ENRICHMENT OF RECOMBINANT GROWTH HORMONE IN DIET CONTAINING DIFFERENT LEVELS OF PROTEIN ENHANCED GROWTH AND MEAT QUALITY OF STRIPED CATFISH (*Pangasionodon hypophthalmus*)

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ABSTRACT

Application of fish recombinant growth hormone (frGH) has been known as one of the applicable method to improve growth performance of culture fish.The purpose of this study was to evaluate the effects of recombinant growth hormone (rGH) supplementation in commercial diet on growth, feed utilization and flesh quality in striped catfish grow out. The rGH was mixed with chicken egg yolk and sprayed on commercial feed with different protein levels (32, 28 and 23%). As control the diets were sprayed with chicken egg yolk without rGH. Striped catfish (body weight: 110,66 ± 1,32 g ind^{-1}) were fed on rGH-supplemented diets two times a week (first and third months), and the rest was on diet without rGH supplementation. Fish were reared for 120 days in 18 hapa (2×1×1,5 m^3) with initial density of 20 fish. The result showed that the highest weight gain, specific growth rate (SGR), and lowest feed conversion ratio (FCR) were obtained in fish fed on 32% protein content of rGH-supplemented diet. Furthermore, there was no significant difference in weight gain, SGR and FCR in rGH treatment group with 28% protein content of rGH supplemented diet and non-rGH control group receiving 32% protein diet (P>0.05). 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 treatment 23% protein content rGH supplemented diet has lower lipid content in fish body and meat were found (P<0.05). It can be concluded that the highest SGR is obtained when fish fed 32% with protein feed combined with r*El*GH. Enrichment with r*El*GH depleted the fat content in meat of fish feed all levels of protein which was the lowest fat found in 23% protein feed.

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

INTRODUCTION

Striped catfish *Pangasianodon hypophthalmus* is a considerable fresh water commodity and shows a great attraction in Indonesia. The average production rate has been reported to increase 31.63% from 2010-2014 (SIDATIK 2015). Commonly, one of main constraints in fish cultivation is increased feed cost, but the fish price tends to stable. In addition, it requires long time to obtain ideal size for consumption, leading to decreased benefits. Fish flour is one of major protein sources. In general, feed quality is determined by its protein content. Higher protein content in feed leads to higher price. Therefore, feed price plays a key factor since it contributes to 30-60% of total production cost (Hasan 2010).

Protein is a pivotal 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). Lovell (1989) stated that the protein was converted into energy when energy produced by

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non-protein sources was low, leading to lower contribution of protein for building tissues. In short, addition of non-protein sources to produce energy reduces the use of protein as energy source (protein sparring effect), thus increasing protein function in improving fish growth (Hasan & Khan 2013).

In order to improve fish growth rate, there are some approaches such as nutrition, reproduction, environmental and genetical approach ie application of recombinant protein, hybridization, triploidization, selection and transgenesis technology. In molecular biotechnology approach, the use of recombinant growth hormone (rGH) has been reported to have advantageous effects on fish growth rate. The growth hormone is peptide hormone from anterior pituitary that acts in controlling growth, metabolism 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 using feed supplementation is more efficient in large quantities of fish. Some studies reported that rGH-supplemented feed successfully increased fish growth rate of channel catfish *Ictalurus punctuatus* (Silverstein *et al.* 2000), common carp *Cyprinus carpio* (Li *et al.* 2003), rabbitfish *Siganus* sp. (Funkenstein *et al.* 2005), eel *Anguilla* sp. (Handoyo *et al.* 2012), tilapia (Muhammad *et al.* 2013), and giant gouramy *Osphronemus goramy* (Budi *et al.* 2014).

Feed supplementation by rGH to improve fish growth has been evaluated by some studies. Budi *et al.* (2014) reported that application of rGH on feed enriched by protein with different levels could increase growth performance and efficiency of protein use for gourami juvenile. Additionally, Rasmussen *et al.* (2001) found that rGH supplementation lowered lipid content, increased protein synthesis. Lower lipid content and increased fish growth indicated protein sparring effect with increasing growth hormone level. Kobayashi *et al.* (2007) showed that growth hormone was capable of increasing protein retention and absorption and reducing ammonia excretion. Meanwhile, studies related to the application of r*El*GH in feed with different and lower levels of protein (23%, 28%, and 32%) in *Pangasius* grow out have yet to be done. This research aims to investigate effects of rGH supplemented-feed with different protein levels on growth and quality product of striped catfish.

MATERIALS AND METHODS

Exprimental Diet and Production rGH

The rGH production was performed using *Escherichia coli* BL 21 containing pCold-1/r*El*GH vector expression. Bacterial culture and rGH collection methods were performed as described by Alimuddin *et al.* (2010).

There are 3 types of test feed (commercial feed), each with a different proportion of protein; 23, 28 and 32%. Result of feed proximate test is shown in 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 (Hardianto *et al.* 2012). Non-supplemented feed was also coated with egg yolk and 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
Carbohydrate (%)*	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 fibre + 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 r*El*GH

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Fish Rearing

Fish (BW 110.66±1.32g) were reared in experimental pond (200 m^2) using 18 hapa (2×1×1.5 m^3) with density of 20 fishes per hapa. The fishes were reared for 120 days and fed (feeding rate at 3% of fish biomass) twice a day at morning and afternoon. Feed containing r*El*GH was given at twice a week, and others treatment were fed with diets without r*El*GH. Fish body weight was measured every 20 days until the end of the experiment. The water quality was measured temperature range between 29-34°C, pH 7.05-7.51, DO (dissolved oxygen) 4.20-7.60 mg L^{-1}, and TAN (total amonium nitrogen) 0.12-0.80 mg L^{-1}.

Data Collection and Statistical Analysis

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

$$\left(\sqrt[t]{\frac{Wt}{W0}} - 1\right) X\ 100$$

SGR = daily 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: FCR = [P/((Wt + Wm)-Wo)] X100; [FCR = feed conversion ratio; 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)]. 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 = 100 x liver weight/body weight). Edible portion was calculated using the equation: edible portion = 100 x weight of meat tobe eaten/body weight). While belly fat was calculated using the equation: belly fat = 100 x weight of belly fat/body weight. A complete proximate analysis of each experiment unit was carried out on the first and last day of the experiment according to the methods of Takeuchi (1988). Fish is taken minimum three fishes per unit experiment for proximate analysis. This experiment was designed into factorial 2x3; two levels of rGH (supplemented and non-supplemented) and three levels of protein. Each treatment performed at 3 replicates. All data were analyzed by two-way ANOVA using Minitab statistical software with p >0.05.

RESULTS AND DISCUSSION

Growth Performance and Survival Rate

In this 120-day experiment, we found that feed treated with r*El*GH 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 r*El*GH, while the lowest biomass gain and SGR and the highest FCR were associated with feed A (protein 23%) without r*El*GH addition. weight gain, SGR, feed consumption, and FCR of fish treated by feed B (protein 28%) with rGH was not significant different (P>0.05) than that of feed C with rGH, and feed C without rGH. In addition, biomass gain, SGR, FCR showed no significant interaction with protein levels and r*El*GH addition (Table 2). Survival rate in all treatments was 100%.

Treatment of 23% protein diets with r*El*GH 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). Budi et al (2014) conducted study about administration of rElGH with different protein level showed different result. A giant juvenile gouramy (size: 15.82± 0.13 g) given feed supplemented with rElGH will experience increased growth and dietary utility compared with gouramy given the same feed without supplementation. Meanwhile, Fu *et al.* (1998) stated that F4 generation of human growth hormone (hGH) transgenic common carp were more efficient in utilizing dietary protein than control. This dissimilarity result may related with

Table 2 Final weight (g fish^{-1}), weight gain (g fish^{-1}), specific growth rate (SGR, % day^{-1}), feed consumption (g fish^{-1}), and feed conversion ratio (FCR, %) after feeding with different protein level and recombinant growth hormone

Dietary protein level		Parameters				
		Final weight	Weight gain	SGR	Feed Consumption	FCR
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}
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}
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

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

different fish species used. Hertz *et al.* (1991) stated age dependent and species specific were remarkable factors that also influence effects of rGH. Furthermore, differences in total energy-protein 21% (4337.76 kcal/kg) and ratio C/P (20.38) of experimental diets used by Budi *et al.* (2014) may contribute to the results. Ratio of protein energy (DE/P) in catfish feed was about 7.4–12 kcal/g (Halver & Hardy 2002). Administration of rGH significantly differ in improving amylase, lipase, and protease in 21% protein diet (Budi 2014).

Feeding with various levels of protein and energy affects fish growth. Halver & Hardy (2002) stated that feed with lower energy caused protein conversion as additional energy source required for metabolic process. Consequently, protein for growth activities is reduced, leading to slow growth rate. Liu *et al.* (2011) also reported that catfish fed with low protein and high energy ratio had lower growth rate. High carbohydrate content in 23% protein diets may also associate with slow growth rate. Yamamoto *et al.* (2001) stated that digestive activity and utilization protein and fat were more efficient compared to carbohydrate, which may be various and depends on its complexity. Mokoginta *et al.* (2004) reported that these phenomena resulted from different enzymatic activities in each fish, and utilization carbohydrate was more limited in carnivorous fish than omnivorous and herbivorous fish. Additionally, fat content in feed is able to influence the fish growth. Guillon *et al.* (1995) found that fat in the fish feed considerably served as energy source, fatty acids, solvent for fat soluble vitamins.

Feed conversion obtained tends to decrease with higher proportion of protein, suggesting that lower feed conversion means more efficiency of feed use. Feed conversion of 23% protein diet without rGH was highest, meanwhile FCR of 28% and 32% protein diets with addition of rGH was not significantly different. This result was in accordance with Liu *et al.* (2011), that FCR decreased with higher level of protein.

Protein and Lipid Retention

Proximate data of fish was presented in Table 3. The results indicated that feed treated with protein and r*El*GH showed significant effects (P<0.05) on lipid content, protein retention and lipid retention. Value of protein and lipid interaction demonstrated interaction (P<0.05) between different protein levels and incorporation of r*El*GH. Lipid retention in feed B and C with addition of r*El*GH was not statistically different compared to control. However significant difference was observed between r*El*GH-supplemented feed A and non-supplemented feed A group.

Table 3 Moisture content, wet protein body content, protein retention, wet lipid body content and lipid retention after feeding with different protein level and recombinant growth hormone

Dietary protein levels		Parameters				
		Moisture (%)	Wet protein body content (%)	Protein Retention (%)	Wet lipid body content (%)	Lipid Retention (%)
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]
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]
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

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

Protein retention represents percentage of protein stored in the body. The highest protein retention was attributed to 28% protein feed with rGH addition. Meanwhile wet lipid body content was significantly reduced in 23% protein feed with rGH compared to control (P<0.05). The same result was also found by Safir (2012), that rGH administration in feed could suppress fat content. Kharisma (2009) studied ratio of carbohydrate and fat content in catfish meat (initial weight: 119.23 g) and found that increased ratio of carbohydrate and fat increased fat retention. The 23% protein diet in this experiment had higher ratio of carbohydrate and fat in comparison with other treatments. Linder (1992) found that higher carbohydrate content and lower fat content in feed were associated with higher lipid retention. Lipid bioconversion from non-lipid compounds (such as carbohydrate) to fatty acids and triglycerides in liver and fat tissues is consequence of limited fat intake. Kersten (2001) showed that fatty acids were able to be synthesized from glucose (derivative product of carbohydrate) in excess amount of glucose.

Meat Quality (fillet) and Belly Fat

Table 4 indicated that different protein levels and r*El*GH administration significantly influenced (P<0.05) fat content in meat, but not significantly influenced protein content, edible portion, and belly fat (P>0.05).

Table 4 Meat quality (fillet, %) and belly fat (%) of striped catfish fed after feeding with different protein level and recombinant growth hormone

Dietary protein level		Parameters				
		Moisture content	Protein	Lipid	Edible portion	Belly fat
23%	Non rGH	75.24±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]
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]
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

*Different superscript in the same column indicates significant difference between treatments (P<0,05).

Protein content in all treatments is not significantly different (15.79-18.89%). This value is slightly higher than previous results by Poernomo *et al.* (2015), that 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. In rGH enriched diet, the fat content were lower by increasing the protein levels. Table 4 showed that fat in fish meat treated with 23% and 28% protein diets supplemented by rGH, and control was low-fat. In addition, moisture content of meat was about 75.24-77.46%. Suryaningrum *et al.* (2010) reported that moisture content of some catfish was 75.53-79.42 %. High water content remarkably influences textural properties of fish meat. Edible portion weight (43,35-47,64%) and belly fat (0,52-0,69%) were not significantly different among treatments. These findings were in accordance with Poernomo *et al.* (2015), that feed with 23-32% of protein for striped catfish seeds (33.61 g) for 60 days of rearing resulted in similar edible portion weight and belly fat. The result of Suryaningrum *et al* (2010) research showed that percentage carcass for some spesies of catfish between 44-49% (*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, Fat and Liver Glycogen

Data obtained from fish liver analysis were exhibited in Table 5. Protein treatment influenced moisture content, liver glycogen, and HSI (P<0.05), but r*El*GH administration had no significant effects on these parameters. The treatments also showed no interaction with moisture content, liver fat, liver glycogen, and HSI (P>0.05). HIS tends to decrease with higher protein content in feed.

Liver is an important organ that serves as fat storage. NRC (2011) stated that liver act as center of metabolic activities. In this study, we found that HSI was negatively correlated with feed protein proportion. Arnason *et al.* (2010) reported that HSI decreased with increased protein content in feed. Poernomo *et al.* (2015) found that fat content in fish liver was not significantly different from other diets with different protein levels (23%-32%). Glycogen is a carbohydrate storage in fish liver and meat (Halver & Hardy 2002). The 23% protein feed enriched with rGH and control resulted higher liver glycogen compared to other treatments. Ali & Jauncey (2005) low protein and high fat content of feed caused fat deposition in all fish body and liver, leading to high liver glycogen content in *Clarias garipinus*.

Table 5 Moisture content, liver fat, glycogen, and hepatosomic index (HSI) after feeding with different protein level and recombinant growth hormone

Feed Treatments		Parameters			
		HSI	Moisture (%)	Liver fat (%)	Glycogen (%)
23%	Non rGH	1.86±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]
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]
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

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

CONCLUSION

Administration of recombinant *Epinephelus lanceolatus* growth hormone of (r*El*GH) in commercial feeds improved the SGR where the highest SGR is obtained when fish fed 32% with protein feed combined with r*El*GH . Enrichment with r*El*GH depleted the fat content in meat of fish feed all levels of protein which was the lowest fat found in 23% protein feed.

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