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Effectiveness of Recombinant Growth Hormone on the Growth of Java Barb (*Barbonymus gonionotus*) with Different Stocking Densities

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ABSTRACT Tawes fish has economic value and affordable price. Growth rate and survival rate are important factors in cultivation productivity. This study aimed to evaluate the impact of recombinant growth hormone derived from kertang grouper (rElGH) on the growth performance of tawes fish seeds under varying stocking densities. A factorial randomized design was employed, incorporating two treatment variables and three replications. The treatments were rElGH (dose 0 mg/kg and 2 mg/kg) and different stocking densities (40 fish/m^3, 80 fish/m^3 and 160 fish/m^3). The tawes fish seeds used weighed 2.33 ± 0.002 g and were 4-5 cm long. Maintenance is carried out for 60 days used a hapa measuring 1.0 × 1.0 × 1.0 m with a water height of ±60 cm. Fish were fed to satiation using commercial feed containing 31–33% protein. The administration of rElGH was carried out on the first 7 days of maintenance and continued with the provision of feed without rElGH. The results of the Two-way ANOVA analysis showed that the use of rElGH and stocking density and their interactions had significant differences. The best treatment was the provision of rElGH stocking density 40 (R40) with survival rate 100%, specific growth rate 3.58%/day, absolute weight gain 8.75 g, and feed conversion ratio 1.12. The combination of the use of rElGH and increasing stocking density significantly increased the biomass production of tawes fish.

Keywords: Biomass production, growth performance, rEIGH, tawes fish

INTRODUCTION

Tawes fish is a type of freshwater fish native to Indonesia and is widely cultivated. It has economic value as a consumable fish and is relatively affordable for the community (Utami *et al*., 2018). The price of tawes fish varies by region, with fresh tawes fish reaching IDR 20,000/kg in Lamongan (Sa'adah, 2021), meanwhile in Central Java, tawes fish for consumption is priced at IDR 25,000. Value of tawes fish can increase up to IDR 35,000/kg when processed into smoked tawes fish (Sa'adah, 2021). Tawes fish farming can be carried out year-round in Indonesia, although production has declined over the years. Based on data from the Ministry of Marine Affairs and Fisheries [KKP] (2024), the production of farmed tawes fish in 2019 reached 20,048.90 tons, decreased to 17,913.27 tons in 2020, and continued to decline to 17,750.88 tons in 2021. Various measures need to be taken to increase production, including intensification through increased stocking density (Puspita & Ratih, 2018) and accelerating the growth rate of fish (Apriliana *et al*., 2017).

Stocking density in fish farming is a crucial factor that must be considered, as it affects the growth rate, feed conversion ratio, and fish survival rate, ultimately impacting overall production (Utami *et al*., 2018). Growth is a key aspect of tawes fish farming since rapid seed growth can shorten maintenance time and increase production. Tawes fish typically take 3-4 months to reach a weight of 300–350 g from an initial size of around 50 g. Maintenance time can be further reduced by stimulating fish growth through the application of recombinant growth hormone (rGH) (Kordi, 2013; Yuniarti *et al*., 2022), which enhances growth and allows for shorter maintenance periods (Apriliana *et al*., 2017).

The bioactivity test of rGH protein in enhancing growth rates has been conducted on various farmed fish species. The growth rate of fish given rGH can increase by up to 100%, resulting in more uniform fish sizes (Sutiana *et al*., 2017), a higher survival rate, and improved resistance to disease infections (Acosta *et al*., 2009). rGH supplementation also increases feed efficiency and accelerates gonad development in fish (Linan-Cabello *et al*., 2013). The application of recombinant growth hormone (rGH) via the mixing method resulted in a growth rate enhancement of clownfish (*Amphiprion percula*) larvae by as much as threefold in comparison to those that were not treated with rGH. Given these findings, research on the addition of rGH to tawes feed at different stocking densities is necessary to evaluate its impact on the growth performance of tawes fish seeds.

MATERIALS AND METHODS

This research was conducted over 75 days, from January to March, at the Aquaculture Experimental Pond, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. The testing of research parameters was carried out at the Aquatic Organism Reproduction and Genetics Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University.

Materials

The tawes fish seeds used in this study had an average length of 4–5 cm and a weight of approximately 2.28±0.002 g. Tawes seeds were sourced from the Sukabumi Freshwater Aquaculture Center and were kept in a 1.0 × 1.0 × 1.0 m hapa placed in a 20 × 10 × 1.5 m pond. The fish were fed commercial artificial pellets with a protein content of 31–33%, supplemented with recombinant *Epinephelus lanceolatus* growth hormone (rEIGH) during the experiment. The tawes fish seeds underwent an adaptation process to the artificial feed before feeding. The rEIGH hormone used in the feed was obtained from a commercial product under the trademark "Mina Grow" From Sukabumi Freshwater Aquaculture Center.

Methods

Experimental design

The study utilized a factorial randomized design consisting of two factors and three replications.

Table 1. Experimental design.

Symbol	Treatments
R40	Feeding with rEIGH at a stocking density of 40/m^3
R80	Feeding with rEIGH at a stocking density of 80/m^3
R160	Feeding with rEIGH at a stocking density of 160/m^3
K40	Feeding without rEIGH at a stocking density of 40/m^3
K80	Feeding without rEIGH at a stocking density of 80/m^3
K160	Feeding without rEIGH at a stocking density of 160/m^3

Fish maintenance

The maintenance of tawes fish during testing was conducted in 18 hapa units, each measuring 1.0×1.0×1.0 m, installed in a pond measuring 20×10×1.5 m. The water level in each container was maintained at approximately 60 cm. Tawes fish were first adapted to artificial feed during the initial week after seed distribution. The feed used was commercial feed with a protein content of 31–33%. The maintenance period lasted for 60 days, during which feed containing rEIGH was provided as treatment for the first week, followed by feed without rEIGH for the remainder of the study. Feed was administered to satiation twice daily, in the morning and evening.

Data collection

Data collection was conducted every two weeks by measuring the fish's weight on days 0, 15, 30, 45, and 60 of the rearing periods. Weight measurements were taken from 100% of the fish population in each hapa. The daily feed amount was recorded and accumulated until the end of the rearing period to calculate feed conversion.

The survival rate (SR) is the percentage comparison between the number of fish that survive at the end of the maintenance period and the number of fish stocked at the beginning. The survival rate is calculated using a specific formula (Effendie, 2002).

$$\mathrm{SR}\ (\%) = \frac{N_t}{N_a} \times 100 \quad \text{......} \quad 1$$

Note:
- SR = Survival rate (%)
- N_0 = Number of fish at the start of maintenance
- N_t = Number of fish at the end of maintenance

Absolute weight gain (AWG) is the difference in weight between the start of maintenance and the end of maintenance, calculated using the following formula (Effendie, 2002):

$$\mathrm{AWG} = W_t - W_0 \quad \text{......} \quad 2$$

Note:
- AWG = Absolute weight gain (g)
- W_t = Weight at the end of maintenance (g)
- W_0 = Weight at the start of maintenance (g)

The calculation of the specific growth rate (SGR) can be done using the formula according to Effendie (2002):

$$\mathrm{SGR}\ (\%/\mathrm{day}) = \frac{(\ln W_t - \ln W_0)}{t} \times 100 \quad \text{......} \quad 3$$

Note:
- SGR = Specific growth rate (%/day)
- W_t = Average weight of fish at the end of the study (g)
- W_o = Average weight of fish at the start of the study (g)
- t = time period (day)

The amount of feed consumption is the accumulation of feed consumed by fish during 60 days of maintenance. The feed conversion ratio (FCR) is the amount of feed needed to produce one kilogram of meat. FCR can be calculated using the formula (Effendie, 1997):

$$\mathrm{FCR} = \frac{F}{(B_t + B_d) - B_0} \quad \text{......} \quad 4$$

Note:
- FCR = Feed conversion ratio
- F = Amount of feed given to seeds (g)
- B_t = Seed biomass at the end of maintenance (g)
- B_d = Dead seed biomass (g)
- B_o = Seed biomass at the beginning of maintenance (g)

Biomass gain is calculated based on the equation (Zonneveld, 1991):

$$\text{Biomass gain} = B_t - B_0 \quad \text{......} \quad 5$$

Note:
- B_t = Biomass at the end of maintenance (g)
- B_0 = Biomass at the start of maintenance (g)

Data analysis

The data obtained were tabulated using Microsoft Excel 2016 and analyzed using One-Way ANOVA to determine differences between treatments and Two-Way ANOVA to assess the effects of treatments and their interactions at a 95% confidence level. Significantly different treatments were further analyzed using the Duncan test with the same confidence level, while comparisons between two treatments were conducted using the Independent T-test. All statistical analyses were performed using SPSS Statistics version 27.0.

RESULTS AND DISCUSSION

The average survival rate (SR), absolute weight gain (AWG), specific growth rate (SGR), feed consumption rate (FOR), feed conversion ratio (FCR), and biomass increase of tawes fish during the study are presented in Table 2. The SR of tawes fish ranged from 96% to 100%, with the R40, K40, and R80 treatments showing the highest SR (100%), surpassing the K80, R160, and K160 treatments. At a stocking density of 80–160 fish/m³ without the provision of rEIGH, the SR decreased.

As shown in Table 2, higher stocking density results in lower absolute weight gain (AWG). The provision of rEIGH significantly increased AWG, with the highest value observed in the R40 treatment and the lowest in the K160 treatment (P<0.05). Compared to their respective controls, the R40 treatment had an AWG 1.52 times higher, R80 was 1.45 times higher, and R160 was 1.15 times higher. The specific growth rate (SGR) followed the same pattern as AWG, where higher stocking density led to lower SGR. Additionally, the provision of rEIGH significantly increased SGR, with the highest value recorded in the R40 treatment and the lowest in the K160 treatment (P<0.05).

As shown in Table 2, the administration of rEIGH significantly increased AWG, with the highest value observed in the R40 treatment and the lowest in the K160 treatment ($P < 0.05$). Compared to their respective controls, the R40 treatment had an AWG 1.52 times higher, R80 was 1.45 times higher, and R160 was 1.15 times higher. The specific growth rate (SGR) followed a similar pattern, where higher stocking densities resulted in lower SGR values. Furthermore, the administration of rEIGH significantly increased SGR, with the highest value recorded in the R40 treatment and the lowest in the K160 treatment ($P < 0.05$). The rEIGH treatment also led to a significant reduction in SGR, with the highest decrease of 41.7% observed at the 160 stocking density, while the lowest decrease of 20.4% was recorded at the 40 stocking density.

rGH protein is a growth hormone that can stimulate fish growth (Putra *et al.*, 2018). rGH enhances growth and survival rates by boosting the immune system and increasing resistance to stress (Ihsanudin *et al.*, 2014). This is evident in the present study, as shown by the higher absolute weight growth and specific growth rate in the rEIGH treatment compared to the control, even at high stocking densities. The superior growth rate observed in the rEIGH-treated group is likely due to rEIGH's ability to suppress the stress response in fish. This finding aligns with Ihsanudin *et al*. (2014), who reported that rGH improves growth and survival rates by strengthening the immune system and enhancing stress resistance.

Stocking density is a crucial factor that can induce physiological stress in fish, ultimately affecting their growth performance and survival rate (Refaey *et al.*, 2018). Stress triggers a series of physiological responses as organisms attempt to counter threats and restore homeostatic balance (Gorissen & Flik, 2016). Prolonged, severe, or constant stress leads to poor welfare and health conditions in fish, negatively impacting growth performance and increasing susceptibility to disease and mortality (Li *et al.*, 2021).

From the data on feed consumption, fish at high densities exhibit a lower appetite compared to those at low densities. The stress response in teleost fish begins with the introduction of external stimuli that activates several neuroendocrine pathways, affecting fish physiology. Appetite-related signals reaching the brain are integrated by the hypothalamus, which responds by releasing orexigenic or anorexigenic neuropeptides, ultimately leading to an increase or decrease in food intake (Conde *et al.*, 2018).

The highest feed consumption was observed in the K160 treatment, which is believed to be due to stress experienced by the fish. Stress affects growth hormones in fish, which in turn influence appetite, swimming activity, and aggressive behavior (Irmawati *et al.*, 2022). Growth hormone (GH) is involved in the processes of protein synthesis and lipid metabolism. The administration of exogenous GH improves the capacity of fish to digest food, absorb nutrients, and convert a larger percentage of their intake into body mass, thereby influencing the feed conversion ratio (Alimuddin *et al.*, 2014).

The decrease in AWG and SGR, along with the increase in FCR, is believed to result from the fish's stress

Table 2. Growth performance of tawes fish after 60 days of maintenance.

Parameters	Treatment						Two-way ANOVA		
	R40	K40	R80	K80	R160	K160	R	T	R*T
SR (%)	100,00±0^{a}	100,00±0^{a}	100,00±0^{a}	97,00±2^{c}	98,33 ±0,58^{b}	96,00 ±1^{c}	*	*	*
AWG (g)	8,75± 0,04^{a}	5,76±0,06^{c}	6,87±0,02^{b}	4,75±0,01^{d}	4,32±0,06^{e}	3,76±0,03^{f}	*	*	*
SGR (%/day)	3,58±0,06^{a}	2,92±0,02^{c}	3,22±0,02^{b}	2,64±0,22^{d}	2,37±0,03^{e}	2,21±0,02^{f}	*	*	*
FOR (g)	9,82±0,10^{a}	11,82±0,31^{c}	8,71±0,13^{e}	10,99±0,51^{b}	6,16±0,18^{f}	8,73±0,54^{d}	*	*	*
FCR	1,12±0,02^{e}	2,05±0,07^{b}	1,26±0,02^{d}	2,31±0,11^{b}	1,43±0,03^{c}	2,32±0,14^{a}	*	*	*
Biomass gain (g)	218,70±1,12^{e}	144,2±1,43^{f}	343,42±0,88^{c}	232,84±5,27^{d}	424,55±8,22^{a}	360,93±6,09^{b}	*	*	*

Note: Mean values ± standard deviations followed by different uppercase letters in the same row indicate significant differences (p<0.05). (-) indicates no significant difference, while () *indicates a significant difference. R represents rEIGH, T refers to stocking density, and R*T denotes the interaction between rEIGH and stocking density. SR stands for survival rate, AWG for absolute weight gain, SGR for specific growth rate, FOR for total feed consumption, and FCR for feed conversion ratio and biomass gain. The treatment groups include R40 (feeding with rEIGH at a stocking density of 40 fish/m³), K40 (feeding without rEIGH at a stocking density of 40 fish/m³), R80 (feeding with rEIGH at a stocking density of 80 fish/m³), K80 (feeding without rEIGH at a stocking density of 80 fish/m³), R160 (feeding with rEIGH at a stocking density of 160 fish/m³), and K160 (feeding without rEIGH at a stocking density of 160 fish/m³).

response to the environment. High stocking density can reduce the growth rate of tilapia due to limited space for movement or physiological stress reactions caused by poor conditions (Costa *et al*., 2017). Stunted growth at high stocking densities is primarily due to crowding stress, which increases energy demands to activate physiological responses for coping with stress, thereby reducing the energy available for growth. Additionally, crowding stress alters energy metabolism, diverting energy toward stress management and leading to decreased feed conversion efficiency (Refaey *et al*., 2018).

The best results were obtained in the R160 treatment, while the lowest was observed in K40, with the R160 treatment yielding 2.95 times more biomass than K40. The higher stocking density and the administration of rEIGH are positively correlated with biomass growth. Stocking density is also positively correlated with aquaculture production, as production increases with higher stocking densities (Moniruzzaman *et al*., 2015). However, with a maintenance period of 60 days and rEIGH administration in the first week, the highest harvest size was achieved in the R40 treatment. A longer maintenance period is still required to reach the desired harvest size, and a booster may be necessary to further stimulate growth. The administration of an rGH booster to eel seeds has been shown to significantly enhance growth (Handoyo *et al*., 2012).

The results of the two-way ANOVA analysis showed that the use of rEIGH, differences in stocking density, and their interaction had a significant effect on SR, AWG, SGR, FOR, and FCR. The best growth performance was observed in the R40 treatment, with SR (100%), AWG (8.75 g), SGR (3.58%/day), and FCR (1.12). However, based on the difference in biomass increase compared to each control, the R80 treatment had the highest value (approximately 110 g), followed by R40 with around 74.5 g and R160 with around 63.6 g. These results indicate that the use of rEIGH and appropriate stocking density can significantly enhance biomass growth.

The administration of rGH through feed is believed to enter the body via the digestive system (Sutiana *et al*., 2017), after which it enters the bloodstream and signals the pituitary gland (Dehkhoda *et al*., 2018). Recombinant growth hormone (rGH) activates the hypothalamus, thereby increasing the secretion of growth hormone-releasing hormone (GH-RH), which subsequently prompts the pituitary gland to synthesize growth hormone (Apriliani *et al*., 2017). rEIGH hormone subsequently enters various internal organs such as the liver, kidneys, muscles, bones, and other tissues, accelerating fish growth. The pituitary gland plays a crucial role in rGH absorption by stimulating the release of GH, which promotes growth, explaining why rEIGH treatment is superior in enhancing growth performance (Kurniawan *et al*., 2017).

CONCLUSION AND RECOMMENDATION

Conclusion

This study demonstrated that the administration of rEIGH significantly improved growth performance, including absolute weight, specific growth rate, and feeding conversion ratio, across various stocking densities compared to the control at the same stocking density. Treatment R40, which involved rEIGH administration and a stocking density of 40 fish/m^3, produced the best results in survival rate, absolute weight gain, specific growth rate, and feed conversion ratio. However, based on the biomass increase parameter, treatment R160 exhibited the highest biomass increase.

Recommendation

Future studies can optimize dosing strategies, evaluate economic feasibility under commercial aquaculture conditions, and assess the environmental impacts of increased biomass production at higher stocking densities, such as in the R160 treatment group. Comparative studies involving different fish species or varying environmental conditions (e.g., temperature, water quality) would also help generalize the applicability of rEIGH in aquaculture.

AUTHORS' CONTRIBUTIONS

SRY as data collection. AMD provided research ideas and as research supervisor. OCM as research supervisor. KAR as a manuscript writer.

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