



Effect of feedings with different protein levels and dietary supplemental rElGH on culture performances of sex reversed *Oreochromis niloticus* (Linnaeus, 1758)

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ABSTRACT

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This study aims to evaluate the culture performance of tilapia (*Oreochromis niloticus*) that have been treated with 17 α -methyltestosterone (MT) and without MT (w-MT), feed with different protein levels (20, 24, and 28%) and recombinant *Epinephelus lanceolatus* growth hormone (rElGH)-diet. The research was conducted in 9 treatments and triplicate. Tilapia larvae were soaked twice, totaling 500 larvae, at the age of 10 days after hatching (DAH) and the age of 14 DAH was soaked for 4 hours using 1/1 MT 2 mg/l solution. Fish maintenance was conducted in an aquarium of 1.0x0.5x0.5 m³ in the first month, and three months later in net cages (2.0x2.0x1.5 m³). Daily growth rate (DGR) and biomass gain (BG) were increased in line with increasing feed protein content and rElGH supplementation. The highest DGR and BG values were MT+28+rElGH (P < 0.05) treatment. The highest feed consumption and the lowest feed conversion ratio were also obtained in the MT+28+rElGH treatment (P < 0.05). Fish survival was ranged from 79.89 to 90.28% (P > 0.05). The highest profit potential was found in the MT+28+rElGH treatment. The efficient aquaculture can be obtained by feeding sex-reversed tilapia at a protein level of 28% and a diet supplemented with rElGH.

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Introduction

Tilapia (*Oreochromis niloticus*) is a kind of freshwater cultured fish. This species has a gonadal maturity of less than 100 g body weight making it easy to breed and its growth is reduced, making a higher feed conversion ratio (FCR) and smaller size at harvest making it highly variable (Chakraborty *et al.*, 2011; Bhujel, 2013). The solution to this problem is monosex cultures of all males, with sex reversal treatment with the 17 α -methyltestosterone hormone (Srisakultiew and Kamonrat, 2013; Loum *et al.*, 2013). 17 α -methyltestosterone treatment will increase growth and decrease feed conversion when compared to mixed nurseries (Chakraborty *et al.*, 2011; Bhujel, 2013).

The protein content of the feed is a major growth factor. Tilapia protein requirements ranged from 21-45% (Loum *et al.*, 2013), while the feed protein in *O. niloticus* ranged from 25 - 37% (Bhujel, 2013). Using

high protein feed in *O. niloticus* culture creates high costs (Borski *et al.*, 2011), and this has the potential to reduce revenue. Feeding with protein less than 25% can reduce the growth of *O. niloticus* (Loum *et al.*, 2013). Feed cost efficiency without affecting biomass is done in various ways. Efficient feed research efforts in Indonesia are carried out using materials from agro-industry by-products and feed additives (Suprayudi *et al.*, 2011). Three types of recombinant growth hormone (rGH), namely common carp rGH, giant gourami rGH, and giant grouper rGH (rElGH) have been produced in our laboratory (Alimuddin *et al.*, 2010; Hardiantho *et al.*, 2012; Irmawati *et al.*, 2012). Among them, the highest level of production by *Escherichia coli* as a host is rElGH (Irmawati *et al.*, 2012).

In a previous study, we carried out rElGH supplementation in the diet of juvenile *O. niloticus* (body length of 2 cm) to determine the appropriate

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dose of rElGH and create higher growth enhancement (Hardiantho *et al.*, 2012). Growth was found to be higher with the addition of feed 2-3 mg/kg (crude protein diet: 31%) (Hardiantho *et al.*, 2012). Growth response of various sizes of *O. niloticus* (BW about 3.5 g, 12.5 g, and 40 g) treated with rElGH with 3 mg/kg feed (crude protein: 35-36%), and the highest growth was found at 12.5 g juvenile (Muhammad *et al.*, 2014). Research previously used mixed-sex *O. niloticus*, and created higher feed efficiency using rElGH. This study was performed to evaluate growth performance and potential gain of 17 α -methyltestosterone sex-reversed *O. niloticus* by feeding with different protein levels and dietary supplementation of rElGH.

Materials and Methods

Sex reversal treatment

Nine-day-old after hatching, *O. niloticus* larvae (weight: 10.2 \pm 5.1 mg, body length: 10.2 \pm 5.1 cm) from the Development and Stock Improvement Agency for Tilapia and Carp, Wanayasa, West Java Province, Indonesia. The fish was acclimatized for 1 day in 100 l (1.0x0.5x0.5 m³) aquariums and each aquarium was provided with two aerators for dissolve oxygen maintenance.

Tilapia monosex population was produced by sex reversal treatment with 17 α -methyltestosterone (MT) following Safir *et al.* (2017a) with minor modifications. A total of 2.0 mg of MT solution was dissolved in 0.25 mL of 100% ethanol, then put into a plastic bag filled with 1 liter of water. After the plastic was filled with water, as much as 500 larvae of *O. niloticus* were added and given oxygen allowing them to stand for 4 hours. On the other hand, the control had the same treatment but without MT (w-MT). In the immersion treatment, the larvae were released and washed three times in fresh water and returned to the aquarium with a density of 500 larvae per aquarium. The second immersion was carried out 4 days after the first treatment (14 days after hatching) with the same method.

Larvae were cultured for 2 weeks before feeding the recombinant *Epinephelus lanceolatus* growth hormone (rElGH)-supplemented diet. In the larvae rearing (including the first immersion), the fish were fed artemia nauplii, bloodworms, and commercial feed (crude protein: 40%), three times a day (morning (8:00 am), afternoon (1:00 pm), and evening (5:00 pm)). The water temperature was measured at 28-30°C and the oxygen level was maintained by using an aerator and each aquarium has one aeration point.

Feed preparation

Feeds formulated with isoenergy (approximately 4,000 kcal GE/kg feed) protein differences of 20, 24, and 28% (Safir *et al.*, 2017b). The feed was supplemented with 3 mg/kg rElGH (Hardiantho *et al.*, 2012; Muhammad *et al.*, 2014; Safir *et al.*, 2017b). The rElGH was obtained from the Center for Aquaculture Development, Sukabumi, West Java Province. The mixture of rElGH and chicken egg yolk was then sprayed onto 1 kg of feed and dried. Feeds received the same procedure without rElGH supplementation for controls and stored at -20°C. Proximate analysis of the experimental feed was carried out using the AOAC method (2007), to investigate the nutrient content of the feed.

Experimental design

This study used a completely randomized design consisting of nine treatments and three replications. The treatment was fish feeding with 17 α -MT and different protein levels (20, 24, and 28%), added with rElGH (designated: MT+20+rElGH, MT+24+rElGH, and MT+28+rElGH), and without rElGH supplementation (MT+20+w-rElGH, MT+24+w-rElGH, and MT+28+w-rElGH). As controls were fish without MT treatment (w-MT), fed with different protein content without rElGH supplementation (designated: w-MT+20+w-rElGH, w-MT+24+w-rElGH, and w-MT+28+w-rElGH).

Fish rearing and feeding

One-month-old larvae of *O. niloticus* (weight: 1.23 \pm 9.26 g, body length: 3.40 \pm 0.55 cm) were reared in 27 aquariums (1.0x0.5x0.5 m³) at a density of 400 individuals during one month. Furthermore, the fish were kept in 2.0x2.0x1.5 m³ net cages placed in a concrete pond (20.0x10.0x1.5 m³) for three months. Samples were taken randomly and transferred to net cages of as many as 120 individuals from each aquarium.

Before rElGH treatment, larvae were adapted for one week by artificial feeding without rElGH supplementation. Feeding with rElGH supplementary feed was carried out twice a week for 4 weeks in the first and third months of rearing, and without supplementary rElGH feed for the rest of the day. The treated fish were fed three times a day (8:00 am, 1:00 pm, and 5:00 pm) at satiation. Aeration in aquariums and ponds was given to maintain dissolved oxygen (DO). The water in the aquarium was replaced 20-40% per day and added because of the evaporation that occurs. Water quality was analyzed periodically and obtained dissolved oxygen of 2.3-4.9 mg/l, pH: 7.3-7.8, and temperature: 28-30°C. The water quality was the same as Chakraborty *et al.* (2011) described.

Test parameters

Biomass measurements were carried out every two weeks until the end. Daily growth rate (DGR) was calculated following Safir et al. (2017a), biomass (BG) gain follows Loum et al. (2013). Fish survival rate (SR), feed consumption (FC), feed conversion ratio (FCR), and male ratio were calculated at the end of the period following Chakraborty et al. (2011). Seventy fish were taken from each treatment at the beginning of the process, and three test fish were taken from each replicate at the end of the rearing period for analysis of protein (PR) and lipid retention (LR). All fish samples were mashed separately on each replicate, and protein and lipid retention were calculated according to Takeuchi (1988).

Statistical analysis

All data obtained including DGR, SR, FC, FCR, PR, LR, and male ratio were statistically analyzed using one-way ANOVA, and in Duncan's further test if there was a difference.

Results

Production performance

Production performance including DGR, SR, BG, FC, FCR, PR, LR, and MR *O. niloticus* is presented in Table 1. DGR of w-MT fish was at 2.79-3.16%, with the highest value ($P < 0.05$) in the treatment w-MT+28+w-rElGH (3.16%) and the lowest was at w-MT+20+w-rElGH (2.79%). Furthermore, the DGR of MT-treated fish without rElGH treatment was at 3.00-3.38%, higher than that of w-MT fish, and the highest DGR ($P < 0.05$) was at MT+28+w-rElGH (3.38%). DGR in MT+rElGH treatment fish was at 3.01-3.41% with the highest DGR ($P < 0.05$) at MT+28+rElGH (3.41%). DGR of treated fish MT+28+w-rElGH was the same as DGR of fish MT+28+rElGH.

There was no difference in the survival rate (SR) at the end ($P > 0.05$), ranging from 79.89 to 90.28% (Table 1). Water quality was considered under conditions suitable for the maintenance of *O. niloticus*. Meanwhile, BG was directly proportional to the increase in feed protein content (Table 1). The highest BG was obtained at MT+28+rElGH (5.57 kg), and the lowest was at 20% (3.12 kg).

Feed consumption (FC) was directly proportional to the increase in feed protein content (Table 1). FC in w-rElGH MT was the same ($P > 0.05$), but FC was higher in rElGH-treated fish compared to the others (Table 1). On the other hand, an increase in the protein content of the feed was inversely related to a decrease in FCR (Table 1). Both MT and rElGH treatments tended to reduce the FCR of tilapia. The average FCR in

w-MT and w-rElGH treated fish was 1.75 (range 1.56-1.96), in MT-treated fish was 1.52 (range 1.45-1.62), and MT+rElGH treatment was 1.44 (range 1.28-1.58). In general, the lowest FCR values were found in fish treated with MT+28+rElGH+MT (FCR 1.28).

The rElGH treatment increased protein and lipid retention (Table 1). PR and LR in rElGH-treated fish were between 50.55-56.37% and 50.47-56.47%, respectively, while in the unenriched rElGH treatment were 42.15-49.32% for PR and 42.88-47.75% for LR.

Male sex ratio

The male ratios in MT-treated *O. niloticus* and controls are presented in Table 1. The MT-treated male ratio was higher ($P < 0.05$) with the w-MT treatment. The mean number of male fish in the MT treatment was 71.10% (range 61.62 to 80.60%), in the w-MT treatment was 43.31% (range 38.58 to 49.17%).

Estimated income

The projected income of tilapia farming is presented in Table 2. The highest potential income is MT+28+rElGH, followed by MT+24+rElGH, MT+24+w-rElGH, MT+20+rElGH, MT+28+w-rElGH, MT+20+w-rElGH, w-MT+28+w-rElGH, w-MT+24+w-rElGH, and the lowest was w-MT+20+w-rElGH. *O. niloticus* culture with rElGH supplemented feed has a higher potential profit than without rElGH supplementation at the same protein content of the feed.

Discussion

Tilapia (*O. niloticus*) needs feed with a protein content of 21-45% (Loum et al., 2013). Increased feed prices, feeding protein content of less than 28% can provide benefits without impacting the level of cultivation. Growth stimulation of juvenile *O. niloticus* given rGH was reported by Hardiantho et al. (2012), Muhammad et al. (2014), and Safir et al. (2017a). Conducted to observe the growth of *O. niloticus* and potential benefits by using different protein content of feeds with and without rElGH supplementation and given to *O. niloticus* which were sex-reversed.

The results show that higher growth performance (Table 1) and potential gain (Table 2) were obtained by feeding reversed MT-sex *O. niloticus* at a protein level of 28% and dietary supplementation of rElGH (MT+28+rElGH). The male ratio of fish increased with MT treatment (Table 1), growth and addition of fish biomass in MT treatment were higher than w-MT (control) (Table 1).

Table 1. Daily growth rate (DGR), survival (SR), biomass gain (BG), feed consumption (FC), feed conversion ratio (FCR), protein retention (PR), lipid retention (LR), male ratio (MR) of the sex-reversed *O. niloticus* which fed by different protein levels (20, 24, and 28%) and supplemented with recombinant *Epinephelus lanceolatus* growth hormone (rElGH).

Parameters	Feeding fish with a different protein levels of diets (%)								
	Without methyltestosterone (w-MT) and without rElGH treatments (w-rElGH)			Methyltestosterone treatment (MT)*					
				Without rElGH treatment (w-rElGH)			With rElGH treatment**		
	w-MT+20+w-rElGH	w-MT+24+w-rElGH	w-MT+28+w-rElGH	MT+20+w-rElGH	MT+24+w-rElGH	MT+28+w-rElGH	MT+20+rElGH	MT+24+rElGH	MT+28+rElGH
DGR(%/day)	2.79±0.19 ^{a***}	2.82±0.13 ^a	3.16±0.12 ^{bc}	3.07±0.17 ^b	3.00±0.12 ^{ab}	3.38±0.11 ^{cd}	3.01±0.05 ^{ab}	3.13±0.10 ^b	3.41±0.12 ^d
SR(%)	80.83±9.39 ^a	80.56±6.68 ^a	79.89±5.86 ^a	81.67±7.95 ^a	80.28±10.72 ^a	80.83±7.12 ^a	90.28±3.94 ^a	85.00±5.20 ^a	87.50±9.61 ^a
BG(kg)	3.12±0.40 ^a	3.77±0.27 ^{abc}	4.10±0.21 ^{bcd}	3.71±0.58 ^{ab}	4.19±0.16 ^{bcd}	4.45±0.09 ^{cd}	4.06±0.27 ^{bcd}	4.58±0.33 ^d	5.57±0.63 ^e
FC(kg)	6.06±0.03 ^a	6.46±0.04 ^{bc}	6.41±0.25 ^{bc}	5.93±0.23 ^a	6.21±0.14 ^{ab}	6.44±0.07 ^{bc}	6.41±0.19 ^{bc}	6.67±0.11 ^c	7.09±0.34 ^d
FCR	1.96±0.07 ^a	1.72±0.08 ^b	1.56±0.07 ^{bc}	1.62±0.20 ^{bc}	1.48±0.09 ^c	1.45±0.04 ^{cd}	1.58±0.12 ^{bc}	1.46±0.09 ^{cd}	1.28±0.08 ^d
PR(%)	46.91±5.07 ^{abc}	43.69±3.39 ^{ab}	41.05±2.13 ^a	49.32±5.03 ^{bc}	45.16±2.33 ^{abc}	42.15±0.76 ^a	56.36±1.40 ^d	51.72±3.16 ^{cd}	50.55±5.56 ^{cd}
LR(%)	39.22±4.79 ^a	45.72±4.21 ^{abc}	44.07±4.09 ^{ab}	42.88±7.98 ^{ab}	47.75±1.92 ^{abc}	45.13±5.54 ^{abc}	50.47±3.40 ^{bcd}	52.87±3.04 ^{cd}	56.47±3.13 ^d
MR(%)	38.58±2.71 ^a	49.17±8.82 ^{ab}	42.18±15.80 ^a	61.62±7.87 ^{bc}	70.93±11.63 ^c	65.33±14.39 ^{bc}	80.60±2.43 ^c	75.07±12.21 ^c	73.13±10.38 ^c

*10 days old and 14 days after-hatched larvae were immersed in water containing 2 mg/l of 17 α -methyltestosterone (MT) hormone. **Feed supplemented by growth hormone with recombinant *Epinephelus lanceolatus* growth hormone (rElGH). The same superscript letters in the same row indicate no significant differences (P > 0.05).

Table 2. Income projections in the sex-reversed *O. niloticus* culture fed different protein levels (20, 24, and 28%) and a recombinant *Epinephelus lanceolatus* (rElGH) supplement diet.

Support Analysis/cycle	Feeding fish with a different protein level of diets (%)								
	Without methyltestosterone (w-MT) and without rElGH treatments (w-rElGH)			Methyltestosterone treatment (MT)*					
				Without rElGH treatment (w-rElGH)			With rElGH treatment**		
	w-MT+20+w-rElGH	w-MT+24+w-rElGH	w-MT+28+w-rElGH	MT+20+w-rElGH	MT+24+w-rElGH	MT+28+w-rElGH	MT+20+rElGH	MT+24+rElGH	MT+28+rElGH
TCP (cycle/happa)*	43.605	49.520	53.392	43.504	48.189	54.111	46.201	51.567	59.392
Income (K)**	57.757	69.782	75.887	68.561	77.626	82.251	75.036	84.638	102.897
Profit (K)***	14.152	20.262	22.495	25.057	29.436	28.140	28.835	33.070	43.505

*TCP; Total cost production (feed cost larvae and sex reverse substance, rElGH and supplement) in one cycle (four months) in IDR (K); **Income (sell market price of *O. niloticus* at the end cultural period); *** Profit (difference between income and cost production)

In the w-MT group (without MT and rElGH treatment) the growth and addition of fish biomass that was fed protein content was 28% higher than the others. A similar pattern in w-MT+28+w-rElGH was also found in the group of fish treated with MT+rElGH. Increased growth and biomass gain of MT+28+rElGH-treated *O. niloticus* as a result of higher male ratio as a result of MT treatment, the appropriate protein content of the diet, and rElGH supplementation. The highest profit is obtained on MT+28+rElGH (Table 2). Fish farmers are advised to keep monosex male tilapia, using feed with a protein content of 28% and enriched with rElGH.

The percentage of male MT treated fish obtained was lower than that reported by Safir *et al.* (2017a), perhaps because our study immersed 500 larvae in one liter of MT solution or about 2.5 times higher than that used in Safir *et al.* (2017a). In another experiment, similar results were obtained as reported by Safir *et al.* (2017a) can be obtained by immersing 300 larvae aged 10 days using a 2 mg/l MT solution at a water temperature of 36°C (Afpriyaningrum *et al.*, 2016).

The results showed high feed consumption, protein, and lipid retention, while low feed conversion rates were found in the MT+28+rElGH treatment. According to Budi *et al.* (2015), feeding added rElGH to gourami increased the utilization of non-protein, indicated by a decrease in the expression of nitrogen metabolism, allowing a better growth rate compared to control. The feed conversion ratio treated with 20% protein content without rElGH had higher values (Table 1); showing that the proportion of feed protein is more catabolized for energy compared to growth (Budi *et al.*, 2015).

Appetite is influenced by increased stimulation of growth hormone inducing by ghrelin (Budi *et al.*, 2015), alpha-melanocyte-stimulating hormone (White *et al.*, 2016), and down-regulated by anorexigenic proopiomelanocortin and orexigenic neuropeptide Y (Dalmolin *et al.*, 2015). The rGH supplementation increased appetite seen in the increase in FC of fish kept in this study (Table 1). In addition, FC was affected by feed palatability related to the protein content of the feed (Table 1). The palatability of the feed was directly related to its attractiveness, determining which fish will seek, catch, and swallow. Feed palatability is related to nutrient content, especially free amino acids such as glycine, alanine, and betaine, as well as energy (Tantikitti, 2014). The increase in feed palatability occurs in feeds with higher protein content. Digestive activity is influenced by the substrate, and

indirectly by GH (Mataruga *et al.*, 2012; Irmawati *et al.*, 2012). Our previous research reported the protein content of the feed and the provision of rGH activates digestive enzymes such as (protease, amylase, and lipase) with increasing protein levels and rGH enrichment in the feed (Safir *et al.*, 2017b). Similar to Budi *et al.* (2015), enzyme activity (protease, amylase, and lipase) treatment with low protein content was the same as compared to treatment with high protein content and rGH gave an effect of increased enzyme activity in feed with a protein content of 21%.

Increased protein and lipid retention due to rGH supplementation has been reported in other studies (Budi *et al.*, 2015). Non-protein energy affects the use of protein for energy, increasing protein retention and a decrease in ammonia (Suprayudi *et al.*, 2014). In our study, rGH supplementation allowed increased protein and lipid retention (Table 1). This suggests that higher protein retention decreases the excretion of nitrogen metabolites (in the form of TAN) due to the protein-reducing effect during energy production. According to Budi *et al.* (2015), in fish receiving rGH treatment, nitrogen retention (protein) was higher, indicating that the increased utilization of non-protein nutrients as an energy source (protein-sparing effect) is a function of rGH. Reducing TAN excretion has also been reported in tilapia studies due to the role of rGH (Vinasyiam *et al.*, 2016; Safir *et al.*, 2017b). An increase in feed protein causes an increase in TAN excretion (Safir *et al.*, 2017b). Previous research by Suprayudi *et al.* (2014) showed that protein and ammonia excretion were directly proportional. Amino acids from protein will be deaminated and excreted as ammonia and used to produce energy rather than being stored for growth when feeding (Ip and Chew, 2010; Budi *et al.*, 2015; Vinasyiam *et al.*, 2016). Low levels of protein content in feed and rGH enrichment reduce ammonia pollution from aquaculture.

The increase in protein and lipid retention that we found was due to rGH supplementation, followed by a decrease in FCR (Table 1). An increase in dietary protein content, rGH enrichment, and a decrease in FCR, indicated that the use of feed was efficient. rGH enrichment can make efficient use of protein and fat in increasing growth. In tilapia, the role of growth hormone in feed efficiency through increased appetite, energy absorption, and increased body weight has been reported (Budi *et al.*, 2015; Vinasyiam *et al.*, 2016; Safir *et al.*, 2017a).

Conclusion

Overall, Sex reversed, and increased dietary protein with rElGH enrichment resulted in a

significant increase in growth and business profit potential in tilapia aquaculture. Growth and utilization of tilapia feed can be increased by sex reversed, and supplementation of recombinant *Epinephelus lanceolatus* growth hormone (rELGH) at different protein levels of the feed.

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