Growth and survival of giant gourami juvenile immersed in different doses of recombinant growth hormone

Pertumbuhan dan kelangsungan hidup juvenil ikan gurami direndam dalam hormon pertumbuhan rekombinan dengan dosis berbeda

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ABSTRAK

Penelitian ini dilakukan untuk meningkatkan pertumbuhan benih ikan gurami (*Osphronemus goramy*) melalui imersi dengan hormon pertumbuhan rekombinan ikan gurami (r-*Og*GH). Imersi dengan larutan mengandung badan inklusi dilakukan dengan dosis r-*Og*GH berbeda, yakni 0 mg/L (kontrol), 10 mg/L, 20 mg/L, dan 30 mg/L, sekali seminggu selama tiga minggu. Ikan dipelihara selama lima minggu dalam akuarium dengan kepadatan 5 ekor/L, dan ikan diberi pakan naupli *Artemia* dan cacing sutera dua kali sehari secara *ad libitum*. Hasil penelitian menunjukkan bahwa rerata bobot tubuh ikan perlakuan 30 mg/L (0,34 g) adalah sekitar 75% lebih tinggi (p<0,05) daripada perlakuan 10 mg/L (0,24 g) dan kontrol (0,19 g). Kelangsungan hidup tertinggi diperoleh pada perlakuan 30 mg/L (100%), diikuti oleh perlakuan 20 mg/L (96%), perlakuan 10 mg/L (94%), dan kontrol (94%). Dengan demikian, imersi menggunakan hormon pertumbuhan rekombinan dapat digunakan untuk meningkatkan pertumbuhan dan kelangsungan hidup benih ikan gurami.

Kata kunci: protein rekombinan, hormon pertumbuhan, imersi, *Osphronemus goramy*

ABSTRACT

This study was aimed at enhancing the growth of giant gourami *Osphronemus goramy* fry by immersion with recombinant giant gourami growth hormone (r-*Og*GH). Immersion with solution containing inclusion bodies with different doses of r-*Og*GH, 0 mg/L (control), 10 mg/L, 20 mg/L, and 30 mg/L was performed on weekly basis for the first three weeks of experimental period. Fish were reared for five weeks in glass aquaria at a stocking density of 7 fish/L, and fed with *Artemia* nauplii and blood worm twice a day ad libitum. The results showed that the average body weight of 30 mg/L r-*Og*GH treated fish (0.34 g) was 75% higher (*p*<0.05) than those of 10 mg/L (0.24 g) and control (0.19 g). The highest survival was obtained at 30 mg/L r-*Og*GH immersed fish (100%), followed by 20 mg/L (96%) and 10 mg/L treated fish (94%), and control (94%). The results indicate that immersion with recombinant growth hormone could be applied to enhance the growth and survival of giant gourami juvenile.

Keywords: recombinant protein, growth hormone, immersion, *Osphronemus goramy*

INTRODUCTION

Giant gourami *Osphronemus goramy* has the highest price compared to that of other freshwater species in Indonesia. Production of giant gourami is targeted to be 48,900 metric ton in 2014, or 27% higher than in 2009 (Ismanadji, 2010). The crucial problem to meet the target is giant gourami performs slow growth, where it spends about one year of rearing to reach its market size of 500 g. Hence, a technique to accelerate its growth is required. The application of growth stimulation technique using recombinant growth hormone (rGH) is considered to be an advantage approach.

Advances in recombinant DNA technology have offered a way to produce large amounts of teleostean growth hormones using bacteria *Escherichia coli* as a vector (Sekine *et al*., 1985; Sato *et al*., 1989; Sugimoto & Yokoo, 1991; Tsai *et al*., 1995; Promdonkoy *et al*., 2004; Funkenstein *et al*., 2005). Furthermore, administration of

recombinant fish growth hormone (rGH) has been reported to considerably increase the growth of finfish (Moriyama & Kawauchi, 1990; Lin *et al.*, 1995; Tsai *et al.*, 1997; Ben-Atia *et al.*, 1999; Promdonkoy *et al.*, 2004; Funkenstein *et al.*, 2005; Acosta *et al.*, 2009) and shellfish (Xu *et al.*, 2000; Moriyama & Kawauchi, 2004; Santiesteban *et al.*, 2010). Treatment of rGH can also enhance fish survival through increasing its resistance to disease (Sakai *et al.*, 1997) and stress conditions such as salinity (reviewed by McCormick, 2001). The used of rGH may also increase the growth and survival of giant gourami.

There are three types of giant gourami juvenile usually offer as market size, namely the size of 'kuku' (about 3 cm in total body length/BL), 'jempol' (BL of ~4 cm), 'silet' (BL of ~6 cm), and 'korek' (BL of ~8 cm). Administration of rGH in larval stage is suggested to be considerably increasing the growth of giant gourami juvenile. Delivery of rGH into larval and juvenile of fish is generally conducted by immersion bath method (Moriyama & Kawauchi, 1990; Moriyama *et al.*, 2008; Acosta *et al.*, 2009; Santiesteban *et al.*, 2010). This research was aimed to stimulate the growth of giant gourami fry by administration of recombinant giant gourami growth hormone (r-*Og*GH) into 1-day after yolk egg absorbed larvae.

MATERIALS AND METHODS

Construction of protein expression vector

Signal peptide sequence of giant gourami growth hormone (Nugroho *et al.*, 2008) was predicted using SignalP 3.0 software (http://www.cbs.dtu.dk/services/signalP). Predicted nucleotide sequence encoding mature growth hormone of giant gourami (*Og*GH) was amplified by PCR using a set primer of *Og*-mGH-F (5'-GGATCC CAG CCA ATC ACA GAC AGC CAG-3') and *Og*-mGH-R (5'-GAATTC CTA CAG AGT GCA GTT AGC TTC TGG-3'), containing *Bam* HI and *Eco* RI recognition sites (underlined) respectively. Subsequently, the PCR products were purified and cloned into the pGEM-T Easy vector (Promega, Madison, USA). *Bam*

HI/*Eco* RI-double digested *Og*GH cDNA fragment was then ligated into the corresponding sites of the expression vector pCold-1 (Takara Bio Inc., Japan) driven by *cspA* promoter. The constructed plasmid was designated as pCold-1/*Og*GH (Alimuddin *et al.*, 2010).

Production of r-*Og*GH

Escherichia coli BL21 (DE3) harboring pCold-1/*Og*GH was inoculated in 4 mL of 2×YT medium containing 100 μg/mL of ampicillin and was incubated overnight at 37 °C. Approximately 1 μL of bacterial culture was transferred into 100 mL of medium containing 100 μg/mL and was subjected to cold shock treatment by incubating the bacterial culture at 15 °C for 30 min. Induction of protein synthesis was performed by adding 1 mM IPTG (isopropyl-b-D-thiogalactopyranoside) and subsequently incubated at 15 °C for 24 hours. The bacterial cells were harvested by centrifugation at 12,000 rpm for 1 min at 4 °C, then was resuspended in phosphate buffer saline (PBS) containing 0.1% Triton X-100, and was washed twice by the same solution. Bacterial cells was lysed by adding 500 μLlysozyme (10 mg/mL lysozyme in TE buffer) and was incubated at 37 °C for 20 min, or was sonicated six times for 30 s on ice. Inclusion bodies were collected by centrifugation at 12,000 rpm for 5 min, and the pellet was washed twice by 1 M NaCl containing 1% Triton X-100, and then was resuspended in PBS containing SDS and β-mercaptoethanol. Sample of inclusion bodies solution was heated at 100 °C for 10 min, and then was chilled on ice and was centrifuged briefly. An aliquot of 10 μL was analyzed by SDS-PAGE with 10% polyacrylamide gel, and protein was visualized using Coomassie Blue dye. The recombinant protein was stored at -80 °C until use.

Immersion of recombinant growth hormone and fish rearing

There are three series of immersion treatments due to concentration of r-*Og*GH, i.e. 10 mg/L, 20 mg/L, and 30 mg/L respectively, and one treatment without r-*Og*GH as the control. In amount of 50 fish

was used for each treatments including for the control treatment. The 50 fish of one day after first feeding were subjected to hyper osmotic shock of 3.5% NaCl for 2 min (this was obtained from our preliminary study), and subsequently immersed for 60 min in 200 mL of r-*Og*GH solution at different concentrations depend on the treatment and without r-*Og*GH as the control. Along with the hormone, 0.9% of NaCl and 0.01% of BSA were also added to the immersion solution. The treatment was performed in duplicate and was performed once weekly for three weeks.

Fish were reared for five weeks in 8 L of glass aquaria with water temperature of 27±1 °C, and fed with *Artemia* nauplii during the first week and blood worms for the next four weeks. Feeding was conducted twice a day ad libitum. Water quality was maintained by daily water replacement at a level of 50% for the first two weeks, and 80% for the remaining three weeks. Fish body weight was measured at seven days interval. Fish feeding response was observed in the 5th week.

Statistical analysis

Statistical analysis were performed by SPSS 16.0 software package. Differences in body weight and growth rate were determined by one-way ANOVA followed by Duncan's test at a significance level of p=0.05. Survival and fish feeding response were descriptively described.

RESULTS

Recombinant growth hormone production

Inclusion bodies containing recombinant giant gourami growth hormone (r-*Og*GH) obtained from 100 mL of bacterial culture was about 390 mg in wet weight. Result of SDS-PAGE analysis (Fig. 1) showed that *E. coli* BL21 carrying pCold-1/*Og*GH expression vector could produce a relatively high level of r-*Og*GH in the size of about 21 kDa.

Fish growth

The changes of body weight of non-treated control and r-*Og*GH treated groups fish is shown in Fig. 2A. Significant increment in body weight of treated fish groups, especially those treated with 30 mg/L r-*Og*GH was initially observed on the third week of experimental period, and this consistently observed until the 3rd week of treatment. By the end of experiment, both 20 and 30 mg/L r-*Og*GH treated fish body weight showed 75% and 64% higher than the control (P<0.05), whereas no significant difference (P>0.05) was observed between fish treated with 10 was observed between fish treated with 10 mg/L r-*Og*GH (0.24 g) and those of control (0.19 g). Furthermore, the growth rate of 30 mg/L (2.86%/day) and 20 mg/L r-*Og*GH treated fish (2.63%/day) was comparable (P>0.05), and both were significantly higher (P<0.05) than those of 10 mg/L r-*Og*GH treated fish (2.14%/day) and the control (1.69%/day). Phenotype of 30 mg/L r-*Og*GH treated fish and those of control fish after five weeks rearing period was shown in Fig. 2B.
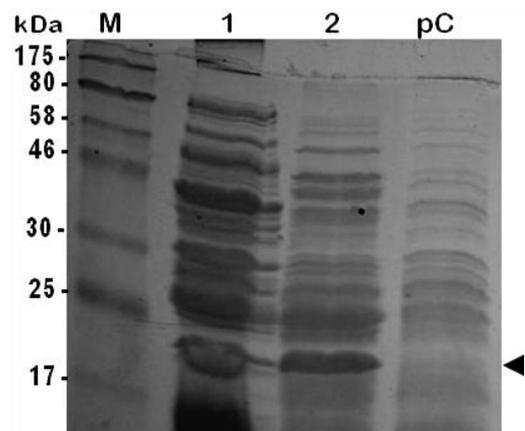

Fig. 1. SDS-PAGE analysis of inclusion bodies containing recombinant giant gourami growth hormone (arrow head) separated in 10% SDS-PAGE gel. Lane 1: inclusion bodies from lysozyme-lysed bacterial cells. Lane 2: inclusion bodies from sonicated bacterial cells. pC: inclusion bodies from bacterial cells carrying pCold-1 empty. M: molecular weight marker.

Fish survival

Recombinant growth hormone (rGH) treatment appears to increase the survival of giant gourami fry (Table 1). The highest survival was shown by the treatment of 30 mg/L r-*Og*GH (100%), followed by the treatment of 20 mg/L (96%), 10 mg/L (94%), and the control (94%).
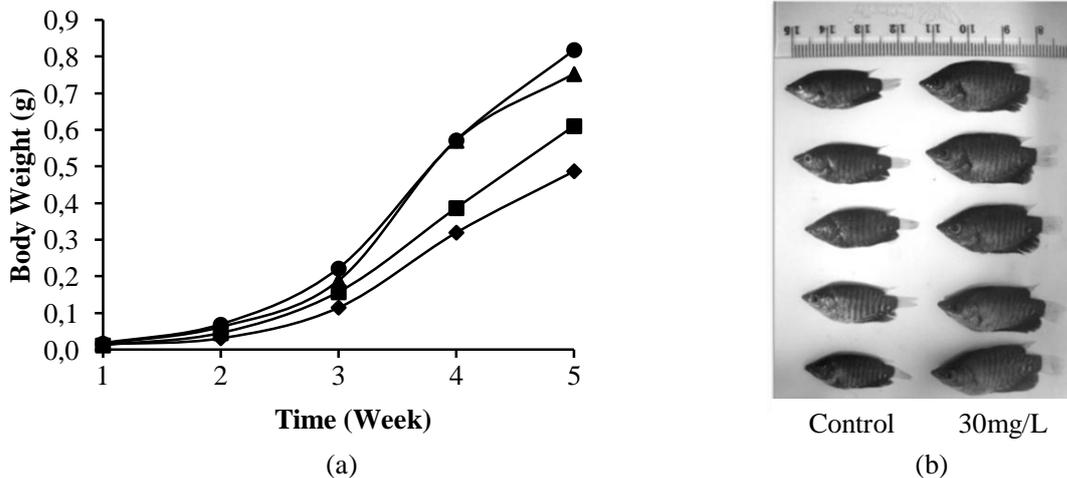
(a)   (b)

Fig. 2. Changes in body weight (g) of giant gourami fry in control (−○−), 10 mg/L (−■−), 20 mg/L (−▲−), and 30 mg/L recombinant growth hormone-immersed groups fish (−●−) for 5 weeks rearing period (a). Fish (n=50) were immersed in the hormone solution for 60 min on weekly basis for the first three of five weeks of experimental period. The photograph shows the phenotype of non-treated control and 30 mg/L recombinant growth hormone-treated groups of fish after five weeks of rearing period in 8-l aquaria (b).

Table 1. Body weight (g), growth (%) and survival (%) rates of giant gourami fry in control and recombinant growth hormone-immersed fish at different doses

r-*Og*GH dose treatments (mg/L)	Body weight (g)	Growth rate (%/day)	Survival rate (%)
0 (control)	0.19 ± 0.001^a	1.69^a	94 ± 2.0
10	0.24 ± 0.023^a	2.14^b	94 ± 2.0
20	0.32 ± 0.012^b	2.64^c	96 ± 4.0
30	0.34 ± 0.004^b	2.86^c	100 ± 0.0

The body weight and survival of fish are given as mean ±SE of duplicated treatments. r-*Og*GH is recombinant giant gourami *Osphronemus goramy* growth hormone. Different superscript letters in the same column indicate significant differences (P<0.05).
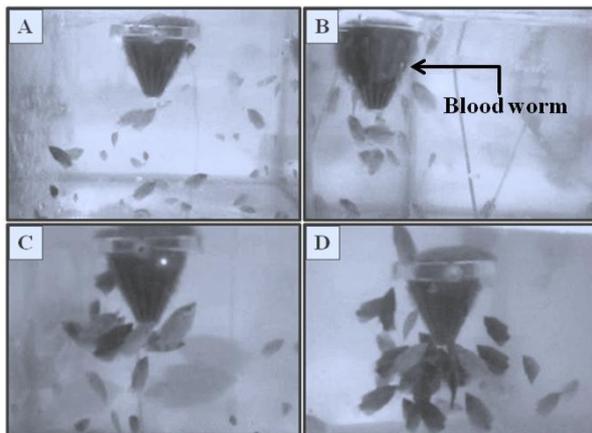

Fig. 3. Different responses to live food (blood worm) of giant gourami with non-treated control (A), 10 mg/L (B), 20 mg/L (C), and 30 mg/L recombinant growth hormone-treated (D). Photographs were taken on the last week of rearing period.

Fish feeding response

Visual observation on fish feeding behavior showed that treatment of rGH in giant gourami fry could increase the fish feeding response. This response was initially observed on the second week and

continuously shown for the remaining weeks of experimental period (Fig. 3). Figure 3 also shows that the number of fish which response to feeding was found to be higher as the doses of r-*Og*GH treatment increases.

DISCUSSION

It is well known that the growth of giant gourami is relatively slower than other freshwater aquaculture species. Several manipulation techniques such as selection have been applied as an effort to improve fish growth. However, to obtain a significant genetic gain this technique is labor intensive and time-consuming. Therefore, another technique such as the application of recombinant growth hormone can be applied as an alternative way with a minimum cost and time. This experiment showed that application of recombinant growth hormone r-*Og*GH for three consecutive weeks with at dose of 30 mg/L and 20 mg/L resulted in significant increase (p<0,05) of 75% and

64% growth rate, respectively, when compared to the control. Furthermore, the growth was still higher at the end of experiment (five weeks) even the treatment had been stopped in the third week (Fig. 2A). Similar results were also reported in salmonid (Moriyama & Kawauchi, 1990), striped bass, channel catfish, grass carp (Sonnenschein, 2001), abalone (Moriyama & Kawauchi, 2004), Nile tilapia, goldfish, and common carp (Acosta *et al.*, 2009).

Inclusion bodies obtained from 100 mL of bacterial culture was about 390 mg in wet weight. With the dose of 30 mg/L and 20 mg/L, three times immersion and 250 fish can be immersed in 1 L water, so that the total numbers of fish that can be treated using 390 mg recombinant protein were about 1,080 and 1,600 fish, respectively. The number of the treated fish per immersion can be increased if its density during the treatment is raised to more than 50 fish per 200 mL, thus improving the efficiency of the use of rGH. Increasing the number of treated fish however may result in lower growth acceleration, therefore an optimum fish density for an efficient recombinant growth hormone immersion treatment still requires further investigation.

Indonesian farmers commonly perform size grading for giant gourami seed production where the price is determined based on total body length, i.e. 'kuku' (2–3 cm, IDR 150–250 in price), 'jempol' (3–4 cm, IDR 500–750), and 'silet' (5–6 cm, IDR 1,500–2,000). When this grading is applied to the result of this experiment at the end of the rearing period, it is found that the size of fry obtained from the treatment with a dose of 30 mg/L r-*Og*GH was mostly categorized into 'jempol' size, while non-treated control was mostly 'kuku' size. Thus, the administration of rGH not only shorten the time required to obtain fry with 'jempol' size, but also improves the farmers income as the price of this size of fry is 2 times higher than the 'kuku' size.

The immersion method has been developed in various fish species, fish stages (larvae, fry, or juvenile), concentrations and GH sources (fish and mammalian origin), purities (purified or crude protein),

frequencies and intervals (once to several times immersion, and once to thrice weekly), methods and periods of fish rearing. Level of growth acceleration is also varied, such as 63% in grass carp, 45% in channel catfish, 35.5% in striped bass and 20% in freshwater prawn using bovine somatotropin (Sonnenschein, 2001), 20% in abalone using salmon GH (Moriyama & Kawauchi 2004), 100% in tilapia juvenile using tilapia GH (Acosta *et al.*, 2009) and 42.4% in Pacific white shrimp using tilapia GH (Santiesteban *et al.*, 2010). In our study, immersion was performed using crude protein as inclusion bodies containing r-*Og*GH resulted a significant growth enhancement of giant gourami larvae approximately 75% at the dose treatment of 30 mg/L.

Survival rate of fish treated by 30 mg/L r-*Og*GH was higher compared to that of other dose treatments and non-treated control (Table 1). Water quality was equally maintained among treatments, and no pathogen infection was visually observed during experiment. Thus, it is more likely that increasing of survival rate is due to response of r-*Og*GH treatment. Furthermore, increasing in survival of the r-*Og*GH treated fish perhaps as a result of an improved resistance of fish to hyperosmotic treatment. In fact, administration of ovine GH increase survival rate of amago salmon *Oncorhynchus rhodurus* in 32‰ seawater (Miwa & Inui, 1985). This effect is due to the capacity of GH to increase the number and size of gill chloride cells, Na^+,K^+-ATPase, and the Na^+, K^+, $2Cl^-$ cotransporter (NKCC), ion transporters involved in salt secretion (McCormick, 2001), and some of the actions of GH are through insulin-like growth factor I (Sakamoto & McCormick, 2006). The same actions of r-*Og*GH may also involve in giant gourami. In addition, administration of rGH enhances resistance of rainbow trout against bacterial pathogen *Vibrio anguillarum* (Sakai *et al.*, 1997). This effect is through stimulation of innate immune system (Sakai *et al.*, 1997; Acosta *et al.*, 2008). A similar stimulation effect of r-*Og*GH may also exist against bacterial pathogen infection, and this remains to study in future.

Growth hormone treatment increased

appetite of giant gourami juvenile. This was clearly seen in fish treated with r-*Og*GH at a dose of 30 mg/L (Fig. 3). Similar response had also been reported in channel catfish treated by recombinant bovine growth hormone (Wilson *et al*., 1988) and goldfish treated with recombinant giant catfish growth hormone (Promdonkoy *et al*., 2004). The appetite stimulation that affects feed consumption had been suggested as a mainly factor in increase of channel catfish growth following rGH treatment (Wilson *et al*., 1988). This maybe also takes place in growth enhancement of r-*Og*GH-treated giant gourami juvenile. In fact, growth rate of 30 mg/L and 20 mg/L r-*Og*GH treated fish was higher than those of 10 mg/L r-*Og*GH treated fish and non-treated fish (Table 1).

In conclusion, we have demonstrated the growth- and survival-promoting effects of r-*Og*GH on giant gourami juvenile following immersion. It suggested that the rearing period of giant gourami to harvesting period can be shorten, and survival of larvae can also be improved. To our knowledge, this is the first study that has achieved the growth enhancement of giant gourami using recombinant growth hormone.

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