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Evaluation of the Growth of G6 Transgenic Mutiara Catfish (*Clarias gariepinus*) after Mixed Feeding of Snail Flour (*Achatina fulica*) and Commercial Feed

Ibnu D. Buwono [a]*, Roffi Grandiosa. H [a], Iskandar [a] and Putri Budi. S [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.

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ABSTRACT

This research was conducted in Hatchery Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The purpose of this experiment was to obtain the optimal ratio of snail flour mixture and commercial feed (Hi Pro Vite 781) to support growth, feed efficiency ratio, protein retention value, and carbohydrate retention in G6 transgenic mutiara catfish. The study was carried out experimentally with four treatments and three replicates (CRD), the treatment given was the

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

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ratio of commercial feed mixture: snail flour, namely A (60%: 40%), B (40%: 60%), C (20%: 80%) for G6 transgenic mutiara catfish and A* treatment (60%: 40%) for non-transgenic catfish (sangkuriang catfish as a control). Significant differences between treatments were tested using the Duncan Multiple Range Test (DMRT) with a confidence level of 95%. Data on absolute weight growth (Wg), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention, and carbohydrate retention were analyzed using Sigmaplot 15.0. The results showed that treatment B using 40% snail flour showed the highest body weight gain (402.357 g), lowest FCR (0.69), protein efficiency ratio (16.91%), best protein retention (53.01%) and lowest carbohydrate retention (11.32%) compared to other treatments. The best results in treatment B were due to Transgenesis-GH inducing *a protein sparing effect*, showing that the efficiency of feed use and protein use in transgenic G6 mutiara catfish increased the conversion of mixed feed carbohydrates of treatment B into protein. As a consequence, the growth of transgenic catfish is increased higher than that of non-transgenic fish, which is indicated by increased retention value and protein efficiency.

Keywords: G6 mutiara ransgenic catfish; snail meal and commercial feed; protein retention and efficiency; carbohydrate retention; absolute growth.

1. INTRODUCTION

The demand for catfish is increasing day by day. This needs to be accompanied by which fairly high production. One of the things that needs to be considered in the availability of catfish production is to accelerate fish growth. Growth in fish is not only influenced by external factors but also by internal factors [1]. Growth acceleration can be done in two ways, namely by genetic improvement and also increasing the daily protein content in feed. Genetic improvements for bes growth can be done by the gene transfer method. The transgenesis technology process basically transfers certain superior genes to related fish which have the advantage of a faster growth rate compared to non-transgenic catfish.

Growth is a complex process and involves a number of hormones, such as growth hormone. Which involves various factors, both endogenous and exogenous factors, including genetic factors, nutrition, hormones, cultivation techniques, pests and diseases, and the environment [2]. Apart from improving genetics, one of the factors to accelerate fish growth performance is by changing the feed formula needed by fish as a source of energy and growth. For several species of catfish, the ideal dietary protein needs have been established for a range of growth stages and environmental factors. Protein is one of the main substances that is important to achieve optimal growth [3]. In addition, protein is also a major source of energy for fish [4].

Fish need feed with good nutrient content for optimal growth, considering that the protein required for optimal fish growth ranges from 30-40% [5]. In addition to protein content, carbohydrates are needed, fat is a non-protein energy source [6]. One way to test the overexpression of the growth of transgenic fish is to conduct a growth test using a mixture of high-protein feed in commercial feed (pellets). As a mixed feed, snails contain 59.28% protein, 3.62% fat, and their continuous availability is ideal for use in alternative feeds to support the growth and production of catfish cultivation [7].

Evaluation of the growth of G6 transgenic fish (formed by the breeding method of brood stock breeding results between G5 transgenic mutiara catfish) using mixed snail can compliment the expression of CgGH hormone and produce the optimal efficiency of mixed feed use for fish growth [8]. The presence of exogenous growth hormone in transgenic fish can increase appetite, feed conversion, stimulate protein synthesis, or stimulate metabolism [9]. Transgenesis techniques are needed so that the production of catfish can be faster than non-transgenic catfish. The use of transgenic technology has been widely carried out to improve fish growth performance. The insertion of growth hormone from dumbo catfish (CgGH, *Clarias gariepinus Growth Hormone*) into mutiara catfish sperm can increase the growth of transgenic fish twice as to the growth of non-transgenic fish [10]. Therefore, a study is needed to determine the optimal ratio of snail flour addition for accelerating the growth of transgenic catfish. This will be evaluated by measuring weight gain (Wg), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention and carbohydrate retention.

2. MATERIALS AND METHODS

This research was carried out from May to August 2023, at the Hatchery 4 Building, Faculty of Fisheries and Marine Sciences, Padjadjaran University. Proximate analysis could be conducted for test feed at the Laboratory of Animal Nutrition and Ruminant Animal Food Chemistry, Faculty of Animal Husbandry, Padjadjaran University at the beginning and end of the study. Materials used in the study: juvenile G6 transgenic mutiara catfish and sangkuriang catfish measuring 6-9 cm, Hi Pro Vite 781 commercial feed with a protein content of 32%, snail (*Achatina fulica*) flour contain 59.28% protein, and *carboxymethyl cellulose* (CMC).

2.1 Research Design

The study was carried out experimentally with four treatments and three replicates (CRD), the treatment given was the ratio of commercial feed mixture: snail flour, namely A (60%: 40%), B (40%: 60%), C (20%: 80%) for G6 transgenic mutiara catfish and A* treatment (60%: 40%) for non-transgenic catfish (sangkuriang catfish as a control). Feed is given as much as 4% of the total biomass weight and is given twice a day. The aim of using transgenic fish is to evaluate fish growth when part of the commercial feed is replaced with snail meal.

2.1.1 Preparation for implementation

Each aquarium is filled with ± 15 L of water, followed by the installation of aeration and heating installations. Then 5 fish with a length of 6-9 cm are put into the aquarium and acclimatized first for 7 days in a fiber tub to adjust to the new environment, maintenance media, use of aeration and heater installations are set at a temperature of 28°C. During the acclimatization process and the fish are fed commercial feed (without treatment) with a frequency of feeding twice a day. Then the fish are weighted to determine the initial weight and the dose of feed to be given to the fish.

2.1.2 Implementation of research

The research was carried out for 60 days and fish growth sampling was conducted every week, including water quality measurements, weighing and measuring body length along with weighing the feed that will be given to adjust the amount of feed in the next maintenance period. Feed is given twice a day, namely at 07.00 a.m. and 05.00 p.m. The amount of feed given is 5% of the fish biomass. The observation parameters for protein growth and efficiency are as follows:

a. Average Weight Gain

Average absolute weight gain is measured by calculating the weight of fish every 7 days. The calculation of absolute weight gain is carried out using the formula for absolute weight gain [11].

$$AWG = Wt - Wo$$

Information:

AWG = average weight gain (g)
Wt = final fish weight after harvesting (g)
Wo = initial fish weight of the first stocking (g)
T = duration of fish reared (days)

b. Food Convertion Ratio

The feed conversion ratio is measured by calculating the amount of feed consumption during culture divided by the total weight gain after fish harvested (if any dead weight is added to the final weight) using the formula feed conversion ratio, which is [12]:

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

Information:

FCR = feed conversion rate
Wo = biomass weight of the test fish at the start of the study (g)
Wt = biomass weight of the test fish after fish harvested (g)
D = weight of dead fish (g)
F = weight of Feed given (g)

c. Protein Efficiency Ratio

The protein efficiency ratio can be determined by comparing weight gain with the amount of feed protein consumed during maintenance. The protein efficiency ratio formula is as follows [13]:

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

Information:

PER= protein efficiency ratio
Wg = weight gain (g)
P = protein intake (g)

d. Protein Retention

Protein retention can be determined by analyzing the proximate protein of the fish's body at the beginning and end of maintenance and dividing by the amount of protein consumed during maintenance. The formula for protein retention is as follows [14]:

$$\mathrm{RP} = \frac{(\mathrm{Fp} - \mathrm{Lp})}{\mathrm{P}} \times 100\%$$

Information:

PR = protein Retention
Fp = amount of fish body protein at the beginning of rearing (g)
Lp = amount of body proteins at the end of maintenance (g)
P = amount of protein consumed during maintenance

e. Carbohydrate Retention

Carbohydrate retention can be known by analyzing the fish's body proximate carbohydrate at the beginning and end of maintenance and divided by the amount of protein consumed during maintenance. The formula for carbohydrate retention is as follows [15]:

$$\mathrm{CR} = \frac{(\mathrm{Ct} - \mathrm{Co})}{\mathrm{C}} \times 100\%$$

Information:

CR = carbohydrate retention
Co = amount of carbohydrates in the fish's body at the beginning of rearing (g)
Ct = amount of body carbohydrates after fish harvested (g)
C = amount of carbohydrates consumed during maintenance

f. Water quality

Observation of water quality parameters is carried out every 10 days, used as supporting data in determining the optimal conditions for the maintenance of test fish. Instrument used for measuring water quality parameters were water temperature with a thermometer, dissolved oxygen (DO) with a DO meter, and pH with a pH meter.

g. Proximate Analysis

At the beginning and end of the experiment, the final weight of the fish is measured, and the total amount of feed consumed is determined to calculate the feed conversion ratio (FCR). Finally, one catfish was taken from each replicate of each treatment for further analysis (used 100g fish for whole-body proximate analysis). Proximate analysis of water, ash, protein, lipid, carbohydrate content in fish follows the AOAC method (1990) [16].

2.2 Data Analysis

The data obtained was analyzed by Analysis of Variance (ANOVA) with a confidence level of 95% and if there is a significant difference, it will be continued with Duncan Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

3.1 Absolute Weight Gain

Absolute weight gain differed significantly between treatments representing final biomass weight and early biomass due to different protein administrations. The value of absolute weight gain (Fig. 1).

The results of the analysis showed that the absolute weight gain of G6 test fish kept for 49 days increased at each treatment between 686.63 g and 4619.1 g (Fig. 1). The highest absolute weight gain value was found in treatment B of 4619.1 g (40:60), followed by treatment C at 4391.9 g (20:80), treatment A at 4274.4 g (60:40). and treatment A* with a value of 139.523 g (60:40). In Fig. 1, it can be seen that the average total weight gain of G6 transgenic mutiara catfish is the highest at 402.357 g, this value is almost 3 times higher than that of sangkuriang catfish, which has an average total weight gain of 139.523 g.

The average specific growth rate of each treatment increased over time, suggesting that the fish were able to take advantage of the addition of snail meat meal. The ability of fish to digest and utilize feed greatly affects the weight gain of the fish itself [9].

Protein absorption in feed formulations in fish digestibility is influenced by the presence of amino acid content in the feed. Snail meat flour has nine types of essential amino acids, namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonin and valine [17]. Meanwhile, in commercial feed containing fishmeal with 10 amino acids, namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonin, tryptophan and valine [18]. There are 1 aromatic amino acids in snail flour and 2 aromatic amino acids in commercial feed. Amino acids that are classified as aromatic amino acids are phenylalanine, tyrosine, and tryptophan [18]. Amino acids are generally needed to support growth and balance the body's metabolic processes (maintenance) in fish which can trigger fish growth.

In addition, the highest score was obtained in transgenic mutiara catfish. The existence of this significant difference in the value of the daily growth rate indicates that the transgenic mutiara catfish has a faster growth rate than the sangkuriang catfish. The results showed that plasma GH and IGF-1 levels in transgenic fish were higher than in non-transgenic fish [19] causing fish growth spurt. The induction of increased growth of transgenic catfish-CgGH is thought to be caused by increased expression of exogenous GH that stimulates the production of IGF-1 (insulin-like growth factor 1) in the liver. This increase in IGF-I secretion further triggers the production of more growth hormone, thus spurring the growth of fish.

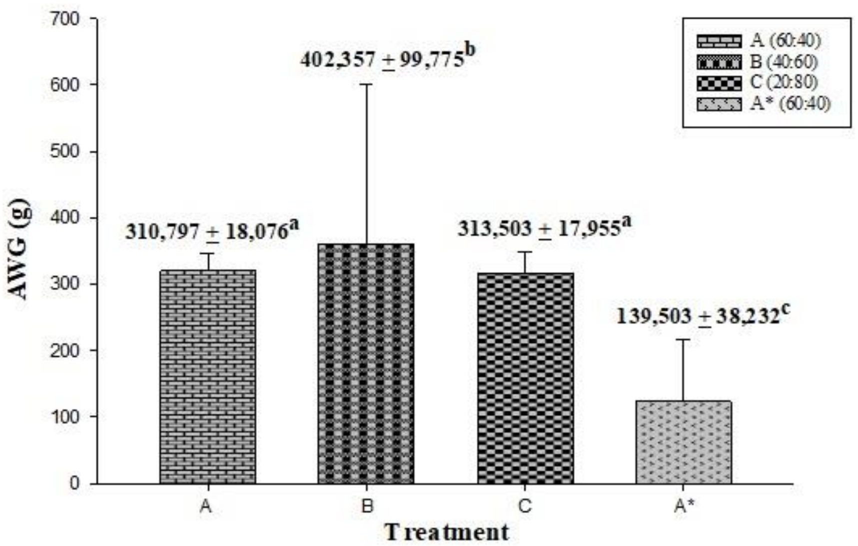

Fig. 1. Absolute weight growth of G6 transgenic mutiara catfish and sangkuriang catfish during 49 days of research. The mean value followed by SD with different letter notation, shows significance ($p<0.05$)

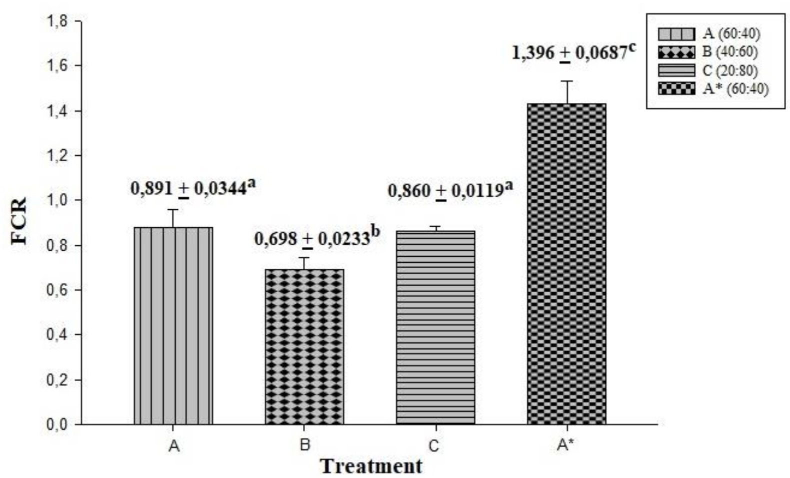

Fig. 2. Feed conversion ratio (FCR) value of G6 transgenic mutiara catfish and sangkuriang catfish During The 49 days of the study. The average value followed by SD with different letter notations indicates significance ($p<0.05$)

3.2 Feed Conversion Rate

Based on the data from the research results that have been carried out (Fig. 2), the feed conversion value ranges from 0.698-1.396. Treatment B gave the lowest feed conversion value (0.698) compared to other treatments, followed by treatment C of 0.860, treatment A of 0.891 and treatment A* at 1.396. This suggests that transgenic fish can use mixed feed more efficiently for growth than non-transgenic fish.

Conversion is influenced by feed protein content, fish genetics and the ability of fish to save protein (the protein sparing effect) which can show the best feed conversion value. The smaller the feed conversion ratio, the food consumed is good for showing fish growth during rearing and vice versa, the larger the feed conversion ratio shows that the feed given is not effective in supporting fish growth [20]. The significant decrease in the value of the feed conversion ratio in the treatments A, B, and C was caused by the expression of exogenous growth hormone, which resulted in changes in the endocrine system and metabolism of the transgenic catfish [21].

Feed response, accompanied by faster growth due to the presence of CgGH inserts leads to lower FCR values. The induction of increased growth of transgenic-*CgGH* is mainly due to increased expression of exogenous GH which stimulates the production of IGF-1 (insulin-like growth factor 1) [22]. The hormone IGF-1 (*insulin-like growth factor 1*) can improve eating behavior, increase swimming activity, and food intake in transgenic fish [23]. Exogenous GH can increase the absorption of feed in the intestine such as protein, thereby increasing feed efficiency [24]. The rapid growth in these transgenic fish will correlate with improved feed conversion (FCR) [25]. This lower FCR can occur because transgenic fish are able to convert carbohydrate or fat from feed nutrients into proteins through interconversion pathways. This lower FCR can occur because transgenic fish are able to convert carbohydrate or fat levels from formula feed into protein through the interconversion pathway, where the energy from this interconversion result is used to support growth and reduce the FCR value. In accordance with Melzer's statement [26], that the use of non-protein energy sources (carbohydrates and fats) will be optimized to replace protein. When the carbohydrates or fats consumed by the fish are less than needed, the protein will be used as an energy source, this process is called gluconeogenesis.

3.3 Feed Efficiency Ratio

The following are the results of the analysis of protein efficiency ratios in transgenic mutiara catfish (treatment A, B, C) and sangkuriang catfish (treatment A* as a control) presented in (Fig. 3).

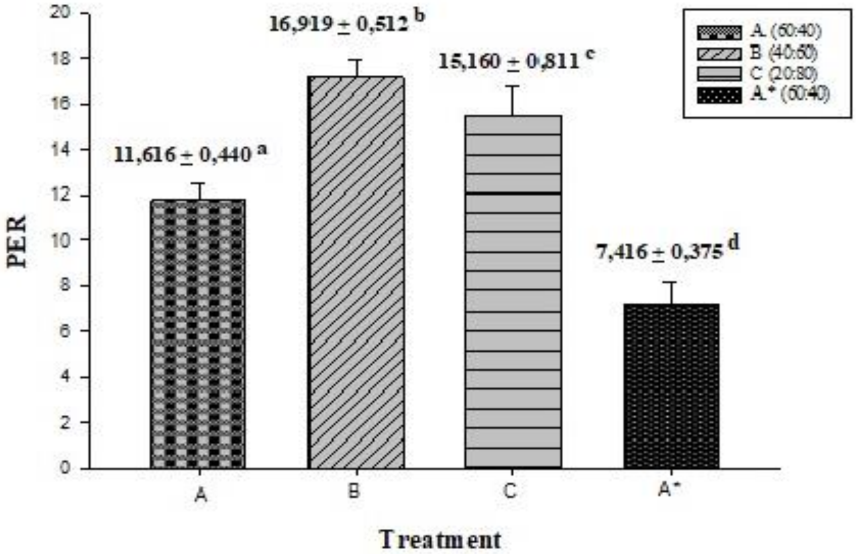

Fig. 3. Protein efficiency ratio values of G6 transgenic mutiara catfish and sangkuriang catfish during 49 days of research. The mean value followed by SD with different letter notation shows significance (p<0.05)

Based on Fig. 3, the comparison test between treatments shows that transgenic mutiara catfish in treatment B (40:60) with mixed protein of 42% has the highest protein efficiency ratio (16,919) and the lowest in non-transgenic catfish treatment A* (60:40) of 7,416. Meanwhile, transgenic mutiara catfish in treatment A (60:40) and treatment C (20:80) were valued at 11,616 and 15,160, respectively. The highest value in treatment B with a ratio of snail meat and pellets of 40% was considered good and still tolerable with its anti-nutrient level. Catfish appear to have limitations given the lower feed utilization efficiency shown by experimental fish at higher dose levels, especially above 50% [27]. The higher the dose of snail flour, it will result in a low ability of fish to digest it, because snail flour contains chitin which is crystalline and insoluble in strong acid solutions, so it cannot be fully digested by the fish's body [28].

Genetic improvements in fish for rapid growth or high feed efficiency can result in fish that are more efficient in using feed protein for body growth. This indication is shown in trasgnenic mutiara catfish which contains exogenous GH (*CgGH),* able to efficiently protein for fish growth. Meanwhile, the low PER value in the A* treatment, namely sangkuriang catfish, is due to more protein being used as an energy source without maximum protein storage [29]. This high PER value is related to *the sparring effect protein.* If *protein sparing effect* is high, the PER value is also likely to increase because less protein is used for energy purposes and more protein is available to meet the body's growth and development needs.

The existence of non-protein energy such as carbohydrates and fats can later reduce the use of feed protein into energy for the body's metabolic process (*protein sparing effect*), so that the protein retention value increases [30]. *Protein sparing effect* has a significant impact on protein retention and PER value. The higher *the protein sparing effect*, followed by increased protein retention, as less protein is used as an energy source [31]. In addition, the PER value also increased, indicating that protein is more efficiently used to meet the body's growth and development needs.

3.4 Protein Retention

Protein retention is a protein stored in the body which is measured based on the protein content of the body at the end of the study minus the protein content of the body at the beginning of the study which shows the amount of protein stored by the body [32], The value of protein retention is influenced by the average weight of the individual at the end of the study and the beginning of the study, where if the difference in protein content between the end and the beginning increases, it will be followed by an increase in protein retention. This result was shown in treatment B that the protein retention value was higher when compared to other treatments (Fig. 4).

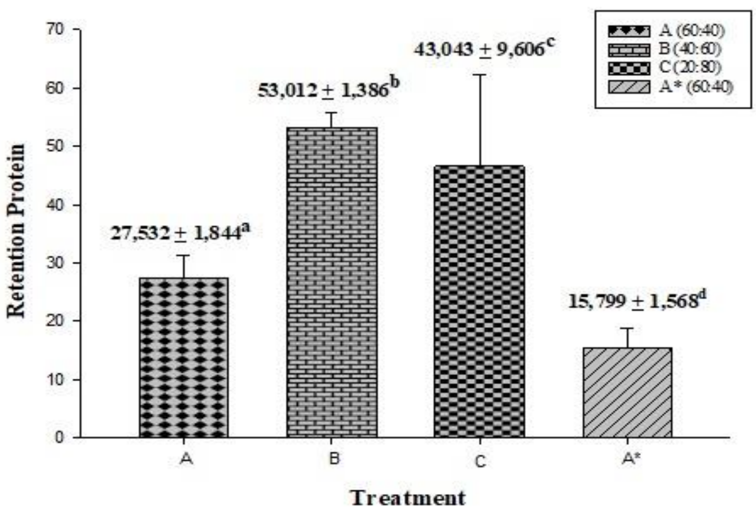

Fig. 4. Protein retention value of G6 transgenic mutiara catfish and sangkuriang catfish during 49 days of research. The Mean value followed by SD with different letter notation shows significance (p<0.05)

Based on a comparative test of protein retention values between transgenic and sangkuriang catfish with mixed treatment of snail meat feed and pellets, it was shown that treatment B (40:60) had the highest protein retention value with a value of 53.01%, treatment C (20:80) was 43.04%, and treatment A (20:80) was 27.532% among transgenic fish. Meanwhile, the D treatment (60: 40) in non-transgenic catfish had the lowest protein retention value of 15.799% (Fig. 4).

The high value of the protein efficiency ratio in fish is influenced by the genetic factors of the fish, and the composition of the compound feed used [33]. The mixed ratio of 40% snail flour and 60% commercial feed increases the protein retention value higher than the mixed ratio of 60% snail use and 60% commercial feed due to the balance of nutrients between snail flour and commercial feed fish meal, considering that the use of 60% snail flour will increase antinutrative substances that inhibit fish growth. When energy and non-protein nutrients are insufficient, protein will be converted into an energy source, reducing its function as a building block or that can be stored [34]. The increase in feed protein consumption in sangkuriang catfish in A* treatment is due to the protein in the feed which is not only used for growth, but also as a source of digestible energy. Therefore, the protein stored in the body of sangkuriang catfish becomes lower and tends to use more protein as an energy source without maximum protein storage [29].

The difference in protein retention between transgenic mutiara catfish and sangkuriang catfish is related to the presence of exogenous GH (*CgGH* insert*).* The function of this exogenous GH provides a protein-saving/*protein sparing* effect as an energy source, by mobilizing alternative energy substrates such as glucose, free fatty acids, and ketones in the same tissue where protein synthesis occurs [10]. The presence of exogenous hormones promotes metabolic activity of overhauling carbohydrates or feed fats through the interconversion pathway in the Krebs cycle for protein synthesis and leads to an increase in protein retention value in transgenic fish from the use of non-protein nutrients as an energy source *(protein sparring effect)* [35]. Therefore, the high protein retention in G6 transgenic transgenic catfish is influenced by the presence of *CgGH* inserts, while in non-transgenik fish (sangkuriang) this effect does not occur.

3.5 Carbohydrate Retention

Carbohydrate retention in fish is the ability of the fish's body to store a certain amount of carbohydrates consumed in the body. The carbohydrate retention values of each treatment are shown in Fig. 5.

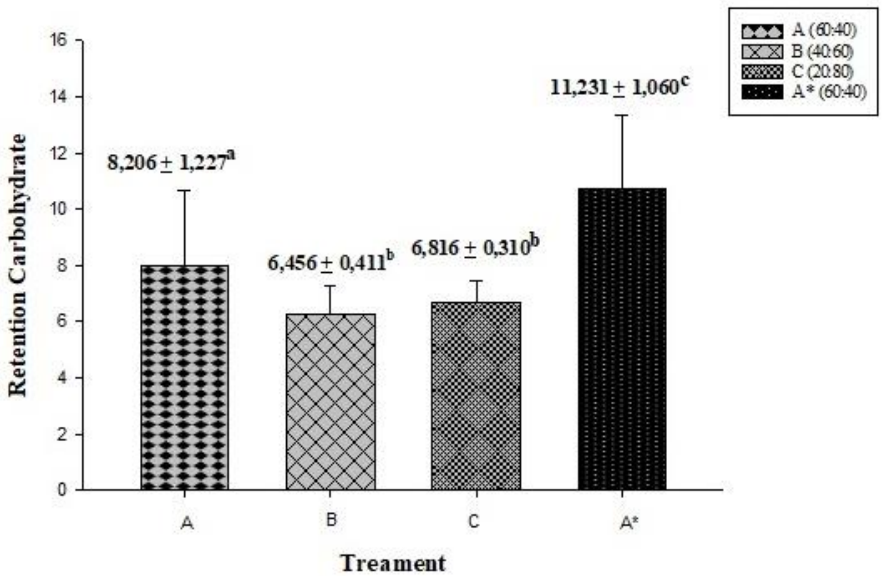

Fig. 5. Carbohydrate retention value of G6 transgenic mutiara catfish and sangkuriang catfish during 49 days of research. The mean value followed by SD with different letter notation shows significance (p<0.05)

Based on the results of data analysis, the highest carbohydrate retention value was 11.23% in sangkuriang catfish (A*) with a mixture ratio of snail flour and pellets of 60 : 40. While the lowest value was in treatment B, namely transgenic mutiara catfish with a ratio of 40 : 60 of 6.456%. Transgenic mutiara catfish in treatment A and C had carbohydrate retention values of 8.2% and 6.816%, respectively (Fig. 5).

Carbohydrate metabolism in fish can be affected by various factors such as activity levels, water quality, and the composition of the ingredients that make up the feed. The hormones insulin and glucagon also play an important role in the regulation of blood glucose levels and carbohydrate metabolism in fish. The small carbohydrate retention value is due to the breakdown of carbohydrates into a source of energy for growth and protein stored by fish. This carbohydrate retention value is inversely proportional to the high protein retention in transgenic mutiara catfish. Mutriara catfish contains exogenous GH while sangkuriang catfish does not contain exogenous GH [36]. Exogenous GH in transgenic fish will cause the conversion of feed carbohydrates into proteins resulting in lower feed carbohydrate levels after the end of the study compared to non-transgenic catfish (sangkuriang) as shown in Fig. 5. The conversion of feed carbohydrates at the mixed ratio of 40:60 and 20:80 was not significantly different, but it was different from the mixed ratio of 60:40 among transgenic fish. These results suggest that the maximum limit that can be converted by exogenous GH in the metabolic interconversion pathway of transgenic fish is 40% snail flour use. More than 40% is suspected to increase the content of antinutrients in snail flour which interferes with the process of interconversion of feed carbohydrates into proteins, causing the retention of treatment A carbohydrates (ratio 60:40) to remain high [37].

Table 1. Water quality range during the riset

Water Quality	Observation Results
Temperature (^{o}C)	28^{o}-30^{o}
pH	6,8-7,3
DO (mg/l)	3,3-4,1

3.6 Water Quality

Water quality plays an important role in supporting the survival of catfish. The water quality measured for 49 days in this study was temperature, pH, and dissolved oxygen (DO) levels. The average value of water quality can be seen in Table 1.

The water quality range values between treatments A, B, C, and A* did not experience significant differences because the four treatments were carried out in the same place and environmental conditions. The water temperature in the maintenance medium ranges from 27-30°C. Referring to SNI, this temperature range is still within the recommended normal threshold value for fish [38]. The pH value obtained during the research ranged from 6,8-7,3. The pH range measured in this study is still classified as the optimum pH to support the life of catfish. According to previous research, the pH suitable for catfish cultivation ranges from 6.5 to 8.5 [39]. A pH high or above 8.5 can cause increased toxicity in the waters, but if the pH is low or below 6.5 it can inhibit the growth rate of sangkuriang catfish. [40] states that microorganisms generally have growing conditions with a pH of 4–9.5 and the the appropriate oxygen concentration for catfish should not be less than 3 mg L-1 [39]. Low oxygen is generally followed by an increase in ammonia and carbon dioxide in the water, which causes the nitrification process to be inhibited, thereby disrupting the metabolic process in fish.

4. CONCLUSION

The conclusion of this study is that the addition of snail flour to commercial feed has a significant effect on improving growth performance, feed conversion ratio, protein efficiency ratio, protein retention and carbohydrate retention in G6 transgenic mutiara catfish. The use of 40% snail flour showed the highest body weight gain (402.357 g), lowest FCR (0.69), protein efficiency ratio (16.91), best protein retention (53.01%) and lowest carbohydrate retention (11.32%) compared to other treatments.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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