 DOI: 10.11598/btb.2019.26.1.710

GROWTH AND MEAT QUALITY ENHANCEMENT OF STRIPED CATFISH (*Pangasianodon hypophthalmus*) USING RECOMBINANT GROWTH HORMONE

RENI AGUSTINA LUBIS[1*], ALIMUDDIN[2**] AND NUR BAMBANG PRIYO UTOMO[2]

[1]*Jambi Freshwater Aquaculture Development Centre (JFADC), Muaro Jambi 36364, Indonesia*
[2]*Department of Aquaculture, Faculty of Fisheries and Marine Science, Institut Pertanian Bogor, Bogor 16680, Indonesia*

Received 19 October 2016 / Accepted 17 May 2018

ABSTRACT

The application of fish recombinant growth hormone (rGH) has been known as one of the methods to improve the growth performance of cultured fishes, one of which is the striped catfish *Pangasianodon hypophthalmus*, a species that is becoming commercially attractive in Indonesia. Hence, this study was aimed to evaluate the effects of rGH supplementation in commercial diet on the growth, feed utilization and flesh quality of *P. hypophthalmus*. The rGH was mixed with chicken egg yolk and sprayed on the commercial feeds with different protein levels (32, 28 and 23%). In the control, the feeds were also sprayed with chicken egg yolk but without rGH. Striped catfish with body weights of 110.66 ± 1.32 g.ind^{-1} were fed on rGH-supplemented diets two times a week during the first and third months, and during the rest of the months they were fed on diet without rGH supplementation. The fishes were reared for 120 days in 18 hapa (2×1×1.5 m^3) with initial density of 20 fishes per hapa. The results showed that the highest weight gain, specific growth rate (SGR), and lowest feed conversion ratio (FCR) were obtained by fishes fed on 32% protein content with rGH-supplemented diet. No significant difference was observed in the weight gain, SGR and FCR in rGH treated group with 28% protein content with rGH supplemented diet and non-rGH control group receiving 32% protein diet. Similar moisture content of meat, protein content of meat, belly fat and edible portion were observed in rGH-supplemented diet and their control. Except in the treatment 23% protein content rGH supplemented diet that has lower lipid content in fish body and meat. The highest SGR was obtained when the fishes were fed on the 32% protein feed combined with rGH. Enrichment with rGH depleted the fat content in the meat of fish fed on all levels of protein in which the lowest fat was found in the 23% protein feed.

Keywords: Dietary protein, grow-out, rGH, striped catfish

INTRODUCTION

Striped catfish *Pangasianodon hypophthalmus* is a considerably large freshwater commodity that is gaining attraction in Indonesia. Its average production rate has increased by 31.63% from 2010-2014 (SIDATIK 2015). One of the main common constraints in fish cultivation is the increasing feed cost coupled with a relatively stable fish price and the longer production time in obtaining the ideal marketable size, leading to decreased profits. Fish flour is one of the major protein sources. Generally, feed quality is determined by its protein content. The higher the protein content the higher the price. Thus, the feed price is a key factor since it contributes 30-60% to the total production cost (Hasan 2010).

Protein is essential nutrient required for growth (Helver 1998). The use of protein for growth depends on balanced amount of other nutrients such as fat and carbohydrate. In unbalanced amount, the protein is utilized as source of energy (Craigh & Helfich 2002). Protein is converted into energy when the energy produced by non-protein sources is low, leading to lower contribution of protein for building tissues (Lovell 1989). Hence, the addition of non-protein sources to produce energy reduces the use of protein as energy source (protein sparring effect), thus increasing

Corresponding author: *reniagustina_lbs@yahoo.co.id; **alimuddin@apps.ipb.ac.id

protein function in improving fish growth (Hasan & Khan 2013).

In order to improve fish growth rate, some approaches are applied, such as nutrition, reproduction, environmental and genetically approach (i.e. application of recombinant protein, hybridization, triploidization, selection and transgenesis technology). In the molecular biotechnology approach, the use of recombinant growth hormone (rGH) has produced positive effects on the fish growth rate. The growth hormone is the peptide hormone from the anterior pituitary that acts in controlling growth, metabolic activities, and energy balance (Bartke 2005).

The rGH can be applied in many techniques such as feed supplementation, injection, and immersion method. The application of rGH in feed supplementation is more efficient in large quantities of fish. Studies showed that rGH-supplemented feed successfully increased the growth rates of channel catfish *Ictalurus punctatus* (Silverstein *et al*. 2000), tilapia (Li *et al*. 2003), rabbitfish *Siganus* sp. (Funkenstein *et al*. 2005), eel *Anguilla* sp. (Handoyo *et al*. 2012), and giant gourami *Osphronemus goramy* (Budi *et al*. 2015).

Other studies also showed the improvement of fish growth by feed supplementation with rGH. The application of rGH on feed enriched by protein with different levels has increased the growth performance and efficiency of protein used for gourami juvenile (Budi *et al*. 2015). The rGH supplementation also lowered the lipid content and increased the protein synthesis (Rasmussen *et al*. 2001). The lower lipid content and increased fish growth indicated a protein sparring effect with increasing growth hormone level. The growth hormone was capable of increasing protein retention and absorption and reducing ammonia excretion (Kobayashi *et al*. 2007). Meanwhile, studies related to the application of rGH in feed with different and lower levels of protein (23%, 28%, and 32%) in *Pangasius* grow out have yet to be done. As such, this research aimed to investigate the effects of rGH supplemented-feed with different protein levels on the growth and meat quality of striped catfish.

MATERIALS AND METHODS

Experimental Diet and Production of rGH

The recombinant growth hormone (rGH) was produced using *Escherichia coli* BL21 containing pCold-1/r*El*GH vector expression. Bacterial culture and rGH collection methods were performed as described by Alimuddin *et al*. (2010).

Three types of commercial feed were used as test feeds, each with a different proportion of protein; 23, 28 and 32% (Table 1). The rGH was coated with 20 mg chicken egg yolk and then applied to feed pellet at a dose of 2 mg/kg (Hardiantho *et al*. 2012). Non-supplemented feed was also coated with egg yolk and phosphate buffer saline (PBS).

Table 1 Proximate composition of test feeds (dry matter)

Parameters	Test feed					
	23%	23%+ rGH	28%	28%+ rGH	32%	32%+ rGH
Crude protein (%)	23.7	24.4	26.9	27.8	31.9	33.4
Lipid (%)	2.2	2.6	5.6	6.2	6.4	6.3
Ash (%)	8.3	7.9	9.5	9.4	10.9	10.8
Crude fiber (%)	7.5	8.3	6.7	6.4	3.8	4.0
NFE (%)*	58.3	56.7	51.2	50.9	46.9	45.5
GE (kkal/kg)**	3924.5	3939.8	4138.6	4184.6	4313.8	4332.29
C/P (kkal/g)	16.5	16.2	15.4	15.5	13.5	13.0

Note: **Nitrogen free extract/NFE = Dry matter - (Crude protein + Lipid + Crude fiber + Mineral matter);
**GE = Gross energy protein 5.6 kcal/g; fat 9.4 kcal/g; carbohydrate 4.1 kcal/g (Watanabe 1988); CP = Ratio of protein energy; 23% = feed protein 23%; 23%+rGH: feed protein 23% with addition of rGH

Fish Rearing

Fishes with body weights (BW) of 110.66±1.32 g were reared in an experimental pond (200 m^2) using 18 hapa (2×1×1.5 m^3) with density of 20 fishes per hapa. The 360 fishes were reared for 120 days and fed at a feeding rate of 3% of fish biomass twice a day, morning and afternoon. Feeds containing rGH were given twice a week during the first and third months, and during the rest of the months they were fed on diet without rGH supplementation. Fish body weight was measured every 20 days until the end of the experiment. The water quality was measured at a temperature range of 29-34 °C, pH range of 7.05-7.51, DO (dissolved oxygen) range of 4.20-7.60 mg L^{-1}, and TAN (total ammonium nitrogen) range of 0.12-0.80 mg L^{-1}.

Data Collection and Statistical Analysis

Variables observed were weight gain, specific growth rate (SGR), feed consumption, and feed conversion ratio (FCR), protein retention, lipid retention, survival rate, proximate component of feed, body and meat, hepatosomatic index (HSI), belly fat, and edible portion. Daily specific growth rate (SGR) was calculated with equation (Huisman 1987):

$$\left(\sqrt[t]{\frac{Wt}{Wo}} - 1\right) \text{ x } 100$$

SGR = daily specific growth rate; Wt = Average weight of an individual at the end of the rearing period (g); Wo = average weight of an individual at the beginning of the rearing period (g); t = length of rearing time (days).

Feed conversion ratio (FCR) was calculated using the equation for feed conversion ratio: FCR = [P/{(Wt + Wm)-Wo}] ×100; where P = amount of feed given during rearing (g), Wt = biomass of fish at the end of the rearing period (g), Wo = biomass of fish at the beginning of rearing (g), Wm = Weight of fish that died during rearing (g). The protein and lipid retention were calculated based on Takeuchi (1988). The liver was removed from each fish and weighed for calculation of the hepatosomatic index, HSI where HSI = 100 × liver weight/body weight). Edible portion was calculated using the equation: edible portion = 100 × weight of meat/body weight). Lastly, the belly fat was calculated using the equation: belly fat = 100 × weight of belly fat/body weight. A complete proximate analysis of each experimental unit was carried out on the first and last day of the experiment according to the methods of Takeuchi (1988). A minimum of three fishes per experimental unit were taken for proximate analysis. The experimental design used is the 2x3 factorial; two levels of rGH (supplemented and non-supplemented) and three levels of protein (23%, 28% and 32%). Each treatment was replicated three times. All data were analyzed by two-way ANOVA using the Minitab statistical software.

RESULTS AND DISCUSSION

Growth Performance and Survival Rate

In this 120-day experiment, the survival rate of striped catfish (*Pangasianodon hypophthalmus*) was 100% in all treatments. Moreover, the feed treated with rGH resulted in higher fish growth (Table 2). The highest biomass and SGR, and the lowest FCR were observed in feed C (protein 32%) enriched with rGH, while the lowest biomass gain and SGR and the highest FCR were observed in feed A (protein 23%) without rGH addition. The weight gain, SGR, feed consumption, and FCR of fish treated with feed B (protein 28%) with rGH was not significantly different (P>0.05) from that of feed C with rGH, and that of feed C without rGH. In addition, biomass gain, SGR, FCR showed no significant interaction with protein levels and rGH addition (Table 2).

Table 2 Growth performance of striped catfish (*Pangasianodon hypophthalmus*) at different protein levels and recombinant growth hormone

Dietary protein level		Growth parameters				
		Final weight (g fish^{-1})	Weight gain (g fish^{-1})	SGR** (% day^{-1})	Feed consumption (g fish^{-1})	FCR***
Feed A 23%	Non rGH	418.67 ± 44.43^{b}	308.00 ± 44.48^{b}	1.11 ± 0.09^{c}	537.33 ± 20.06^{c}	1.77 ± 0.25^{a}
	rGH	436.60 ± 29.87^{b}	325.92 ± 29.95^{b}	1.15 ± 0.06^{bc}	553.67 ± 72.63^{bc}	1.69 ± 0.07^{ab}
Feed B 28%	Non rGH	547.71 ± 20.52^{ab}	437.06 ± 20.52^{ab}	1.34 ± 0.03^{ab}	604.07 ± 37.31^{abc}	1.38 ± 0.06^{bc}
	rGH	646.81 ± 57.22^{a}	536.13 ± 57.23^{a}	1.48 ± 0.07^{a}	685.43 ± 52.67^{ab}	1.28 ± 0.07^{c}
Feed C 32%	Non rGH	608.45 ± 74.75^{a}	497.80 ± 74.69^{a}	1.43 ± 0.11^{a}	664.73 ± 36.71^{abc}	1.35 ± 0.14^{c}
	rGH	690.94 ± 65.36^{a}	580.26 ± 65.28^{a}	1.54 ± 0.08^{a}	700.28 ± 76.04^{a}	1.21 ± 0.00^{c}
Two-way ANOVA						
Feed Protein (P)		P<0.00	P<0.00	P<0.00	P<0.00	P<0.00
rGH (R)		P<0.02	P<0.02	P<0.02	P<0.10	P<0.10
PxR (Interaction)		P<0.39	P<0.39	P<0.52	P<0.57	P<0.89
Standard Error		52.30	52.30	0.08	53.19	0.13

Note: ***Different superscript in the same column indicates significant difference among treatments (P<0.05)
***SGR = specific growth rate
***FCR = feed conversion ratio

Treatment of 23% protein diets with rGH has lower total energy (3924.53 kcal/kg) and high protein energy ration (16.17 kcal/g) resulted in low weight gain and SGR in comparison with other treatments (P<0.05), and insignificant effects compared to control (P>0.05). A study on the administration of rGH with different protein levels showed different results (Budi *et al.* 2015). A giant gourami juvenile with sizes 15.82± 0.13 g given with feed supplemented with rGH experienced an increase in growth and dietary utility compared with gourami given the same feed without supplementation. The F4 generation of human growth hormone (hGH) transgenic carp was more efficient in utilizing the dietary protein than the control (Fu *et al.* 1998). This dissimilarity result may be related with the presence of different fish species used. Age dependent and species specific were remarkable factors that also influenced the effects of rGH (Hertz *et al.* 1991). Furthermore, differences in total energy-protein 21% (4337.76 kcal/kg) and C/P ratio (20.38) of experimental diets that were used by Budi *et al.* (2015) may have contributed to the results. The ratio of protein energy (DE/P) in catfish feed was about 7.4–12 kcal/g (Halver & Hardy 2002). The administration of rGH significantly differed in improving amylase, lipase, and protease in 21% protein diet (Budi *et al.* 2015).

Feeding with various levels of protein and energy affected the fish growth. Feeds with lower energy caused protein conversion as additional energy source required for metabolic process (Halver & Hardy 2002). Consequently, protein for growth activities is reduced, leading to slow growth rate. For example, the catfish that was fed with low protein and high energy ratio had lower growth rate (Liu *et al.* 2011). High carbohydrate content in the 23% protein diets may also be associated with slow growth rate. The digestive activity and utilization of protein and fat were more efficient compared to carbohydrate, which was varied and depended on its complexity (Yamamoto *et al.* 2001). These phenomena resulted from different enzymatic activities in each fish, and so the utilization of carbohydrate was more limited in carnivorous fishes than omnivorous and herbivorous fishes (Mokoginta *et al.* 2004). Moreover, the fat content in feeds influenced the fish growth as it considerably served as energy source, and fatty acids (Koskela *et al.* 1998).

Feed conversion ratio (FCR) decreased with higher proportion of protein, suggesting that lower feed conversion means a more efficient feed use. FCR of 23% protein diet without rGH was highest, meanwhile FCR of 28% and 32% protein diets with addition of rGH were lower and did not differ significantly. This confirmed the results obtained by Liu *et al.* (2011) that FCR decreased with higher level of protein.

Table 3 Protein and lipid retention of striped catfish (*Pangasianodon hypophthalmus*) fed on different protein levels and recombinant growth hormone

Dietary protein levels		Moisture content (%)	Wet protein body content (%)	Protein retention (%)	Wet lipid body content (%)	Lipid retention (%)
		Parameters				
Feed A 23%	Non rGH	66.48±0.00[ab*]	16.52±0.22[ab]	46.67±5.76[b]	14.09±0.06[bc]	456.25±52.70[a]
	rGH	63.95±0.24[a]	16.64±1.02[ab]	47.28±2.28[b]	12.44±0.42[d]	334.12±16.97[b]
Feed B 28%	Non rGH	64.24±1.00[ab]	17.52±1.59[ab]	54.14±2.36[ab]	14.41±0.06[ab]	221.02±9.60[c]
	rGH	62.92±1.07[b]	18.80±1.56[a]	62.42±3.02[a]	13.20±0.45[cd]	192.18±9.23[c]
Feed C 32%	Non rGH	63.22±1.11[a]	14.52±0.22[b]	35.05±3.44[c]	15.32±0.15[a]	210.13±18.26[c]
	rGH	66.49±0.28[b]	17.35±0.66[ab]	50.35±0.02[b]	14.40±0.09[ab]	213.76±0.35[c]
Two-way ANOVA						
Feed Protein (P)		0.05	0.06	0.00	0.00	0.00
rGH (R)		0.68	0.06	0.00	0.00	0.00
PxR (Interaction)		0.00	0.26	0.01	0.22	0.00
Standard Error		0.77	1.04	3.29	0.26	24.45

Note: *a-c = different superscript in the same column indicates significant differences among treatments ($P<0.05$)

Protein and Lipid Retention

The addition of rGH showed significant effects on lipid content, protein retention and lipid retention (Table 3). Protein and lipid interaction also demonstrated significant effects on different protein levels and incorporation of rGH. Lipid retention in feed B and C with addition of rGH was not statistically different compared to control. However, significant difference was observed between rGH-supplemented feed A and non-supplemented feed A groups.

Protein retention represents percentage of protein stored in the body. The highest protein retention (62.42±3.02) was at 28% protein feed with rGH addition (Table 3). The wet lipid body content (12.44±0.42) was significantly reduced at 23% protein feed with rGH compared to control. In a similar study, the rGH administration in feed also suppressed the fat content (Safir 2012). A study on the ratio of carbohydrate and fat content in catfish meat (initial weight: 119.23 g) found that the increased ratio of carbohydrate and fat resulted in an increased fat retention (Kharisma 2009). The 23% protein diet in this experiment had higher ratio of carbohydrate and fat in comparison with other treatments. The higher carbohydrate content and lower fat content in feed were also associated with higher lipid retention (Linder 1992). Lipid bioconversion from non-lipid compounds (such as carbohydrate) to fatty acids and triglycerides in liver and fat tissues is a consequence of limited fat intake. Fatty acids were synthesized from glucose (derivative product of carbohydrate) in conditions where glucose is in excess (Kersten 2001).

Meat Quality (fillet) and Belly Fat

Different protein levels and rGH administration significantly influenced fat content in meat, but did not significantly influenced protein content, edible portion, and belly fat (Table 4).

Protein content in all treatments was not significantly different (15.79-18.89%) (Table 4). However, these values are slightly higher than previous results of Poernomo *et al.* (2015) where 23-32% protein diets without hormone addition resulted in slightly higher protein content (15.27-16.17%) in meat. Low fat content (2.72%) was observed in 23% protein diet enriched with rGH. Fat content was also lowered as protein levels increased in rGH enriched diet. Fat content in fish meat treated with 23% and 28% protein diets supplemented by rGH, and fat in the control was low. In addition, moisture content of meat was about 75.24-77.46% showing similarity with that of Suryaningrum *et al.* (2010) where moisture content of some catfish was at 75.53-79.42%. High water content remarkably influenced the textural properties of fish meat. Edible portion weight (43.35-47.64%) and belly fat (0.52-0.69%) did not significantly differ among treatments. These findings were in accordance with Poernomo *et al.* (2015) that used feed with 23-32% of protein for striped catfish seed (33.61 g) for 60 days. The rearing resulted in similar amounts of edible portion weight and belly fat. The results of Suryaningrum *et al.* (2010) showed that percentage carcass was at 44-49% for some species of catfish like, *P. hypophthalmus, P. djambal, Pasupati, Nasutus* and hybrid of Siam and Nasutus catfish. The thicker meat is associated with larger edible portion, but bigger fish head and thinner meat are attributed to smaller proportion of edible portion.

Hepatosomatic Index (HSI), Moisture Content, Liver Fat and Glycogen

The protein treatment significantly influenced moisture content, liver glycogen, and hepatosomatic index (HSI) of the catfish, but rGH addition on the diet had no significant effects on these parameters (Table 5). The treatments also showed no interaction with moisture content, liver fat, liver glycogen, and HSI of the catfish. HSI decreased with higher protein content in the feed.

Table 4 Meat quality (fillet %) and belly fat (%) of striped catfish (*Pangasianodon hypophthalmus*) fed on different protein levels and recombinant growth hormone

Dietary protein level		Parameters				
		Moisture content	Protein	Lipid	Edible portion	Belly fat
Feed A 23%	Non rGH	$75.24 \pm 1.26^{a*}$	16.33 ± 0.96^{a}	4.93 ± 0.29^{a}	43.45 ± 1.37^{a}	0.58 ± 0.12^{a}
	rGH	76.52 ± 0.92^{a}	15.79 ± 1.54^{a}	2.72 ± 0.22^{c}	44.90 ± 1.32^{a}	0.52 ± 0.11^{a}
Feed B 28%	Non rGH	76.52 ± 0.59^{a}	16.39 ± 0.78^{a}	3.41 ± 0.29^{bc}	44.91 ± 1.33^{a}	0.60 ± 0.07^{a}
	rGH	77.46 ± 0.83^{a}	16.33 ± 1.12^{a}	3.70 ± 0.02^{b}	47.16 ± 3.09^{a}	0.69 ± 0.17^{a}
Feed C 32%	Non rGH	74.02 ± 2.61^{a}	18.89 ± 2.39^{a}	4.71 ± 0.44^{a}	46.24 ± 1.07^{a}	0.53 ± 0.07^{a}
	rGH	76.16 ± 0.07^{a}	16.78 ± 0.21^{a}	4.76 ± 0.08^{a}	47.64 ± 2.80^{a}	0.61 ± 0.21^{a}
Two-way ANOVA						
Feed Protein (P)		P<0.08	P<0.09	P<0.00	P<0.01	P<0.48
rGH (R)		P<0.04	P<0.18	P<0.00	P<0.44	P<0.57
PxR (interaction)		P<0.72	P<0.41	P<0.00	P<0.75	P<0.59
Standard Error		1.31	1.35	0.26	2.95	0.13

Note: *a-c = Different superscripts in the same column indicates significant differences among treatments (P<0.05)

Table 5 Moisture content, liver fat, glycogen, and hepatosomic index (HSI) of the catfish (*Pangasianodon hypophthalmus*) fed on different protein levels and recombinant growth hormone

Feed Treatments		Parameters			
		HSI	Moisture (%)	Liver fat (%)	Glycogen (%)
Feed A 23%	Non rGH	$1.86 \pm 0.31^{a*}$	74.91 ± 0.51^{ab}	3.90 ± 0.22^{a}	0.07 ± 0.02^{a}
	rGH	1.88 ± 0.30^{a}	74.80 ± 0.53^{ab}	3.51 ± 0.51^{a}	0.05 ± 0.03^{ab}
Feed B 28%	Non rGH	1.66 ± 0.04^{ab}	75.70 ± 0.33^{ab}	3.40 ± 0.21^{a}	0.01 ± 0.00^{c}
	rGH	1.73 ± 0.11^{ab}	74.66 ± 1.19^{b}	3.69 ± 0.29^{a}	0.02 ± 0.00^{c}
Feed C 32%	Non rGH	1.26 ± 0.10^{b}	77.45 ± 0.90^{a}	3.48 ± 0.24^{a}	0.01 ± 0.00^{c}
	rGH	1.35 ± 0.02^{ab}	76.59 ± 0.21^{ab}	3.65 ± 0.24^{a}	0.01 ± 0.00^{c}
Two-way ANOVA					
Feed Protein (P)		P<0.00	P<0.00	P<0.65	P<0.00
rGH (R)		P<0.53	P<0.06	P<0.87	P<0.41
PxR (Interaction)		P<0.95	P<0.44	P<0.19	P<0.24
Standard Error		0.20	0.73	0.32	0.01

Note: *a-c = different superscripts in the same column indicates significant differences among treatments (P<0.05)

Liver is an important organ that serves as fat storage and acts as the center of metabolic activities of the body (NRC 2011). HSI was negatively correlated with feed protein proportion (Table 5). This confirmed the results of Arnason *et al*. (2010) that HSI decreased with increased protein content in feed. In another study (Poernomo *et al*. 2015), fat content in fish liver did not significantly differ among diets with different protein levels (23%-32%). Glycogen is a carbohydrate stored in fish liver and meat (Halver & Hardy 2002). The 23% protein feed enriched with rGH and control resulted in higher liver glycogen compared to other treatments. The low protein and high fat content of feed caused fat deposition in the fish body and liver that led to a high liver glycogen content in *Clarias gariepinus* (Ali & Jauncey 2005).

CONCLUSION

Application of recombinant growth hormone (rGH) in commercial feed improved the specific growth rates (SGR) of the striped catfish (*Pangasianodon hypophthalmus*) where the highest SGR was obtained when the fishes were fed with the 32% protein feed combined with rGH. Enrichment with rGH depleted the fat content in the meat of the fishes fed with different levels of protein with the lowest fat found in 23% protein feed.

ACKNOWLEDGEMENTS

This report is part of the thesis of the first author, which was funded by BPSDM scholarship award from the Ministry of Marine and Fisheries Affairs of Indonesia. Thanks to Suardi Laheng, and Hasan Nasrullah for assisting in the preparation and technical implementation of this research.

REFERENCES

Alimuddin, Lesmana I, Sudrajat AO, Carman O, Faisal I. 2010. Production and bioactivity potential of three recombinant growth hormones of farmed fish. Indonesian Aquaculture Journal 5 (1):11-7.

Ali MZ, Jauncey K. 2005. Approaches to optimizing dietary protein to energy ratio for African catfish *Clarias gariepinus* (Buchell, 1982). Aquac Nutr 11:95-101.

Arnason J, Rannveig B, Arnarsson I, Arnadottir GS, Thorarensen H. 2010. Protein requirements of Atlantic cod *Gadus morhua* L. Aquac Res 41:385-93.

Bartke A 2005. Role of the growth hormone/insulin-like growth factor system in mammalian aging. *Minireview*. Endocrinol 146:3718-23.

Budi DS, Alimuddin, Suprayudi MA. 2015. Growth response and feed utilization of giant gourami *Osphronemus gouramy* juvenile feeding different protein level of the diets supplemented with recombinant growth hormone. HAYATI J Biosci 22:12-9.

Centre of Statistic Data and Information of the Ministry of Marine and Fisheries Affairs [SIDATIK] [Internet]. 2015. Marine and Fisheries Affairs in figures 2015. [download on 26 September 2016]. Available from: www.kkp.go.id

Craigh S, Helfrich LA. 2002. Understanding fish nutrition, feed, and feeding. Virginia (US): Department of Fisheries and Wildlife Science, Virginia Tech.

Fu C, Cui Y, Hung SSO, Zhu Z. 1998. Growth and feed utilization by F4 human growth hormone transgenic carp fed diets with different protein levels. J Fish Biol 53(1):115-29.

Funkenstein B, Dyman A, Lapidot Z, de Jesus-Ayson EG, Gertler A, Ayson FG. 2005. Expression and purification of a biologically active recombinant rabbitfish *Siganus guttatus* growth hormone. Aquac 250:504-15.

Halver JE, Hardy RW. 2002. Fish nutrition 3rd edition. London (UK): Academic Press Inc.

Handoyo B, Alimuddin, Utomo NBP. 2012. Growth, feed conversion and retention, and proximate of eel juvenile treated by immersion of recombinant giant grouper growth hormone. Indonesian Aquaculture Journal 11(2):132-40.

Hardiantho D, Alimuddin, Prasetiyo AE, Yanti DH, Sumantadinata K. 2012. Performance of Nile tilapia juvenile fed diet containing different dosages of recombinant common carp growth hormone. Indonesian Aquaculture Journal 11:17-22.

Hasan MR. 2010. On-farm feeding and feed management in aquaculture Manila, the Philippines. FAO Aquaculture Newsletter 45(1):48-9.

Hasan AJZ, Khan U. 2013. Protein sparring effect and the efficiency of different composition of cabohydrate, lipid and protein on the growth of rohu *Labeo rohita* fingerlings. World J Fish Mar Sci 5(3):244-50.

Helver JE. 1998. Fish nutrition. New York (US): Academic Press Inc.

Hertz Y, Tchelet A, Madar Z, Gertler A. 1991. Absorption of bioactive human growth hormone after oral administration in the common carp and its enhancement by deoxycholate. J Comp Physiol 161:159-63.

Huisman EA. 1987. Principle of fish production. Wageningen (NL): Department of Fish Culture and Fisheries, Wageningen University. 187 p.

Kersten S. 2001. Mechanism of nutritional and hormonal regulation of lipogenesis. EMBO Reports 21:282-6.

Kharisma NH. 2009. Effect of dietary carbohydrate to lipid ratio on growth and muscle lipid retention of *Pangasius hypophythalmus* for grow out [Thesis]. Bogor (ID): Institut Pertanian Bogor.

Kobayashi S, Alimuddin, Morita T, Miwa M, Lu J, Endo M, … Yoshizaki G. 2007. Transgenic Nile tilapia *Oreochromis niloticus* over-expressing growth homone show reduced ammonia excretion. Aquac 270:427-35.

Koskela J, Jobling M, Savolainen R. 1998. Influence of dietary fat level on feed intake, growth and fat deposition in the whitefish *Coregonus lavaretus*. Aquac Int 6:95-102.

Li Y, Bai J, Jian Q, Ye X, Lao H, Li X, … Liang X. 2003. Expression of common carp growth hormone in the yeast *Pichia pastoris* and growth stimulation of juvenile tilapia *Oreochromis niloticus*. Aquac 216:329-41.

Linder MC. 1992. Nutrisi dan metabolisme karbohidrat [Carbohydrate nutrition and metabolism]. In: Linder MC (editor). Biokimia nutrisi & metabolisme [Biochemistry of nutrition & metabolism]. Jakarta (ID): UI Press. 781 p.

Liu XY, Wang Y, Ji WX. 2011. Growth, feed utilization and body composition of Asian catfish *Pangasius hypophthalamus* feed at different dietary protein and lipid level. Aquac Nutr 11:578-84.

Lovell RT. 1989. Nutrition and feeding of fish. New York (US): New York Van Nostrand Reinhold. 217 p.

Mokoginta I, Takeuchi T, Hadadi A, Jusadi D. 2004. Different capabilities in utilizing dietary carbohydrate by fingerling and subadult giant gouramy *Osphronemus gouramy*. Fish Sci 70:996-1002.

Nutritional Research Council [NRC]. 2011. Nutrient requirement of fish and shrimp. Washington DC (US): National Academic Press.

Poernomo N, Utomo NBP, Azwar ZI. 2015. The growth and meat quality of Siames catfish feed different level of protein. Indonesian Aquaculture Journal 14(2):104-11.

Rasmussen RS, Ronsholdt B, Ostenfeld TH, McLean E, Byatt JC. 2001. Growth, feed utilization, edible portion composition and sensory characteristics of rainbow trout treated with recombinant bovine placental lactogen and growth hormone. Aquac 195:367-84.

Safir M. 2012. Response giant gourami *Osphronemus goramy* on oral administration in different dose of recombinant growth hormone. [Thesis]. Bogor (ID): Institut Pertanian Bogor.

Silverstein JT, William RW, Munetaka S, Walon WD. 2000. Bovine growth hormone treatment of channel catfish: Strain and temperature effects on growth, plasma IGF-I level, feed intake and efficiency and body composition. Aquac 190:77-8.

Suryaningrum DT, Muljanah I, Tahapai E. 2010. Sensori profile and nutritional value of several species of catfish and Nasutus hybrid. Jurnal Pascapanen, Bioteknologi Kelautan dan Perikanan 5(2):153-64.

Takeuchi T. 1988. Laboratory work - chemical evaluation of dietary nutrient. In Watanabe T (Ed). Fish Nutrition and Mariculture. Tokyo (JP): JICA Kanagawa International Fisheries Training Centre. p. 179-233.

Watanabe T. 1988. Fish nutrition and mariculture. Tokyo (JP): Department of Aquatic Bioscience, Tokyo University of Fisheries, JICA. 233 p.

Yamamoto T, Konishi K, Shima T, Furuita H, Suzuki N, Tabata M. 2001. Influence of dietary fat and carbohydrate levels on growth and body composition of rainbow trout *Oncorhynchus mykiss* under self-feeding conditions. Fish Sci 67:221-7.