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by Timo Tiwu

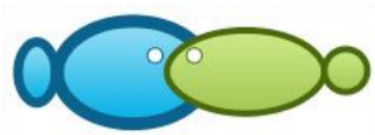
Submission date: 14-Nov-2022 11:09AM (UTC-0500)

Submission ID: 1880166512

File name: bioencapsulation_of_Artemia_with_recombinant_growth_hormone.pdf (226.06K)

Word count: 5300

Character count: 28133



Growth performance and sex ratio of *Channa striata* through immersion and bioencapsulation of *Artemia* with recombinant growth hormone

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Abstract. The aim of this study was to evaluate the effect of different doses of recombinant giant grouper (*Epinephelus lanceolatus*) growth hormone (r-EIGH) on both growth performance and sex ratio of *Channa striata* juveniles. The r-EIGH treatments were conducted in two experiments, each using a specific method. In the first experiment, using immersion method, four kinds of treatments were used: one was without salinity shocked and no hormone treatments (negative control); and the others were with salinity shocked and hormone treatments (0 mg L⁻¹ (positive control), 2 mg L⁻¹ (P1), 20 mg L⁻¹ (P2)). In the second experiment, using *Artemia* bioencapsulation method, five kinds of treatments were used: one was *Artemia* without r-EIGH, BSA and NaCl (negative control), and the others were *Artemia* with r-EIGH, BSA and NaCl (0 mg L⁻¹ (positive control), 2 mg L⁻¹ (P1), 4 mg L⁻¹ (P2), and 6 mg L⁻¹ (P3)). All treatments in both experiments were replicated three times. The results showed that the r-EIGH increases specific growth rate, absolute body weight and length, while it decreases feed conversion ratio and it does not affect sex ratios. Furthermore, the ratio of female to male was 1:1.5 at five months old.

Key Words: *Channa striata*, juvenile, recombinant growth hormone, specific growth rate, sex ratio.

Introduction. *Channa striata* (striped snakehead) is one of carnivore freshwater fish of Channidae family and ordo Perciformes, an original species in Asia and Africa region (Nakkrasae et al 2015) which has high economic value because its price is relatively high at about IDR 40,000-70,000 kg⁻¹ (Directorate General of Strengthening Competitiveness of Fisheries and Marine Products 2019). It occupies the top ten species of national household preference with 2.40% consumption level per year in 2013 (Directorate General of Domestic Trade 2013), has firm and tasty flesh (Khanna 1978; Muntaziana et al 2013), and has a rich source of albumin 63-107 mg g⁻¹ from its body weight (Chasanah et al 2015). Therefore, this fish is one of potential commodities that need to be studied intensively regarding its production technology.

The main problem of current production technology is decreasing growth rate after three months old, from 1.3-3.0 to 0.3-0.9 mm day⁻¹ (Murugesan 1978; Boonyaratpalin et al 1985; Courtenay & Williams 2004; Muntaziana et al 2013). Besides, the feed conversion ratio (FCR) was high (Hien et al 2016) and it takes more than 13.5 months to reach market-size (Murugesan 1978) and two years to reach the length of 30 cm, where it sexually matures (Talwar & Jhingran 1992). Then, particular studies to increase growth rate, improve the FCR, and observe the sex ratio are needed.

Growth rate in fish can be increased through applying recombinant growth hormone (rGH), which is derivative of growth hormone (GH). Previously, rGH was produced from the human body tissues with the help of microorganism (Ayyar 2011).

1 However, due to the limited resources, now rGH is produced from fish pituitary gland, whose function resembles growth hormone: to increase growth, feed efficiency, and gonadal development processes (Berishvili et al 2006; Funkenstein 2006; Linan-Cabello et al 2013). rGH can increase growth rate of fish up to 11 times faster than normal fish in *Oncorhynchus* spp.; 3.7 times in *Cyprinus carpio*; 2 times in *Oreochromis niloticus* (Alimuddin et al 2003).

In the last three decades, intensive uses of rGH in cultivated fishes have been done both on consumption and ornamental purposes. According to Alimuddin et al (2010) rGH produced from *Epinephelus lanceolatus* (r-ElGH), from *C. carpio* (r-CcGH), and from *Osphronemus gouramy* (r-OgGH) has an ability to induce the best growth in *O. niloticus* by 20.94%, by 18.09% and by 16.99%, respectively. Use of r-ElGH through immersion method has been applied in some species likes *Anguilla bicolor* (Handoyo et al 2012), *Litopenaeus vannamei* (Saputra et al 2015), and *Chromobotia macracanthus* (Permana et al 2018), whereas the use of r-ElGH through the *Artemia* bioencapsulation method had not existed yet. In advance, *Artemia* bioencapsulation has been applied using n-3 HUFA to *Farfantepenaeus paulensis* (Mutti et al 2017), using essential fatty acids (EFA) to *Acipenser gueldenstaedtii* (Kamaszewski et al 2014), and using lipid to *Lebbeus groenlandicus* (Park et al 2016). So far, the use of r-ElGH through immersion and or *Artemia* bioencapsulation to *C. striata* to increase growth rate had not been studied.

It is also known that the growth and gonadal differentiation occurred together in the early life phase of fish. Previous studies showed that there was unbalanced sex ratio and the reproductive pattern was hermaphroditic protogyni in the *C. striata* adults caught from nature (Irmawati et al 2017; Musdalifah 2018). The control of sex ratio is a valuable biotechnological approach for the optimization of the quality of fish products according to particular commercial needs. So far, sex ratio of *C. striata* had not been studied in the juvenile phase. The aim of this study was to examine the effect of different doses of r-ElGH on both growth performance and sex ratio of *C. striata* juveniles.

Material and Method

Experimental design. The research was conducted for five months from January to June 2020. Two separate experiments were conducted simultaneously in the Aquaculture Laboratory of Lampung University. Both experiments used an immersion and bioencapsulation of r-ElGH for larvae, which were conducted for 42 days, then the juvenile were maintained without treatments for 56 days. After that, for observing sex ratio the fish were maintained until five months old. The r-ElGH was commercially purchased from The Hall for Development of Freshwater Aquaculture (BBPBAT) Sukabumi, Indonesia. In both experiments, two weeks old *C. striata* larvae were obtained at hatchery of Lampung State Polytechnic with initial body weight and body length are 0.08 ± 0.04 g and 20.48 ± 3.80 mm, respectively. Approximately 100 larvae were stocked in 50-L tanks ($60 \times 40 \times 40$ cm³). The immersion and bioencapsulation methods experiments used 12 and 15 tanks, respectively. All larvae were fed with *Artemia* sp. and acclimatized to the experimental condition for 4 days. Larvae were reared under natural photoperiod, and dissolved oxygen level (3.20 - 5.82 mg L⁻¹) of water were monitored routinely and maintained within standard limits. The water exchange was performed up to a third removal of tanks water every week and also tanks were siphoned in the morning and evening every day. After larvae reached 14 days old, the juveniles were fed with pellet (crude protein: 37%) three times a day at 08.00, 12.00 and 16.00 West Indonesia Time (zone) to apparent satiation. But the day before the treatment, the fish were not siphoned as well as not fed to maintain their metabolism.

In the first experiments, larvae were treated by immersion method with four treatments and three replications: without salinity shocked and no hormone treatments (0 mg L⁻¹ (negative control, K-)), with salinity shocked and hormone treatments (0 mg L⁻¹ (positive control, K+), 2 mg L⁻¹ (P1), and 20 mg L⁻¹ (P2)). In K+, P1 and P2 treatments, larvae were treated first with salinity shocked at 25 ppt for two minutes then immersed

1 with different doses of *r-EIGH* in 0.01% Bovine Serum Albumin (BSA) and 0.9% NaCl L⁻¹. Further, larvae from each treatment were moved into plastic bags filled with oxygen for 60 minutes, then were moved back to the rearing aquarium. This immersion was conducted once a week for six weeks.

In the second experiment, larvae were treated by bioencapsulations of *Artemia* method with five doses of *r-EIGH* and three replications: without salinity shocked and no hormone treatments (0 mg L⁻¹ (negative control, K-)), with salinity shocked and hormone treatments (0 mg L⁻¹ (positive control, K+), 2 mg L⁻¹ (P1), 4 mg L⁻¹ (P2), and 6 mg L⁻¹ (P3)). One hundred milliliter of *Artemia* nauplii were obtained by hatching 3.5 g of cyste *Artemia*, then it divided into five cup each 20 mL. For K- treatment, *Artemia* nauplii were mixed with 980 mL of water in the dark bottle. On the other hand, for other treatments each 20 mL of *Artemia* nauplii were mixed with 80 mL of mixed solutions containing different doses of *r-EIGH*, 0.01% BSA, 0.9% NaCl, and 900 mL of water in each dark bottle. The process of bioencapsulation took one hour. After that *Artemia* nauplii were harvested, washed and divided into 3 replications for each treatment (33 mL assumption each aquarium) to be given to larvae. *Artemia* bioencapsulation of *r-EIGH* was given twice a week in the morning for six weeks.

After being treated with immersion and bioencapsulation, fish were reared without hormones for 56 days and fed with *Artemia* and tubifex four times a day until three weeks old, then fed with pellet (crude protein: 37%) three times a day at 08.00, 12.00 and 16.00 WIB by satiation feeding.

Fish sampling, growth and sex ratios parameters. At the end of the experiment fish were sampled for analysing growth performance (n = 20 per group) by measuring standard body length and weight of each fish, and sex ratios (n = 10 per group). To determine the specific growth rate (SGR) and feed conversion ratio (FCR), they were calculated by the equations:

$$\text{SGR (\% day}^{-1}\text{)} = [\sqrt{(\text{time \& final weight of fish} / \text{initial weight of fish}) - 1}] \times 100$$

$$\text{FCR} = \text{weight of feed eaten} / [\text{final weight of live fish} - \text{initial weight of fish} + \text{weight of dead fish}]$$

Then, the sex ratios was sampled by histology analysis. For this analysis, fish were anesthetized in 1% of clove oil, fixed in 10% formalin fixative for 24 hours, and stored in 70% etanol. Briefly, specimens were then dehydrated, embedded in ParaplastPlus, cut in 6 μ thick serial sections, and stained with hematoxylin-eosin. Because this was in pioneer project, histological preparations were examined under a microscope to identify females using the presence of primary oocytes, perinucleolar oocytes, and ovarian cavity, while males with the presence of blood vessel and spermatogonia as criteria (Haugen et al 2012; Moallem et al 2015; Chen et al 2017) (Figure 1).

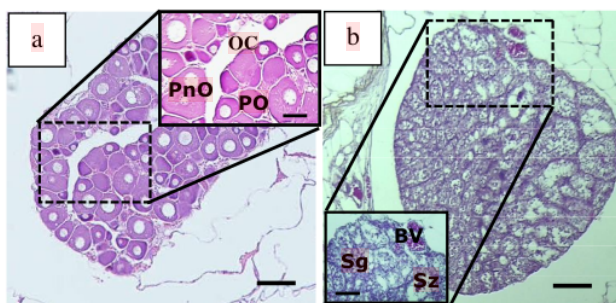


Figure 1. Five months old of snakehead gonad. (a) Female gonad, length of 68 mm, weight 2.20 g, complemented by primary oocytes (PO), perinucleolar oocytes (PnO), and ovary cavity (OC); (b) Male gonad, length of 78 mm, weight 4.16 g, complemented by spermatogonia (Sg), spermatozoa (Sz) and blood vessel (BV) (The magnification of a and b are 10x and 40x magnification, scale bar 50 μ m and 20 μ m, respectively).

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Statistical analysis. Data of growth performance and FCR were analyzed using analysis of variance (ANOVA), the data were further analyzed with Duncan's Multiple Range Tests (DMRT), while the sex ratio was analyzed using z test proportion of two samples ($\alpha = 5\%$).

Results. The results in this study indicate that the administration of r-E/GH had an effect on growth performance and the FCR of *C. striata*, both on immersed and bioencapsulation methods. This can be shown based on the growth parameters observed during 98 days of maintenance (Table 1 and Table 2). The first experiment with further analysis of Duncan's test found that SGR, absolute body weight and length between P1, P2, and K+ treatments were not significantly different. However, they were significantly different to K- ($p < 0.05$). For the FCR, it was found that the hormone treatments had a lower value than the control treatment ($p < 0.05$).

Table 1
 Specific growth rate, absolute body weight (ABW), absolute body length (ABL), feed conversion ratio, and sex ratio of *C. striata* through r-E/GH immersion method

Parameters	K-	K+	P1	P2
SGR (% day ⁻¹)	3.57±0.05 ^b	3.66±0.04 ^{a,b}	3.74±0.09 ^a	3.75±0.05 ^a
ABW (g)	2.42±0.12 ^b	2.64±0.10 ^a	2.84±0.25 ^a	2.86±0.14 ^a
ABL (mm)	50.64±2.05 ^b	53.80±0.80 ^{a,b}	55.88±2.89 ^a	54.90±1.12 ^a
FCR	1.40±0.09 ^a	1.44±0.09 ^a	1.18±0.15 ^b	1.16±0.10 ^b
Female:male ratio	16:23 (1:1.5)			

*Different superscript in the same column indicates significant difference between treatments ($p < 0.05$) (DMRT).

Table 2
 Specific growth rate, absolute body weight (ABW), absolute body length (ABL), feed conversion ratio, and sex ratio of *C. striata* through r-E/GH bioencapsulation method

Parameters	K-	K+	P1	P2	P3
SGR (% day ⁻¹)	3.77±0.10 ^c	3.75±0.16 ^{b,c}	4.13±0.04 ^a	3.93±0.02 ^b	3.99±0.14 ^{a,b}
ABW (g)	2.95±0.29 ^c	2.89±0.47 ^{b,c}	4.12±0.14 ^a	3.42±0.07 ^b	3.65±0.47 ^{a,b}
ABL (mm)	48.00±0.17 ^b	46.70±0.78 ^{a,b}	54.49±0.13 ^a	49.58±0.05 ^b	51.47±0.38 ^{a,b}
FCR	1.67±0.12 ^a	1.63±0.19 ^{a,b}	1.15±0.03 ^c	1.30±0.06 ^b	1.35±0.15 ^b
Female:male ratio	15:22 (1:1.5)				

*Different superscript in the same column indicates significant difference between treatments ($p < 0.05$) (DMRT).

The second experiment, based on Duncan's tests showed that the effect of r-E/GH addition through *Artemia* bioencapsulation to the SGR and ABW and ABL were the same in P1 and P3 treatments, however P1 were significantly different to K-, K+ and P2 ($p < 0.05$). For the FCR, it was found that the hormone treatments (P1 and P2) had a lower value than the control treatment (K- and K+) and highest dose (P3) ($p < 0.05$). Interestingly, experiment of r-E/GH addition with the *Artemia* bioencapsulation showed the growth rate of *C. striata* 10.42% higher and the weight gain 43.97% higher than the immersion.

Meanwhile, sex ratio of *C. striata* statistically on z test proportion of two samples, showed that ratio between both of methods were same, where the r-E/GH did not affect sex ratio of *C. striata* juvenile. Sex ratio of female:male to five months old juvenile on both methods were 1:1.5 (Table 1 and Table 2).

1 Discussion. In this study, growth performance and sex ratios analysis were performed in both immersion and bioencapsulation of *Artemia* with r-EIGH on *C. striata* larvae to clarify the effect of this recombinant hormone on growth performance and sex ratios. The results of this study showed that the r-EIGH increases SGR, ABW and ABL of *C. striata* juveniles, while it decreases FCR and it does not affect sex ratios. The most effective dose from both experiments was 2 mg L⁻¹. It is generally known that the improper dose of hormone causes negative feedback. This is due to the biphasic characteristic of hormone (Caraty et al 1989; Debnath 2010) which gives a negative response of growth if the doses given is too low or too high.

Besides that, the BSA used in the treatment of both experiments was estimated to affect the growth performance by its albumin content. In advance, it is known that *C. striata* contains high albumin levels in their bodies. It is assumed that serum albumin contained in BSA solution increases albumin levels in *C. striata* body. BSA is a biotechnology application in the form of protein transport (albumin) which is osmotically active to support cell growth through binding and transporting the body metabolites such as fatty acid, hormone, and growth factor (Andreeva 2010; Gaharwar et al 2013; Susilowati et al 2015), so that nutrients can be absorbed more optimally and further can be used for growth purposes.

Then, there is a possible mechanism that r-EIGH affects on growth performance of *C. striata* juveniles. It can be realized from the growth rate of fish with hormone treatments 5.04-10.20% higher than fish without hormone treatments. In the immersion method, it is suspected that the mechanism for r-EIGH entry into the body of *C. striata* is through the gills and epidermal. According to Habibi et al (2003) epidermal layer of *Oncorhynchus mykiss* is able to absorb BSA solution as a solvent for r-EIGH. The BSA particle size is around 67 kDa (Andreeva 2010) bigger than 22 kDa particle size of r-EIGH (Arsene et al 2014). Thus, r-EIGH is also able to be absorbed into *C. striata* body. r-EIGH which has been absorbed subsequently with the help of BSA will spread through blood plasma and the cell fluid of *C. striata* body. Whereas, in the bioencapsulation method, r-EIGH will enter through *Artemia* that is non-selective filter feeder (Dhont & Dierckens 2013; Riisgard et al 2015), then *Artemia* is consumed by fish and will be involved in *C. striata* body metabolism, so that r-EIGH can be utilized by the body for growth.

Based on the results of the experiments, *Artemia* bioencapsulation method with r-EIGH gives better growth performance than immersion method. This is due to the mechanism of the r-EIGH in bioencapsulation method which enter directly into the intestine. This can increase the absorption of nutrients to be circulated throughout the body (Chen et al 2019). Meanwhile, in immersion method it was estimated that not all of r-EIGH enter the body, but only a part that is absorbed by the fish's epidermal. Even more, the immersion method is more risky of being affected by stress which can inhibit growth metabolism (Handoyo et al 2012). Therefore, r-EIGH utilization through bioencapsulation method gives better growth results than immersion method. In its mechanism, rGH worked directly and indirectly. For indirectly, rGH involves IGF-1 whereas the IGF-1 utilizes towards the target organ. IGF-1 stimulates growth hormone produced by pituitary gland which increases the growth of body tissues (Yamaguchi et al 2006) and interacting with steroid hormones to trigger the spermatogenesis and oocytes proliferation and maturation (Reinecke 2010).

Based on the results of ABW, it was found that r-EIGH is able to increase *C. striata* growth 17.35-42.50% than control fish. The same as to *A. bicolor* increases 37.40% (Alimuddin et al 2014) and *L. vannamei* 37.77% (Subaidah et al 2012). Based on the increase in length and weight, the exponential growth rate is obtained when the *C. striata* is less than three months old, however after that, *C. striata* growth slows down (Murugesan 1978; Boonyaratpalin et al 1985; Courtenay & Williams 2004; Muntaziana et al 2013). SGR on *C. striata* decreased from 4.30-5.25% day⁻¹ (2 months), 4.86-4.92% day⁻¹ (3 months), 3.57-3.77% day⁻¹ (4 months) to 2.83-3.16% day⁻¹ (5 months). Therefore, booster of r-EIGH could be given before three months old or during their exponential growth phase to increase *C. striata* growth.

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The addition of hormone also has a significant effect on FCR, so that r-*E*/GH is considered to be able to increase the feeding efficiency of *C. striata*. Low FCR observed in larvae treated by r-GH might be caused by the increase of digestion and absorption processes of feed nutrient, through protein and lipid metabolism (Kobayashi et al 2007; Lubis et al 2019). Therefore, fish with hormone treatments has more ability to digest food, absorb nutrients, and convert food proportions to form fish body composition, thus feed can be used for good growth (Kling et al 2012). The role of r-*E*/GH in improving the feed efficiency is also reported in *Anguilla bicolor bicolor* (Alimuddin et al 2014) and *Cromileptes altivelis* (Antoro et al 2015).

Sex ratios observed in both methods are the same. It indicates that both experiments do not affect the process of gonadal development. Also, it is assumed that growth hormone through IGF-1 does not affect the gonadal development of *C. striata* juvenile, where female and male gonadal development in hormone treatments did not significantly differ from it in control treatment.

Conclusions. *Artemia* bioencapsulation method with r-*E*/GH increases specific growth rate and absolute body weight of *C. striata* juveniles, higher than immersion method. Furthermore, the ratio of female to male in *C. striata* juveniles at five months old from both methods was 1:1.5. Further studies are on histological differentiation as effect of a recombinant growth hormone instead of growth performance. Also, the effect of environmental factor on growth performance and histological sex differentiation in snakehead needs to be studied.

Acknowledgements. This project was funded by LPPM Lampung University. Also, thanks to Mrs. Dwi Mulyasih, M.Si for helping during the technical process of this research.

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Received: 20 August 2020. Accepted: 05 October 2020. Published online: 29 October 2020.

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How to cite this article:

Vahira A. D., Riadin A. H., Sarida M., Utomo D. S. C., Setiawan W. A., 2020 Growth performance and sex ratio of *Channa striata* through immersion and bioencapsulation of *Artemia* with recombinant growth hormone. *AACL Bioflux* 13(5):2928-2936.

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