone gene (*growth hormone*) from Dumbo catfish (CgGH,*Clarias gariepinus Growth Hormone*) which was inserted into the sperm of male mutiara catfish [6]. The inserted GH gene from the Dumbo catfish will express increased growth compared to the non-transgenic catfish. The use of this technology has produced G0 transgenic Mutiara catfish through transgenesis technology with a growth increase of two and a half times (125%) compared to non-transgenic catfish. The production of each generation of transgenic Mutiara catfish followed the breeding scheme in Figure 1 where CgGH transmission reached 70% in G3 and 74% in G4 [7] and G5 production was through crosses between G4 transgenic fish.

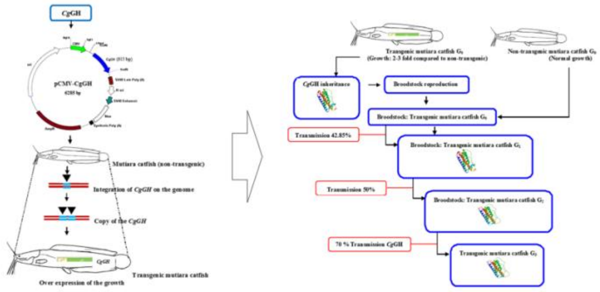

Figure 1 Breeding Scheme of Transgenic Mutiara Catfish Production [7]

The process of transgenesis technology basically transfers certain superior genes to related fish. Several other advantages produced by transgenic Mutiara catfish include stress-resistant fish, high appetite, adaptability to natural and artificial feeds, so that their growth rate is faster than non-transgenic Mutiara catfish.

With the superiority of transgenic fish which have super growth characters, it is necessary to test using low protein feed. The results of previous studies showed that channel catfish (*Spotted ictalurus*) which was tested with 16% protein feed resulted in normal growth during a maintenance period of 155 days [8]. There are no studies that have tested feeds with a protein content below 16%. The use of commercial feed tests that have different protein content, namely Prima feed (39% protein), Hi Pro Vite (31% protein), and Supra (14% protein) needs to be applied to evaluate the performance of this growth character, whether it can still grow normally or not. To test the growth performance of G5 transgenic Mutiara catfish, a test was carried out using commercial feed containing different levels of protein, and the results can be represented by the absolute growth value, FCR (Feed conversion ratio), protein efficiency ratio and protein retention. The presence of CgGH inserts in transgenic catfish fed low protein diets is expected to support normal fish growth during 42 days of rearing.

2. MATERIALS AND METHODS

This research was carried out in November - December 2022, at the Hatchery Building 4 of the Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. The tools used in this research are: Aquarium, aerator installation, heater, DO meter, ph meter, thermometer, digital scale, filter, label paper, ruler, and siphon tool. The materials used in the research were the seeds of the transgenic Mutiara catfish and the Sangkuriang catfish measuring 3.5-5 cm (1 month old), commercial feed (Prima Feed-1000, Hi ProVite 781-2, and Supra).

2.1 Research Design

The research was carried out experimentally with four treatments and three replications, the treatments given were: commercial feed Prima Feed 1000 feed protein 39% (A), commercial feed Hi ProVite 781-2 protein feed 31% (B), commercial feed Supra protein feed 14 % (C). and commercial feed Prima Feed 1000 protein feed 39% (D). Feed is given 5% of the total weight of biomass and given twice a day. The research design used was Completely Randomized Design (CRD).

2.1.1 Preparation for implementation

Fill each aquarium with a water level of ± 30 cm, followed by setting up the aeration installation and installation*heater*, then put 10 test fish into each aquarium and the aquarium was given a cover in the form of a net and labeled according to the treatment.

2.1.2 Implementation of research

The study was conducted for 42 days and observations were made every 7 days, each aquarium was filled with 10 fish and the initial weight was measured before being put into the aquarium, feed was given 2 times a day, at 09.00 and 16.00 WIB. Water quality measurements are carried out every 7 days including pH, temperature, and DO (dissolved oxygen) parameters. Parameters observed to assess growth performance and feed efficiency are as follows:

a. Absolute Growth

Absolute growth was measured by calculating the body weight of the fish and measuring the body length of the fish every 7 days. The absolute growth calculation was carried out using the formula for absolute weight growth and absolute length growth, namely [9].

$$\Delta W = Wt - W_0$$

Information:
ΔW = Growth in absolute weight (g)
Wt = Fish weight at the end of rearing (g)
IN_0 = Initial fish weight rearing (g)

Absolute length growth can be calculated using the formula:

$$\Delta L = Lt - L_0$$

Information:
ΔL = absolute length growth (cm)
Lt = Length of fish at the end of rearing (cm)
L_0 = initial fish length (cm)

b. Feed Conversion Ratio (FCR)

The feed conversion ratio is measured by calculating the total feed consumption during maintenance divided by the total weight gain at the end of the study (if any die, the final weight is added) using the feed conversion ratio formula, namely [10].

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

Information:
FCR = Feed Conversion Ratio
F = Amount of food eaten by fish
Wt = Final weight of fish (g)
IN_0 = initial weight of fish (g)
D = Weight of dead fish (g)

c. Protein retention

Protein retention can be determined by analyzing the proximate protein body of fish at the beginning and end of rearing and dividing it by the amount of protein consumed during rearing. The protein retention formula is as follows [11] :

$$PR = \frac{(Fp - Lp)}{P} x\ 100\%$$

Information:
PR = Protein retention
Fp =Amount of fish body protein at the beginning of rearing (g)
Lp =Amount of body protein at the end of maintenance (g)
P =Total protein consumption during rearing

d. Protein Efficiency Ratio

The protein efficiency ratio can be determined by comparing the weight gain with the amount of feed protein consumed during rearing. The protein efficiency ratio formula is as follows [12] :

$$PER = \frac{Wt - Wo}{P}$$

Information:
PER =Protein efficiency ratio
Wt = Total final weight during the study (g)
Where = Total initial weight during the study (g)
P =Amount of protein consumption during maintenance (g)

e. Water Quality

During maintenance, water quality measurements were carried out, namely the degree of acidity (pH), dissolved oxygen (DO) and temperature. These measurements are carried out every 7 days.

2.2 Data Analysis

The data obtained were analyzed with *Analysis Of Variance* (ANOVA) with a confidence level of 95% and if there is a difference in reality will be continued with the test *Duncan Multiple Range Test* (DMRT) using software *Sigmaplot 15.0.*

3. RESULTS AND DISCUSSION

3.1 Absolute Growth

The results of the ANOVA test showed that the growth in absolute weight and length through feeding with different protein content had a significant effect on the weight and length gain of the Transgenic Mutiara catfish. The absolute weight and length growth values can be showed in Figure 2 and Figure 3.

3.1.1 Absolute weight gain

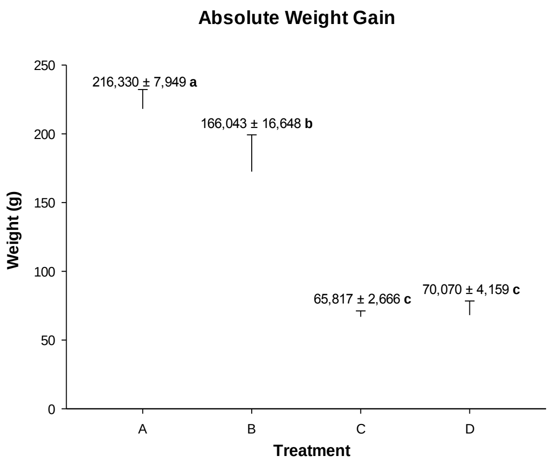

Figure 2. Absolute Weight gain

In Figure 2. Based on the results of Duncan's advanced test, treatment C gave results that were not significantly different from treatment D, but significantly different from treatments A and B. The absolute weight growth values for all treatments averaged 65.81-216.33 g.

The absolute weight growth rate is the total increase in fish biomass/weight during rearing expressed in g. Based on the results of the research, it showed an increase in catfish weight in each treatment. The highest absolute weight value was obtained in treatment A (39% protein), while the lowest absolute weight value was in treatment C (14% protein). Differences in protein content of all treatments gave significantly different results from one another. Factors affecting the high absolute weight value in treatment A were due to the protein content of the feed and supporting genetic factors in transgenic mutiara fish, where this could meet the fish's need for protein and maximize the nutrition received. In treatment C, the provision of low protein feed to the Transgenic Mutiara catfish gave growth results that were not significantly different from the Sangkuriang catfish in treatment D. This was due to the expression of endogenous GH and the addition of exogenous GH in the form of*Cg*The GH added causes higher growth compared to the Sangkuriang catfish which only has one GH. This exogenous GH causes growth stimulation and additional protein synthesis in transgenic fish, so that the utilization of feed for growth is higher [13].

3.1.2 Absolute length growth

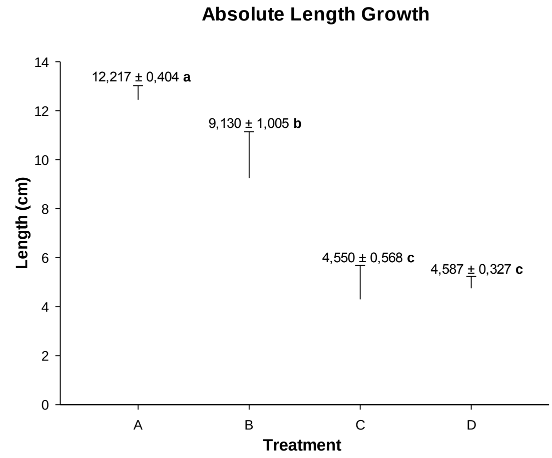

Figure 3 Absolute length growth

In Figure 3, based on the results of Duncan's advanced test, treatment C gave results that were not significantly different from treatment D, but significantly different from treatments A and B. The absolute length growth values for all treatments averaged 4.5-12.2 cm.

The absolute length growth rate is the increase in the total length of fish during rearing expressed in cm. The results showed that the highest absolute length value was obtained in treatment A, which was 12.2 cm, and the lowest was in treatment C, which was 4.5 cm. Based on Duncan's further test results, the absolute length value for treatment C was not significantly different from treatment D but significantly different from treatments A and B. This shows that the use of feed with different protein content can affect the growth rate of absolute length in fish. Body length growth in fish can be influenced by the genetics of each individual as well as the protein intake used to support growth obtained from feed [14]. In treatment C, namely transgenic Mutiara catfish with low protein content feed resulted in length gain which was not significantly different from Sangkuriang catfish.

3.2 Feed Conversion Ratio (FCR)

The results of the ANOVA test showed that feeding with different levels of protein had a significant effect on the FCR of Transgenic Mutiara catfish and Sangkuriang catfish. The FCR values for each treatment can be seen in Figure 4.

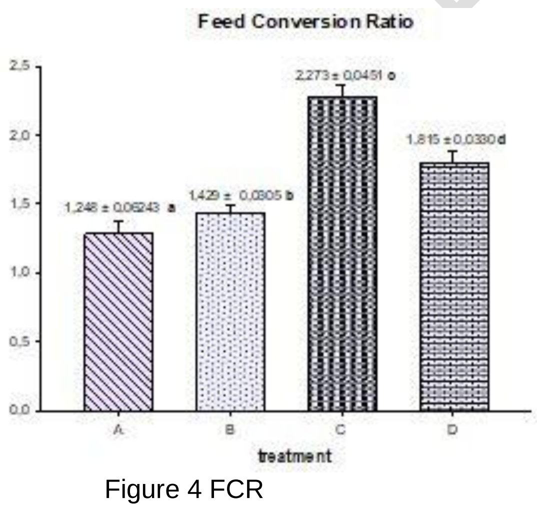

Figure 4 FCR

In Figure 4. Based on the results of Duncan's advanced test, all treatments gave significantly different results between one treatment and another. The average FCR value for all treatments ranged from 1.25 to 2.27.

FCR is the ratio between the amount of feed given and the amount of weight produced or measures how efficiently a feed can be converted into growth. The lower the feed conversion value indicates the more efficient use of feed, and vice versa. The value of the feed conversion ratio is determined by the ability of the fish to digest the nutrients in the feed and minimize the nutrients being wasted in the feces.

Treatment A (39% feed protein) was the lowest with an FCR value of 1.24 and the highest was treatment C with a value of 2.27. Treatment C resulted in a high FCR value because the feed used in treatment C was low protein feed (14% protein), while the fish needed minimal protein to meet normal growth.*catfish* by 20% [15]). When viewed further, the high FCR in treatment C resulted in an absolute body weight growth which was not significantly different from treatment D. This illustrates that if the transgenic mutiara catfish were fed with low protein feed, the average weight growth was not much or could be offset by the treatment. D where non-transgenic catfish were given high protein feed (39% protein) with an FCR value of 1.81.

The low value of the feed conversion ratio in treatment A was caused by the faster growth of the fish and indicated that the transgenic Mutiara catfish were optimal in digesting and absorbing the nutrition of the feed given during rearing, so that it can convert the feed into meat optimally. This was also due to genetic improvements in growth performance and feed conversion in the transgenic Mutiara catfish, because the transgenic Mutiara catfish contained exogenous GH (CgGH) inserts. On the other hand, the Sangkuriang catfish does not contain exogenous GH. The lower the value of the feed conversion ratio produced, the better the fish for cultivation, because a low feed conversion ratio value can help reduce feed production costs. Fish that have the ability to properly digest nutrients from feed will have faster growth [16].

3.3 Protein Retention

The results of the ANOVA test showed that feeding with different levels of protein had a significant effect on the protein retention values of the transgenic Mutiara catfish and Sangkuriang catfish. The protein retention values of each treatment can be seen in Figure 5.

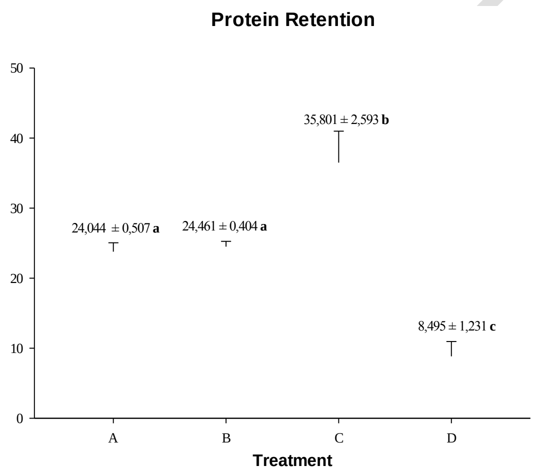

Figure 5 Protein Retention

Protein retention is used as a parameter that can describe the effectiveness of using feed protein to be converted into growth and is obtained by analyzing fish protein content at the beginning and end of the study [17]. Based on the results of Duncan's further test, all treatments gave significantly different results between one treatment and another (Figure 5). The average protein retention value in all treatments ranged from 8.49 to 35.80.

Based on the results of the comparative test results of the data analysis performed, the C treatment using Supra feed (14% protein) in the transgenic Mutiara catfish had the highest protein retention value of 35.80%, because the feed protein contained in the C treatment was low (this because the amount of feed consumption on protein feed consumed is low) so that the transgenic Mutiara catfish dismantles a lot of non-protein energy sources (fats and carbohydrates). This also does not reflect that treatment C has a higher weight growth than the other treatments (Figure 2). In treatments A and B, there were differences in protein retention results produced in transgenic Mutiara catfish, this was due to differences in protein content in the feed which caused differences in protein retention values in treatments A and B with treatment C. To calculate retention, namely by comparison between the increase in body protein divided by the protein consumed. The high value of protein retention in treatment C, is caused by the feed protein content which is below 20%, this illustrates that the feed protein absorbed by fish is lower than treatment A (39% feed protein) and B (31% feed protein). Protein retention values in treatments A and B were above 20%, namely 24.04% and 24.46%. This is supported by research results which state that using feed with a protein content of 38-40% results in protein retention of at least 20% [18]. This shows that the non-protein energy in the feed at the protein content is available in sufficient quantities and in a balanced ratio so that most of the feed protein can be used for growth. The balance of the protein-energy ratio will encourage fish to use fat and carbohydrates as non-protein energy sources [19]. If fish lack protein obtained from feed, fish tend to convert non-protein nutrients (carbohydrates and fats) into protein, where fat is stored in the form of triglycerides, while carbohydrates are stored in the form of glycogen in the body [20].

3.4 Protein Efficiency Ratio

The results of the ANOVA test showed that feeding with different levels of protein had a significant effect on the protein retention value of the transgenic Mutiara catfish and Sangkuriang catfish. The protein efficiency ratio values in each treatment can be seen in Figure 6.

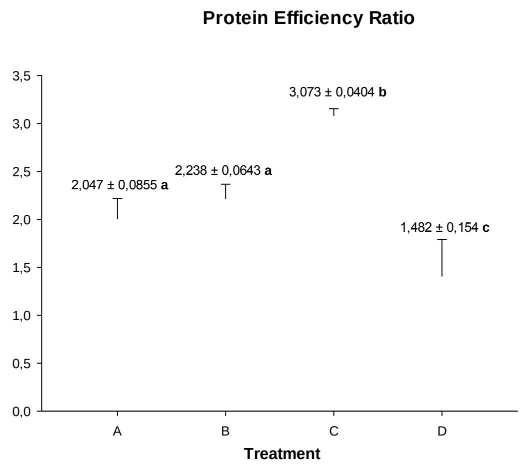

Figure 6 PER

In Figure 6. Based on the results of Duncan's advanced test, all treatments gave significantly different results between one treatment and another. The protein efficiency ratio value in all treatments averaged from 1.48 to 3.07. Based on the test results between treatments after rearing, the transgenic Mutiara catfish in treatment C had the highest protein efficiency ratio of 3.07 and the lowest in Sangkuriang catfish treatment D with feed protein of 39% (control) of 1.48. Meanwhile, the transgenic mutiara catfish in treatment A with 39% feed protein and treatment B with 31% feed protein were 2.04 and 2.23, respectively.

In treatment C, the protein efficiency ratio was high, not reflecting that treatment C had good efficiency, because in treatment C the feed protein content was low (14%), only giving an average weight gain of 65.81 g (Figure 2). lower when compared to the weight gain in treatments A and B (216.33 g and 166.04 g). Because the fish treated with C were transgenic fish containing CgGH, they still grew normally even though they were fed with 14% protein. This is a compensation for transgenic catfish being able to convert feed carbohydrate nutrients into protein to cover the lack of feed protein [21].

3.5 Water quality

Water quality plays an important role in supporting the survival of catfish. The water quality measured during the 42 days of the study was temperature, pH, and dissolved oxygen (DO) levels. The average value of water quality can be seen in Table 1.

Table 1 Water Quality

Water quality	Observation result
Temperature (^{O}C)	28 – 32
pH	6,9 – 7,3
DO (mg/L)	3,8 – 4,3

Temperature is very influential in the process of fish growth. As the water temperature increases, the fish appetite also increases. The temperature values obtained during the study ranged from 28-32^{O}C. The temperature values obtained during the study were within the normal range for the life of catfish fry. The results of other studies are good for supporting catfish survival, which ranges from 25-32°C [22]. The degree of acidity (pH) is the ability of a waters to produce mineral salts, if the value is not in accordance with the needs of the organisms being maintained, this will have a negative impact on these organisms. The results showed that the average pH value for each treatment ranged from 6.9 to 7.3. The value of the degree of acidity obtained is categorized as good for the growth of catfish. The optimal pH value for the growth and survival of catfish (Clarias sp.) is in the range of 6.5-8 [23]. Besides temperature and degree of acidity, dissolved oxygen (DO) is a water quality parameter that is no less important in supporting fish growth. Dissolved oxygen is needed for respiration and metabolism as well as survival in an organism. The results of the study obtained dissolved oxygen values ranging from 3.8

to 4.4 mg/l. The results of these measurements indicate that the quality of the water used during the study is feasible for catfish farming because it is within the range recommended by SNI, namely DO> 2 mg/l [23].

4. CONCLUSION

The use of Supra commercial feed for transgenic Mutiara catfish provides the same growth or offsets normal growth with the use of Prima Feed commercial feed for Sangkuriang catfish. Transgenic mutiara catfish on commercial feed with feed protein content of 39% (treatment A) gave the best absolute weight growth and feed conversion ratio.

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