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Research Article

# Growth Performance of Nile Tilapia Immersed in 17α-methyltestosterone and r*El*GH, and Fed a Diet Enriched with r*El*GH

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***ABSTRACT***

The aim of the present study was to investigate the growth performance of Nile tilapia immersed in 17α-methyltestosterone (MT) and recombinant giant grouper growth hormone (r*El*GH), and fed a r*El*GH-enriched diet. A total of 200 tilapia larvae aged 10 days and 14 days post hatching was immersed in 1 liter of 30 g/L saline water for 3 minutes, then in freshwater containing either MT, MT+r*El*GH, or r*El*GH for 4 hours. The MT dose was 2.0 mg/L and r*El*GH was 2.5 mg/L. As control was treatment without MT and r*El*GH. Fish were maintained in 250-L aquariums for 6 weeks and then moved to net cages for 8 weeks of rearing. The r*El*GH-enriched diet was fed to the 6-week-old fish for a month, and then fed a non-enriched diet. Each treatment was triplicates. The result showed that the daily growth rate (DGR), biomass gain (BM), survival (SR) and feed (artemia nauplii) consumption during maintenance in the aquariums in the r*El*GH and MT+r*El*GH treatments were higher (P<0.05) than in the control. The higher DGR and BM at net cage rearing and lower feed conversion ratio (P<0.05) were also found in MT and MT+r*El*GH treatments. None of the treatments showed any difference in survival (P>0.05). Profits estimation in the MT and MT+r*El*GH treatments were 63.38% and 57.91% higher than the control. The present study concluded that higher Nile tilapia farming performance could be obtanied by MT immersion in the larval phase and feeding juvenile on the r*El*GH-enriched diet.

***Keywords**: growth, Nile tilapia, sex reversal, 17α-methyltestosterone, rElGH.*

## 1. Introduction

The growth of male Nile tilapia is faster than that of females (Sudrajat et al., 2007; Chakraborty 2011), making the cultivation productivity of monosex male Nile tilapia is higher and more profitable. One of the efforts that could be done to produce monosex male Nile tilapia is through sex reversal which could be done using the hormone 17α-methyltestosterone (MT) (Srisakultiew and Kamonrat, 2013; Megbowon and Mojekwu, 2014; Afpriyaningrum et al., 2017). The fish growth rate can also be accelerated using a recombinant growth hormone (rGH). The rGH has been studied in several fish species, including eel (Handoyo et al., 2012), giant gourami (Irmawati et al., 2012; Budi et al., 2015), humpback grouper (Antoro *et al.,* 2014), and Nile tilapia (Muhammad et al., 2014; Vinasyiam et al., 2016). During the larval phase, rGH treatment can be delivered by immersion method. Administration of rGH through immersion has been proven to increase gourami larvae biomass by 79.8% compared to the control (Irmawati et al., 2012). In Nile tilapia larvae, using the same method at a dose of 2.5 mg $L^{-1}$, it could increase growth by 49.6% compared to the control (Setyawan *et al.,* 2014). In addition, in the previous study, the productivity and potential profit of aquaculture using feeds with different protein concentrations and enrichment with rElGH in Nile tilapia rearing in ponds was tested (unpublished data). In that study, the best performance was found in treatment using feed with a protein content of 28% and enriched with giant grouper recombinant growth hormone (r*El*GH).

The administration of MT and r*El*GH through immersion in larvae followed by the administration of r*El*GH through feed is

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assumed to give better results. In fact, treatment using a combination between rGH and MT via injection has been reported to give a response in the growth of juvenile Nile tilapia (Linan-Cabello et al., 2012). Therefore, this study was conducted to assess the performance of Nile tilapia given a combination treatment MT & r*El*GH via immersion method for larvae followed by administration of r*El*GH-enriched diet.

## 2. Materials and Method

*Nile tilapia larvae procurement*

Nine-day-old Nile tilapia larvae (weight: 13.23±0.43 mg, length: 0.92±0.16 cm) post hatching were procured from the Field Station, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University. The Nile tilapia larvae were adapted for two days in an aquarium before given the treatment.

*Research design*

This study was designed in two steps, and each used a complete randomized design by applying 4 treatments and triplicates. The first step involved these treatments: 1) immersion in the 17α-methyltestosterone (MT) solution, 2) immersion in r*El*GH, 3) immersion in MT and r*El*GH, and 4) the control (without MT and r*El*GH treatments). Then the fish were kept in a net cage and fed diet with a protein content of 28% and enriched with r*El*GH, except for the controls which were fed on diet not enriched with r*El*GH.

*Immersion of larvae with MT and rElGH*

The MT (17α-methyl-4-androsterone-17α-ol-3one,$C_{20}H_{30}O_2$; Argent, Philippines) immersion method and dosage used referred to Wassermann and Afonso (2003). An amount of 2.0 mg of MT was dissolved in 0.25 mL of 100% ethanol. The r*El*GH (Mina Grow: Center for Freshwater Aquaculture Sukabumi and Department of Aquaculture-Faculty of Fisheries and Marine Sciences- Bogor Agricultural University) dosage referred to Setyawan *et al.* (2014), and the r*El*GH immersion method used referred to Syazili et al. (2012), with slight modifications. An amount of 2.5 mg r*El*GH was dissolved in 150 mL PBS *(phosphate buffer salin),* and each of the solutions [MT & r*El*GH; MT; r*El*GH; control (ethanol and PBS)] were placed inside plastic bags that had been filled with 1 liter of water and given strong aeration for homogenization. Then 200 larvae of 10-day-post hatching were placed in each plastic bag containing 30 g/L of saline water for 3 minutes. Then the plastic bags were oxygenated (30% oxygen : 70% water), then let to stand for 4 hours for the immersion process. The second immersion was conducted after the larvae had reached the age of 14 days post hatching, using the same procedure and larvae as the first immersion. All of the treatments and control were conducted triplicate.

*Fish maintenance and feeding treatment*

The Nile tilapia larvae were kept in 12 aquariums (sized 1 × 0.5 × 0.5 $m^3$) at densities of 200 larvae/aquarium for six weeks. The next phase was maintenance in a net cage (sized 2 × 2 × 1.5 $m^3$) placed in a concrete pond (sized 20 × 10 × 1.5 $m^3$), for eight weeks. Before the fish were placed in the net cage, the density was made uniform by taking 160 fish randomly from each aquarium. The rest of the fish were kept in aquariums for sexing.

The feed given during the 6 week maintenance in the aquarium was artemia nauplii (for 12 days), tubifex (for 14 days), 40% protein commercial feed (for 14 days), and 28% protein commercial feed (for 15 days) as adaptation feed before entering the next phase. Each change from one feed to another was done gradually. After the adaptation and after the fish were placed in the net cages, the fish were fed 28% protein treatment feed enriched with r*El*GH at a dosage of 3.0 mg/kg feed following the method Hardiantho et al. (2012). The control (without MT immersion and r*El*GH) were given diet not enriched with r*El*GH. Diet enriched r*El*GH was administered every three days for four weeks during the first month. The feeding frequency was three times a day (morning, noon, and afternoon) *at satiation.* To maintain water quality, in each aquarium was placed one aeration unit and 40-75% of the water was replaced every other day, while the pond was equipped with a blower (100 points capacity) and water was only added when the volume decreased due to evaporation. The quality of water where the Nile tilapia were kept both in the aquariums and in the pond was: the temperature ranged 28-30°C, the pH 6.51-7.23, dissolved oxygen 3.7-5.8 mg/L and amonium ($NH_4$) 0.21-0.33 mg/L. The water quality parameters were all within a range suitable for Nile tilapia rearing (Chakraborthy et al., 2011).

*Parameters and data analysis*

The fish biomass was measured every two weeks, and the daily growth rate (DGR) was calculated based on Lugert et al. (2014). The feed conversion ratio (FCR) and survival (SR) were evaluated at the end of the maintenance period (aquarium and net cage) referring to Chakraborthy et al. (2011). Potential profits were calculated referring to Alimuddin et al. (2017, not yet published) which the difference between income (sell market price of nile tlapia) and cost production (feed cost larvae and sex reverse substance, rElGH and supplement) in one cycle rearment**.** Percentage of males (M) was only calculated once at the end of the second phase of fish maintenance, including the rest fish kept in the aquariums. Samples were randomly taken 40% of the fish from each net cage for sex identification for all treatments. Sexing was done using a histological method with acetocarmine staining (Chakraborty et al., 2011).

The DGR, FCR, and SR, biomass gain (BM) and M data 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.

## 3. Results

*Maintenance in the aquarium*

*Growth performance*

The growth performance of the Nile tilapia larvae demonstrated by each treatment during maintenance in the aquariums (6 weeks) is presented in Table 1. Results showed that the DGR of fish treated with r*El*GH and those treated with MT+r*El*GH were the same and both were higher (P<0.05) than the control and MT treatments. This was also the case with the resulting BM, but the BM in the MT+r*El*GH treatment was lower (P<0.05) than in the r*El*GH treatment.

The larvae survival during maintenance in the aquarium is presented in Table 1. The analysis results demonstrated that the SR of the fish larvae treated with r*El*GH (r*El*GH+MT, and r*El*GH) was higher (p<0.05) than those treated without r*El*GH (MT and control). The SR of larvae treated with MT and the controls was the same, ranging between 81.67 and 82.33%.

Table 1. Daily growth rate (DGR), biomass gain (BM), and survival (SR) of the Nile tilapia larvae during maintenance in the aquariums

| Parameters | Immersion Treatment | | | |
|---|---|---|---|---|
| | Control | MT | r*El*GH | MT+r*El*GH |
| DGR (%/day) | $12.44 \pm 0.10^{a}$ | $12.38 \pm 0.08^{a}$ | $12.80 \pm 0.12^{b}$ | $12.68 \pm 0.10^{b}$ |
| BM (kg) | $0.37 \pm 0.00^{a}$ | $0.36 \pm 0.01^{a}$ | $0.42 \pm 0.00^{c}$ | $0.39 \pm 0.01^{b}$ |
| SR (%) | $82.33 \pm 2.80^{a}$ | $81.67 \pm 0.29^{a}$ | $89.17 \pm 1.53^{b}$ | $87.50 \pm 1.32^{b}$ |

Control: immersion without the hormone 17α-methyltestosterone (MT) and without the recombinant giant grouper growth hormone (r*El*GH), MT: Immersion in MT without r*El*GH, r*El*GH: Immersion in r*El*GH without MT, MT+r*El*GH: Immersion in MT and r*El*GH. During rearing in the net cage, the fish were fed on r*El*GH-enriched diet, except for the controls which were fed diet not enriched with r*El*GH. The values are presented in the form of the average ± standard deviation (n=3). Different superscript letters on the same row indicate significantly different values (p<0.05).

Table 2. Feed consumption in the Nile tilapia during maintenance in the aquariums

| Feed consumption (grams) | Immersion Treatment | | | |
|---|---|---|---|---|
| | Control | MT | r*El*GH | MT + r*El*GH |
| Artemia nauplii | $19.65 \pm 0.72^{a}$ | $20.90 \pm 1.47^{a}$ | $24.15 \pm 0.50^{b}$ | $23.01 \pm 1.08^{b}$ |
| Tubifex | $163.37 \pm 12.78^{a}$ | $142.00 \pm 19.14^{a}$ | $148.57 \pm 6.41^{a}$ | $150.60 \pm 16.87^{a}$ |
| Commercial feed-1 | $89.33 \pm 5.35^{a}$ | $93.67 \pm 21.36^{a}$ | $94.83 \pm 15.20^{a}$ | $92.07 \pm 3.37^{a}$ |
| Commercial feed-2 | $170.75 \pm 32.88^{a}$ | $176.19 \pm 30.06^{a}$ | $192.95 \pm 18.90^{a}$ | $200.41 \pm 19.51^{a}$ |

Control: immersion without the hormone 17α-methyltestosterone (MT) and without the recombinant giant grouper growth hormone (r*El*GH), MT: Immersion in MT without r*El*GH, r*El*GH: Immersion in r*El*GH without MT, MT+r*El*GH: Immersion in MT and r*El*GH. During rearing in the net cage, the fish were fed on r*El*GH-enriched diet, except for the controls which were fed diet not enriched with r*El*GH. The values are presented in the form of the average ± standard deviation (n=3). Different superscript letters on the same row indicate significantly different values (p<0.05).

Table 3. Daily growth rate (DGR), biomass gain (BM), survival (SR), and feed conversion ratio (FCR) of Nile tilapia fed feed enriched with rElGH during rearing in the net cage

| Parameters | Treatment | | | |
|---|---|---|---|---|
| | Control | MT | rElGH | MT+rElGH |
| DGR (%/day) | $3.94 \pm 0.14^{a}$ | $4.36 \pm 0.08^{c}$ | $4.17 \pm 0.05^{b}$ | $4.39 \pm 0.06^{b}$ |
| BM (kg) | $2.75 \pm 0.13^{a}$ | $13.53 \pm 0.10^{bc}$ | $3.34 \pm 0.13^{b}$ | $3.62 \pm 0.18^{a}$ |
| FCR (kg) | $1.65 \pm 0.06^{c}$ | $1.25 \pm 0.04^{a}$ | $1.42 \pm 0.03^{b}$ | $1.22 \pm 0.09^{a}$ |
| SR (%) | $94.79 \pm 1.91^{a}$ | $94.38 \pm 2.25^{a}$ | $95.83 \pm 3.55^{a}$ | $94.37 \pm 2.50^{a}$ |

Control: immersion without the hormone 17α-methyltestosterone (MT) and without the recombinant giant grouper growth hormone (r*El*GH), MT: Immersion in MT without r*El*GH, r*El*GH: Immersion in r*El*GH without MT, MT+r*El*GH: Immersion in MT and r*El*GH. During rearing in the net cage, the fish were fed on r*El*GH-enriched diet, except for the controls which were fed diet not enriched with r*El*GH. The values are presented in the form of the average ± standard deviation (n=3). Different superscript letters on the same row indicate significantly different values (p<0.05).

*Feed consumption*

Feed consumption (FC) of the Nile tilapia larvae during maintenance in the aquariums for the four types of feed given is presented in Table 2. The analysis results demonstrated that a difference in FC was only found in those fed artemia nauplii, while the rest were the same (P>0.05). The FC of those fed artemia and given the r*El*GH and MT+rElGH treatments were the same, and both were higher than the other treatments (Table 2).

*Rearing in net cages*

*Growth, feed conversion ratio, and survival*

The growth performance of Nile tilapia kept in net cages for 8 weeks and fed diet enriched with r*El*GH is presented in Table 3. The analysis results demonstrated that the the DGR of fish treated with MT+r*El*GH and those treated with MT were similar, and both were higher than those treated with r*El*GH and the controls (P<0.05). The highest BM was also demonstrated by treatment using MT+r*El*GH, followed by treatment using MT, r*El*GH and the lowest was the control (P<0.05). The BM of fish treated with MT+ r*El*GH, MT, and r*El*GH were 31.66%, 28.31% and 21.42%, respectively, all higher than that of the control.

Table 3 also revealed that feeding diet enriched with r*El*GH could significantly (P<0.05) reduce FCR in fish treated with MT+r*El*GH, MT, and r*El*GH each by 26.27%, 24.19% and 14.23% compared to the control, respectively. The FCR for fish treated with MT+r*El*GH, and MT were not significantly different (P<0.05), whereas the SR for all treatments was the same (P>0.05), ranging between 94.38 and 95.83% (Table 3).

*Percentage of males*

The male percentage (M) of Nile tilapia treated with MT+r*El*GH, r*El*GH, MT and control is presented in Figure 1. Based on the analysis results, it was discovered that the M in fish treated with MT and MT+r*El*GH were similar, and both were higher (P<0.05) than those treated without MT. The M in fish treated with r*El*GH and the control were not sigificantly different (P>0.05).

*Potential profit*

The potential profit for Nile tilapia aquaculture activities which calculated in a simple way is presented in Figure 2. The profits from fish treated with MT and with MT+r*El*GH were higher than those treated with r*El*GH and the control. The profits were 63.68% and 57.91%, respectively, higher than the profit from the control.

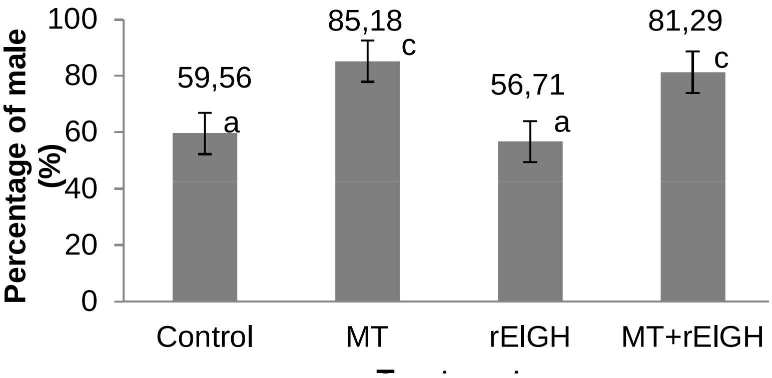


Figure 1. The percentage of male Nile tilapia at the end of the maintenance period for each treatment. Control: immersion without the hormone 17α-methyltestosterone (MT) and without the recombinant giant grouper growth hormone (r*El*GH), MT: Immersion in MT without r*El*GH, r*El*GH: Immersion in r*El*GH without MT, MT+r*El*GH: Immersion in MT and r*El*GH.

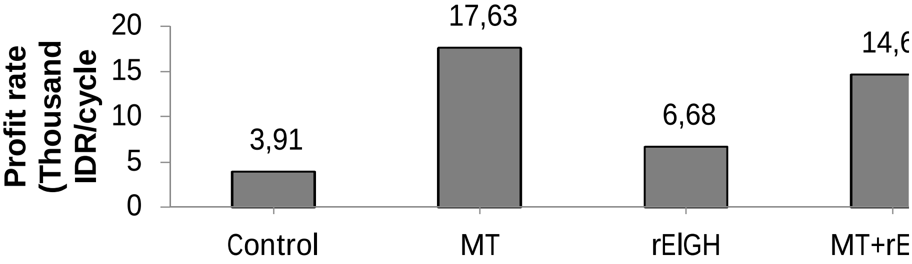


Figure 2. The potential profit rate from Nile tilapia at the end of the maintenace period for each treatment. Control: immersion without the hormone 17α-methyltestosterone (MT) and without the recombinant giant grouper growth hormone (r*El*GH), MT: Immersion in MT without r*El*GH, r*El*GH: Immersion in r*El*GH without MT, MT+r*El*GH: Immersion in MT and r*El*GH. During rearing in the net cage, the fish were fed on r*El*GH-enriched diet, except for the controls which were fed diet not enriched with r*El*GH. The income is listed in thousand IDR. One cycle is assumed to be 3.5 months.

## 3. Discussion

Immersion with r*El*GH both on its own (r*El*GH) and combined with MT (MT+r*El*GH) were proven to increase the weight growth performance, biomass gain (BM), and survival (SR) of the Nile tilapia larvae (Table 1). The DGR of fish treated with r*El*GH and treated with MT+r*El*GH were not significantly different (P>0.05), but both were higher than treatment using MT and the control. This demonstrated that the growth of Nile tilapia larvae was more influenced by the hormone r*El*GH than the MT. GH accelerates growth through the involvement of its receptor (GHRs) in the target tissue and could also involve the insulin-like growth factor (IGF-1) (Debnanth, 2010). Expression of IGF-1 was higher in fish treated with rElGH than in the control, demonstrated by Hardiantho et al. (2012).

The hormone MT in the larval stadium functions in directing sexual differentiation towards the male attributes through the inhibition of the aromatase which changes estrogen from androgen so that the percentage of males increases (Sudrajat et al*.,* 2007). The results of this study were in line with the results reported in Wassermann and Afonso, (2003) that the use of MT through the immersion of Nile tilapia larvae has been proven to increase the percentage of males by 91.6%; even though there were no differences in DGR in the first month post treatment (Chakraborty et al*.,* 2011). While the application of rGH through immersion has been proven to increase the DGR in each test fish (Moriyama and Kawauchi, 1990; Acosta et al., 2007; Irmawati et al., 2012). Moreover, it was reported by Linan-Cabello et al., (2012) that the administration of rGH and MT (rGH and MT+rGH) through injections in juvenile Nile tilapia both gave responses that were not significantly different (P>0.05) from the DGR, but both were higher (P<0.05) than those treated with MT or the control 45 days post treatment.

In addition, the increase in DGR was followed by an increase in the larvae's appetite post immersion with r*El*GH. This was revealed by the FC (artemia nauplii) which was higher ($P<0.05$) in the treatment using r*El*GH (r*El*GH and MT+r*El*GH) compared to treatment using MT and the control (Table 2), while the feed consumption in the others (Tubifex, commercial feed 1 and 2) were the same ($P>0.05$). The increase in FC was assumed to be an effect of r*El*GH on the larvae in increasing the DGR, causing the need for nutrients to increase. This was in line with the statement by Irmawati et al. (2012) that in accelerating growth, rGH can act through an anabolic action using the protein metabolism pathway if adequate energy is available. This was supported by Antoro et al. (2014) stating that the function of rGH in humpback grouper, in addition to increasing growth, was to increase the appetite which was demonstrated by the increase in the feed consumption.

In addition to an increased FC in the treatment using r*El*GH, the SR was also higher than that of the treatments without r*El*GH (MT, and control) (Table 1). This was in line with the results reported by Syazili et al. (2012) who found that the SR of giant gourami larvae treated with immersion with rGH was higher (96.67%) than that of the the control (77.33%). Increased in SR was likely to be related to the fish increased resilience to stress which was thought to be caused by the change of feed type and physical disturbances during rearing. This was supported by Acosta et al. (2009) and Syazili et al. (2012) who stated that stress could be caused by the salinity treatment, physical disturbances during maintenance such as collection of the larvae for treatment and changing water during rearing.

The increased DGR and SR in Nile tilapia larvae given the r*El*GH treatment would have a direct correlation with the BM (Table 1). In this study, the BM for the MT+r*El*GH treatment was lower ($P<0.05$) than the sole r*El*GH treatment. Different results were reported by Linan-Cabello et al. (2012) where juvenile Nile tilapia given the rGH and MT treatment through the injection method experienced increases in biomass that were not significantly different ($P>0.05$) and both were higher ($P<0.05$) than the treatment using MT or the control 45 days post treatment. The BM value was lower in the treatment using MT+r*El*GH; this was believed to be caused by a competition in absorbing r*El*GH and MT in the larval bodies, or a limit in the amount of liquids that could be absorbed by the larvae. A lower r*El*GH dosage did not give an optimum effect, while an overly large dosage would trigger the negative feedback mechanism, causing growth to be slow (Promdonkoy et al., 2004).

Then the fish were moved to the net cages and given the treatment feed enriched with r*El*GH and kept for 8 weeks, and they demonstrated better DGR, BM, and FCR responses than those of the control (Table 3). The DGR of the MT and MT+r*El*GH treatments were not significantly different ($P>0.05$), but both were higher ($P<0.05$) than those of the r*El*GH treatment and control. This proved that the Nile tilapia DGR, besides being influenced by the r*El*GH treatment, was also influenced by the MT treatment which caused a higher percentage of males (Figure 2). The high male percentage would influence growth because the males of this spesies have a faster growth rate than the females (Phelps and Popma, 2000; Sudrajat et al., 2007; Chakraborty et al., 2011). Administration of feed enriched with rGH was proven to increase the DGR in eels (Handoyo et al., 2012), humpback grouper (Antoro et al., 2014), and giant gourami (Budi et al., 2015).

The high DGR in the r*El*GH treatments (especially the MT+r*El*GH and MT treatments) was followed by a low feed conversion rate (FCR), which had a direct positive correlation with higher biomass gain (Table 3). The low FCR followed by a higher BM showed that the r*El*GH administered through feed could stimulate the nutrient efficiency for the growth. This was in line with the reports from a number of researchers such as Hardiantho et al. (2012) in Nile tilapia and Budi et al. (2015) in giant gourami. In addition, Vinasyiam et al. (2016) reported that the addition of rGH in Nile tilapia feed could decrease the FCR through the increased nutrient utilization (protein and fats) for growth, resulting in a high BM.

During the rearing phase, the SR for all treatments at the end of the maintenance period was the same ($P>0.05$). This indicated that the environmental conditions during maintenance were within a suitable range, and there was no effect of the application of r*El*GH through feed on the SR.

The increased DGR, BM, and FCR in the MT, and MT+r*El*GH treatments had a positive correlation with the potential income rate at the end of the rearing period. At the income calculation, because the fish had not yet reached >200 gram/fish (IDR 27,000/kg) (http://wpi.kkp.go.id/: for West Java), the price of the fish was assumed to be at the lowest (IDR 18,500/kg) for all fish sizes. Based on that assumption, the highest profit rate was at the MT and MT+r*El*GH treatments, each 63.68% and 57.91% higher than the control. The higher profit rate for the MT treatment compared to the MT+r*El*GH treatment was due to the higher cost for using r*El*GH, raising the production cost. This differed from the results reported by Alimuddin et al. (2017, not yet published). The potential profit for fish farmers who cultivated sex reversed Nile tilapia and used feed enriched with rElGH was higher than those who raised fish receiving only the sex reversal treatment. This was most likely because the rearing period in that particular study was 14 weeks post immersion (MT) treatment and the treatment with feed enriched with r*El*GH was only conducted for four weeks (the second month post initial treatment).

## 5. Conclusion

The administration of r*El*GH to the larval and juvenile Nile tilapia accelerates growth and improves feed conversion. However, the highest potential profit was demonstrated by the sex reversal treatment without the application of rElGH during the larval phase. Therefore, the highest Nile tilapia aquaculture performance was found in the sex reversal treatment during the larval phase, followed by feeding feed enriched with r*El*GH at the rearing phase.

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