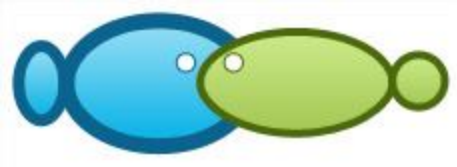

Recombinant growth hormone supplemented on feed to the growth performance of *Barbodes binotatus*

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Abstract. Recombinant growth hormone (rGH), a gene product isolated and transformed into microbes, such as *Escherichia coli*, *Bacillus* sp., *Streptomyces* sp., and *Saccharomyces* sp., is to accelerate growth and increase the fish endurance. This study aimed to examine the effect of the commercial rGH "Mina Grow" on the growth performances of *Barbodes binotatus* by oral administration. In brief, *B. binotatus* was administered with commercial rGH "Mina Grow" at 1 mg kg^{-1}, 2 mg kg^{-1}, 3 mg kg^{-1}, and 4 mg kg^{-1} supplemented to commercial feed. The specific growth rate (SGR), daily length growth (DLG), feed conversion ratio (FCR), and survival rate (SR) were assessed to discover *B. binotatus* growth responses. The present study showed that 2 mg kg^{-1} rGH "Mina Grow" had the growth performance responses (GR, SGR, DLG, and FCR) and SR of *B. binotatus* significantly higher compared with the control group. The present findings suggest that commercial rGH "Mina Grow" at the concentration of 2 mg kg^{-1} enhanced the growth performance responses (GR, SGR, DLG, and FCR) and SR in *B. binotatus* using oral administration.
Key Words: oral administration, rGH, SGR, wader fish.

Introduction. Wader fish (*Barbodes binotatus*), fish endemic of Indonesia, is one of the most familiar freshwater fish commodities (Suryanti et al 2017). These fish have been found in several Asian countries, including Indonesia, Brunei Darussalam, Cambodia, Laos, Malaysia, Philippines, Myanmar, Thailand, Vietnam, Palau, and Singapore (Lim et al 2013). The demand for *B. binotatus* continues to increase and make aquaculture farms provide *B. binotatus* in high quantity (Raharjo 2019). Aquaculture could be applied as one of the solutions to reach market demand, which natural catches continually cannot meet market demand (Deutsch et al 2007). According to Lim et al (2013), there is no information on the culture of *B. binotatus,* and probably it is not an essential aquacultural species in any other region. Indonesia has a different story in which *B. binotatus* has a potential market as fish consumption and even as an ornamental fish (Batubara et al 2019).

Besides, the growth of *B. binotatus,* which is rather slow with a relatively small size, is one of the obstacles. This obstacle reduced the interest of farmers to cultivate this fish (Budiharjo 2003). Accelerated growth of *B. binotatus* is a way to increase production. The recombinant Growth Hormones (rGH) may stimulate the growth of *B. binotatus* that is supplemented into the feed. The rGH is a technological innovation in the field of fisheries that has the potential as a feed supplement that can provide acceleration of growth in cultured fish (McLean et al 1999). rGH is one of the polypeptide hydrophilic hormones composed of amino acids that can be used to stimulate fish growth (Alimuddin et al 2010). Besides being able to increase growth, the administration of rGH can also improve the survival of fish through a system of boosting the body's immunity against disease and stress (McCormick 2001). The rGH hormones are expected to accelerate the growth of *B. binotatus* so that they can meet market demand.

Based on the description above, we aimed to determine the effect of rGH in feed on the growth performance of *B. binotatus*.

Material and Method

Fish and feed preparation. *B. binotatus* (1±0.2 cm) was obtained from UPT PBAT Umbulan, East Java, Indonesia, and cultivated in fisheries laboratory, Department of aquaculture, University of Muhammadiyah Malang, East Java, Indonesia. One hundred eighty *B. binotatus* (two fish/liter) were grown in an aquarium (40 cm × 30 cm × 25 cm) containing 30 L of water and fed with commercial feed at 3% of fish body weight per day.

Feed preparation used commercial feed supplemented with commercial rGH that was provided by the "Mina Grow" brand isolated from grouper fish. The handmade-feed was prepared following Ihsanudin et al (2014) and Lubis et al (2018) with some modification. Briefly, rGH powder (1 mg kg^{-1}, 2 mg kg^{-1}, 3 mg kg^{-1}, and 4 mg kg^{-1} of commercial feed) was dissolved in 100 mL Phosphate Buffer Saline (PBS) and 20 mg of egg yolks. The solution was gently homogenized and transferred to the sprayer bottle. The mixture was then spread to commercial feed and dried at room temperature for 10-15 minutes until ready to use. Supplemented feed was given 3% of body weight twice per day at 09.00 and 15.00 for 60 days, June 12 to August 11 2019.

Experimental design. An experimental evaluation with a completely randomized design (CRD) was used for this study with four treatments and three repetitions.

T0 = 100% commercial feed;
T1 = commercial feed + commercial rGH (1 mg kg^{-1});
T2 = commercial feed + commercial rGH (2 mg kg^{-1});
T3 = commercial feed + commercial rGH (3 mg kg^{-1});
T4 = commercial feed + commercial rGH (4 mg kg^{-1}).

Effect of commercial rGH on the growth performance of B. binotatus. The sampling of fish was held every ten days within 60 days. The sampling method of fish was carried out for 10 percent of the total fish. Specific growth rate (SGR), daily length growth (DLG), feed conversion ratio (FCR), and survival rate (SR) were used to asses the growth performances of *B. binotatus* (Susatyo et al 2016; Shah et al 2018).

$$\mathbf{SGR} = \frac{\ln Wt - \ln Wo}{t}$$

where: SGR = specific growth rate (% day^{-1});
ln Wo = initial fish weight (g);
ln Wt = final fish weight (g);
t = time (day).

$$\mathbf{DLG} = \frac{\ln Lt - \ln Lo}{t}$$

where: DLG = fish body length (% day^{-1});
ln Lo = initial fish body length (cm);
ln Lt = final fish body length (cm);
t = time (day).

$$\mathbf{FCR} = \frac{F}{Wt - Wo}$$

where: FCR = feed conversion ratio;
F = feed consumption during cultivation (kg);
W0 = initial total weight of fish (kg);
Wt = final total weight of fish (kg).

$$\mathbf{SR} = \frac{Nt}{No}$$

where: SR = survival rate (%);
No = initial number of fish;
Nt = final number of fish.

Statistical analysis. The collected data were analyzed using ANOVA (One-way analysis of variance) to determine the variation of data. The measure of significant changes among treatments used Duncan test provided by SPSS 17. Data were performed as the mean±SD, $p < 0.05$ considered significant.

Results and Discussion. The feed supplemented with commercial rGH "Mina Grow" affected the growth performances of *B. binotatus* after 60 days of cultivation (Table 1). The feeding with commercial feed completed with commercial rGH "Mina Grow" showed a significant difference compared with the control group ($p > 0.05$).

Table 1

Growth performance and survival of *B. binotatus* fed with different dosages of rGH "Mina Grow" after 60 days

Parameter	*T0* *(0 mg kg^{-1})*	*T1* *(1 mg kg^{-1})*	*T2* *(2 mg kg^{-1})*	*T3* *(3 mg kg^{-1})*	*T4* *(4 mg kg^{-1})*
SGR (% day^{-1})	2.88±0.10^{a}	2.98±0.19^{a}	3.62±0.17^{b}	3.43±0.18^{b}	3.11±0.13^{a}
DLG (% day^{-1})	1.12±0.09^{a}	1.37±0.06^{b}	1.51±0.02^{d}	1.47±0.03^{c}	1.41±0.02^{c}
FCR	2.10±0.11^{a}	1.77±0.12^{b}	1.32±0.13^{c}	1.51±0.10^{c}	2.02±0.06^{a}
SR (%)	69.44±2.55^{a}	76.11±2.55^{a}	77.22±2.55^{b}	74.44±3.47^{b}	72.22±2.55^{a}

Note: Different superscripts on the same row reveal a significant difference ($p < 0.05$)

Growth performances. The T2 and T3 (3.62% and 3.43% per day, respectively) showed optimum dosage to enhance SGR of *B. binotatus,* followed by T4, T1, and T0 (no significant difference). While the fish body length was optimum enhanced by T2 (2 mg kg^{-1}) with 1.51±0.02% day^{-1} followed by T3 and T4 (1.47 and 1.41% day^{-1}, respectively). Based on it, the commercial rGH "Mina Grow" supplemented to feed by 2 mg kg^{-1} to 3 mg kg^{-1} could boost the growth of *B. binotatus* by increasing their metabolic process. The rGH can regulate body growth (Bartke 2005; Alimuddin et al 2010), reproduction (Tsai et al 2007), immune system (Leedom et al 2002) and regulate osmotic pressure in Teleostei fish, and regulate metabolism including lipolytic activity and protein anabolism of vertebrates (Company et al 2002; Pérez-Sánchez 2000).

Many studies had been conducted to evaluate the effect of the rGH to some fishes such as tilapia (*Oreochromis niloticus*) (Robles Basto et al 2016; Vinasyiam & Suprayudi 2016), *Cromileptes altivelis* (Antoro et al 2016), and striped catfish (*Pangasianodon hypophthalmus*) (Lubis et al 2019). The recent research by Apriliani et al (2018) showed that the use of 2 mg kg^{-1} of rGH supplemented to commercial feed induced growth and survival of Tawes fish (*Puntius* sp.). Interestingly, the present study revealed that a higher dosage of the rGH influenced negative feedback, such as lower fish growth performances. According to Cameron et al (2007) and McLean & Clarke (1997), negative feedback occurs hormonally, i.e., IGF-1 will suppress pituitary in producing growth hormone (GH) when GH concentration in the fish body is excessive. Based on our findings, rGH "Mina Grow" brand is recommendable to be applied and supplemented with commercial feed for increasing fish growth performances.

Feed convention ratio (FCR). The FCR results of *B. binotatus* within 2 months could be seen in Table 1 . The feed supplemented with commercial rGH "Mina Grow" affected the growth performances of B. binotatus after 2 months of cultivation (Table 1). The feeding with commercial feed completed with commercial rGH "Mina Grow" showed a significant difference compared with the control group ($p > 0.05$).

The present study showed that the addition of the rGH into commercial feed stimulated fish more efficient in optimizing the amount of feed consumption. The 2 mg kg^{-1} treatment was the optimal dosage in feed efficiency, where the FCR was the lowest (1.32) followed by T3, and T1 (1.51 and 1.77, respectively).

A similar study by Putra et al (2016) showed that commercial feed supplemented with rGH decreased the FCR of *Osphronemus gouramy* from 1.35 to 1.05. Apriliani et al (2018) also observed that the FCR of Tawes fish (*Puntius* sp.) without rGH treatment (0 mg kg^{-1} of feed) showed the highest FCR value of 2.75, while using rGH treatments

reduced FCR to 1.86 and 1.60, 4 mg kg^{-1} of feed treatment being the optimal dosage. The commercial rGH (in amount of 2.5 mg kg^{-1}) also reduced the FCR in koi carp (*Cyprinus carpio*) from 2.71 to 1.92. According to Kling et al (2012), a factor that can increase the value of FCR in fish is the provision of GH, which plays a vital role on different levels, such as digestion and absorption processes, allocation and utilization of nutritional energy. GH can support the distribution of power to muscle and bone growth through its effects on protein and lipid metabolism (Yousefian & Shirzad 2011). Based on our findings, rGH "Mina Grow" brand is recommendable to be applied and supplemented with commercial feed for reducing fish FCR.

Survival rate (SR). The feed supplemented with commercial rGH "Mina Grow" affected the growth performances of B. binotatus after 2 months of cultivation (Table 1). The feeding of commercial feed completed with commercial rGH "Mina Grow" showed a significant difference compared with the control group ($p > 0.05$).

Based on Table 1, the survival rate of *B. binotatus* found that in the treatment of commercial feed with the addition of 1 mg kg^{-1}, 2 mg kg^{-1}, 3 mg $kg^{-1,}$ and 4 mg kg^{-1} the RGH had higher survival than the control (0 mg kg^{-1}) ($p < 0.05$). The T2 with 2 mg kg^{-1} of commercial rGH supplemented on feed showed optimum dosage with 77.22% survival rate of *B. binotatus* followed by T1, T3, and T4 (76.11%, 74.44%, and 72.22%, respectively).

According to Liana (2007), the survival rate is influenced by optimal environmental conditions and adequate feed. Our results revealed that the administration of rGH could improve fish survival by increasing health status and the immune system of *B. binotatus* against disease and environmental changes. The rGH could stimulate lysozyme activity and hemagglutinating activity of tilapia (*Oreochromis hornorum*) that relates to its survival rate (Acosta et al 2009). Another explanation of how rGH could stimulate the immune system was demonstrated by Franz et al (2016). GH was believed to be a stimulator of innate immunity such as leucocyte (Kajita et al 1992; Meazza et al 2004) and phagocyte (Sakai et al 1995) production in both mammals and teleost fish.

A similar study by Etoh et al (2011) showed that 30 mg of recombinant giant gourami growth hormone (r-OgGH) assisted 100% survival rate of *O. goramy* for 5 weeks cultivation. In another study, the addition of rGH supplemented with feed could increase *Trachinotus blochii* survival rate (Putra & Raza'i 2018). Based on our findings, rGH "Mina Grow" brand is recommendable to be applied and supplemented with commercial feed for increasing fish survival rate.

Conclusions. The results of this study indicated that the addition of the rGH hormone to commercial feed could affect the growth rate performance of *B. binotatus*, in terms of the SGR, DLG, SR and FCR by 2 mg kg^{-1} of rGH "Mina Grow" brand which was the optimal dosage. It was concluded that four treatments supplemented with the rGH could accelerate the growth performance of *B. binotatus*.

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