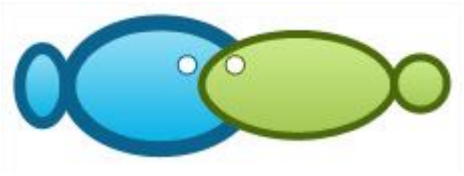

Biochemical responses and feed digestibility in the sex reversed Nile tilapia fed different protein levels and r*El*GH enriched diet

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Abstract. This study was aimed to evaluate the response of sex reversed Nile tilapia fed different protein level feeds (20%, 24%, and 28%) and a recombinant *Epinephelus lanceolatus* growth hormone (r*El*GH) enriched diet based on biochemical tests and feed digestibility in supporting growth performance. This study employed four-month-old tilapia produced by sex reversal using 17α-methyltestosterone (MT) and non-sex reversed tilapia fed different protein levels and feeds enriched with r*El*GH and without r*El*GH. This study consisted of nine treatments and three replications. The results of this study demonstrated that the r*El*GH treatment increased the activity of protease, lipase and amylase enzymes. The highest ($p < 0.05$) enzyme activity was found in the treatment using 28%+r*El*GH+MT, but amylase activity was similar ($p > 0.05$) among r*El*GH treatments. At identical protein levels, the value of protein digestibility, total digestibility, and liver and muscle glycogen contents of fish treated with r*El*GH were higher ($p < 0.05$) than those of the control. The blood glucose level of fish treated with r*El*GH increased then decreased faster than that of control. The highest hepatosomatic index ($p < 0.05$) was found in the treatment using 28%+r*El*GH+MT. Ammonia excretion in fish treated with r*El*GH was lower ($p < 0.05$) than that of control at identical levels of protein, but was similar among r*El*GH treatments ($p > 0.05$). In conclusion, sex reversed tilapia fed a 28% protein content feed enriched with r*El*GH demonstrated the best biochemical responses and a higher level of feed digestibility.

Key Words: food glucose, dietary protein level, enzyme activity, glycogen, TAN excretion.

Introduction. Utilization of recombinant growth hormones (rGH) has significantly increased growth in several fish species. These findings were reported by a number of researchers, among them Acosta et al (2009) in Nile tilapia (*Oreochromis niloticus*) and goldfish (*Carassius auratus*), Irmawati et al (2012) in giant gourami (*Osphronemus goramy*), Handoyo et al (2012) in eel (*Anguilla* sp.), Alimuddin et al (2010), Hardiantho et al (2012), Muhammad et al (2014) in Nile tilapia, and Antoro et al (2016) in humpback grouper (*Cromileptes altivelis*). Among the three rGH application methods (immersion, injection, and oral), the oral method is more commonly and effectively used for raising fish, that is by mixing it with feed (Promdonkoy et al 2004; Hardiantho et al 2012; Antoro et al 2016; Muhammad et al 2014). In addition, it was reported by Budi et al (2015) that the oral method through rGH enrichment of low protein feed was proven to be able to stimulate the utilization of non-protein energy which resulted in an increased growth in giant gourami juvenile.

The function of rGH is similar to natural GH, aside from being involved in the regulation of somatic growth, this hormone is also involved in the metabolism of organisms including fish (Vijayakumar et al 2010). Some metabolic responses related to the application of rGH had been reported by several researchers, including an increase in lipase activity in the liver (Irmawati et al 2012) and digestive enzymes (amylase, lipase,

and protease) in giant gourami (Budi et al 2015), an increased ratio of trypsin and chymotrypsin (T:C), and an increased feed digestibility in tilapia (Vinasyiam et al 2016). Furthermore, the application of rGH was also able to increase the level of blood glucose, liver and muscle glycogen, the hepatosomatic index, and protein and fat retention in fish (Antoro et al 2016; Budi et al 2015). In addition, application of rGH in fish also had positive correlation to the decline in ammonia excretion. This reflects a decrease in the breakdown rate of protein into energy, optimizing the function of protein for growth (Kobayashi et al 2007; Budi et al 2015).

Studies related to enrichment of feed using rGH in tilapia concerning the biochemical and physiologic responses in promoting growth until this day are only focused on one level of feed protein and mostly on high protein levels such as studies conducted by Hardiantho et al (2012) at a protein level of 31%, Muhammad et al (2014) at a protein level of 35%, and Vinasyiam et al (2016) at a protein level of 28%. Those studies also used non-sex reversed tilapia (without 17α-methyltestosterone immersion; NMT) as test fish. Meanwhile, studies related to the application of r*El*GH in feed with different and lower levels of protein (20%, 24%, and 28%) in sex reversed tilapia (17α-methyltestosterone immersion; MT) have yet to be done. On this note, this study specifically assessed the response of sex reversed Nile tilapia fed different protein levels (20, 24, and 28%) and r*El*GH enriched diets based on biochemical and digestibility tests.

Material and Method. This study was conducted on September-November 2015 at Fish Nutrition Laboratory, Laboratory of Reproduction and Genetics of Aquatic Organisms and Field Station, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University.

Formulation and preparation of experimental diets. The feed formulation was prepared based on the protein level of experimental diets, namely 20%, 24% and 28% (Table 1).

Table 1

Formulation and chemical composition of the experimental diets

Ingredients (%)	*Dietary protein level*		
	20%	*24%*	*28%*
Fish meal	10.00	17.00	21.00
Soybean meal	5.00	14.00	29.00
Pollard	74.00	58.00	39.00
Corn oil	2.00	2.00	2.00
Fish oil	2.00	2.00	2.00
Tapioca	4.00	4.00	4.00
Vitamin and mineral mix	3.00	3.00	3.00
Proximate (% dry weight) and gross energy (GE)			
Crude protein	20.03	24.94	28.45
Fat	9.65	9.11	8.89
Ash	8.27	9.96	11.21
Crude fibre	11.93	12.78	11.69
Carbohydrate[1]	50.12	43.21	39.75
GE (kcal kg^{-1})[2]	4083.68	4024.73	4059.04
Energy:protein (kcal g^{-1})	20.39	16.14	14.27

[1]Carbohydrate = dry weight - (crude protein + fat + crude fibre + ash), [2]GE = gross energy protein 5.6 kcal g^{-1}, fat 9.4 kcal g^{-1}, carbohydrate 4.1 $kcal^{-1}g$ (Watanabe 1988).

Feed enrichment with r*El*GH (Mina Grow; Center for Freshwater Aquaculture Sukabumi and Department of Aquaculture-Faculty of Fisheries and Marine Sciences-Bogor Agricultural University) was done according to the method of Hardiantho et al (2012) with a dose of 3.0 mg kg^{-1} feed (Muhammad et al 2014). Control feed was prepared by coating the feed using the same procedure without r*El*GH enrichment. Then, for the feed

digestibility test, an amount of 0.5% Cr_2O_3 kg^{-1} of experimental diet was added. Proximate analysis of the feed composition was done using the AOAC method (2007). Water content was measured by calculating the difference between feed weight before and after the feed was dried overnight using an oven at a temperature of 105-110°C (Takeuchi 1988). Protein content was determined based on the Kjeldahl method. Total fat content was measured using the Soxhlet method. Result of proximate analysis is presented in Table 1.

Experiment design. This study consisted of nine treatments with three replications. The treatments tested were the administration of different protein level feeds (20%, 24%, and 28%) enriched with r*El*GH on sex reversed fish (coded: MT), and three treatments without r*El*GH enrichment (20%+MT, 24%+MT, and 28%+MT). Control fish were non sex reversed fish (coded: NMT) and given different protein level feeds without r*El*GH enrichment (20%+NMT, 24%+NMT, and 28%+NMT).

Preparation and maintenance of test fish. Four-month-old tilapia (weight: 52.67±1.70 g) post sex reversal treatment using 17α-methyltestosterone (MT) hormone (Wassermann & Afonso 2003), and without sex reversal (NMT) treatment were obtained from the Field Station of the Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University.

Thirty fish were put into each hapa net (sized 2 × 2 × 1.5 m^3) which were placed in concrete ponds (sized 20 × 10 × 1.5 m^3), nine hapa nets for NMT fish and 18 hapa nets for fish treated with MT. Fish were adapted for a week by providing normal feed without r*El*GH according to the protein level of the experimental diets. Feeding of treatment feed enriched with r*El*GH was conducted once every three days for four weeks, with a feeding frequency of three times a day (morning, noon, and afternoon) *at satiation,* and the rest of the time the fish were fed a r*El*GH-unenriched feed. This was also performed on the control treatment. All measurements of the test parameters were done in the aquarium except for the measurement of liver and muscle glycogen levels, and hepatosomatic index (HSI).

Test parameter and data analysis. Measurement of digestive enzyme activity was done by collecting three fish from each hapa net for all treatments. The fish were put into prepared aquariums (sized 1 × 0.5 × 0.5 m^3). Treatment feeding was done after test fish were adapted for one week. Sampling for measurement of digestive enzyme activity referred to the procedure applied by Vinasyiam et al (2016).

The digestibility test was done by collecting nine fish at random from each hapa net for all treatments. Then the fish were put into their respective aquariums (sized 1 × 0.5× 0.5 m^3). The r*El*GH enriched diet was given after fish were adapted until an appropriate condition was achieved. Feeding frequency was twice a day (morning and afternoon), *at satiation.* The process of feces sample collecting and preparation was done according to the study by Vinasyiam et al (2016). Measurements of total digestibility and protein referred to method by Takeuchi (1988).

Measurement of blood glucose was conducted by collecting four fish from each hapa net for all treatments. Then the fish from each replication of the same treatment were collected together in one aquarium (sized 1 × 0.5 × 0.5 m^3) prepared for each treatment. Adaptation was carried out for two weeks by giving r*El*GH-unenriched diet at a frequency of three times a day. To prevent fish from experiencing stress during blood sampling, four aquariums were provided for each treatment with a density of three fish each aquarium. Fish were given the experimental diet after being fasted for 24 hours. Samples for blood glucose were taken at hour 0, 2, 4 and 6 after consuming the treatment feed, referring to Vinasyiam et al (2016). Measurement of blood glucose level was done using the enzymatic calorimetry test method by liquicolor glucose test (*Human mbH*, German). Measurements of liver and muscle glycogen were done by collecting the liver and muscle samples from three fish for each replication and storing the samples separately. Measurement of the glycogen level was conducted according to the method in Peungvicha et al (1998).

Measurement of HSI was performed by collecting all the remaining test fish from each replication at the end of the maintenance period. The fish's body and liver were weighed separately. Calculation of the HSI value was done referring to Budi et al (2015). The ammonia excretion test was carried out based on the procedure in Kobayashi et al (2007) with slight modifications. Four fish were taken from each hapa net for all treatments. Fish from each replication of the same treatment were collected together in one aquarium (sized 1 × 0.5 × 0.5 m^3) which had been prepared for each treatment. Fish were adapted for two weeks and were given r*El*GH-unenriched diet. Feeding frequency was three times a day (morning, noon, and afternoon). Treatment feeding was done *at satiation* after fish were fasted for 24 hours. After the test fish were rested for one hour and their weights had been recorded, the fish were put into new aquariums (sized 0.4 × 0.3 × 0.5 m^3) with a total of 27 units (the water volume of each aquarium was 50 L and the aquariums had been given strong aeration for 24 hours). Maintenance was done for six hours without aeration, feeding, or water exchange. Samples of maintenance water were collected every two hours (at hour 0, 2, 4, and 6) after the fish consumed the treatment feed. Measurement of total ammonia nitrogen (TAN) concentration was done using the Phenate method according to APHA (2012), while calculation of TAN excretion was conducted by referring to Suprayudi et al (2014).

All data obtained, including the digestibility level, digestive enzyme activity, blood glucose level, muscle and liver glycogen, HSI, and the level of TAN excretion, were analyzed using analysis of variance (One-Way ANOVA). If there was an influence that was significantly different at a significance level of 5%, it was followed by the Duncan test. The potential profit was analyzed descriptively. Data analyses were conducted using the statistical software SPSS 16.0.

Results

Digestive enzyme activity. The activity of digestive enzymes in sex reversed tilapia is presented in Table 2. The results showed that MT treatment in larval tilapia did not affect the digestive enzyme activity. In contrast, treatments using different dietary protein levels affected the activity of digestive enzymes in tilapia except for of lipase and amylase activity and the trypsin:chymotrypsin (T:C) ratio ($p < 0.05$). Furthermore, r*El*GH treatment in each dietary protein level indicated a significant effect ($p < 0.05$) on increasing digestive enzyme activity in tilapia compared to the treatments without r*El*GH.

The highest activity of digestive enzymes in the protease group (pepsin, trypsin, and chymotrypsin) among the r*El*GH treatments was found in treatment using 28%+r*El*GH+MT. Lipase activity in 28%+r*El*GH+MT treatment was higher ($p < 0.05$) than that of 24%+r*El*GH+MT treatment, yet it was not significantly different from that of the 20%+r*El*GH+MT treatment. Amylase activity in tilapia between r*El*GH treatments was similar ($p > 0.05$). Different results were found in the T:C ratio with the highest value ($p < 0.05$) shown by the 20%+r*El*GH+MT treatment.

Feed digestibility. Protein digestibility (PD) and total digestibility (TD) of feed in r*El*GH treatment fish was higher ($p < 0.05$) than treatment without r*El*GH (sex reversed and non sex reversed fish) at the identical levels of protein (Table 3). The PD value between fish of r*El*GH treatment at different levels of protein was similar ($p > 0.05$). TD between fish treated with r*El*GH continued to increase in line with the increase in dietary protein level, and the highest value ($p < 0.05$) was found in the 28%+r*El*GH+MT treatment.

Blood glucose, and liver and muscle glycogen contents. The levels of blood glucose in test fish during fasting (at hour 0) and after consuming experimental diets (at hours 2, 4, and 6) are presented in Figure 1. The blood glucose level in all test fish during fasting was relatively the same (ranging between 51.51 and 53.71 mg dL^{-1}). An increase in blood glucose occurred after the fish consumed the feed. The highest increase in blood glucose ($p < 0.05$) was demonstrated by fish given the r*El*GH treatment at hour 4, but the peak occurred at hour 6 in test fish given feed without r*El*GH (Figure 1). After the level of

blood glucose reached its peak both in fish treated with r*El*GH (hour 4) and without r*El*GH (hour 6), the blood glucose level of r*El*GH-treated fish decreased.

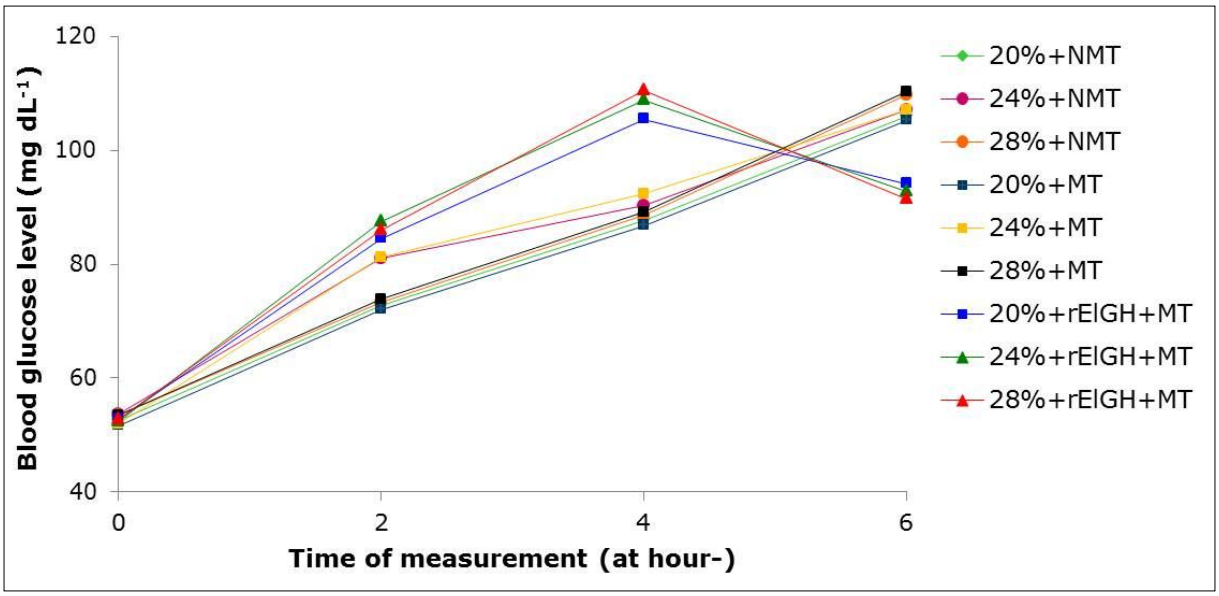

Figure 1. The pattern of blood glucose level in sex reversed and non sex reversed tilapia up to six hours after being fed feed with different levels of protein, with and without rElGH enrichment.

The levels of liver and muscle glycogen in all the test fish are presented in Table 3. The results demonstrated that the levels of liver and muscle glycogen of fish given the r*El*GH treatment were higher ($p < 0.05$) than those of fish treated without r*El*GH (treatment NMT, and MT) at identical levels of protein. The highest ($p < 0.05$) levels of liver and muscle glycogen among r*El*GH treatments were found in the treatment using 20%+r*El*GH+MT, followed by 28%+r*El*GH+MT.

Hepatosomatic index. The hepatosomatic index (HSI) of sex reversed tilapia in each treatment is presented in Table 3. The HSI of sex reversed fish (20%+MT, 24%+MT, and 28%+MT), and non sex reversed (20%+NMT, 24%+NMT, and 28%+NMT) were not significantly different ($p > 0.05$) at all dietary protein levels. However, the HSI of sex reversed fish given the r*El*GH treatment was higher ($p < 0.05$) than that of without r*El*GH treatment. The highest HSI was demonstrated by the 28%+r*El*GH+MT treatment.

Total ammonia nitrogen (TAN) excretion. The TAN excretion in the test fish during the six hour maintenance is presented in Table 3. The results indicated that TAN excretion increased in line with increases in dietary protein level ($p < 0.05$). The TAN excretion in NMT and MT treatment fish (without r*El*GH) at identical levels of protein were not significantly different. However, TAN excretion in fish treated with r*El*GH was lower ($p < 0.05$) that that of fish without r*El*GH treatment. The lowest TAN excretion was found in the 20%+r*El*GH+MT treatment.

Table 2

Activity of protease (pepsin, trypsin, chymotrypsin), lipase, amylase, and the ratio of trypsin and chymotrypsin (T:C) of sex reversed tilapia (*Oreochromis* sp.) fed different levels of protein (20%, 24%, and 28%) and enriched with recombinant *Epinephelus lanceolatus* growth hormone (r*El*GH)

Enzyme activity ($U\ mL^{-1}\ minute^{-1}$)	*Treatment using different levels of protein (%)*								
	Without sex reversal treatment (NMT), and without rElGH enrichment			*Sex reversal treatment (MT)*					
				Without rElGH enrichment			*With rElGH enrichment*		
	20	24	28	20+MT	24+MT	28+MT	20+r*El*GH+MT	24+r*El*GH+MT	28+r*El*GH+MT
Pepsin ($\times 10^{-1}$)	$0.17\pm0.005^{a*}$	0.19 ± 0.007^{b}	0.32 ± 0.004^{d}	0.17 ± 0.002^{a}	0.20 ± 0.008^{b}	0.32 ± 0.006^{d}	0.26 ± 0.003^{c}	0.33 ± 0.003^{d}	0.42 ± 0.006^{e}
Trypsin ($\times 10^{-1}$)	0.02 ± 0.001^{a}	0.02 ± 0.002^{a}	0.03 ± 0.001^{b}	0.02 ± 0.001^{a}	0.02 ± 0.001^{a}	0.03 ± 0.001^{b}	0.04 ± 0.00^{c}	0.04 ± 0.004^{c}	0.05 ± 0.001^{d}
Chymotrypsin ($\times 10^{-2}$)	0.12 ± 0.003^{a}	0.13 ± 0.001^{b}	0.15 ± 0.002^{c}	0.12 ± 0.003^{a}	0.13 ± 0.003^{b}	0.15 ± 0.002^{c}	0.19 ± 0.002^{d}	0.22 ± 0.002^{e}	0.24 ± 0.001^{f}
Lipase ($\times 10^{-1}$)	1.23 ± 0.073^{a}	1.23 ± 0.004^{a}	1.25 ± 0.042^{a}	1.21 ± 0.015^{a}	1.20 ± 0.018^{a}	1.26 ± 0.042^{a}	1.42 ± 0.018^{bc}	1.37 ± 0.026^{b}	1.44 ± 0.011^{c}
Amylase	3.49 ± 0.44^{b}	3.07 ± 0.07^{a}	3.04 ± 0.02^{a}	3.33 ± 0.15^{ab}	3.14 ± 0.03^{a}	3.12 ± 0.25^{a}	3.94 ± 0.01^{c}	3.93 ± 0.05^{c}	3.87 ± 0.05^{c}
T:C ratio	1.80 ± 0.09^{a}	1.89 ± 0.13^{abc}	1.94 ± 0.05^{abc}	1.84 ± 0.13^{ab}	1.78 ± 0.07^{a}	1.86 ± 0.05^{abc}	2.27 ± 0.03^{d}	2.00 ± 0.18^{bc}	2.04 ± 0.04^{c}

*Different superscript letters on the same row indicate significantly different values ($p < 0.05$). MT = Treatment with 17α-metiltestosterone, NMT = Treatment without 17α-methyltestosterone. The values are presented in the form of the average±standard deviation (n = 3).

Table 3

Protein digestibility (PD), total digestibility (TD), liver glycogen (LG), muscle glycogen (MG), hepatosomatic index (HSI), and total ammonia nitrogen (TAN) excretion, of sex reversed tilapia (*Oreochromis* sp.) fed different levels of protein (20%, 24%, and 28%) and enriched with recombinant *Epinephelus lanceolatus* growth hormone (r*El*GH)

Parameters	*Treatment using different levels of protein (%)*								
	Without sex reversal treatment (NMT), and without rElGH enrichment			*Sex reversal treatment (MT)*					
				Without rElGH enrichment			*With rElGH enrichment*		
	20	24	28	20+MT	24+MT	28+MT	20+r*El*GH+MT	24+r*El*GH+MT	28+r*El*GH +MT
PD (%)	$79.50\pm3.23^{a*}$	80.47 ± 2.85^{a}	81.93 ± 2.75^{ab}	80.92 ± 1.31^{a}	81.09 ± 3.21^{a}	82.09 ± 1.74^{ab}	85.87 ± 0.97^{bc}	86.01 ± 1.09^{bc}	87.29 ± 2.79^{c}
TD (%)	52.27 ± 0.95^{a}	54.00 ± 2.91^{a}	55.01 ± 1.46^{ab}	53.30 ± 0.93^{a}	53.88 ± 0.62^{a}	54.37 ± 1.97^{ab}	57.00 ± 0.93^{bc}	57.85 ± 1.33^{cd}	60.05 ± 1.19^{d}
LG (mg g $sample^{-1}$)	4.96 ± 0.25^{b}	4.80 ± 0.12^{b}	4.21 ± 0.064^{a}	4.83 ± 0.082^{b}	4.91 ± 0.10^{b}	4.13 ± 0.08^{a}	7.39 ± 0.010^{d}	6.10 ± 0.30^{c}	5.09 ± 0.052^{b}
MG (mg g $sample^{-1}$)	0.30 ± 0.007^{a}	0.31 ± 0.007^{a}	0.35 ± 0.021^{b}	0.31 ± 0.001^{a}	0.30 ± 0.004^{a}	0.36 ± 0.011^{b}	0.37 ± 0.006^{b}	0.38 ± 0.019^{b}	0.47 ± 0.005^{c}
HSI (%)	1.85 ± 0.08^{a}	1.82 ± 0.14^{a}	1.93 ± 0.11^{a}	2.03 ± 0.15^{ab}	2.05 ± 0.02^{ab}	2.04 ± 0.30^{ab}	2.29 ± 0.09^{bc}	2.28 ± 0.05^{bc}	2.35 ± 0.20^{c}
TAN excretion**	59.72 ± 1.21^{c}	62.90 ± 1.55^{de}	70.67 ± 2.15^{f}	60.07 ± 1.47^{cd}	63.48 ± 1.77^{e}	72.18 ± 1.34^{f}	50.98 ± 1.97^{a}	54.04 ± 1.67^{b}	62.14 ± 1.98^{ede}

*Different superscript letters on the same row indicate significantly different values ($p < 0.05$). MT = Treatment with 17α-metiltestosteron, NMT = Treatment without 17α-methyltestosterone. **TAN excretion (ng TAN kg $fish^{-1}$ $hour^{-1}$). The values are presented in the form of the average±standard deviation (n = 3).

Discussion. Increasing the level of dietary protein significantly ($p < 0.05$) increased the activity of digestive enzymes, particularly pepsin, trypsin and chymotrypsin. This finding is in line with the results reported by Eusebio & Coloso (2002) on Asian sea bass (*Lates calcarifer*), Debnath et al (2007) on *Labeo rohita*, Mohanta et al (2008) on silver barb (*Puntius gonionotus*), and Tu et al (2015) on gibel carp (*Carassius auratus gibelio*), that an increase in the protein level of feed given up to a certain level could increase protease activity. On the other hand, the activity of amylase continued to decrease along with the increased level of dietary protein. This is in accordance with the report by Mohanta et al (2008) on silver barb, and Liu et al (2009) on Jian carp (*Cyprinus carpio* var. Jian), that the decline in amylase activity occurred due to the decreased level of dietary carbohydrate, along with the increase in dietary protein level. Lipase activity in the present study was not significantly different, as with the findings reported by Liu et al (2009). Similarly, the ratio between trypsin and chymotrypsin (T:C) was not significantly different ($p > 0.05$) in all treatments without r*El*GH.

The activity of pepsin, trypsin, chymotrypsin, lipase and amylase continued to increase along with the addition of r*El*GH in feed compared with treatments without r*El*GH. This finding is in line with the opinions of Debnath et al (2010), Antoro et al (2016) and Budi et al (2015), that rGH treatment in test fish was expected to induce ghrelin hormone in the stomach which increases the appetite. Results of study by Mataruga et al (2012) proved that the addition of ghrelin in insects (*Lymantria dispar*) succeeded in increasing feed intake and the activity of digestive enzymes.

The ratio of T:C increased in treatments with r*El*GH enriched feed. The increase in the T:C ratio in fish treated with r*El*GH has also been reported by Vinasyiam et al (2016) in tilapia at a protein level of 28%. However, in this study, the highest T:C ratio between r*El*GH treatments was demonstrated by the lowest protein level treatment. This was assumed to be due to the low level of dietary protein which resulted in the lack of specific substrates for the activity of chymotrypsin (Table 2). As reported by Eusebio & Coloso (2002), Debnath et al (2007), Mohanta et al (2008), and Tu et al (2015), the activity of an enzyme is strongly affected by the availability of substrates in the digestive tract. Hedstrom et al (1992) stated that each enzyme has specific characteristics, for example chymotrypsin only cuts hydrophobic peptide bonds such as thyroxine, tryptophan and phenylalanine, while trypsin cuts peptide bonds after lysin and arginin on the carboxyl side.

Based on the results of the present study, an increase in digestive enzyme activity in all r*El*GH treatments had a positive effect on the nutrient digestibility value. This was supported by the PD and TD values in fish treated with r*El*GH which were higher than those of fish treated without r*El*GH (Table 3). The increase in PD and TD values is presumed to be caused by the action of intestinal microvilli which were affected by the rGH treatment. Walker et al (2004) reported that the application of rGH through feed proved to increase the length and density of intestinal microvilli in test fish which resulted in a longer contact period between intestinal microvilli and nutrients, thus increasing nutrient absorption.

The level of blood glucose in fish treated with r*El*GH increased, but then it decreased faster than that of fish treated without r*El*GH (Figure 1). This increase was due to the role of intestinal microvilli which enabled more an optimum absorption (Walker et al 2004). Increase in the blood glucose level in fish given the rGH treatment was also reported by Antoro et al (2016) in humpback grouper and Budi et al (2015) in giant gourami. This demonstrated that the energy for metabolic requirements was available more quickly because glucose is more quickly utilized as an energy source (Hemre et al 2002). However, the faster decrease in blood glucose reflected that the glucose absorbed from feed was not only transformed into energy, but was also absorbed into energy reserves in the form of glycogen in the liver and muscles which is mediated by the hormone insulin (Kersten 2001; Debnath et al 2007). It is evident from the results of this study that the levels of liver and muscle glycogen in fish treated with r*El*GH were higher than in fish treated without r*El*GH (Table 3). The increase in the liver glycogen level is assumed to be the cause of the increase in the test fish's HSI (Table 3). This was in line with the statement by Yang et al (2002) that the increase in liver glycogen levels had a

positive correlation to the increase in HSI. This was supported by Yandes et al (2003) who stated that the high absorption and metabolism of nutrients (protein, fat, and carbohydrate) could increase the HSI in fish because the liver is the center of nutrient metabolism (Ighwela et al 2014). The increased HSI in fish treated with r*El*GH was also reported by Antoro et al (2016) in humpback groupers, Budi et al (2015) in giant gourami, and Vinasiyam et al (2016) in tilapia.

The rate of TAN excretion in fish treated with r*El*GH was lower than that of treatment without r*El*GH (Table 3). The low TAN excretion in fish treated with r*El*GH was assumed to be due to the utilization of non-protein nutrients as a higher energy source. This was in line with the results reported by Farmanfarmaian & Sun (1999) who found that the application of GH in striped bass (*Perca saxatilis*) proved to increase the absorption of amino acids and to decrease the excretion of ammonium (NH_4^+) compared to the control treatment. Furthermore, Perez-Sanchez (2000) stated that in sea bream (*Sparus aurata*), Budi et al (2015) in giant gourami, and Vinasiyam et al (2016) in tilapia, GH could stimulate the utilization of non-protein energy derived from feed, thus increasing protein retention in the test fish, and would later have a positive effect on decreasing TAN excretion (Kobayashi et al 2007; Suprayudi et al 2014). The low TAN excretion at low protein level was caused by the increasing of carbohydrate and fat level in feed (Table 1). This increase will make a reduction in the activities of amino acid-degrading enzymes in the hepatopancreas and resulted in a low ammonia excretion rate (Shimeno et al 1981; Engin & Carter 2001; Yang et al 2002; Tu et al 2015). On the other hand, TAN excretion continued to increase along with the increasing level of dietary protein (Table 2). In previous studies it was reported that protein intake had a positive correlation with TAN excretion (Yang et al 2002; Guo et al 2012; Suprayudi et al 2014; Budi et al 2015). This shows that the absorption of amino acids from high levels of dietary protein, in addition to being used for growth and development, are also utilized as a source of energy, and the excess would be deaminated and secreted as ammonia in the form of TAN (Mohanta et al 2008; Budi et al 2015). This result also indicated that the application of r*El*GH in feed could reduce ammonia excretion, and that a dietary protein level of 28% was the level of protein that resulted in the best response.

Based on the description above, the application of r*El*GH through feed at different levels of protein in sex reversed tilapia is proven to provide better biochemical responses and higher digestibility compared with the control. These responses could increase the growth of tilapia as reported by Antoro et al (2016) in humpback groupers, Budi et al (2015) in giant gourami, and Vinasiyam et al (2016) in tilapia.

Conclusions. Every feed protein levels that was enriched with r*El*GH and had been given to sex reversed Nile tilapia (*Oreochromis* sp.) could increase protein digestibility, total digestibility, liver glycogen, muscle glycogen, hepatosomatic index, and decreased total ammonia nitrogen excretion. Feed with a protein level of 28% enriched with r*El*GH resulted in the best biochemical responses and a higher level of feed digestibility.

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