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Growth Response of Four Nile Tilapia Strains Fed on Diet Containing a Recombinant Teleostean Growth Hormone

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Abstract

Various Nile tilapia strains arecultured in Indonesia. This study was conducted to examine the growth response of four Nile tilapia strains, namely SULTANA (superior selected tilapia strain from Selabintana), NIRWANA (tilapia strain from Wanayasa), SRIKANDI (salinity resistant improvement from Sukamandi), and red tilapia fed on diet containing recombinant *Epinephelus lanceolatus* growth hormone (r*El*GH). In the first study, red tilapia were reared in 200-L glass aquaria and fed on artificial diet with different doses of crude r*El*GH protein, namely 0 (control), 0.03, 0.30, and 3.00 mg/kg diet. The results showed that growth, specific growth rate (SGR), and feed conversion ratio (FCR) were notsignificantly differentamongtreatments ($p>0.05$), but significantly different when comparedto the control ($p<0.05$).The second study was performed in the hapa ($2x1x1m^3$) settled in a concrete pond ($20x10x1.5\ m^3$). By using 3 mg r*El*GH/kg diet, the results ofthesecond study showed that the highest ($p<0.05$) SGR was obtained in SULTANA (3.73%), followed by NIRWANA (3.41%). SRIKANDI (3.36 %),and red tilapia (3.14%) strain. Lowest FCR ($p<0.05$) was found in SULTANA (0.84), followed by NIRWANA (0.99), SRIKANDI (1.02), and red tilapia (1.22) strains. Survival of the four strains were similar, ranging from 84.67 to 90.00% ($p>0.05$). The highest ($p<0.05$) liver and muscle glycogen, protein and fat retention, and RNA/DNA ratio were found in SULTANA, followed by NIRWANA, SRIKANDI, and red tilapia.

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As conclusion, SULTANA Nile tilapia strain showed high growth response on r*El*GH oral administration, and farming of this strain can be helpful to increase aquaculture production level.

***Keywords*:** Nile tilapia;recombinant growth hormone; RNA/DNA ratio; strains.

1. Introduction

Growth hormone (GH) plays an important role in the growth of vertebrate organisms. Natural GH levels are relatively low and isolation method is complicated making its application uneconomical and impractical, therefore the recombinant DNA technology has been appliedto produce recombinant growth hormone (rGH) [1]. Recombinant growth hormone (rGH) from a variety of vertebrate species, including fish has been produced in large quantity using a bioreactor such as bacterium *Escherichia coli* [2,3,4],and the yeast *Pichia pastoris*[5]. Studies investigating the use of rGHbioactivity for promoting growth have been performed on fish, shrimp, and oyster [4,5,6,7]. Furthermore, studies investigating the use of rGH for promoting growth have been performed in various species, including tilapia [4,5,8,9], salmonids[1], goldfish [2], *rainbow trout* [10], *black seabream*[11], rabbitfish [12], Japanese flounder [13], Indonesian eel [14], giant gouramy [15,16], and white shrimp *Litopenaeus vannamei* [17].

The rate of growth due to rGH administration can be influenced by the type of rGH [4,18], species and age of the target fish (species-specific and age-dependent) [19], dose [9,15,16], and the method of administration by injection, immersion, oral, or a combination of immersion and oral [14]. Three types of rGH has been made in Indonesia, namely carp rGH (r*Cc*GH), giant gouramy rGH (r*Og*GH) and giant grouper rGH (r*El*GH). In addition, weight gain improvement of Nile tilapia juvenile after injected using r*El*GH (20.94%) was higher than injected using r*CcG*H (18.09%), and r*Og*GH (16.99%) [4]. Moreover, orally dose of 30 mg r*Cc*GH per kg of feed which was given twice times a week for sequential three weeks resulted weight gain improvement 35% higher than control [9].

Various varieties of tilapia (*Oreochromis niloticus*) breeding program with the main purpose of growth promotion has been launched in Indonesia with the decree of the Minister of Marine and Fisheries, including tilapia strain from Wanayasa, NIRWANA (KEP.45/MEN 2006, 14th December, 2006), and superior selected tilapia strain from Selabintana, SULTANA (KEP.28/MEN/2012, 7th June, 2012). Strain SULTANA and NIRWANA were produced using family selection. In addition, hybridization between NIRWANA and blue tilapia (*O. aureus*) produced salinity resistant improvement from Sukamandi, SRIKANDI (KEP.09/MEN/2012, 1stMay, 2012) with the main target was salinity tolerant. Red tilapia strain was hybridization between *Oreochromis niloticus* and *Oreochromis honorum.* Body color of SULTANA, NIRWANA, and SRIKANDI strains are gray-black, gray-green, and gray bluish green, while red tilapia body color rangging from reddish to reddish yellow.

Effective dose of giant grouper growth hormone (r*El*GH) administration and growth response of different strains to the rGH administration in tilapia is still unknown. The aim of this study was to evaluate theoptimum dose of

r*El*GH usage and the growth response of four tilapia strains (SULTANA, NIRWANA, SRIKANDI, and red tilapia) on r*El*GH oral administration.

2. Methods

Production of rElGH and feed preparation

*Escherichia coli*BL21strain harboring the pCold-*El*GH protein expression vector was used as a bioreactor for producing r*El*GH. Bacterial culture, extraction and verification of inclusion body containing r*El*GH were performed according to [4].Bacterial cells were lisated by lisozym, inclusion bodies containing r*El*GH were washed several times by PBS (16 mM Na2HPO4·2H2O, 4 mM NaH2PO4·2H2O, 120 mMNaCl, pH 7.4), freezed dried and then kept at -20 °C until used. The r*El*GH supplemented diet was prepared according to [9]. The r*El*GH were dissolved in 15 mL PBS solution, mixed with chicken yolk egg of 2 mg/kg diet, and then sprayed onto 100 g commercial feed (protein content:32%). Diets were air dried and then kept at -20^{o}C until it given to the fish.

2.1. First Study

Four level of r*El*GH doses were tested, namely 0 (control diet; CD), 0.03 (test diet 1; TD1), 0.3 (TD2) and 3.0 mg/kg diet (TD3) with nutrient composation are presented inTable1. Completely randomized design using three treatments, two controls with three replications was applied in this study.

Table 1. Proximate fed diet containing recombinantgiant grouper growth hormone (r*El*GH) and control

Nutrition composition	r*El*GH fed diet			Control	
	3.00 mg/kg	0.30 mg/kg	0.03 mg/kg	Control-1	Control-2
Protein (%)	31.62	31.52	31.17	30.81	32.35
Fat (%)	3.97	4.18	4.22	4.13	3.40
Crude Fiber (%)	1.20	1.02	1.03	1.35	1.70
NFE (%)	41.07	41.75	41.55	41.72	44.63
Ash (%)	8.32	8.38	8.76	8.57	8.78
Water content (%)	13.82	13.15	13.27	13.42	9.14
DE (kcal/kg diet)	2455.02	2485.53	2471.52	2455.88	2523.40
Ratio C/P(kcal/g protein)	7.76	7.89	7.93	7.97	7.80

Control-1: commercial diet was sprayed by chicken yolk egg only. Control-2 original commercial feed diet.NFE : Nitrogen free extract. DE (digestible energy) is calculatedfrom energy in protein 1 g = 3.5 kcal, fat 8.1 kcal, and carbohydrate 1 g = 2.5 kcal.

Red tilapia juvenile (average body weight: 3.5±0.5 g) were obtained from Experimental Station of Aquaculture Department, IPB. Fish (*n*= 25) were reared in the 200 L glass aquaria equipped with aeration for eight weeks. Adaptation to commercial diet was performed for one week before treatments began. Fish were fed with TDat interval of two days three times daily (07.30-08.30, 12.00-13.00,and 16.30-17.30 o'clock) *at satiation* for one month, and CD for the rest days.Feces and uneaten diet were siphoned every day, and water was changed 100% of volume in every 2 days.

The effectiveness of r*El*GHadministration was determined based on the average weight gain (ΔW), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR). Total body weights were measured every 2 weeks. Survival was calculated at the end of the study. Data are presented as mean ± standard deviation (SD). Statistical significance was assessed by a one-way analysis of variance followed by Fisher test using Minitab 16.

2.2. Second Study

In the second study, completely randomized design using four treatments namely (A) SULTANA, (B) NIRWANA (C) SRIKANDI, and (D) red tilapia with three replications was applied in this study.SULTANA strain was obtained from Sukabumi Main Center for Freshwater Aquaculture Development, NIRWANA strain from Wanayasa Center for Freshwater Fish Seed Development, SRIKANDI strain from Sukamandi Research Center for Fish Genetic Improvement, and red tilapia from Experimental Station of Aquaculture Department, IPB Bogor. The fish (avarage body weight 5.80±0.19 g) were reared in the hapa (2x1x1 m^3) settled in a concrete pond (20x10x1.5 m^3) at density of 50 fish/hapafor ten weeks. Similar at the first study, the fish were adapted for one week before treatment, than fed with diet containing3 mg/kg r*El*GH at satiation, at interval two days, three time daily for one month, than commercial diet was given at the rest days. The condition of water qualities during teen weeks experiment were temperature: 24 – 31 ^oC, pH: 6.0 - 6.5, and DO: 6-7 mg/L.

The effectiveness of fed on diet containing r*El*GH determined based onΔW, SGR, FCR, SR, protein and fat retention, liver and muscle glycogen, and RNA/DNA ratio. Total body weights were measured every two weeks,survival rate and feed conversion ratio was calculated at the end of the study. Protein retention, fat retention, liver glycogen and muscle glycogen were determined according to[20]obtained from three individual duplicate pooled samples. RNA and DNA concentration were measured using the gene quant calculator. Total RNA and DNA were extracted from the liver of fish after ten weeks rearing periode using isogen (Nippon Gene, Tokyo, Japan) and Puregene®Core Kit A kit (QIAGEN Science Marylan USA). Statistical significance was assessed by a one-way analysis of variance followed by Fisher Test using Minitab 16.

3. Results and Discussion

3.1. First Study

The result of the first study showed that all r*El*GH treatments allowed higher in biomass, ΔW, SGR, and FCR compared to controls ($p<0.05$), while the survival rate was not significanly different ($p>0.05$; Table 2). Furthermore the biomass, ΔW, SGR, and FCR among the treatments were similar ($p> 0.05$).

Table 2. Initial biomass (IB), final biomass (FB), growth (ΔW), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) of red tilapia fed on diet containing recombinant giant grouper growth hormone (r*El*GH)

Parameter	Treatment			Control	
	3.00 mg/kg	0.30 mg/kg	0.03 mg/kg	Control-1	Control-2
IB (g)	104.85±1.34[a]	104.72±0.99[a]	105.72±1.01[a]	105.08±1.54[a]	105.15±0.92[a]
FB (g)	703.60±21.80[a]	685.20±41.35[a]	669.91±19.15[a]	562.24±60.67[b]	559.89±60.56[b]
ΔW (g)	589.74±21.69[a]	580.48±42.01[a]	564.17±18.12[a]	457.16±60.67[b]	454.70±60.25[b]
SGR (%)	3.46±0.06[a]	3.41±0.13[a]	3.35±0.04[a]	3.03±0.21[b]	3.02±0.20[b]
FCR	1.07±0.04[a]	1.14±0.06[a]	1.14±0.03[a]	1.38±0.17[b]	1.40±0.19[b]
SR (%)	93.33±2.31[a]	93.33±2.31[a]	90.67±2.31[a]	93.33±2.31[a]	93.33±2.31[a]
ΔW:C2 (%)	31.68	27.66	24.07	0.54	0.00

Fish were reared in the 200-L glass aquaria for 8 weeks. Control-1: commercial diet was sprayed by chicken yolk egg only. Control-2: original commercial feed diet.Different superscript letters in the same line indicate significantly difference ($p<0.05$).

The results of these experiments indicate that the administration of r*El*GH orally at a dose of 0.03 to 3.00 mg/kg feed gave the same growth response and could increase growth of 24.07 to 31.68 %. Based on the value of feed conversion ratio, to produce 1 kg of biomass r*El*GH oral administration at dose 0.03 to 3.00 mg/kg feed can save 18.57 to 23.57 % amount of feed. When referring to [9] who reported recombinant common carp growth hormone (r*Cc*GH) administration on tilapia, this experiment showed that the administration of recombinant giant grouper growth hormone (r*El*GH) more effective and efficient by reducing 10 to 100 times of the dose. Similar finding was reported by [4] who concluded that oral administration of recombinant giant grouper growth hormone (r*El*GH) was the best compared to oral administration of recombinant common carp growth hormone (r*Cc*GH) and recombinant giant gouramy growth hormone (r*Og*CH).

Among the three level doses tested in this experiment can be argued that the administration of r*El*GH to increase the growth of red tilapia at dose of 0.03 mg/kg feed showed efficiently the best.

3.2. Second Study

SULTANA strain showed the best growth (ΔW and SGR) than other strains ($p<0.05$; Table 3). Meanwhile, the survival rate of all strains were not significantly different ($p>0.05$), so the differences in growth between the strains were not caused by differences in the density of culture.

Fish were reared in the same pond and placed randomly in hapa. Thus, the differences growth response betweenstrains on r*El*GH administration allegedly associated with their superiority due to historical breeding program. The difference between the growth response to administration of rGH strains have also been reported

by [21] on different catfish strains (strains Norris and USDA-103) were given recombinant bovine growth hormone (rbGH).

Table 3. Initial biomass (IB) and final biomass (FB), growth (ΔW), specific growth rate (SGR), survival rate (SR) of four different Nile tilapia strains fed on diet containing recombinant *Epinephelus lanceolatus* growth hormone (r*El*GH).

Parameter	Strain			
	SULTANA	NIRWANA	SRIKANDI	Red tilapia
IB (g)	276.04±5.84[a]	293.25±5.88[a]	294.29±2.21[a]	296.74±3.68[a]
FB (g)	3584.69±32.53[a]	3069.86±172.50[b]	2987.13±180.87[b]	2581.06±105.48[c]
ΔW (g)	3308.65±32.17[a]	2776.61±177.57[b]	2692.84±182.33[b]	2284.32±108.10[c]
SGR (%)	3.73±0.03[a]	3.41±0.11[b]	3.36±0.10[b]	3.14±0.08[c]
SR (%)	91.33±2.31[a]	9.33±1.16[a]	88.67±1.16[a]	88.67±2.31[a]

Fish were reared in the hapa(2x1x1 m^3) settled in a concrete pond (20x10x1.5 m^3) for 10 weeks. ΔW: final biomass – initial biomass. Different superscript letters in the same line indicate significantly difference ($p<0.05$).

In line with the growth response to r*El*GH administration (Table 3). SULTANA strain have metabolic response (feed conversion ratio, levels of liver glycogen, muscle glycogen, protein retention, fat retention) and molecular (ratio of RNA/DNA) were highest compared to the other three strains ($p <0.05$). NIRWANA and SULTANA feed conversion rate were not different ($p>0.05$), and both of them were better than SRIKANDI and red tilapia strains ($p<0.05$). Levels of glycogen, protein and fat retention, and the ratio of RNA/DNA in NIRWANA and SRIKANDI strains were not different ($p>0.05$), but higher than the red tilapia strain (Table 4).

The highest retention of protein and fat in SULTANA strain indicated that protein and fat were not much overhauledfor energy, and bioconversion of carbohydrates occur. [22] reported that the main biological effects of GH are mediated growth, especially the growth of body and bone, stimulates protein synthesis in several tissues, mobilize lipids from adipose tissue via gluconeogenesis, increase glycogen deposits in the liver. The results of study [23,24]proved that one of the biological effects of GH is to stimulate protein synthesis in several tissues of the body as well as reducing the need for amino acids to suppress proteolysis process.

The growth rate can be predicted using the value of the ratio of RNA/DNA [25]. In this study SULTANA strain showed highest growth rate and ratio of RNA/DNA value than the other three strains. Nevertheless, total RNA does not directly indicate a specific gene that induced by the administration of rGH. By using a semi-quantitative method, gene expression of insulin-like growth factor-1 (IGF-1) increased after administration of rGH in tilapia [9]. With these facts have alleged that the expression of IGF-1 in SULTANA strain is highest than other strains. Further research is needed to clarified more information about endogenous factors that differentiate between strains of tilapia in response to administration of r*El*GH.

Tabel 4. Feed conversion ratio (FCR), liver and muscle glycogen, protein and fat retention, and RNA/DNA ratio of four different Nile tilapia strains fed on diet containing recombinant giant grouper growth hormone (r*El*GH).

Parameter	Tilapia Strain			
	SULTANA	NIRWANA	SRIKANDI	Red tilapia
FCR	0.84±0.02^{c}	0.99±0.07^{c}	1.02±0.07^{b}	1.22±0.06^{a}
Liver glycogen (mg/100 mL)	0.49±0.02^{a}	0.34±0.01^{b}	0.26±0.06^{bc}	0.17±0.03^{c}
Muscle glycogen (mg/100 mL)	4.10±0.07^{a}	3.65±0.14^{b}	2.51±0.65^{bc}	2.35±0.50^{c}
Protein Retention (%)	59.56±1.35^{a}	53.72±0.51^{b}	49.78±0.86^{b}	43.94±3.02^{c}
Fat Retention (%)	206.52±14.48^{a}	157.63±6.77^{bc}	168.11±0.87^{b}	138.33±8.86^{c}
RNA/DNA ratio	0.57±0.01^{a}	0.47±0.01^{b}	0.47±0.00^{b}	0.24±0.00^{c}

Fish were reared in the hapa(2x1x1 m^3) settled in a concrete pond (20x10x1.5 m^3) for 10 weeks. Different superscript letters in the same line indicate significantly difference ($p<0.05$).

Considering growth rate and feed conversion ratio values, culture of SULTANA strain has potential to increase production and efficiency of tilapia culture. Generally the culture periode of tilapia in freshwater pondto reach marketable size (300-500 g/fish) ranging from four to six months. In this experiment administration of r*El*GH for one month, then the fish cultured for 1.5 months without administration r*El*GH (Figure 1). According to [14,26]who were studied Indonesian eel and white shrimp respectively, reported that twice administration of recombinant giant grouper growth hormone (larva and nursery stages) indicated better than single administration (at larva or nursery stage only). Therefore, second administration of recombinant giant grouper growth hormone (r*El*GH) in tilapia SULTANA strains can allegedly trigger the growth and production becomes higher.

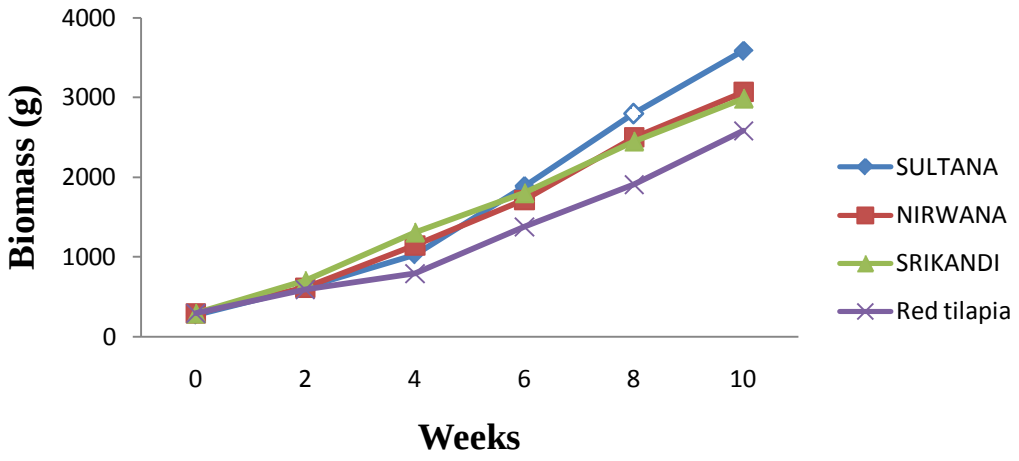

Fig 1. Weight gain biomass of four Nile tilapia strains fed on diet containing a recombinan giant grouper growth hormone (r*El*GH)

4. Conclusions

1. Oral administration of recombinant giant grouper growth hormone (r*El*GH) at dose 0.03 to 3.00 mg/kg of feed was effective in improving the growth of red tilapia by increasing 24.07 to 31.68% of weight gain.
2. SULTANA, NIRWANA, SRIKANDI, and red tilapia strains revealed different growth response to oral administration of recombinant giant grouper growth hormone (r*El*GH). Among four tested of tilapia strains, SULTANA strain showed the best growth response and increased 44.84 % of weight gain compared to Red tilapia.

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References

[1] S. Sekine, T. Mizukami, T. Nishi, Y. Kuwana, A. Saito, M. Sato, S. Itoh, H. Kawauchi. 1985. Cloning and expression of cDNA for salmon growth hormone in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA.* 82:4306-4310.

[2] B. Promdonkoy, S. Warit, S. Panyim. 2004. Production of a biologically active growth hormone from giant catfish (*Pangasianodon gigas*) in *Escherichia coli*. *Biotechnol Lett*. 26: 649-653.

[3] H.P. Sørensen, K.M. Kim. 2005. Advanced genetic strategies for recombinant protein expression in *Escherichia coli.* J. Biotechno 115:113–128.

[4]Alimuddin, I. Lesmana, A.O. Sudrajat, O. Carman, I. Faizal. 2010. Production and bioactivity potential of three recombinant growth hormones of farmed fish. *Indones Aquac J*. 5:11-16.

[5]J. Acosta, R. Morales, A. Morales, M. Alonso, M.P. Estrada. 2007. *Pichia pastoris* expressing recombinant tilapia growth hormone accelerates the growth of tilapia. *Biotechnol Lett.* 29: 1671-1676.

[6] S. Moriyama and H. Kawauchi. 2001. Growth regulation by growth hormone and insulin-like growth factor-I in teleosts. *Otsuchi Mar Sci.* 26:23-27

[7] D. Santiesteban, L. Martín, A. Arenal, R. Franco, J. Sotolongo. 2010. Tilapia growth hormone binds to a receptor in brush border membrane vesicles from the hepatopancreas of shrimp *Litopenaeus vannamei*. *Aquaculture.* 306: 338–342.

[8] Y. Li, J. Bai, Q. Jian, X. Ye, H. Lao, X. Li , J. Luo, X. Liang. 2003. Expression of common carp growth hormone in the yeast *Pichia pastoris* and growth stimulation of juvenile tilapia (*Oreochromis niloticus*). *Aquaculture*. 216 : 329-341.

[9] D. Hardiantho, Alimuddin, A.E. Prasetyo, D.H. Yanti, K. Sumantadinata. Aplikasi Rekombinan Growth Hormon (rGH) Ikan Mas pada Ikan Nila Melalui Pakan Buatan. Makalah yang disampaikan dalam Pertemuan Broodstock Center Nila & Temu Koordinasi Perekayasa Kementerian Kelautan dan Perikanan, Sukabumi Indonesia, 15-17 November 2011. (in Indonesian).

[10] S. Moriyama, H. Yamamoto, S. Sugimoto, T. Abe, T. Hirano, H. Kawauchi. 1993. Oral administration of recombinant salmon growth hormone to rainbow trout, *Oncorhynchus mykiss. Aquaculture.* 112: 99–106.

[11] H.J. Tsai, M.H. Hsih, J.C. Kuo. 1997.*Escherichia coli* produced fish growth hormone as a feed additive to enhance the growth of juvenile black seabream (*Acanthopagrusschlegeli*). *J.Appl.Ichthyol*. 13: 78-82.

[12] B. Funkenstein, A. Dyman, Z. Lapidot, E.G. de Jesus-Ayson, A. Gertler, F.C Ayson. 2005. Expression and purification of a biologically active recombinant rabbitfish, *Siganus guttatus* growth hormone. *Aquaculture.* 250 : 504-515.

[13] S. Liu, X. Zhang, X. Zang, B. Liu, K.K.I.U. Arunakumara, D. Xu, X. Zhang, 2008. Growth, feed efficiency, body muscle composition, and histology of flounder (*Paralichthys olivaceus*) fed GH transgenic *Synechocystis. Aquaculture* 277: 78–82.

[14] B. Handoyo .2012.ResponsBenihIkanSidatterhadapHormonPertumbuhanRekombinanIkanKerapu Kertang Melalui Perendaman dan Oral. [Thesis]. Institut Pertanian Bogor.Bogor (ID) (in Indonesian with English abstract).

[15]Irmawati, Alimuddin, M. Zairin Jr, M.A. Suprayudi, A.T. Wahyudi. 2012. Peningkatanlajupertumbuhanbenihikangurame (*Osphronemusgoramy*Lac.) yang direndamdalam media yang mengandunghormonpertumbuhanikan mas. *J IktiolIndones*.12(1).(in Indonesian with English abstract).

[16] M. Safir. 2012. Respons Benih Ikan Gurami (*Osphronemus goramy*) yang diberi Hormon Pertumbuhan Rekombinan Melalui Oral pada Dosis Berbeda. [tesis]. Institut Pertanian Bogor. Bogor (ID) . (in Indonesian with English abstract).

[17] S. Subaidah, O. Carman, K. Sumantadinata, Sukenda, Alimuddin, 2012. Respons pertumbuhan dan ekspresi gen udang vaname *Litopenaeus vannamei* setelah direndam dalam larutan hormon pertumbuhan rekombinan ikan kerapu kertang. *J. Ris. Akuakultur*. 7 (3): 337 – 352. (in Indonesian with English abstract).

[18]Irmawati 2013. Respons Fisiologis, Biokimia, dan Molekuler Ikan Gurame yang diberi Hormon Pertumbuhan Rekombinan. [Disertasi]. Institut Pertanian Bogor. Bogor (ID). (in Indonesian with English abstract).

[19] Y. Hertz, A. Tchelet, Z. Madar , A. Gertler. 1991. Absorption of bioactive human growth hormone after oral administration in the common carp and its enhancement by deoxycholate. *J Comp Physiol*, 161:159-163.

[20] AOAC. 2000. In: K. Helrich. (Ed). *Official Methods of Analysis*. 15th ed. Association of Official Analytical Chemist, Arlington, VA. USA.

[21] J.T. Silverstein, W.R. Wolters, M. Shimizu, W.W. Dickhoff . 2000. Bovine growth hormone treatment of channel catfish: strain and temperature effects on growth, plasm IGF-I levels, feed intake efficiency and body composition. *Aquaculture.* 190:77–88.

[22] G. Walsh. Biopharmaceuticals, Biochemistry and Biotechnology. Second Edition. England, John Wiley & Sons Ltd. 2003.p 551.

[23] R.S. Rasmussen, B. Rønsholdt, T.H. Ostenfeld, E. McLean, J.C. Byatt. 2001. Growth, feed utilization, carcass composition and sensory characteristics of rainbow trout treated with recombinant bovine placental lactogen and growth hormone. *Aquaculture.* 195:367-384.

[24] A. Vijayakumar, R. Novosyadly, Y. Wu, S. Yakar, D. LeRoith. 2010. Biological effects of growth hormone on carbohydrate and lipid metabolism. *Growth Horm IGF Res*. 20:1-7.

[25] H. Glémet, M.A. Rodriguez. 2007. Short-term growth (RNA/DNA ratio) of yellow perch (*Percaflavescens*) in relation to environmental influences and spatio-temporal variation in a shallow fluvial lake. *Can. J. Fish Aquat. Sci*. 64: 1646 – 1655.

[26] S. Subaidah. 2013. Respons Pertumbuhan dan Imunitas Udang Vaname *Litopenaeus vannamei* terhadap Pemberian Hormon Pertumbuhan Rekombinan Ikan Kerapu Kertang [disertasi). Institut Pertanian Bogor. Bogor (ID). (in Indonesian with English abstract).