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THE EFFECT OF RECOMBINANT GROWTH HORMONE ADMINISTRATION ON BONYLIP BARB (OSTEOCHILUS VITTATUS) FRY AT DIFERENT STOCKING DENSITIES

Pengaruh Penambahan Recombinant Growth Hormone Pada Benih Ikan Nilem (*Osteochilus Vittatus*) Pada Padat Tebar Berbeda

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ABSTRAK

Ikan nilem (*Osteochilus vittatus*) memiliki nilai ekonomi yang tinggi, namun laju pertumbuhannya relatif lambat. Salah satu solusi potensial untuk mempercepat masa budidayanya adalah dengan penggunaan hormon pertumbuhan rekombinan (rGH). Penelitian ini bertujuan untuk mengevaluasi pengaruh pemberian rGH dari Epinephelus lanceolatus (rElGH) secara oral terhadap laju pertumbuhan benih ikan nilem pada kepadatan tebar yang berbeda. Rancangan acak lengkap dengan tiga ulangan digunakan dalam penelitian ini. Benih ikan dengan panjang 4,43±0,03 cm dan berat 0,96±0,03 g dipelihara dalam akuarium berukuran 100 × 50 × 50 cm dengan ketinggian air 20 cm selama 30 hari. Ikan diberi pakan komersial yang mengandung 31–33% protein, dengan pakan yang mengandung rElGH diberikan hingga kenyang dua kali sehari (pukul 08.00 dan 16.00) selama tujuh hari pertama, kemudian dilanjutkan dengan pakan biasa tanpa rElGH. Kinerja produksi terbaik diperoleh pada perlakuan R30 (30 ekor per 100 L dengan rElGH) dengan pertumbuhan bobot mutlak (PBM) 3,4 g, tingkat kelangsungan hidup (TKH) 98,89% dan laju pertumbuhan spesifik (LPS) sebesar 4,8% hari^{-1} sehingga pertumbuhan ikan nilem yang diberi hormon *rEIGH* lebih cepat.

ABSTRACT

Nilem fish (Osteochilus vittatus) has high economic value, but its growth rate is relatively slow. One potential solution to accelerate its cultivation period is by using recombinant growth hormone (rGH). This study aims to provide the effect of oral administration of rGH from Epinephelus lanceolatus (rElGH) on the growth rate of nilem fish seeds at different stocking densities. A completely randomized design with three replications was used in this study. Fish seeds with a length of 4.43±0.03 cm and a weight of 0.96±0.03 g were kept in an aquarium measuring 100×50×50 cm with an air height of 20 cm for 30 days. Fish were fed commercial feed containing 31–33% protein, with feed containing rElGH given until full twice a day (at 08.00 and 16.00) for the first seven days, then continued with regular feed without rElGH. The best production performance was obtained in the R30 treatment (30 fish per 100 L with rElGH) with an absolute weight growth (PBM) of 3.4 g, a survival rate (SGR) of 98.89% and a specific growth rate (SGR) of 4.8% day-1 so that the growth of tilapia given rEIGH hormone was faster.

Kata Kunci	Ikan Nilem, *Recombinant Growth Hormone*, Padat Tebar
Keywords	*Tilapia, Recombinant Growth Hormone, Stocking Density*
Tracebility	Submission: 2/6/2025. Published : 27/6/2025
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INTRODUCTION

Nilem fish has a very high protein content, reaching 50.23% of dry weight and consists of eight types of essential amino acids (Said *et al.,* 2020). Nilem fish harvesting can be done after the fish reaches a size suitable for sale or has a weight of 100 grams/fish, usually after eight months of maintenance, with a selling price of IDR 25,000/kilogram (Handayani, 2021). The increase in feed prices has increased production costs for cultivation efforts. Steps are needed to increase production results, including increasing stocking density and accelerating the growth rate of fish. By achieving the ideal density, production results can be optimized and profits can be obtained. If the stocking density has been achieved optimally, freshwater resources and existing facilities can be utilized efficiently so that production will be optimal (Saputra *et al.,* 2018).

The problem experienced by tilapia fish farmers is relatively slow growth, this is in accordance with Handayani's research (2021) that tilapia fish harvesting can be done for 8 months of maintenance or reaching a weight of 100 g/tail with a selling price of IDR 25,000 kg-1. The use of recombinant hormones is an alternative in reducing production costs and solutions for optimal fish growth (Pratama et al., 2021) and is able to increase fish growth so that it can shorten the maintenance time (Apriliana et al., 2017). The use of growth hormones is recognized as an effective approach to improving the growth performance of farmed fish. The addition of recombinant growth hormone (rGH) can increase the growth rate of fish faster than usual and almost 100% of the fish produced are more uniform in size (Sutiana et al., 2017). In addition, fish that are given additional rGH hormone are not included in genetically modified organism (GMO) products because rGH is not inherited by subsequent generations (Acosta et al., 2008; Apriliana et al., 2017).

The rGH protein with a gene sequence derived from the grouper fish (recombinant Epinephelus lanceolatus Growth Hormone/rElGH) is produced more in Escherichia coli bacteria than other types of fish rGH hormones and can be used universally, not just for one type of fish (Alimuddin et al., 2010). Administration of rGH can be done through feed (Moriyama et al. 1993), injection (Promdonkoy et al., 2004), and immersion (Acosta et al. 2007). Research on rGH in Indonesia has been conducted on several types of fish, including eel (Handoyo, 2012), and tilapia (Latar, 2013). Several fish growth hormones that have been successfully produced besides grouper (Alimuddin et al. 2010), include rGH from giant catfish (Promdonkoy et al., 2004), salmon (Moriyama and Kawauchi, 1990), tilapia (Acosta et al., 2007), and gourami (Alimuddin et al., 2010). The most widely used rGH hormone is rElGH because the protein production level is higher compared to growth hormones from carp (rCcGH) and gourami (rOgGH) (Irmawati *et al.,* 2012).

High stocking density will cause stress to fish that interfere with survival rate, feed conversion ratio, and specific growth rate. Therefore, the optimal density must be determined to allow maximum profit. Research on the stocking density of tilapia fish seeds with the addition of rElGH hormone using the oral method is still not widely carried out so that research is needed on the stocking density of tilapia fish with the addition of rElGH.

METHODS

Materials

Nilem fish seeds as test fish have an average length of 4.43±0.03 cm and an average weight of 0.96±0.03 g totaling 900 fish from the Sukabumi Freshwater Aquaculture Center (BBPBAT). The feed used during maintenance is commercial artificial feed in the form of pellets with a protein content of 31-33% with a size of 1 mm. The recombinant growth hormone used is the recombinant growth hormone of kertang grouper (rElGH) developed by Alimuddin et al. (2010) and produced by the Sukabumi Freshwater Aquaculture Center, West Java.

Research Design

This study will use a completely randomized design. This study consists of six treatments with three replications, the details are as follows:
Treatment K30: Stocking density 30 fish 100 L-1 feeding without rElGH;
Treatment R30: Stocking density 30 fish 100 L-1 feeding with rElGH;
Treatment K50: Stocking density 50 fish 100 L-1 feeding without rElGH;
Treatment R50: Stocking density 50 fish 100 L-1 feeding with rElGH;
Treatment K70: Stocking density 70 fish 100 L-1 feeding without rElGH;
Treatment R70: Stocking density 70 fish 100 L-1 feeding with rElGH.

Container Preparation

The containers used were aquariums measuring 100 × 50 × 50 cm, 18 units with a water height of 20 cm in each container. Each container was cleaned with a brush and rinsed with water until clean. After the cleaning stage, each container was filled with water as high as 20 cm. Aeration was provided in the middle of the container to increase oxygen levels in the water. The water in the maintenance container was left for 24 hours to check for leaks, and the test fish could be spread.

Hormonal Feed Manufacturing

The feed used was commercially made feed measuring 781-1 with a protein content of 31-33%. The coating method was carried out by adding rElGH to commercial feed. Making hormone-containing feed by preparing 50 mL kg-1 of water, 2 mL kg-1 of phosphate buffer saline (PBS), and 2 mg kg-1 of rElGH hormone, then put into a sprayer and homogenized. The binder used was CMC as much as 2 g kg-1 of feed, then spread evenly on the feed. The rElGH solution was sprayed evenly onto 1 kg of feed, air-dried for 30 minutes, the feed was transferred into a labeled storage plastic and the feed was ready to use. The feed was stored at room temperature in a closed container. The control feed was prepared without giving rElGH, but was still given additional binder, water, and PBS.

Seed maintenance

The feeding method was carried out at satiation and the frequency of feeding was twice a day, namely in the morning and evening. Feeding with the addition of rElGH hormone was given every day for the first week of the study and then the nilem fish seeds were fed without rElGH as in the control treatment. Aquarium cleaning (siphon) was carried out every afternoon, while water changes were carried out every 4 days as much as 50% of the water was discarded and refilled with water with the original volume. Measurement of water quality parameters tested included temperature, pH, and DO with a frequency of checking once a week every morning and evening. The tools used in measuring temperature were thermometers, pH using a pH meter, and DO using a DO meter. The results of water quality measurements during maintenance are presented in Table 1.

Fish sampling

Sampling was conducted once every two weeks during the maintenance period, namely on days 0, 15, and 30. Length measurements were conducted by measuring the length of the fish from the mouth to the tip of the tail fin with the help of millimeter blocks expressed in centimeters (cm). The tool used in measuring length in this study was a millimeter block that had been coated with plastic (laminating), while the weight of the fish was measured with the help of a digital scale with an accuracy of two digits behind the decimal point in grams. The number of fish measured for length and weight for each sampling was 30 fish/aquarium.

Test parameters

Feed conversion ratio (FCR) is the amount of feed needed to produce one kilogram of meat. The FCR value is calculated using the following formula (Goddard, 1996), namely:

$$RKP = \frac{F}{(Bt + Bd) - B0}$$

Description

RKP = Feed conversion ratio

F = Amount of feed consumed

Bt = Seed biomass at the end of maintenance (g)

Bd = Dead seed biomass (g)

B0 = Seed biomass at the beginning of maintenance (g)

The specific growth rate can be calculated using the formula according to Effendie (2002):

$$LPS = \frac{LnWt - LnWo}{t} \times 100$$

Description

LPS = Specific growth rate (% day-1)

Wt = Average weight at the end of maintenance (g)

Wo = Average weight at the beginning of maintenance (g)

t = Time period (days)

Absolute length growth is the difference between the final length and the initial maintenance. Length growth is measured from the tip of the head to the tip of the tail. Absolute length growth is calculated using the following formula (Effendie, 1997):

$$PPM = Lt - Lo$$

Description
PP = Absolute length growth (g)
Lt = Average length of fish at the end of the study (g)
Lo = Average length of fish at the beginning of the study (g)

The absolute growth of tilapia fish is calculated using the formula of Hu et al. (2008), namely:

$$PPM = Wt - Wo$$

Description
PBM = Absolute weight growth (g)
Wt = Average length of fish at the end of the study (g)
Wo = Average length of fish at the beginning of the study (g)

Biomass increase is the difference between the initial biomass of maintenance and the end of maintenance. Biomass increase is calculated using the formula:

$$Pertambahan\ Biomassa = Bt - B0$$

Description
Bt = Average biomass at the end of maintenance (g)
B0 = Average biomass at the beginning of maintenance (g)

The survival rate or what is called the survival rate (SR) is the percentage of the number of nilem fish seeds that live at the end of maintenance compared to the number of nilem fish seeds at the beginning of maintenance. The survival rate can be calculated using the following formula (Sinaga and Mukti, 2022):

$$TKH = \frac{Nt}{No} \times 100$$

Description
TKH = Survival rate (%)
Nt = Number of fish at the end of maintenance (tail)
No = Number of fish at the beginning of maintenance (tail)

Data analysis

Parameter data obtained during the study were tabulated using Microsoft Excel 2016. Furthermore, the tabulated data were analyzed using descriptive analysis.

RESULTS

The feed conversion ratio (FCR) of Nilem fish seeds during maintenance is presented in Figure 1. The FCR value in fish treated with rElGH with different stocking densities was lower than the control.

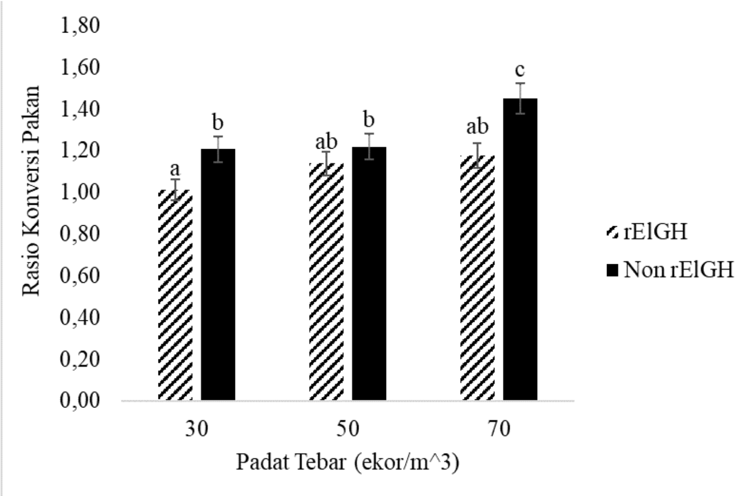

Figure 1 Feed conversion ratio (FCR) of Nilem fish seeds with different stocking densities and administration of rEIGH hormone

The specific growth rate (SGR) of nilem fish fry during maintenance is presented in Figure 2. The provision of rElGH in all stocking density treatments had higher SGR than the control without rElGH, and the SGR value decreased with increasing stocking density. The R30 treatment had the highest SGR.

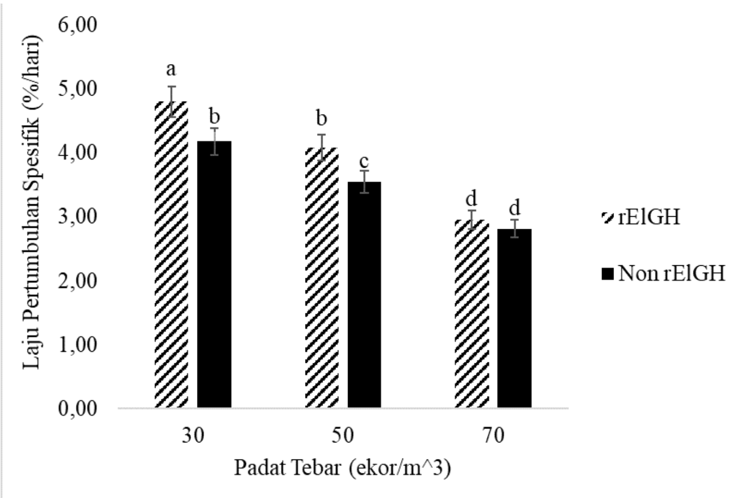

Figure 2 Specific growth rate (SGR) of tilapia seeds with different stocking densities and administration of rEIGH hormone

The results of absolute length growth (PPM) of nilem fish seeds during maintenance are presented in Figure 3. The provision of rEIGH showed better growth compared to its control. The R30 treatment had the highest growth during the treatment.

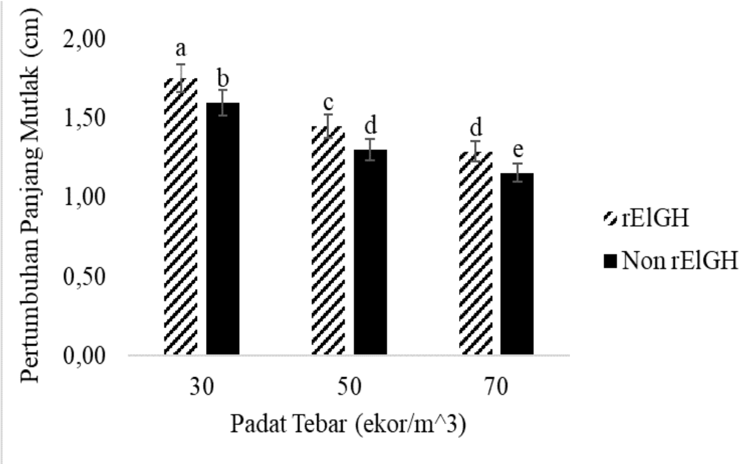

Figure 3 Absolute length growth (PPM) of tilapia fish seeds with different stocking densities and administration of rEIGH hormone

The results of absolute weight growth (PBM) of Nilem fish seeds during maintenance are presented in Figure 4. Administration of rEIGH was able to provide better weight growth compared to the control treatment.

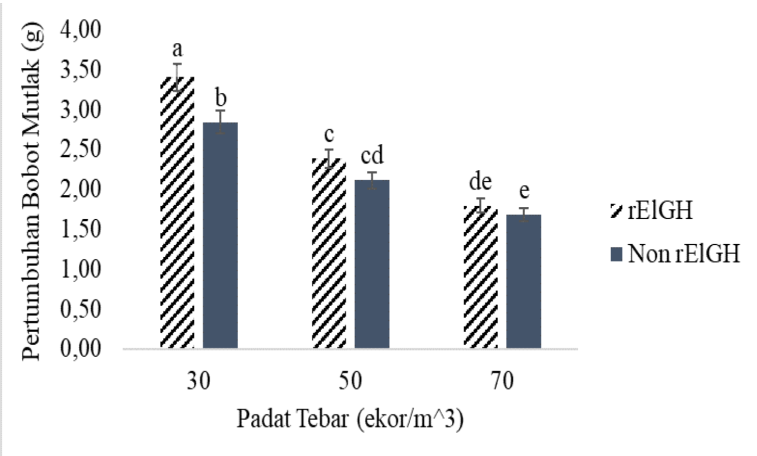

Figure 4 Absolute weight growth (PBM) of tilapia fish seeds with different stocking densities and administration of rEIGH hormone

The results of the increase in biomass of nilem fish seeds during maintenance are presented in Figure 5. The increase in biomass increased with increasing stocking density, and the provision of rElGH had a better biomass increase than the control. Compared to the control, the highest biomass increase was obtained in the R30 treatment.

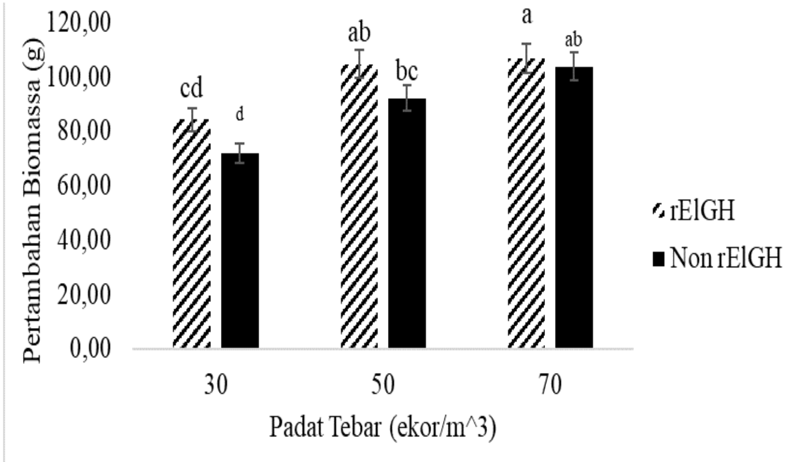

Figure 5 Increase in tilapia seed biomass with differences in stocking density and administration of rEIGH hormone

The results of the survival rate of nilem fish seeds during maintenance are presented in Figure 6. The survival rate of nilem fish seeds ranged from 90-98.89%. The TKH value of rElGH treated fish in all stocking density treatments was higher than the control, and the TKH decreased with increasing stocking density.

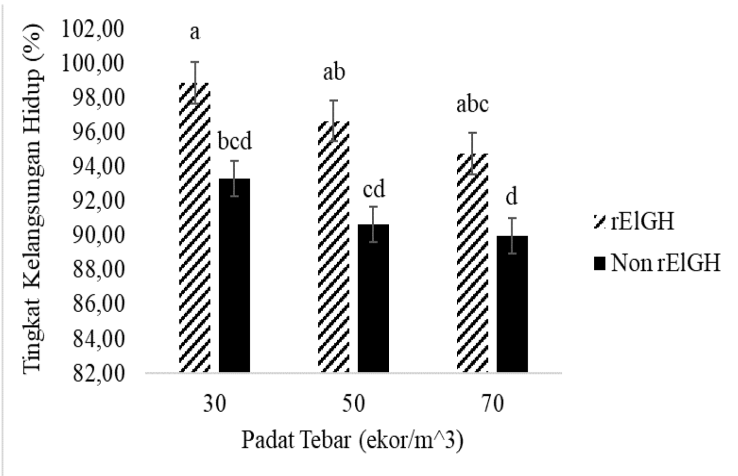

Figure 6 Survival rate (SVR) of tilapia seeds with different stocking densities and administration of rEIGH hormone

Water quality parameters observed during maintenance include temperature, pH, and DO (Dissolved Oxygen). Table 1 shows the results of water quality parameter observations for 30 days.

Table 1. Results of water quality measurements during maintenance

Parameter	Unit	Results	Reference
Suhu	°C	28-31,5	24-34[a]
pH	-	7,3-8,3	6,5-9[b]
DO	mg L^{-1}	3,6-7,8	>5[c]

[a]Nurkarina, 2013, [b]Wicaksono, 2005, [c]Boyd, 2015.

Based on Table 1, the water quality parameters are still within normal limits. The temperature in this study is still in the range of 24-34˚C (Nurkarina, 2013), pH in the range of 6.5-9 (Wicaksono, 2005), and DO in the range of >5 mg L-1 (Boyd, 2015).

DISCUSSION

The highest value of feed conversion ratio (FCR) of nilem fish seeds during maintenance was obtained in the K70 treatment, while the lowest was in the R30 treatment. The smaller the value of the feed conversion ratio, the more efficient the level of feed consumption, conversely if the feed conversion value is high, the level of feed utilization is less good (Iskandar and Elrifadah, 2015). This is proven that the addition of rElGH using the oral method can reduce the FCR value. According to Yuniarti et al. (2022), feed containing the rElGH hormone will be better digested by the fish's digestive system with the help of enzymes stimulated by GH can convert complex compounds into simpler compounds in a shorter time, so that the utilization of feed consumed by fish is more optimal for growth. GH affects protein synthesis and lipid turnover, so that exogenous GH treatment causes fish to have a greater ability to digest food, absorb nutrients, and convert a larger proportion of food to form body composition, thus affecting the feed conversion ratio (Alimuddin et al., 2014).

Administration of rElGH can increase the specific growth rate, absolute length growth, and absolute weight growth of tilapia through oral methods and different stocking densities. These test parameters are in line with the increase in stocking density, where the higher the stocking density, the lower the LPS, LPH, PPM, and PBM values of tilapia. This is because high stocking densities cause competition for movement space and feed provided between individuals so that the growth rate per individual produced is smaller (Ritonga, 2020). The decrease in LPS, LPH, PPM, and PBM is thought to be due to the stress response of fish to stocking densities which affect the quality of the water environment of the maintenance container, thus affecting production performance (Rafaey et al., 2018). The rElGH hormone can increase somatic growth by optimizing the function of the hypothalamus in regulating energy balance in metabolic changes and increasing the efficiency of utilization of absorbed nutrients (Hardianto et al., 2012; Atmojo et al., 2017).

The mechanism of rElGH hormone in influencing the growth rate of tilapia fry is divided into two mechanisms, namely direct and indirect mechanisms (Moriyama et al., 2000; Caputo et al., 2021). The direct mechanism occurs when GH directly affects organ growth without going through the intermediary of insulin-like growth factor 1 (IGF-1) in the liver, while the indirect mechanism occurs through the influence of GH mediated by IGF-1 in the liver (Wong et al., 2006; Debnath, 2010). The direct mechanism begins with oral administration of rElGH which is then absorbed by the digestive organs, especially the intestines (Putra et al., 2015), then rElGH enters the fish's body and triggers the hypothalamus to release growth hormone releasing hormone (GHRH). GHRH then enters the fish's organs such as the liver, kidneys, muscles, bones, and other organs, causing the fish to grow faster. The second mechanism is the indirect mechanism; rGH will go through the IGF-1 media produced by the liver to carry out the function of GH in the growth of fish fry. rGH that enters the fish's body will bind to GH receptors in the blood vessels and stimulate the expression of IGF-1 which will then bind to insulin-like-growth-factor-binding-proteins (IGFBPs) and be carried to the target organ. IGF-1 in the target cells will then bind to the IGF-1 receptor.

The best biomass increase results of nilem fish seeds during the study were in the R70 treatment but had the lowest TKH and LPS when compared to all treatments. The highest TKH and LPS values were found in the R30 treatment. The decrease in the survival rate of nilem fish is thought to be due to an increase in stocking density. Rakhfid et al. (2017) stated that a high increase in stocking density causes fish death due to stress and a decrease in water quality in the maintenance container, resulting in a decrease in survival rate. According to Mumpuni et al. (2022), stocking density will affect growth indirectly, namely through the mechanism of energy requirements, food competition, and water quality. Fish kept at low densities show better growth because they have more room to move compared to fish at high densities. Wide space to move encourages greater physical activity, which requires more energy. This increase in activity spurs increased energy needs and encourages body metabolism. A higher metabolic rate has a positive impact on the specific growth rate. The energy from this metabolism is used for basic body needs, daily activities, growth, and reproduction processes (Putra, 2015).

Increased stocking density causes stress to fish and even death. Stress in fish can reduce the secretion and availability of hormones related to the GH/-IGF-1 axis, the main endocrine growth regulator. Decreased plasma GH levels are associated with an early increase in cortisol levels under stress conditions (Conde et al., 2018). Cortisol is the main corticosteroid hormone and is an important indicator of stress response, chronic stress conditions, increased serum cortisol levels can suppress the GH/IGF-I axis response (Long et al. 2019). Crowding stress can reduce serum GH and IGF-1 protein levels, as well as GH mRNA expression levels in the brain and GH receptor (GHr) in the liver. can result in decreased GHr and IGFR protein synthesis and consequently IGF/IGFR and GH/GHr binding capacity and ultimately inhibit growth. increased IGFOP-1 mRNA levels can reduce IGFBP-1 synthesis and inhibit IGF activity (Zheng et al., 2024).

CONCLUSION

Administration of rEIGH hormone at a dose of 2 mg/kg feed at a stocking density of 30 fish/100 L-1 has a positive effect on the performance of fish seed production with an absolute weight growth (PBM) of 3.4 g, a survival rate (SGR) of 98.89% and a specific growth rate (SGR) of 4.8% day-1 so that the growth of tilapia fish given rEIGH hormone is faster.

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