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Effectiveness of recombinant growth hormone through oral application on growth of snakehead fish juvenile *Channa striata*

Fajar Maulana[1*], *Afrilia Bagus Putri* Hapsari[1], *Yolanita Naomi* Semesta[1], *Harton* Arfah[1], *Ichsan Achmad* Fauzi[1], and Alimuddin[1]

[1]Departement of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University

Abstract. Snakehead fish, or *gabus* in local name (*Channa striata*) is a species that Indonesia could use for aquaculture due to the extensive maintenance period and expensive high protein feed as an obstacle in the culture process. This research aims to evaluate the effectiveness of recombinant growth hormone (rGH) through artificial feed on the growth performance of snakehead juveniles. The study consisted of the addition of rGH at a dose of 5 mg/kg of feed and the control feed without the addition of rGH. Tests were applied on two different sizes of juvenile, namely: 4.29 ± 0.12 cm with the body weight of 0.54 ± 0.05 g (5 replicated groups, 60 days maintenance; 7 days rGH-contained feed administration at satiation) and 5.30 ± 0.03 cm with the body weight of 0.70 ± 0.05 g (4 replicate groups, 90 days maintenance, seven days 4% rGH-contained feed per day). Each rGH treated fish was compared with the control at the same seed size. Fish is reared in a cage net system with a stocking density 30 fish/m3. The parameters were survival rate (SR), length growth, weight growth, and feed conversion ratio (FCR). The results showed the SR was similar, while the absolute growth in the weight and length of fish treated by rGH grew significantly in comparison to the control condition (P<0.05). This was also the case in FCR in the control treatment, which significantly compared to the treatment (P<0.05). Thus, it concluded that providing feed containing rGH improves the growth performance of snakehead fish in nursery activities.

1 Introduction

The snakehead fish or *gabus* in the Indonesian local name (*Channa striata*), is one of the freshwater carnivorous fish from the Channidae family and the Perciformes order [1]. It has a wide distribution ranging from China, India, Sri Lanka, East India, the Philippines, Nepal, Burma, Pakistan, Singapore, Malaysia, and Indonesia [2]. In Indonesian waters, this fish can be found in various river basins, such as those in Sumatra, Java, and Kalimantan. The snakehead fish is considered a potentially significant species for cultivation [3] due to its high economic value, reflected in its relatively high market price. According to the Ministry of Marine Affairs and Fisheries (KKP) in 2019 [4], snakehead fish production in Indonesia

* Corresponding author: fajarmaulana@apps.ipb.ac.id

increased from 6,940 tons in 2015 to 21,987 tons in 2019. This indicates that snakehead fish is a popular consumer fish commodity with a growing market demand. The local market price for snakehead fish varies widely, ranging from Rp35,000 to Rp50,000 per kilogram, depending on the size or quantity of fish per kilogram [5]. This fish has firm and delicious flesh, high nutritional value [6], and a rich source of albumin, ranging from 63–107 mg/g of its body weight [7]. The presence of albumin in snakehead fish is utilized in treating hypoalbuminemia, recovering plasma volume in patients, and aiding in wound healing for burns or post-surgery patients [8].

Snakehead fish are carnivorous and predatory by nature, requiring a diet with higher protein content compared to other freshwater fish. This poses a challenge in their cultivation as the escalating prices of high-protein feed can increase production costs. According to Aliyu-Paiko et al. [9], snakehead fish exhibit good growth response to fish pellets containing 45% protein, 4,440 kcal/kg energy, and 6.5% fat content. Yulfiperius et al. [10] also stated that feeding artificial feed with 39–40% protein content results in good growth for snakehead fish fry. Another challenge in snakehead fish cultivation is the lengthy cultivation period required to reach marketable size, which takes around 8 months with only one harvest cycle per year. The slow growth rate of snakehead fish can lead to higher production costs, coupled with the risks associated with the extended maintenance period, potentially resulting in lower production yields [11]. Optimal growth of snakehead fish fry that does not require an extended period is crucial, prompting efforts to enhance their growth. One approach involves the use of recombinant growth hormone (rGH) [12].

The rGH protein is one of the recombinant hormones capable of stimulating fish growth due to its composition of amino acids found in fish. Recombinant growth hormone contains GH sequences inserted into *Escherichia coli* bacteria, enabling the incorporation of these sequences by the body's receptors indirectly, aided by IGF-1, for various physiological actions [13]. While concerns persist regarding the use of *E. coli* bacteria as an rGH vector for human health, it has been widely published that *E. coli* strains DH5α and BL21 are safe for human health, as they lack toxicity common in other *E. coli* strains that are generally harmful to humans, such as *E. coli* O157. Strains DH5α and BL21 have also been produced in laboratories under strict international standards and have reduced levels of toxicity, having lost their pathogenic mechanisms, making them safe for use as rGH vectors [14]. The rGH can be administered through feed [15], or immersion [16], or injection for big size fish [17].

The growth of fish can be improved by using rGH in growing operations by reactively manipulating the fish, meaning rGH creates a biological environment that supports explosive biochemical reactions within the fish's body [18]. The effectiveness of rGH depends on the fish species, dosage, and method of administration. According to earlier research, giving fish fry growth hormone can improve their development, growth, and survival rate. Hendriansyah et al. [19] stated that administering rGH at a concentration of 6 mg/kg of feed was the optimal dosage+ for the growth of humpback grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*). Fissabela et al. [20] also mentioned that mixing rGH with commercial feed at a dose of 2 mg/kg of feed resulted in higher feed efficiency, specific growth rate, and survival rate in catfish (*Pangasius* sp.). Administering rGH through feed at a dose of 2 mg/kg of feed also had a major impact on feed conversion ratio and feed consumption, weight growth, and the absolute length of Java barb fry (*Puntius* sp.) [21]. The ratio of protein to energy in feed is one dietary balance that might affect growth. A high protein concentration in the feed may not always speed up fish growth if the diet's overall energy level is inadequate. The cost of feed constitutes a significant portion of fish farming production costs, so using low-protein feed can reduce feed prices. The combination of protein levels and rGH in feed is expected to yield positive results by increasing the protein-sparing effect in the feed and addressing growth issues [11]. Based on the above description, research on the addition of recombinant growth hormone to artificial feed is necessary to evaluate its influence on the snakehead fish fry's growing performance.

2 Methods

2.1 Time and location

There were two periods of research: sixty and ninety days, respectively, starting from November 2022 to May 2023, at the Experimental Pond, Faculty of Fisheries and Marine Sciences, Department of Aquaculture, IPB University, Indonesia.

2.2 Test material

Snakehead fish fry of different sizes were used, with an average length of 4.29±0.12 cm, an average weight of 0.54±0.05 g, and an average length of 5.03±0.02 cm with an average weight of 0.70±0.01 g. The snakehead fish fry were raised in mesh cages with a volume of (1.0 x 1.0 x 1.0) m^3 with 30 fish per cage. They were acclimated to artificial feed before receiving feed containing rGH. The study utilized commercial pellets as the feed source, with a protein content of 31% for the small seeds and 39% for the higher seeds. The rGH protein used was a commercial product containing rGH under the trademark "Mina Grow".

2.3 Experimental design

The research was designed with two treatments:
1. Control treatment: Feeding without rGH
2. rGH treatment: Feeding with rGH at a dosage of 5 mg/kg of feed

There were two treatments and four replications in a completely randomized design.

2.4 Research procedure

2.4.1 Container preparation

The preparation of the maintenance containers began with setting up the net cages with a volume of (1.0 x 1.0 x 1.0) m^3, which were installed in the Experimental Pond, Faculty of Fisheries and Marine Science, IPB University, Indonesia. Eight cages were used and arranged according to the research design.

2.4.2 Making feed containing rGH

The addition of rGH to the feed was done by coating it onto commercial feed. The preparation of the rGH solution for 1 kg of feed started by weighing 5 mg of rGH, which was then dissolved in phosphate-buffered saline (PBS) solution. The binder used, under the trade name Agribind, was added at a rate of 5 g/kg of feed, which had been previously dissolved in 125 ml of water. The rGH solution was then transferred to a sprayer. One kilogram of feed was weighed and placed on a tray, and then the rGH solution in the sprayer was evenly sprayed onto the feed. After allowing the feed to air-dry for 1–2 hours, it was transferred to labeled storage plastic bags and stored in the freezer.

2.4.3 Maintenance of fry

Maintenance began with preparing snakehead fish fry that had been acclimated to artificial feed for one week. Snakehead fish fry with an average length of 4.29±0.12 cm and an average weight of 0.54±0.05 g were raised for 60 days with the feeding of 31% protein feed to satiation, twice a day. Snakehead fish fry with an average length of 5.03±0.02 cm and an average weight of 0.70±0.01 g were raised for 90 days with the feeding of 39% protein feed at a restricted rate of 4%, three times per day. The rGH treatment was administered daily for the first week of the study, after which the snakehead fish fry was given non-rGH feed, as in the control treatment.

2.4.4 Sampling

Sampling was conducted every 2 weeks during the maintenance period, on days 15, 30, 45, and 60. Fish length was measured from the mouth to the tip of the tail fin using a millimeter block, expressed in centimeters. In this study, measurements were taken using a laminated millimeter block. A digital scale was used to weigh the fish. In this study, a digital scale with accuracy to two decimal places was used to measure total weight. All test fish were measured for length and weight at each sampling.

2.5 Test parameters

2.5.1 Survival rate

The percentage of fish that remain after the cultivation phase when compared to the initial stocking is known as the survival rate (SR). SR calculated using the Effendie formula [22]:

$$SR = (Nt / No) \times 100 \tag{1}$$

where: SR = Survival Rate (%), Nt = Number of fish at the end of the cultivation period (individuals), No = Number of fish at the beginning of the cultivation period (individuals).

2.5.2 Fish growth (length and weight)

The growth in length of the snakehead fish fry was measured by determining the total length from the tip of the mouth to the end of the tail fin. The fish weight was calculated on a digital scale that had a 0.01 g accuracy. Measurements of length and weight were taken every two weeks during the cultivation period.

2.5.3 Daily growth rate

The calculation of the daily growth rate was done using the Steffens formula [23]:

$$DGR\ (g/day) = (Wt - Wo) / t \tag{2}$$

where: DGR = Daily Growth Rate (g/day), Wt= Average weight of fish at the end of the study (g), Wo= Average weight of fish at the beginning of the study (g), t= Time period (days).

2.5.4 Feed conversion ratio

The Feed Conversion Ratio (FCR) was calculated using the formula by Watanabe [24]:

$$FCR = F / (Wt - Wo) \tag{3}$$

where: FCR= Feed Conversion Ratio, F=Amount of feed consumed, Wt=Average weight of fish at the end of the study (g), Wo=Average weight of fish at the beginning of the study (g)

2.5.5 Absolute growth

The absolute growth of snakehead fish was calculated using the formula by Hu et al. [25]:

$$W = Wt - Wo \tag{4}$$

where: W= Absolute weight growth (g), Wt= Average weight of fish at the end of the study (g), Wo= Average weight of fish at the beginning of the study (g).

2.5.6 Specific growth rate

The specific growth rate was calculated using the formula by Effendie [22]:

$$SGR\ (\%/day) = ((LnWt - LnWo) / t) \times 100 \tag{5}$$

where: SGR=Specific Growth Rate (%/day), Wt=Average weight of fish at the end of the study (g), Wo=Average weight of fish at the beginning of the study (g), t=Time period (days).

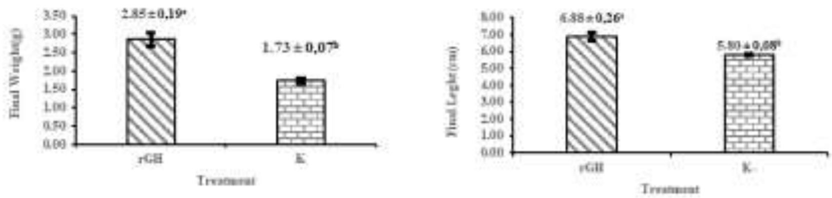
2.6 Data analysis

The data obtained during the research will be analyzed using Microsoft Excel 2016 and analyzed for variance with a 95% confidence interval using the SPSS Statistics version 26.0 application.

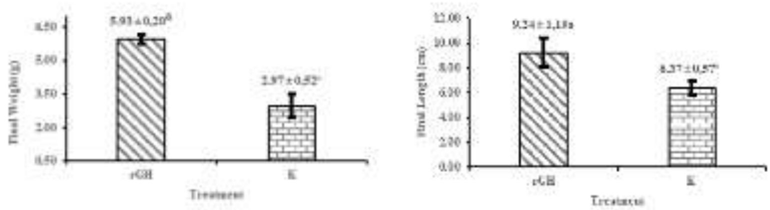
3 Results

3.1 Final weight and length

The average weight and total length refer to the mean values at the end of the cultivation period. As shown in Figure 1a, the treatment with an average fry length of 4.29±0.12 cm and an average weight of 0.54±0.05 g resulted in an average total length of 6.88±0.26 cm (rGH) and 5.80±0.08 cm (control), while the average final weight was 2.85±0.19 g (rGH) and 1.73±0.07 g (control). The treatment with an average fry length of 5.03±0.02 cm and an average weight of 0.70±0.01 g produced an average total length of 9.24±1.19 cm (rGH) and 6.37±0.57 cm (control), with an average final weight of 5.93±0.20 g (rGH) and 2.97±0.52 g (control) (Figure 1b).



(a)

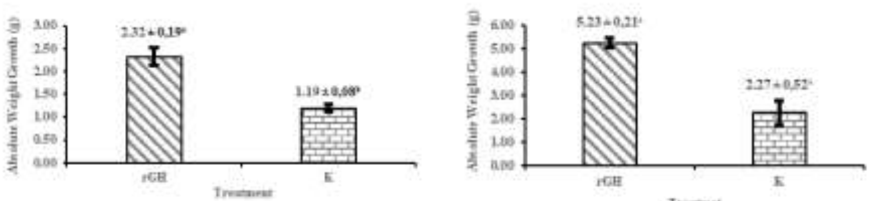


(b)

Fig. 1. Average final body weight and length of snakehead fish during 60 days feed with rGH compared to the control without rGH (K). (a) Fry with an average length of 4.29±0.12 cm and an average weight of 0.54±0.05 g. (b) Fry with an average length of 5.03±0.02 cm and an average weight of 0.70±0.01 g. K: Control treatment, Feeding without rGH; rGH : treatment Feeding with rGH at a dosage of 5 mg/kg of feed.

3.2 Absolute weight growth

The results of absolute weight growth of snakehead fish during the study are presented in the figure below. The absolute weight growth of snakehead fish in the treatment with an average weight of 0.54±0.05 g resulted in 2.32±0.19 g (rGH) and 1.19±0.08 g (control) (Figure 2a), while in the treatment with an average weight of 0.70±0.01 g, it resulted in 5.23±0.21 g (rGH) and 2.27±0.52 g (control) (Figure 2b). The analysis of variance results indicates that the addition of rGH in the feed significantly affected the absolute weight growth of snakehead fish.



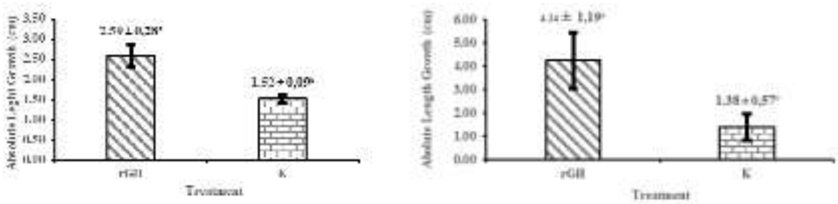
(a) (b)

Fig. 2. Absolute weight growth of snakehead fish during 60 days feed with rGH compared to the control without rGH (K). Initial fry with an average weight of (a) 0.54±0.05 g and (b) 0.70±0.01 g. K: Control treatment, Feeding without rGH; rGH: treatment Feeding with rGH at a dosage of 5 mg/kg of feed.

3.3 Absolute length growth

The results of absolute length growth of snakehead fish during the study are presented in the figure below. The absolute length growth of snakehead fish in the treatment with an average length of 4.29±0.12 cm resulted in 2.59±0.28 cm (rGH) and 1.52±0.09 cm (control) (Figure

3a), while in the treatment with an average length of 5.03±0.02 cm, it resulted in 4.24±1.19 cm (rGH) and 1.38±0.57 cm (control) (Figure 3b). The analysis of variance results indicates that the addition of rGH in the feed significantly affected the absolute length growth of snakehead fish.

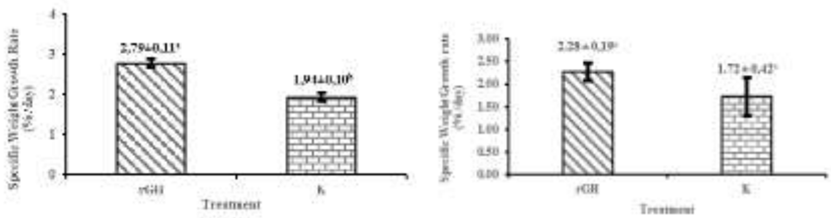


(a) (b)

Fig. 3. Absolute length growth of snakehead fish during 60 days feed with rGH compared to the control without rGH (K). Initial fry with an average length of (a) 4.29±0.12 cm and (b) 5.03±0.02 cm. K: Control treatment, Feeding without rGH; rGH: treatment Feeding with rGH at a dosage of 5 mg/kg of feed.

3.4 Specific weight growth rate

The average specific weight growth rates of snakehead fish during the study are presented in the figure below. The specific weight growth rate in the treatment with an average weight of 0.54±0.05 g resulted in 2.79±0.1 %/day (rGH) and 1.94±0.10 %/day (control) (Figure 4a), while in the treatment with an average weight of 0.70±0.01 g, it resulted in 2.28±0.19 %/day (rGH) and 1.72±0.42 %/day in the control (Figure 4b). The analysis of variance results indicates that the addition of rGH in the feed significantly affected the specific weight growth rate of snakehead fish.

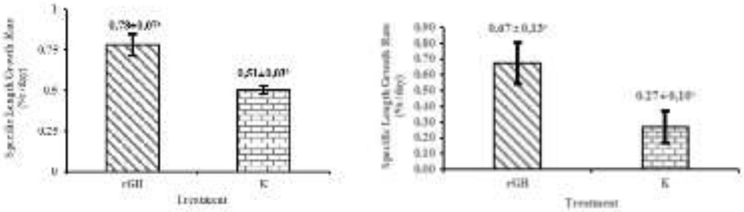


(a) (b)

Fig. 4. Specific weight growth rate of snakehead fish during 60 days feed with rGH compared to the control without rGH (K). Initial fry with an average weight of (a) 0.54±0.05 g and (b) 0.70±0.01 g. K: Control treatment, Feeding without rGH; rGH: treatment Feeding with rGH at a dosage of 5 mg/kg of feed

3.5 Specific length growth rate

The average specific length growth rates of snakehead fish during the study are presented in the figure below. The specific length growth rate in the treatment with an average length of 4.29±0.12 cm resulted in 0.78±0.07 %/day (rGH) and 0.51±0.03 %/day (control) (Figure 5a), while in the treatment with an average length of 5.03±0.02 cm, it resulted in 0.67±0.13 %/day (rGH) and 0.27±0.10 %/day (control) (Figure 5b). The analysis of variance results indicates that the addition of rGH in the feed significantly affected the specific length growth rate of snakehead fish.

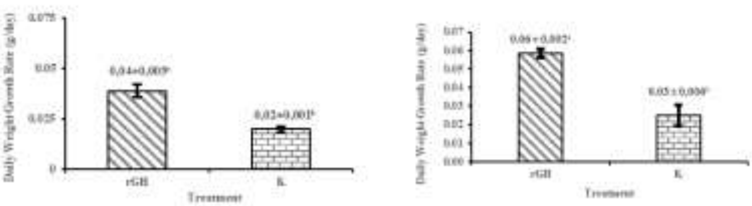


(a) (b)

Fig. 5. Specific length growth rate of snakehead fish during 60 days feed with rGH compared to the control without rGH (K). Initial fry with an average length of (a) 4.29±0.12 cm and (b) 5.03±0.02 cm. K: Control treatment, Feeding without rGH; rGH : treatment Feeding with rGH at a dosage of 5 mg/kg of feed.

3.6 Daily weight growth rate

The average daily weight growth rates of snakehead fish during the study are presented in the figure below. The daily weight growth rate in the treatment with an average weight of 0.54±0.05 g resulted in 0.04±0.003 g/day (rGH) and 0.02±0.001 g/day (control) (Fig. 6a), while in the treatment with an average weight of 0.70±0.01 g, it resulted in 0.06±0.002 g/day (rGH) and 0.03±0.006 g/day (control) (Fig. 6b). The analysis of variance results indicates that the addition of rGH in the feed significantly affected the daily weight growth rate of snakehead fish.

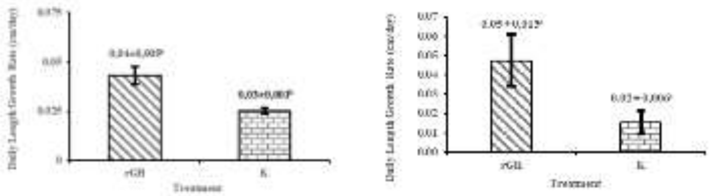


(a) (b)

Fig. 6. Daily weight growth rate of snakehead fish during 60 days feed with rGH compared to the control without rGH (K). Initial fry with an average weight of (a) 0.54±0.05 g and (b) 0.70±0.01 g. K: Control treatment, Feeding without rGH; rGH: treatment Feeding with rGH at a dosage of 5 mg/kg of feed.

3.7 Daily length growth rate

The average daily length growth rates of snakehead fish during the study are presented in the figure below. The daily length growth rate in the treatment with an average length of 4.29±0.12 cm resulted in 0.04±0.005 cm/day (rGH) and 0.03±0.001 cm/day (control) (Figure 7a), while in the treatment with an average length of 5.03±0.02 cm, it resulted in 0.05±0.013 cm/day (rGH) and 0.02±0.006 cm/day (control) (Figure 7b). The analysis of variance results indicates that the addition of rGH in the feed significantly affected the daily length growth rate of snakehead fish.

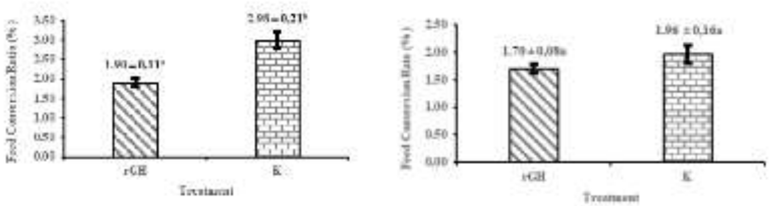


(a) (b)

Fig. 7. Daily length growth rate of snakehead fish during 60 days feed with rGH compared to the control without rGH (K). Initial fry with an average length of (a) 4.29±0.12 cm and (b) 5.03±0.02 cm. K: Control treatment, Feeding without rGH; rGH: treatment Feeding with rGH at a dosage of 5 mg/kg of feed.

3.8 Feed conversion ratio

The average feed conversion ratios of snakehead fish during the study are presented in the figure below. The average feed conversion ratio in the treatment with an average length of 4.29±0.12 cm and an average weight of 0.54±0.05 g resulted in 1.90±0.11 (rGH) and 2.98±0.21 (control) (Figure 8a), while in the treatment with an average length of 5.03±0.02 cm and an average weight of 0.70±0.01 g, it resulted in 1.70±0.08 (rGH) and 1.96±0.16 (control) (Figure 8b). These results indicate that the addition of rGH in the feed significantly affected the feed conversion ratio of snakehead fish.

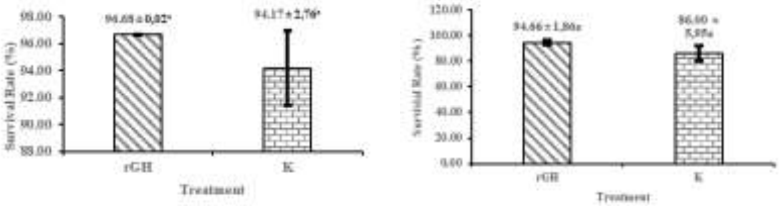


(a) (b)

Fig. 8. Feed conversion ratio of snakehead fish during 60 days feed with rGH compared to the control without rGH (K). Initial fry with an average length of (a) 4.29±0.12 cm and (b) 5.03±0.02 cm. K: Control treatment, Feeding without rGH; rGH: treatment Feeding with rGH at a dosage of 5 mg/kg of feed.

3.9 Survival rate

The average survival rates of snakehead fish during the study are presented in the figure below. The survival rate of snakehead fish in the treatment with an average length of 4.29±0.12 cm and an average weight of 0.54±0.05 g resulted in 96.68% (rGH) and 94.17% (control) (Figure 9a), while in the treatment with an average length of 5.03±0.02 cm and an average weight of 0.70±0.01 g, it resulted in 94.66% (rGH) and 86.00% (control) (Figure 9b). These results indicate that the feed did not have a significantly different effect on the survival rate of snakehead fish.



(a) (b)

Fig. 9. Survival rate of snakehead fish during 60 days feed with rGH compared to the control without rGH (K). Initial fry with an average length of (a) 4.29±0.12 cm and (b) 5.03±0.02 cm. K: Control treatment, Feeding without rGH; rGH: treatment Feeding with rGH at a dosage of 5 mg/kg of feed.

4 Discussion

Based on the observations, it is evident that the administration of rGH through feed to snakehead fish fry (*Channa striata*) with different sizes of initial fry during a 60-day and 90-day cultivation period had a significant effect ($P<0.05$) on absolute weight growth, absolute length growth, specific weight growth rate, specific length growth rate, daily weight growth rate, daily length growth rate, and feed conversion ratio. It has been demonstrated that the use of rGH in aquaculture operations promotes snakehead fish development. This study demonstrates that the addition of rGH to artificial feed with varying protein content, specifically at a dose of 5 mg/kg of feed, can enhance the absolute weight and length growth as well as the specific growth rate of snakehead fish compared to the treatment without rGH addition (control). The rGH can stimulate somatic growth by optimizing the hypothalamus function in regulating energy balance during metabolic changes, thereby increasing the efficiency of nutrient utilization.

The increase in growth of snakehead fish is attributed to the optimal absorption of rGH administered through feed. This aligns with the statement by Silalahi et al. [26] that the direct mechanism begins with orally administered rGH being absorbed in the digestive organs, especially in the intestines of fish fry. Setyawan et al. [27] claim that when growth hormones enter the body of a fish, the hypothalamus is stimulated, improving the activity of the growth hormone releasing hormone (GHRH), which then enters various organs include the kidneys, liver, muscles, bones, and other organs, which causes fish to grow faster. In addition to GHRH, the hypothalamus also releases somatostatin, which regulates the release of GH in the pituitary gland. In the indirect mechanism, rGH influences growth through the The liver produces insulin-like growth factor (IGF-1) pathway, which is responsible for the functions of growth hormone in fish growth. This mechanism involves rGH binding with GH receptors in the bloodstream and stimulating the expression of IGF-1 in the liver. Subsequently, Insulin-like growth factor binding proteins (IGFBPs) bind IGF-1 and transported to target organs. IGF-1 then attaches itself to the IGF-1 receptor in the target cells [28]. Insulin supplementation can influence somatic cell growth by controlling hypertrophy and hyperplasia in fish. This is evident in the significantly increased somatic growth in fish [29]. By boosting feed intake and enhancing the fish's body's ability to metabolize nutrients, rGH administrations can accelerate fish growth rates. Feed with high nutritional value and digestibility supports optimal fish growth. In addition to oral feeding, rGH is also influenced by factors such as fish species, environment, and feed [30].

Snakehead fish that received rGH had lower feed conversion ratio values than the control group, according to the data. This suggests that the addition of 5 mg/kg of rGH to the feed can enhance metabolism and physiological functions in snakehead fish, thereby increasing appetite, as evidenced by increased feed consumption and optimizing nutrient absorption. According to Yulfiperius et al. [10], with a protein content of 39–40%, snakehead fish have a feed conversion ratio of 2.05. This indicates that the use of rElGH in artificial feed with low protein content is still effective in stimulating growth and improving feed conversion. The value of the feed conversion ratio indicates the feed's efficiency, where a higher ratio indicates poorer feed efficiency [31]. Feed containing rGH is believed to be better digested by the fish's digestive system, aided by enzymes stimulated by GH, which can convert complex compounds into simpler compounds in a shorter period, allowing the fish to utilize the feed more efficiently for growth [21]. Setyawan et al. [27] also state that rGH can increase protein content, reduce nitrogen excretion, stimulate metabolism and fat oxidation, and enhance insulin synthesis and release. The higher the digestibility of the feed, the more effectively the fish use the feed's nutrients [32].

The application of recombinant growth hormone (rGH) on giant grouper to snakehead fish through oral administration with different compositions of pellet feed mixed using a binder as an adhesive is being conducted for the first time. The dosage of rGH added to snakehead fish feed has been determined through previous research, specifically by a company that aimed to ascertain the optimal rGH dosage for the growth of snakehead fish fry. Survival rate is one of the key parameters in this study. According to Effendie [33], the survival rate is the probability of an individual's survival over a specific period. Snakehead fish fry fed rGH-rich feed showed a survival rate that was not significantly different compared to the control group. This suggests that the administration of recombinant growth hormone (rGH) has the capability to enhance the immune system of snakehead fish fry. This is in line with the statement by Apriliana et al. [18] that the administration of recombinant growth hormone can enhance fish survival rate by improving the immune system's response to diseases. This is further supported by the statement by Acosta et al. [34] that the administration of growth hormone to fish fry can increase survival rate and enhance the fish's resistance to infectious diseases. Throughout the cultivation period, the survival rate data indicates favorable outcomes. Referring to Mulyani et al. [35], a survival rate of $\geq 50\%$ is considered good, 30–50% is moderate, and less than 30% is considered poor. Aprilia [36] asserts that the high percentage of survival rate is influenced by several factors, including human handling, organism adaptation ability, age, diseases, density, and competitors.

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