Asian Journal of Biotechnology and Bioresource Technology

Volume 9, Issue 3, Page 10-19, 2023; Article no.AJB2T.105111
ISSN: 2457-0125

Growth Performance in Sangkuriang Catfish (*Clarias gariepinus Var. Sangkuriang*) and Transgenic Mutiara Catfish (*Clarias gariepinus*) using Low Protein Feed

Ibnu D. Buwono [a*], Asep A. H. S. [a], Iskandar [a] and Azahra I. [a]

[a] *Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Indonesia.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJB2T/2023/v9i3184

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105111

Original Research Article

Received: 24/06/2023
Accepted: 28/08/2023
Published: 06/09/2023

ABSTRACT

This research was conducted at the hatchery building 4, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. The implementation starts from November to December 2022. This experiment aims to test the growth performance of the G5 transgenic mutiara catfish by providing low protein feed. The treatment design used the completely randomized design method with four 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 as comparison) with three replications. Data were

**Corresponding author: E-mail: ibnu.dwi.buwono@unpad.ac.id;*

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) at 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 mutiara catfish can grow on a low protein diet is a new finding from this study.

Keywords: Absolute growth; FCR; PER; PR; transgenic fish.

1. INTRODUCTION

Fish growth 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 genetic factor will later describe the growth rate of fish.

Quality of feed 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 (GH) 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 protein requirement for catfish feed to support fish growth is at least 20% [4].

The mutiara catfish is a fish resulting from fish breeding research carried out by the Sukamandi Fish Breeding Research Institute (SFBRI) 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. Considering that the mutiara catfish was formed from a hybrid cross of four catfish strains (Paiton, Egypt, Dumbo, Sangkuriang), its growth tends to be unstable and is maintained in the next generation [6].

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 [7]. The inserted GH gene from the dumbo catfish will express increased growth 2-3 fold compared to the non-transgenic catfish in G0. The use of this technology has produced G1, G2, G3, G4 transgenic mutiara catfish through transgenesis technology with a *CgGH* transmission 42.85% (G1), 50% (G2), 70% (G3) and 74% (G4). The production of each generation of transgenic mutiara catfish followed the breeding scheme in Fig. 1. where *CgGH* transmission reached 70% in G3 [8] and 74% in G4 [9] and G5 production was through crosses between G4 transgenic fish.

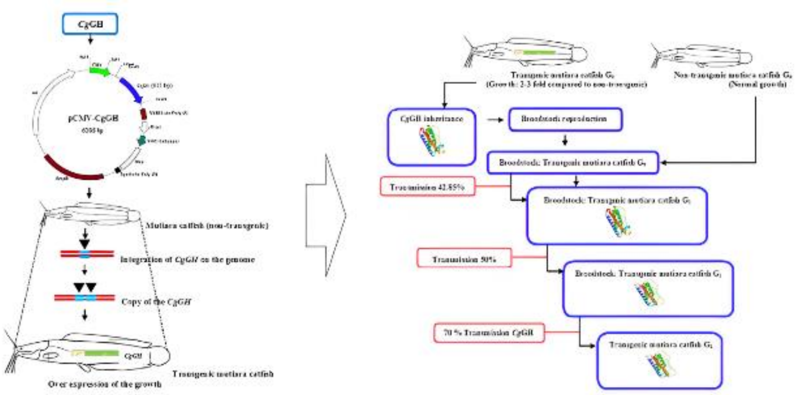

Fig. 1. Breeding scheme of G3 transgenic mutiara catfish production [8,9]

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, and 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 (*Ictalurus punctatus*) which was tested with 16% protein feed resulted in normal growth during a maintenance period of 155 days [10]. 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 ProVite (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 low protein, and the results can be represented by the absolute growth value, 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 (70 x 50 x 60 cm), aerator installation, heater (Atman), DO meter (Lutron), pH meter (Mediatec), thermometer (Lutron), digital scale (Ohaus). The materials used in the research were the seeds of the transgenic mutiara catfish and the sangkuriang catfish as a comparison measuring 3.5-5.0 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 (treatment A, feed protein 39%), commercial feed Hi ProVite 781-2 (treatment B, feed protein 31% B), commercial feed Supra (treatment C, feed protein 14 %) for transgenic catfish and commercial feed Prima Feed 1000 (treatment D, feed protein 39%) for non transgenic catfish. Feed formulations A and D are the same, only feed D is given to non-transgenic fish to test whether the growth of G5 transgenic mutiara catfish is higher than non-transgenic fish when fed with the same protein. 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

Each aquarium was filled with water up to a level of ± 30 cm, followed by setting up the aeration installation and heater, then put 10 test fish into each aquarium and the aquarium was covered with a net and 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 two times a day, at 09.00 and 16.00 WIB. Water quality measurements are carried out every days including pH, temperature, and DO (dissolved oxygen) parameters. Parameters observed to assess growth performance and feed efficiency are as follows:

2.1.2.1 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 [11].

$$\Delta W = Wt - W_0$$

Information:

ΔW = Growth in absolute weight (g)
Wt = Fish weight at the end of rearing (g)
W_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)

2.1.2.2 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 [12].

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

Information:

FCR = Feed Conversion Ratio
F = Amount of food eaten by fish
Wt = Final weight of fish (g)
W_0 = initial weight of fish (g)
D = Weight of dead fish (g)

2.1.2.3 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 [13]:

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

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

2.1.2.4 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 [14]:

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

Information:

PER =Protein efficiency ratio
Wt = Total final weight during the study (g)
W_0 = Total initial weight during the study (g)
P = Amount of protein consumption during maintenance (g)

2.1.2.5 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 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 significant difference, further analysis will be done with the Duncan Multiple Range Test (DMRT) using software *Sigmaplot 15.0.*

3. RESULTS AND DISCUSSION

3.1 Performance Growth

The results of the observations weekly weight average showed that the growth in average 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 average weight and length growth values can be showed in Fig. 2 and Fig. 3.

3.1.1 Average weight gain

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 (Fig. 2). The average weight growth values for all treatments averaged 65.81-216.33 g.

Observations weekly on the results of the research, it showed an increase in catfish weight in each treatment. The highest average weight value was obtained in treatment A (39% protein), while the lowest average 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 average 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 te *CgGH* added causes higher growth compared to the sangkuriang catfish which only has one GH [15]. This exogenous GH causes growth stimulation and additional protein synthesis in transgenic fish, so that the utilization of feed for growth is higher [16].

3.1.2 Absolute length growth

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 (Fig. 3). The absolute length growth values for all treatments averaged 4.5-12.2 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. There was no difference in absolute length values between treatments C and D, but significantly different from treatments A and B based on Duncan's test.

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 [17]. 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 (non transgenic catfish). The FCR values for each treatment can be seen in Fig. 4.

The FCR value of G5 fish in all treatments was significantly different based on Duncan's test (Fig. 4). 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.

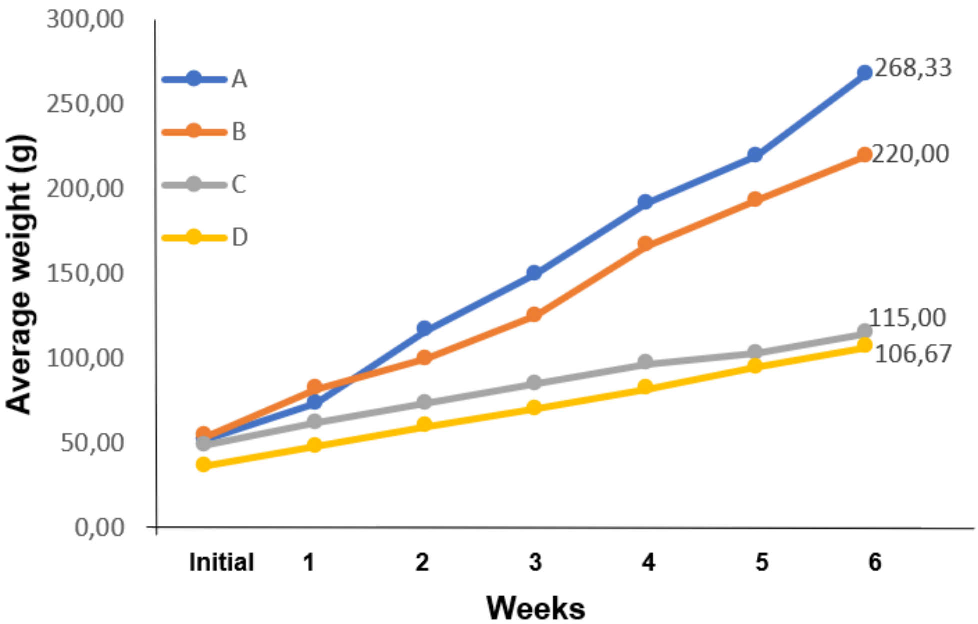

Fig. 2. Average weight gain during experiment (weekly observations)
Feeds A, B, C (protein 39%, 31%, 14%) were fed to transgenic catfish, while feed D (protein 39%) was fed to non-transgenic catfish

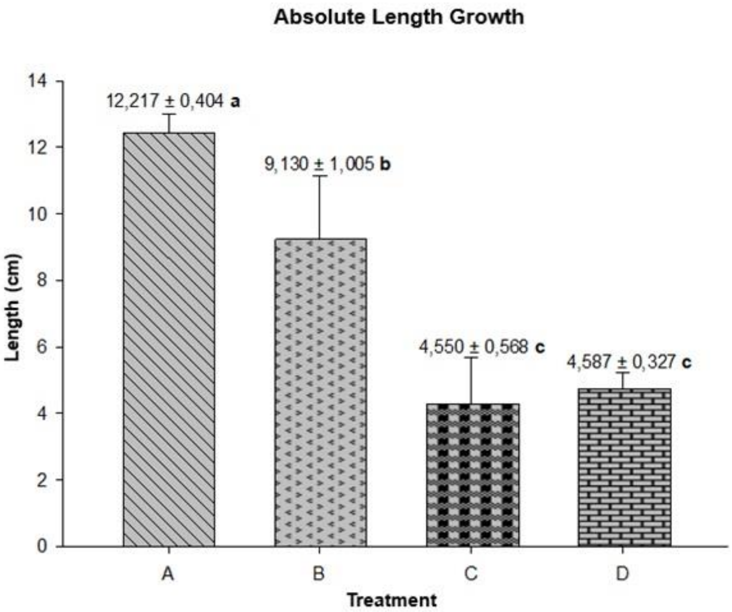

Fig. 3. Absolute length growth of G5 mutiara transgenic catfish (treatment A-C) and sangkuriang catfish) (treatment D)

Data are presented as means ± SD. Across rows, means followed by the same letter are not significantly different (p ≥ 0.05). Feeds A, B, C (protein 39%, 31%, 14%) were fed to transgenic catfish, while feed D (protein 39%) was fed to non-transgenic catfish

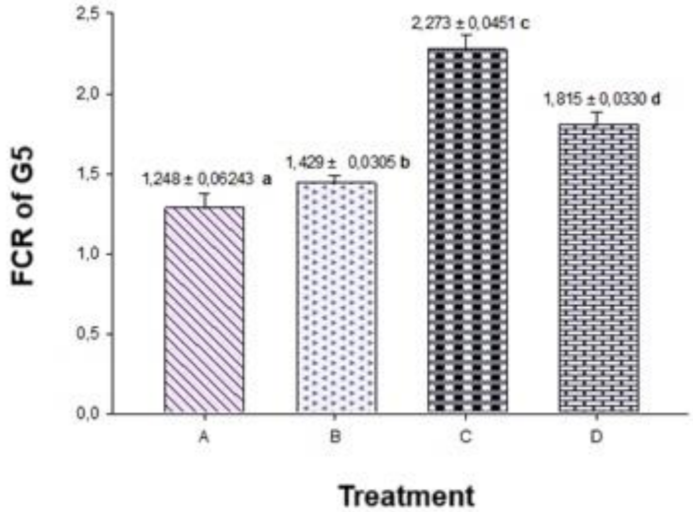

Fig. 4. Feed conversion ratio of G5 transgenic mutiara catfish (feed A-C) and non transgenic catfish (feed D)

Data are presented as means ± SD. Across rows, means followed by the same letter are not significantly different (p ≥ 0.05). Feeds A, B, C (protein 39%, 31%, 14%) were fed to transgenic catfish, while feed D (protein 39%) was fed to non transgenic catfish

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. The increase in the FCR value in treatment C was affected by the low protein content of the feed (14%). As compensation, fish consume more feed, to overcome the lack of feed protein to support their growth, which requires a minimum of 20% protein feed for fish growth needs [4]. When viewed further, the high FCR in treatment C resulted in an average weight gain at the end of the study (115.00 g) which was not significantly different from treatment D (106.67 g) but much different than treatment A (268.33 g) (Fig. 2). This illustrates that the transgenic mutiara catfish fed a low-protein diet (treatment C) gained on average less body weight than those fed a high-protein diet (treatment A).

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 [18].

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 Fig. 5.

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 in fish followed the methods of AOAC [19,20]. Based on the results of Duncan's further test, all treatments gave significantly different results between one treatment and another (Fig. 5). The average protein retention value in all treatments ranged from 8.49 to 35.80.

The low protein feed in treatment C (protein 14%) caused the amount of feed consumed by fish to increase and resulted in the amount of feed protein consumed also increasing. Because the protein content of feed C was low, and the amount of feed protein consumed increased, it resulted in a high protein retention value for treatment C (Fig. 5) 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 (Fig. 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. 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% [8]. 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 [21]. The suggested effect of GH (include CgGH in treatment C) was its importance in affecting the breakdown of carbohydrates and lipids through a complex enzymatic pathways into pyruvic acid and then converted to amino acids into proteins [22].

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 efficiency ratio of the transgenic mutiara catfish and sangkuriang catfish. The protein efficiency ratio values in each treatment can be seen in Fig. 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 (Fig. 6). 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% (comparison) 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 115.00 g (Fig. 2). lower when compared to the average weight gain in treatments A and B (268.33 g and 220.00 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. Through the metabolic pathways in the Krebs cycle, carbohydrates (in the form of glycogen) are converted into glucose and then converted into phosphoglyceral dehyde, then converted into pyruvic acid and converted into amino acids which are formed into proteins [22].

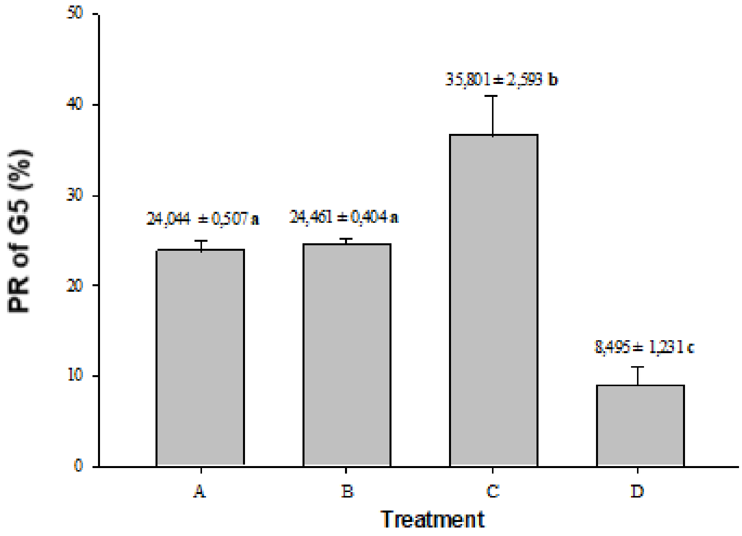

Fig. 5. Protein retention of G5

Data are presented as means ± SD. Across rows, means followed by the same letter are not significantly different (p ≥ 0.05). Feeds A, B, C (protein 39%, 31%, 14%) were fed to transgenic catfish, while feed D (protein 39%) was fed to non-transgenic catfish

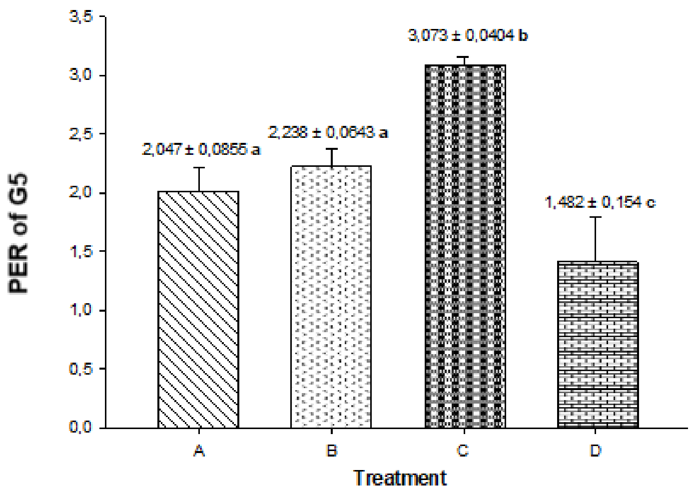

Fig. 6. Protein efficiency ratio of G5

Data are presented as means ± SD. Across rows, means followed by the same letter are not significantly different (p ≥ 0.05). Feeds A, B, C (protein 39%, 31%, 14%) were fed to transgenic catfish, while feed D (protein 39%) was fed to non-transgenic catfish

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 during rearing 42 days

Water quality	Observation result
Temperature (°C)	28 – 30
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 28-30ºC. 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 25-30ºC [23]. 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 [24]. 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 Indonesian National Standard (INS), namely DO> 2 mg/l [24].

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. The use of commercial feed containing 14% protein (treatment C) for transgenic mutiara catfish provided the same growth or compensating for normal growth with the use of commercial feed containing 39% protein for non-transgenic catfish (sangkuriang catfish).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Effendi MI. Fisheries biology method. Dewi Sri Foundation. Bogor; 1979.
2. Iswanto B, Suprapto R, Marnis H, Imron I. Reproductive performance of mutiara catfish (*Clarias gariepinus*). Aquaculture Media. 2016;11(1):1-9.
3. Widiyanto W. Exercise and growth hormone secretion. Medikora. 2007;3(2): 173-188.
4. Jayant M, Muralidhar AP, Sahu NP, Jain KK, Pal AK, Srivastava PP. Protein requirement of juvenile striped catfish, *Pangasianodon hypophthalmus*. Aquaculture International. 2018;26:375-389.
5. Iswanto B, Imron I, Suprapto R, Marnis H. Assembly of catfish strains *(Clarias gariepinus)* grow rapidly through individual selection: formation of the first generation population. Aquaculture Research Journal. 2014;9(3):343-352.
6. Scribner KT, Page KS, Bartron, ML. Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. Reviews in Fish Biology and Fisheries. 2000;10(3):293-323.
7. Buwono ID, Iskandar MUK, Agung U. Subhan. Catfish assembly *(Clarias Sp.)*transgenic with sperm electroporation technique. Journal of Biology. 2016;20(1): 17-28.
8. Buwono ID, Iskandar I, Grandiosa R. Growth hormone transgenesis and feed composition infuence growth and protein and amino acid content in transgenic G3 mutiara catfsh (*Clarias gariepinus*). Aquaculture International. 2021;29:431–451.
9. Buwono ID, Iskandar I, Grandiosa R. CgGH and IGF-1 expression level and growth response of G4 transgenic mutiara strain catfish (*Clarias gariepinus*) reared at different stocking densities. Aquaculture International. 2023; 31(2):827-846.

10. Robinson EH, MH Li. Low protein diets for channel catfish *(Ictalurus punctatus)* raised in earthen ponds at high density 1. Journal of the World Aquaculture Society. 1997; 28(3):224-229.
11. Hidayat D, Sasanti AD. Survival, growth and feeding efficiency of cork fish *(Streaked channa)* given feed made from golden snail flour *(Pomacea sp)*. Journal of Indonesian Swamp Aquaculture. 2013; 1(2):161-172.
12. Djarijah AS. Natural feed. Canisius, Yogyakarta. 1995;87.
13. Takeuchi T. Laboratory work – Chemical evaluation of Dietary nutrients. In: Watanabe, T. (Ed). Fish nutrition and Mariculture Jica textbook. The General Aquaculture Course. Kanagawa international Fisheries Training Centre. Japan international Cooperation Agency (JICA). 1988;233:179-233
14. Zeitoun IH, Ullrey DE, Mages WT. Quantifying nutrient requirement of fish. J. Fish Res. Board of Canada. 1976;33:167-172.
15. Buwono ID, Junianto J, Iskandar I, Alimuddin A. Growth and expression level of growth hormone in transgenic mutiara catfish second generation. Journal of Biotech Research [ISSN: 1944-3285]. 2019;10:102-109.
16. Laksana DP, Subaidah S, Junior MZ, Alimuddin Carman O. Growth of vaname prawn pascalarva given recombinant growth hormone solution. Indonesian Journal of Aquaculture. 2013;20(2):95-100.
17. Estriyani A. The effect of adding turmeric solution *(Turmeric longa)* on feed on the growth of dumbo catfish *(Clarias gariepinus)*. Thesis. Semarang: IKIP PGRI Semarang; 2013.
18. Mewakani S, Pasaribu H. Growth response of sangkuriang catfish seeds *(Clarias sp.)* Effects of addition of probiotics to commercial feed with different doses. TABURA Journal of Fisheries and Marine Sciences. 2019; 1(1):32–42.
19. Viola S, Rappaport. The extra caloric effect of oil in the nutrition of carp. Bamigdeh. 1979;31(3):51-69.
20. AOAC. The official methods of analyses 15th edn. Association of Official Analytical Chemists International. Arlington, Virginia, USA; 1990.
21. Hernandez MD, Egea MA, Rueda FM, Aguado F, Martinez FJ, Garcia B. Effects of commercial diets with different P/E ratios on l sharpsnout seabream *(Diplodus puntazzo)* growth and nutrient utilization. Aquaculture. 2001;195:321-329.
22. Melzer K. Carbohydrate and fat utilization during rest and physical activity. European e-Journal of Clinical Nutrition and Metabolism. 2011;6:45–52.
23. Anis MY, Dyah H. Commercial feeding with the addition of EM4 (Effective Microorganism 4) to increase the growth rate of catfish *(Clarias sp.).* Journal of Research in Biology and Its Applications. 2018;1(1):1–8
24. Indonesian National Standard (INS). Dumbo catfish *(Clarias sp).* National Standardization Body. Jakarta. SNI. 2014; 6484:3.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/105111