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Growth Response and Feed Utilization of Giant Gourami (*Osphronemus goramy*) Juvenile Feeding Different Protein Levels of the Diets Supplemented with Recombinant Growth Hormone

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The purpose of this study was to examine the effect of dietary supplementation with recombinant growth hormone (rGH) on the growth and dietary utility of juvenile giant gourami. The rGH was mixed with chicken egg yolk and sprayed on to artificial feed with different protein levels (34, 28, and 21%; isoenergy). Each treatment group of gourami was paired with a control group that received feed of the same protein level, but without rGH supplementation. Juvenile of giant gourami (weight 15.83 ± 0.13 g) were fed diets containing rGH, to apparent satiation, 2 times a week. Fish were reared from less than 2 months old for 42 days in 100 L glass aquaria at an initial density of 10 fish per aquarium. At the end of this period, the biomass and daily growth rate (SGR) of the fish were measured and the feed conversion ratio calculated and compared. Our data showed that fish fed rGH-supplemented diets experienced higher growth than fish in the control groups and showed that fish with higher protein diets experienced higher growth than the groups with less protein diets. The group with the highest biomass gain, SGR, and lowest feed conversion ratio (FCR) was the group fed a 34% protein diet supplemented with rGH. Furthermore, biomass gain, SGR, and FCR in the rGH treatment group with a 28% protein diet matched the measurements of the non-rGH control group receiving a 34% protein diet (P > 0.05). We conclude that giant juvenile gourami given feed supplemented with recombinant growth hormone will experience increased growth and dietary utility compared with gourami given the same feed without supplementation.

Key words: feed protein levels, growth, giant gourami, rGH

INTRODUCTION

Giant gourami (*Osphronemus goramy*) is an important aquaculture commodity in Indonesia. Giant gourami grows at a relatively slow rate, which can hinder efforts to increase giant gourami production to meet high market demand. However, if increasing the production of giant gourami is difficult, it may be possible to increase the growth of each fish. There are several known strategies for increasing fish growth rate. The use of recombinant growth hormone (rGH) in fish diet is an easy method that can yield quick results in fish growth. Many studies have examined the effect of rGH supplementation on fish species such as Nile tilapia (Acosta *et al*. 2007), rainbow trout (Llorente *et al*. 2004), abalone (Moriyama *et al*. 2008), and yellow tail (Pedroso *et al*. 2009). Regarding giant gourami specifically, prior research has shown that rGH application via immersion method significantly increases the growth rate of giant gourami (Irmawati *et al*. 2012). The application of rGH with 3 mg/kg dose in gourami feed also resulted in higher growth than control groups without rGH (Safir 2012). This study aims to examine the combined effect of rGH application and variation of dietary protein levels, which has not yet been explored.

The rGH can increase metabolic capacity, especially lipid metabolism and optimization of protein synthesis, on giant gourami. Fish with a faster relative growth rate exhibited the protein sparring effect that occurs with increased growth hormone levels (Rasmussen *et al*. 2001; Irmawati *et al*. 2012). Growth and development of body tissue in fish are influenced by the balance of protein and energy in their feed. Feed with high protein content will not necessarily accelerate growth if the total energy content is low. The energy content of feed is

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mainly used for primary metabolic activities, such as respiration, ion transport/metabolites, regulation of body temperature and other physiological functions. Energy for these activities generally derives from non-protein nutrients (fats and carbohydrates). If the energy contribution from non-protein material is relatively low, protein must be tapped to produce energy. This reduces the amount of protein available for building body tissues. In other words, the addition of non-protein nutrients for energy production can minimize the use of protein as an energy source and maximize its use for growth and body mass (protein sparring effect). This improves the function of protein in supporting the growth of fish (Furuichi 1988; Hasan & Khan 2013). The rGH supplementation is expected to increase the efficiency of protein utilization in the diet. The purpose of this study was to examine the effect of different dietary protein levels supplemented with recombinant growth hormone (rGH) on the growth and dietary utility of juvenile giant gourami.

MATERIALS AND METHODS

Experimental Diets and Production of rGH. We used 3 types of experimental feed, each with a different proportion of protein; i.e. 21, 28, and 34%. Gross energy (GE) for each type of feed was the same, approximately 4.200 kcal GE/kg of feed. Feed formulation is shown in Table 1. rGH production was performed using *Escherichia coli* BL21 containing pCold-1/rElGH vector expression. Bacterial culture and rGH collection methods were performed as described by Alimuddin *et al*. (2010).

Table 1. Formulation and chemical composition of the experimental diets

Ingredients (%)	Protein diets (%)		
	21	28	34
Fish meal	10.00	19.00	26.00
Soybean meal	10.00	23.00	39.00
Pollard	73.00	51.00	28.00
Fish Oil	2.00	2.00	2.00
Tapioca	3.00	3.00	3.00
Vitamin and mineral mix	2.00	2.00	2.00
Proximate (% dry matter) and gross energy (GE)			
Crude protein	21.28	28.19	34.29
Lipid	6.81	6.03	6.14
Mineral matter	6.94	8.59	10.66
Crude fibre	3.85	6.18	5.33
Carbohydrate*	61.12	51.02	43.58
GE (kcal/kg)**	4,337.76	4,236.78	4,284.19

*Carbohydrate = 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).

The rGH was coated with 20 mg chicken egg yolk and then applied to feed pellets at a dose of 3 mg/kg (Safir 2012). The 3 treatment groups that received feedat each protein level, coated with rGH, were paired with control groups that received the feed at the same protein level but without rGH, for a total of 6 experimental groups. We conducted 3 repetitions of the experiment for all 6 groups.

Fish Rearing. Juvenile of giant gourami (BW 15.83 ± 0.13 g) were reared for 42 days in 100 L glass aquaria at initial density of 10 fish per aquarium. Water was changed 50% per day each afternoon. Oxygen levels were maintained using aeration. Fish were fed three times a day (morning, afternoon, and evening) until apparent satiation. Treatment groups were given feed containing rGH 2 times a week, at an interval of 3 days.

Data Collection and Statistical Analysis. Fish body weight was measured every 14 days until at the end of the experiment. For each fish, we measured hepatosomatic index (HSI), proximate composition, excreted total ammonia nitrogen (TAN), blood glucose and triglycerides levels, liver and muscle glycogen, and enzyme activity, at the end of experiment. All data were analyzed by two-way ANOVA using SPSS statistical software with $P > 0.05$. Daily growth rate (SGR) was calculated by the equation:

$$SGR = \left(\sqrt[t]{\frac{Wt}{W0}} - 1 \right) x\ 100\%$$

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 time maintenance (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).

Measurement of Parameters. Analysis of total ammonia excretion was determined by measuring total ammonium nitrogen (TAN) in the culture media using a test kit NH_3/NH^{4+} (API © Mars Fishcare North America Inc., USA). Initial measurement was carried out after the fish had fasted for 24 h and the aquaria

were filled with new water (0.19 ± 0.08 mg TAN/L). This was performed 24 h after the end of the fish were fed to satiation. TAN was calculated for each unit of feed consumed per fish biomass, as modified from Suprayudi *et al*. (2014). Blood glucose levels were measured by enzymatic colorimetric method using a liquicolor GLUCOSE test (Human mbH, Germany). A complete proximate analysis of each fish was carried out on the first and last day of the experiment according to the methods of Takeuchi (1988). Blood triglycerides were measured via enzymatic colorimetric test with lipid clearing factor (LCF) using liquicolor^{mono}TRIGLYCERIDES (Human mbH, Germany). Liver and musle glycogen was measured using the procedure in Wedemeyer and Yasutake (1977). Enzyme activity, such as protease, amilase, and lipase, were measured according to Bergmeeyer and Grassi (1983), Worthington (1993), and Borlongan (1990).

RESULTS

Our study results showed that rGH supplementation of fish diets resulted in higher growth than diets without rGH supplementation, at each protein level tested (Table 2). The highest biomass gain, SGR, and feed consumption, and the lowest FCR were found in the group given a 34% protein diet supplemented with rGH. The lowest biomass gain, SGR, and feed consumption, with the highest FCR were found in the group given a 21% protein diet without rGH supplementation. Furthermore, biomass gain, SGR, feed consumption, and FCR in the group given a 28% protein diet supplemented with rGH was comparable to that of the control group receiving a 34% protein diet without rGH supplementation. In addition, biomass, SGR, feed consumption and FCR responses showed no interaction between dietary protein levels and rGH supplementation; but each of them independently to affect are parameters (Table 2).

We found a significant correlation between dietary protein levels and rGH supplementation, and protein and lipid retention, and also protein and lipid body content. There was an interaction between dietary protein levels and rGH supplementation. Protein and lipid body content and retention in rGH treatment groups at all dietary protein levels were higher than that of the groups at corresponding dietary protein levels who did not receive rGH supplementation, except for the groups receiving a 28% protein diet (Table 2).

We found that HSI was inversely correlated to dietary protein levels, but that rGH supplementation

Table 2. Biomass gain, specific growth rate (SGR), feed consumption, feed convertion ratio (FCR), wet protein body content, protein retention, wet lipid body content, and lipid retention of the giant gourami (*Osphronemus goramy*) after feeding with different protein levels and supplemented with recombinant fish growth hormone (rGH)

Dietary protein levels		Parameters							
		Biomass gain (g)	SGR (%)	Feed consumption (g)	FCR	Wet protein body content (%)	Protein retention (%)	Wet lipid body content (%)	Lipid retention (%)
21%	Non rGH*	159.17 ± 13.92^{e**}	1.56 ± 0.13^{e}	301.54 ± 40.44^{c}	1.89 ± 0.09^{a}	17.45 ± 0.10^{bc}	44.56 ± 0.78^{b}	8.65 ± 0.53^{b}	92.06 ± 4.05^{c}
	rGH	214.88 ± 12.31^{d}	1.93 ± 0.05^{d}	356.86 ± 39.10^{abc}	1.66 ± 0.09^{b}	18.14 ± 0.34^{a}	55.39 ± 4.79^{a}	10.05 ± 0.25^{a}	123.40 ± 8.99^{ab}
28%	Non rGH	250.36 ± 11.59^{e}	2.13 ± 0.08^{c}	338.70 ± 13.48^{bc}	1.35 ± 0.04^{c}	18.50 ± 0.42^{a}	54.67 ± 2.22^{a}	8.04 ± 0.09^{c}	121.63 ± 1.73^{ab}
	rGH	298.88 ± 10.54^{b}	2.37 ± 0.04^{b}	399.59 ± 41.53^{ab}	1.34 ± 0.10^{c}	18.08 ± 0.57^{ab}	53.47 ± 1.25^{a}	8.47 ± 0.25^{bc}	130.46 ± 5.50^{a}
34%	Non rGH	290.33 ± 14.93^{b}	2.32 ± 0.06^{b}	392.14 ± 17.00^{ab}	1.35 ± 0.02^{c}	16.87 ± 0.34^{c}	37.38 ± 3.05^{c}	6.64 ± 0.22^{e}	88.31 ± 3.51^{c}
	rGH	339.37 ± 9.69^{a}	2.59 ± 0.03^{a}	405.95 ± 27.419^{a}	1.20 ± 0.05^{d}	18.67 ± 0.23^{a}	49.40 ± 5.07^{ab}	7.41 ± 0.09^{d}	116.38 ± 3.83^{b}
Two-way ANOVA									
Protei diets (P)		P < 0.000	P < 0.000	P < 0.009	P < 0.000	P < 0.043	P < 0.000	P < 0.000	P<0.000
rGH (R)		P < 0.000	P < 0.000	P < 0.014	P < 0.001	P < 0.001	P < 0.001	P < 0.000	P<0.000
PxR (Interaction)		P < 0.854	P < 0.262	P < 0.407	P < 0.064	P < 0.001	P < 0.008	P < 0.035	P<0.035
Standard Error		2.898	0.017	7.544	0.017	0.171	0.776	0.066	0.066

*Non rGH is the dietary without rGH supplementation; **Different superscript letters in the same column indicate significant different at P < 0.05 based on Duncan's multiple range test.

significantly increased the HSI value of groups at each dietary protein levels (Table 3). Furthermore, the HSI of the group receiving the rGH supplemented 28% protein diet was comparable with the HSI of the group receiving the 34% protein diet without rGH supplementation. In this study we did not find that dietary protein levels and rGH supplementation had any influence on each other, nor on the effect of either factor in regards HSI. We found a significant increase in TAN excretion with the increase of dietary protein levels (Table 3). The rGH suplementation decreased TAN excretion, except in the group receiving an rGH supplemented 28% test diet compared to the control group without rGH supplementation. As with HSI, we found no evidence that dietary protein levels and rGH supplementation worked in any way to alter the effect that each factor would have independently on TAN excretion. TAN excretion in the group given the rGH-supplemented 28% protein diet was comparable to the TAN excretion measured in the group given the 34% protein diet without the rGH supplementation, while TAN excretion in the group that received the rGH-supplemented 21% protein diet was comparable to that measured in the group that received the 28% protein diet without rGH supplementation. The lowest TAN excretion was observed in the group receiving the rGH-supplemented 21% protein diet supplemented with rGH, and the highest TAN excretion was found in the group that received the 34% protein diet without rGH supplementation.

The rGH supplementation significantly increased the levels of blood glucose in treatment groups at all protein levels, but we did not find that dietary protein levels and rGH supplementation had any influence on each other, nor on the effect of either factor in regards to blood glucose levels (Figure 1). Blood glucose levels in groups receiving rGH-supplemented feed (60.67-63.17 mg/dL) were on average, across all protien levels, 20.67% higher than those for groups without rGH supplementation treatments (50.00-54.00 mg/dL). However, triglyceride levels in all groups, both treated and control, were comparable, with the exception of the group receiving a 21% protein diet without rGH supplementation, whose triglycerides levels were significantly lower than all treatments (Figure 2).

Both dietary protein levels and rGH supplementation significantly affected liver and muscle glycogen levels (Table 3). Unlike the other measurements in this study, we did find that dietary protein level and rGH supplementation had an interactive effect on each other, increasing the impact each factor would ordinarily have independently. Liver and muscle

Table 3. Hepatosomatic index (HSI), total ammonia nitrogen (TAN) excretion, liver glycogen, muscle glycogen, amilase activity, lipase activity, and protease activity of the giant gourami (*Osphronemus goramy*) after feeding with different protein levels and supplemented with recombinant fish growth hormone (rGH)

Dietary protein levels		Parameters					
		HSI	TAN excretion (mg/g feed/g fish)	Liver glycogen (mg/g sample)	Muscle glycogen (mg/g sample)	Amilase (U/mg substrate)	Lipase (U/mg substrate)	Protease (U/mg substrate)
21%	Non rGH*	$1.70 \pm 0.11^{b**}$	0.095 ± 0.020^{e}	3.12 ± 0.37^{b}	0.91 ± 0.37^{b}	2.80 ± 0.08^{b}	7.71 ± 0.47^{bc}	0.019 ± 0.004^{b}
	rGH	1.91 ± 0.05^{a}	0.057 ± 0.015^{d}	7.04 ± 0.93^{a}	2.26 ± 0.53^{a}	4.22 ± 0.58^{a}	12.01 ± 0.91^{a}	0.040 ± 0.004^{a}
28%	Non rGH	1.28 ± 0.02^{d}	0.114 ± 0.015^{b}	1.98 ± 0.47^{bc}	0.23 ± 0.01^{c}	2.10 ± 0.02^{c}	6.71 ± 0.16^{bc}	0.018 ± 0.006^{b}
	rGH	1.48 ± 0.13^{c}	0.098 ± 0.015^{bc}	2.83 ± 0.66^{bc}	0.82 ± 0.10^{b}	2.22 ± 0.06^{c}	6.16 ± 0.89^{c}	0.023 ± 0.003^{b}
34%	Non rGH	1.10 ± 0.03^{e}	0.172 ± 0.016^{a}	1.74 ± 0.56^{c}	0.27 ± 0.06^{c}	2.91 ± 0.27^{b}	7.66 ± 1.32^{bc}	0.024 ± 0.008^{b}
	rGH	1.26 ± 0.04^{d}	0.134 ± 0.111^{b}	1.86 ± 0.66^{c}	0.53 ± 0.11^{bc}	2.77 ± 0.29^{b}	8.47 ± 1.57^{b}	0.019 ± 0.002^{b}
Two-way ANOVA								
Protein diets (P)		$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.013$
rGH (R)		$P < 0.000$	$P < 0.001$	$P < 0.000$	$P < 0.000$	$P < 0.005$	$P < 0.007$	$P < 0.009$
PxR (Interaction)		$P < 0.866$	$P < 0.387$	$P < 0.001$	$P < 0.014$	$P < 0.001$	$P < 0.004$	$P < 0.002$
Standard Error		0.018	0.004	0.149	0.065	0.0.068	0.237	0.001

*Non rGH refers to the diet without rGH supplementation; **Different superscript letters in the same column indicate significant differences at $P < 0.05$ based on Duncan's multiple range test.

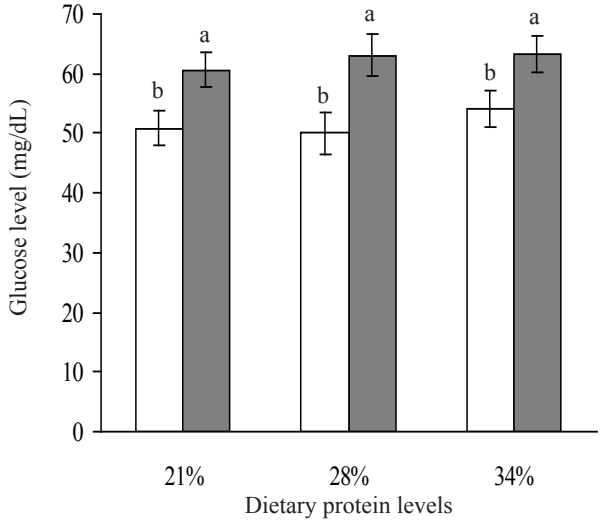

Figure 1. Glucose level in blood (mg/dL) of giant gourami (*Osphronemus goramy*) juvenile after feeding with different protein levels and enriched fish recombinant growth hormone (rGH). The different letters above the bar indicate significant difference ($P < 0.05$) based on Duncan's multiple range tests. □ without rGH suplementation, ■ with rGH suplementation.

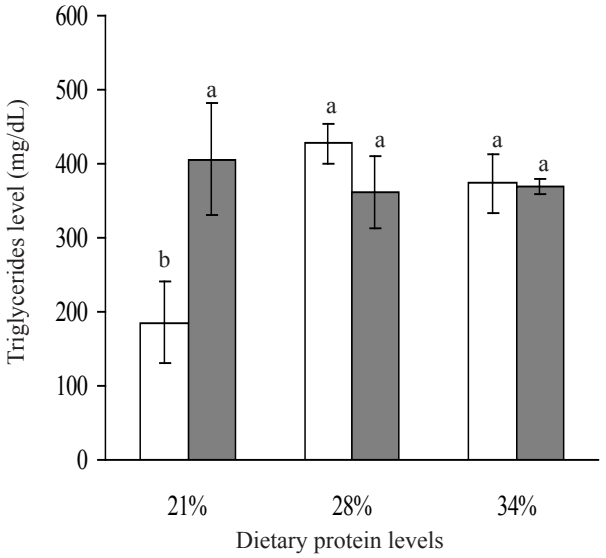

Figure 2. Triglycerides level in blood (mg/dL) of giant gourami (*Osphronemus goramy*) juvenile after feeding with different protein levels and enriched fish recombinant growth hormone (rGH). The different letters above the bar indicate significant difference ($P < 0.05$) based on Duncan's multiple range tests. □ without rGH suplementation, ■ with rGH suplementation.

glycogen levels of fish fed rGH supplemented 21% protein diets were higher than those in fish fed 21% protein diets without rGH supplementation treatments. The rGH supplementation in 21% protein diets treatment significantly increased the activity of amilase, lipase, and protease; however rGH supplementation did not significantly affect this activity for the 28 and 34% protein treatments. There was an interaction between dietary protein levels and rGH supplementation (Table 3).

DISCUSSION

Administration of rGH Increased Fish Appetite. Appetite is thought to be influenced by increased growth hormone stimulation induced by the hormone ghrelin (Volkoff *et al*. 2005; Debnanth 2010). The effect of rGH supplementation in increasing appetite was apparent in the increased feed consumption of fish reared in this research (Table 2). In others, feed consumption also was affected by feed palatability related to dietary protein levels (Table 2). Palatability of feed relates directly to its attactiveness to fish, determining whether fish will seek, capture, and ingest (acceptability) of feed. Palatability of feed relating to nutrient content, especially free amino acids such as glycine, alanine, and betaine; as well as energy of feed (Shamushaki *et al*. 2007). In this research, the increase in palatability of feed occurs in the feed with higher protein content.

Food intake will affect the activity of digestive enzymes. Digestive activity is influenced by the substrate directly and by growth hormone (GH) indirectly (Debhnant 2010; Mataruga *et al*. 2012; Irmawati *et al*. 2012). This was supported by our results: dietary protein levels and rGH administration triggered digestive enzyme activity, with a trend that enzyme activity (protease, amylase, and lipase) of lower dietary protein levels treatments were the same compared to higher dietary protein levels treatments; rGH giving effect an increase in enzyme activity at 21% protein feed (Table 3).

The increase in blood glucose in groups receiving rGH supplementation was comparable at all dietary protein treatment levels (Figure 1); protein composition of feed did not seem to affect blood glucose levels. Glucose functions as a source of energy (Hemre *et al*. 2002). Blood glucose levels were higher in the groups receiving rGH treatments than in those without, regardless of protein levels, and we measured higher energy availability in the blood after the fish in these groups after they fasted for 24 h. The high blood glucose levels were followed by an increase in liver and muscle glycogen (Table 3). This is consistent with the findings of Kersten (2001), that unused glucose will be stored as glycogen and the excess will be converted into triglycerides. We confirmed this process in our finding of an increase in triglyceride levels in groups given rGH supplementation, especially in the group receiving the 21% protein diet (Figure 2). Increased triglycerides were measured in the rGH treated fish, after 24 h fasting, due to endogenous synthesis of triglycerides derived from glucose (lipogenesis) as

the result of liver glycogen mobilization and the transport of free fatty acids from adipose tissue to the liver (Groff & Gropper 2000).

Glycogen levels rise initially because of an incrase in liver volumes (Table 3). Yang *et al*. (2002) similarly saw increases in hepatosomatic index (HSI) due to the accumulation of glycogen in the liver of silverperch (*Bidyanus bidyanus*). The rise in HSI is also related to increased body fat content (Table 2 & 3), with HSI and body fat content highest in the treatment group receiving 21% protein feed. This is in line with the Cheng *et al*. (2006) study of grouper (*Epinephelus coioides*) in which increases in body fat content, followed by an increase in liver fat content, resulted in an increase in the HSI.

Increased protein and lipid retention due to rGH supplementation has been reported in other studies (Promdonkoy *et al*. 2004; Haghighi *et al*. 2011). The presence of non-protein energy (carbohydrates and lipids) can reduce the use of protein for energy (protein sparring effect) resulting in increased protein retention and decreased ammonia excretion (Suprayudi *et al*. 2014). In our study, rGH supplementation allowed for increased protein retention (Table 2) and decreased TAN excretion (Table 3). This indicates that higher protein retention decreases metabolite excretion of nitrogen (in the TAN form) due to the protein sparring effect during the production of energy in fish. This is consistent with Perez-Sanchez (2000), who found higher nitrogen (protein) retention in fish receiving rGH treatment, indicating that rGH functions to increase the utilization of non-protein nutrients as a source of energy (protein sparring effect). The role of rGH in reducing TAN excretion has also been reported in studies of transgenic tilapia (Kobayashi *et al*. 2007). On the other hand, an increase in dietary protein causes TAN excretion to increase (Table 3). Previous research on Australian short-finned eel juveniles (Guo *et al*. 2012) and blue fin trevally (Suprayudi *et al*. 2014) showed a positive relationship between protein intake and ammonia excretion. This suggests that amino acids from protein, deaminated and excreted as ammonia, are used to produce energy rather than stored for growth, when fish are given protein supplemented diets (Mohanta *et al*. 2008). These results also indicate that low levels of dietary protein and rGH supplementation can reduce ammonia pollution from aquaculture.

The increases in protein and lipid retention we found due to rGH supplementation, were followed by improved FCR measurements (Table 2 and 3). Increased dietary protein along with rGH supplementation, resulted in a decrease in FCR, suggesting more efficient feed utilization. This indicates that rGH supplementation can improve the efficiency of protein and fat utilization to increase fish growth. The role of growth hormone in improving feed efficiency has been reported in salmon (Devlin *et al*. 2004), muchloach (Nam *et al*. 2004), and tilapia (Kobayashi *et al*. 2007).

Furthermore, all the parameters that have been described are direct effects of the growth of juvenile giant gourami. The results of this study showed that the growth measures of juvenile giant gourami increased in correspondence to increases in dietary protein and to rGH supplementation. Yang *et al*. (2002) suggests that low dietary protein content results in low growth in many species of fish. Meanwhile, the success of oral administration of rGH in boosting fish growth has also been reported in several previous studies (Promdonkoy *et al*. 2004; Haghighi *et al*. 2011). In our study, we found the highest biomass gain and best SGR in the treatment group receiving the 34% protein diet supplemented with rGH. Biomass gain and SGR in the treatment group receiving the 28% protein diet supplemented with rGH was comparable to that measured in the group receiving the 34% protein diets without rGH supplementation.

Overall, increased dietary protein, as well as rGH supplementation each produced significant improvements in the growth of juvenile giant gourami, but we found no interaction between the effects of these two treatment variables. The interaction between dietary protein levels and rGH supplementation instead was evident in parameters that supporting growth, such as protein and lipid retention, blood triglyceride levels, liver and muscle glycogen, and the activity of digestive enzymes. In conclusion, the growth and feed utilization of juvenile giant gourami can be increased by recombinant growth hormone supplementation in different protein levels of the diets.

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