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The Effect of Different Thyroxine Hormone (T_4) Concentration on The Growth, Survival, and Pigment Development of Pink Zebra Fish Larvae (*Brachydanio reiro)*

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ABSTRACT

The thyroxin hormone plays an important role in the process of metabolism, yolk sac absorbsion, and growth of fish. The aims of this research were to observe the effect of various concentration of thyroxin (T_4) on the absolute growth weight, total length, survival rate, and pigment development of pink zebra fish larvae (*Brachydanio reiro*) after being reared 42 days. The pink zebra fish larvae at 4 day age with the average weight of 0,002 – 0,003g and average length of ± 3.10 – 3.43 mm were immersed at various concentration of T_4 for 24 hours. Prior the treatment fish larvae were dipped into 1 ppt salinity for 2 minutes then transferred into 1 Liter, 1ppt saline and various T_4 in plastic bags. The thyroxin concentrations were A (0 mg / L); B (0.05 mg / L); C (0.10 mg / L) and D (0.15 mg / L) respectively. The stocking density was 40 fish/L. After that they were transferred into aquariums and reared for 42 days. Completely Randomized Design (RAL) with four treatments and three replications were used. The variables observed were absolute and specific growth, total length, survival rate and hue degree. The results showed that the thyroxin hormone had significant effect on absolute growth weight, total length and specific growth rate. The dosage 0.1 mg/L was the best treatment on absolute growth, total length, and specific growth rate. Whilee the survival rate showed no significant differences across the treatments. Treatment C also demonstrated the best ppigment development (14.60 $\pm$ 0.36° hue) compared to others.

Keywords: *thyroxin, growth, survival, pigment, brachydanio reiro*

1. Introduction

Zebra fish (*Brachydanio rerio)* is an ornamental fish that have a high economic value. The zebra fish is a small ornamental fish. The *Brachydanio rerio* on this study is commonly called pink zebra. The total trade of zebra fish in the year 2013 was over 1.04 billion tails (MMAF, 2014). This fish was originated from Myanmar, India, and Srilanka (Tamaru *et al*,1997). Furthermore, maturation was reached around 5 months old at the size of around 4-5 cm. Rematuration occurred almost every 2-3 weeks and at this fish were ready to re spawned. The number of eggs was around 400 – 500 and took several days to release eggs and it's called partial spawning (MeinIt *et al*, 1999). The high demand of Zebra fish and its fast reproduction cycle encouraged ornamental fish hobbies to used stimulated growth and color. Hormone was used to promote growth. Khalil et al (2011) stated that injection of female *Oreochromus niloticus* at concentration of 1 or 10 μg T_4/g body weight significantly enhanced larval and digestive tract development. They further stated that at some extent exogenous L-thyroxin (T_4) in the female brood stock was transferred into oocytes and larvae. Pebriyanti *et al,* (2015) also stated that immersion of thyroxin solution to Betok fish larvae (*Anabas testudineus*) significantly increased growth. Nacario (1983) previously approved that Thyroxin hormone was able to improve growth *Sarrotherodon niloticus* fry and larvae.

The aims of this research were to observe the effect of various concentration of thyroxin (T4) immersion on the absolute growth weight, total length, rate, and pigment development of pink zebra fish larvae

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(*Brachydanio reiro*) after being reared 42 days.

2. Material and Methods

Experimental animal

Four hundred eighty of four days old zebra fish larvae (*Brachydanio reiro*) or first feeding fry were used in this experiment. The body weight was around 0.002 – 0.003 g and total length 3.10 – 3.43 mm. The larvae was obtained from own spawning between 2 of 1.26 and 1.58 g female and 4 of 0.74, 0.30, 0.59 an 0.29 g males respectively at hatchery of Semarang ornamental fish trader and breeder association (APPIHIS).

Thyroxin hormone

Thyroxin hormone (T_4) used was a commercial L-thyroxin (thyrax) tablet. Each tablet tyrax contains 100 µg or equal to 0.1 mg thyroxin. Total of 9 tablets were required to meet various concentration required. Each tablet was diluted in distillate water according to the treatment.

Immersion of Thyroxin hormone

Four hundred and eighty pink zebra fish larvae were firstly shocked in 1ppt saline for 2 minutes to force the larvae to drink. After that they were divided into twelve and put them in 5 liter plastic bags. Each bag contained 40 pink zebra larvae and then immersed in 1 L various concentration of thyroxin for 24 hours in 1ppt immersion media. The concentration of thyroxin hormone were as follows; (A) 0.0 mg/L, (B) 0.05 mg/L, (C) 0.10 mg/L and (D) 0.15 mg/L. After 24 hours immersion in various treatments, they were transferred into 8 liter aquarium each and then reared for 42 days.

Experimental design and rearing

Completely randomized design with 4 treatments and three replication was used in this experiment. During the treatments pink zebra fish larvae were fasted for 24 hours.

During the rearing period, the pink zebra fish larvae were fed with *Rotifer* at the first 12 days. After that they were fed with mixture of *Rotifer* and *Daphnia* for another 7 days. *Daphnia* became a main diet on the day 19 until day 21. After that blood worms (*Tubifex*) were used as a main diet until 42 days. Feed was given at libitum twice a day 08.00 in the morning and 16.00 in the afternoon. Half of rearing media was exchange everyday whilst siphon was started at day 10 and afterward siphoned was done every day.

Variables observed

Growth in terms of absolute weight and total length were observed on the first day, day 14, 28 and 42 respectively. Survival rate was observed every day. Absolute weight was calculated at the end of research according to Wheaterly and Gill (1987) while specific growth rate, total length, and survival rate were calculated according to Effendi (2003).

Pigment development was observed according to °Hue using Adobe Photoshop CC software (Figure 1). This was done by comparing color development of experimental fries at 3 sites i.e. head, dorsal, and tail. One pink zebra from each treatment were put in 50 mL bowl with black background at the bottom of the flask. Pink zebra then snapped with camera 10 megapixel. After that stored in Adobe Photoshop CC software. The software was set the foreground colour to 100% saturation and brightness to meet constant °Hue value (Figure 2). The pink zebra photos of each treatment were put in the foreground setting then click the eyedropper tool to get the °hue value at head, dorsal and tail.°Hue value was the average of 3 pink zebra fries of each treatment and from 3 sites above respectively. Water quality such as temperature, dissolved oxygen, pH, and ammonia were also observed.

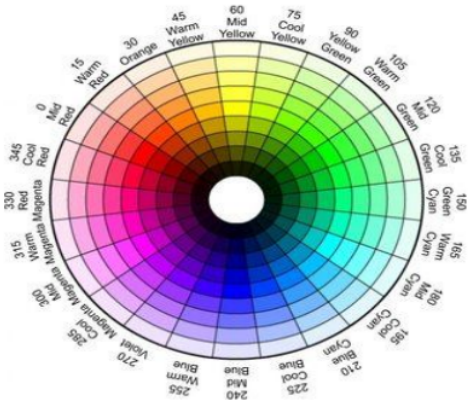

Figure1. Standard pigment according to Hue Degree Value (°Hue).
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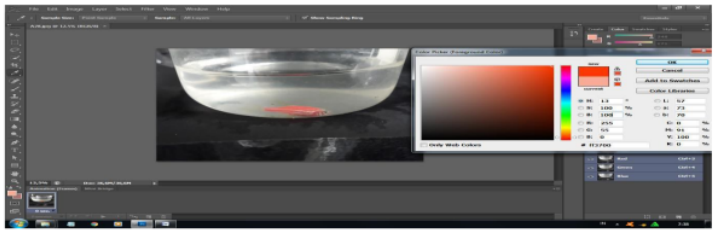

Figure 2. Foreground setting to measure ^{0}Hue of the colour development of Fish

3. Results and Discussion

Absolute body weight

Immersion of pink zebra fry in the various concentration of thyroxin for 24 hours and reared 42 days demonstrated that thyroxin significantly improved absolute weight performance of pink zebra. Immersion in thyroxin solution for 24 hours almost double the absolute growth rate where the control only reach 0.10 $\pm$ 0.005 g while at 0.05 mg (0.15 $\pm$ 0.006 g); 0.10 mg (0.24 $\pm$ 0.16 g) and at 0.15 (0.20 $\pm$ 0.008 g) (Figure 3).From the data above it can be seen that immersion of T_4 at concentration of 0.1 mg/L for 24 hours was able to double (240%) the absolute weight gain of pink zebra compared to control. Duncan test demonstrated that the used of 0.10 mg thyroxin (T_4) was the best treatment in terms of absolute growth weight performance. Pebriyanti *et al*, (2015) found that Immersion of Thyroxin hormone at concentration of 0.1 mg/L for 48 hours was the optimum concentration to gain the best yolk sac absorbsion, growth and survival rate of Betok fish (*Anabas testudineus*). Different study conducted by Siccardi *et al*, (2009) demonstrated that lenght, weight and survival of zebra fish almost similar when they feed with proper artificial diet. However, natural diet is still needed especially during early first feeding fry.

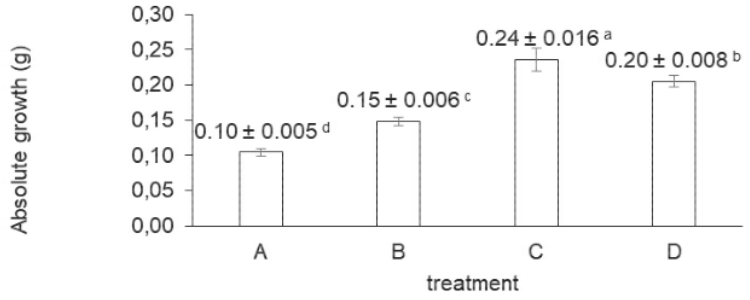

Figure 3. Absolute growth weight of pink zebra larvae post immersion in various T_4 concentration after being reared 42 days

Total length

Observation of total length of pink zebra larvae showed that experimental larvae that have been immersed in various Thyroxine (T_4) solution showed consistent trend. Treatment C exhibited the highest total length (26.45±0.58 mm) followed by treatment D (24.19± 0.37 mm), B (23.10±0.23 mm), and A (20.98 ± 0.26 mm) respectively, and significantly different each other respectively (Figure 4).

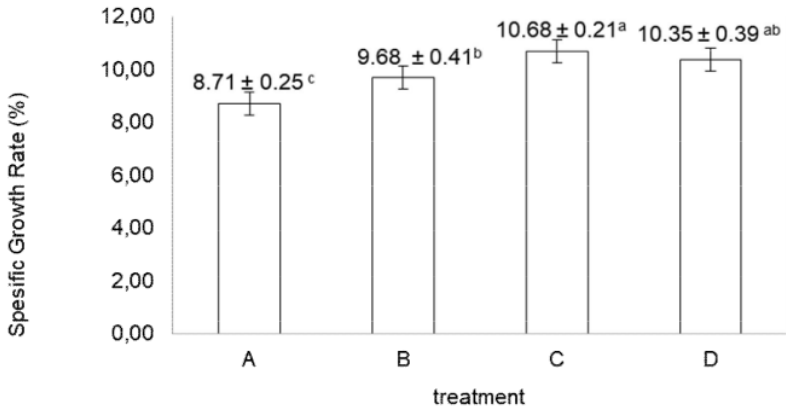

Figure 4. The total length of pink zebra post immersion in various T_4 concentration after being reared 42 days.

Sudrajat *et al* (2013) found in their study that immersion in mixture of 0.1 mg/L thyroxin and 10 mg/L rGH hormones significantly improved total length of catfish (*Pangasianodon hypopthalmus*) 14.41% than control. These results indicated that each fish species might have their optimum concentration of thyroxin (T_4) concentration to promote growth (absolute weight gain and total length).

Specific growth rate

Specific growth rate (SGR) of pink zebra (*Brachydanio reiro*) in Thyroxin (T_4) solution at 0.10 mg/L (C) demonstrated the highest percentages (10.68±0.21%) compared to treatment D (10.35±0.39%), B (9.68±0.41%), and A (8.71±0.25%) (Figure 5). Statistical analysis indicated that the usage of Thyroxin (T_4) at concentration 0.10 mg/L and 0.15 mg/L did not showed any significant differences. However, they exhibited around1.0% - 1.97% higher compared to control and treatment B (0.05 mg/L T_4). This study expressed that pink zebra larvae growth faster from 1.0% to 1.97% daily.

From these three growth variables indicated that the immersion of 4 days old pink zebra fry in Thyroxin (T_4) solution for 24 hours

significantly increased growth performance. This is because Thyroxin hormones (TH) in fish are involved in the control of osmoregulation, metabolism, somatic growth, skin pigmentation, reproduction, and post-hatching (Schnitzler et al., 2011; Yu et al., 2015). Furthermore, Bernier et al, (2009) added that the thyroid cascade involved two components. First, thyroxin (T_4) biosynthesis and secretation that controled by brain-pituitary- tyroid axis and secondly, a convertion of T_4 to its biologically active form T_3 (3,5,30-triiodothyronine). Pebriyanti et al, (2015) stated that the immersion of thyroxine at 0.10 mg/L for 48 hours sgnificantly improved yolk sac absorbsion, growth and survival of Climbing Perch *(Anabas testudineus)* larvae. Similar study conducted by Goswami and Goswami (2015) found that T_3 and T_4 deficiency inhibited the metabolism of vitamin A of grass carp (*Ctenopharyngodon idella*). It means that thyroid hormone affected retinoids reserves of fish.

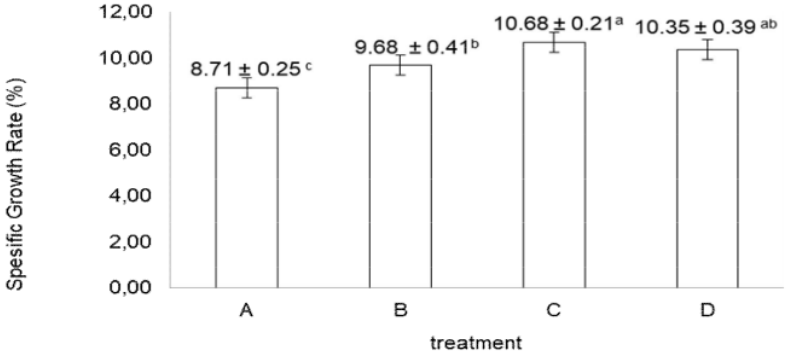

Figure 5. Specific growth rate of pink zebra larvae post immersion in various T_4 concentration after being reared 42 days

Survival rate

The survival rate of pink zebra larvae during the experiment were as follows; treatment B (87.50±2.50%), C (86.67±1.44%), A (86.67±3.82%), and D (85.83±1.44%) respectively (Figure 6). Statistical analysis demonstrated that there were no significant differences among the treatments. This means that immersion of T_4 for 24 hours did not threaten the survival of pink zebra larvae. Rosyadi (2015) on his similar study of the Selais fish (*Kryptopterus lais*) found that addition of thyroxine hormone 0.08 mg/kg feed significantly improve growth and 100% survival of experimental fish. Effendi (2003) added that survival was closely related to feed availability, good environment. This study indicated that experimental fish larvae werereared in good natural feed availability, no parasites and disease, and in an optimum rearing media.

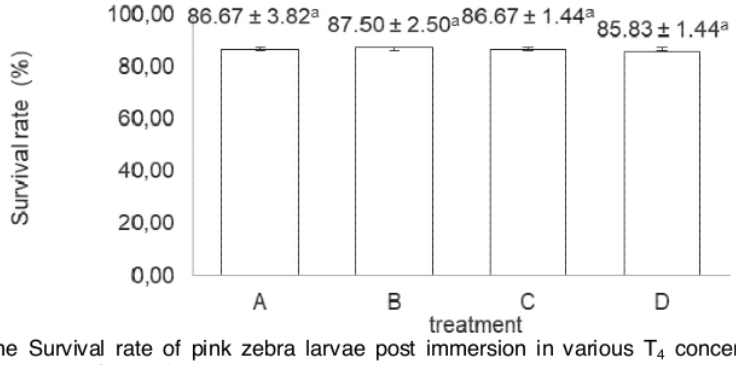

Figure 6. The Survival rate of pink zebra larvae post immersion in various T_4 concentration after being reared for 42 days

Pigment development

Pigment development of pink zebra larvae after reared 42 days according to ° Hue were at the range of 12.72 ± 0.59 ° to 14.60 ± 0.36 ° (Figure 8). Treatment C was the highest (14.60 ± 0.36 °) then followed by B (13.83± 0.34 °), A (12.84±0.17 °), and D (12± 0.59 °) respectively. This result was in contrast with previous research conducted by Putri (2012). The pigment development was significantly spread evenly over the Botia fish larvae (*Chromobotia macrachantus*) at 0.01 mg/L thyroxin compared to control and other treatments. She further stated that pigmentation of botia larvae was started 72 hours post hatched. Whilst in this study pigment development of pink zebra larvae (*B. reiro*) was clearly observed using °Hue after 42 days.

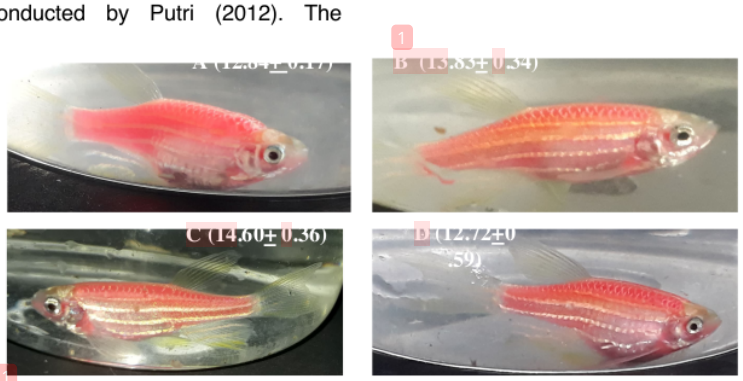

Figure 7. Pigment development (°Hue) of pink zebra larvae post immersion in various T_4 concentration after being reared 42 days

Water quality

During the experiment water temperature was range from 27°C to 29°C.Whilst pH range between 7.2 and 7.7. Dissolved oxygen was measured between 4.2 mg/L and 5.2 mg/L, whilst ammonia detected from 0.009 to 0.07 mg/L (Table 1). Healthy fish required minimum dissolved oxygen 3.0 mg/L and tended to cause mortality at 1.0 mg/L. In contrast, unhealthy fish required at least 4 mg/L dissolved oxygen. Unhelathy fish exhibits stress at 2.0 – 2.5 mg/L dissolved oxygen caused mortality. Water quality during the experiment within optimum range for pink zebra larval rearing up to 42 days. These range were within standard values of Helfman *et al* (1997), Timmons *et al* (2002), and Lewis and Moris (1986).

Table 1 . Water quality performance on the rearing aquarium

Variable	Value	Standard
Temperature (°C)	27 – 29	24 – 29[a]
pH	7.2 – 7.7.	7 – 8[b]
DO (mg/L)	4.2 – 5.2	> 3.0[c]
NH_3(mg/L)	0.0093 – 0.071	< 0.2[d]

References

a.Timmons *et al* (2002)
b.Timmons *et al* (2002)
c.Helfman *et al* (1997)
d.Lewis and Morris (1986)

4. Conclusion

From this study it can be concluded that immersion of thyroxin hormone (T_4) for 24 hours to pink zebra larvae (*Brachydanio reiro*) at concentration of 0.10 mg/L and reared for 42 days significantly improved absolute weight gain (0.24 ± 0.016 g), total length (26.45 ± 0.58 mm) , and specific growth rate (10,68 ± 0,21%). Thyroxin did not affect the survival rate of pink zebra larvae at both control and treated fish larvae. Immersion in Thyroxin solution (T_4) was also did not prevent pigment development. °Hue value was at 14.60 ± 0.36. Water quality was in a good performance throughout the study.

Acknowledgement

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