The effect of immersion time in thyroxine hormone on growth and survival of koi (*Cyprinus carpio*)

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Abstract. Koi (*Cyprinus carpio*) is an ornamental fish with a high potential to be developed. The production of an aquaculture commodity is strongly influenced by the availability of good quantity and quality fry. Therefore, increasing the fry's growth rate is necessary, shortening production time. The growth rate of fish can be increased by applying thyroxine hormone. The thyroxine hormone is known to speed up metabolic processes and stimulate fish movement. This study aimed to determine the effect of thyroxine hormone immersion of koi fry on the on their growth and survival and to determine the best immersion time. The research consisted of three consecutive steps: (1) preparation of fish and feed, (2) experimental design and biological analyses, (3) performance tests without immersion in thyroxine hormone (T0), with immersion in thyroxine hormone for 24 h (T1), with immersion in thyroxine hormone for 48 h (T2), and with immersion in thyroxine hormone for 72 hs (T3). The dose of the hormone used was 0.25 mg L^{-1}. After 45 days of experiment, the length growth, weight growth, specific growth rate, feed conversion ratio, and survival in T1 (2.97±0.06 cm, 5.31±0.16 g, 2.92±0.04% day^{-1}, 1.60±0.12, 100±0%), T2 (3.03±0.06 cm, 5.35±0.09 g, 3.02±0.04% day^{-1}, 1.53±0.08, 100±0%), and T3 (3.07±0.06 cm, 5.43±0.06 g, 3.08±0.04% day^{-1}, 1.47±0.05, 100±0%) were significantly different from the control values (1.77±0.06 cm, 3.16±0.23 g, 2.11±0.16% day^{-1}, 2.26±0.22, 100±0%) ($p<0.05$). The results of this study indicate that the use of thyroxine hormone significantly affects the growth and survival of koi fry compared to those not immersed in thyroxine hormone. At the same time, the immersion time did not give different results on all research parameters.
Key Words: accelerated growth, aquaculture, fry, increased production.

Introduction. Koi (*Cyprinus carpio*) is an ornamental fish with a high potential to be developed in Indonesia. High quality koi fish with a size of 30 cm can reach prices of 61.55 USD per head (Utomo 2020). This fish occupies the top position in ornamental fish sales with an average monthly farmer income of 369.3 USD. In 2020, the total production of koi fish in Indonesia was 443855 fish, and then in 2021, it increased to 458059 fish (Ministry of Marine Affairs and Fisheries 2021, 2022).

The production of an aquaculture commodity is strongly influenced by the availability of good quantity and quality fry. The availability of fry in sufficient quantity and good quality is the starting point for achieving fish production targets. Therefore, the hatchery business is has a great potential. One of the obstacles experienced in the hatchery is the fry's slow growth rate. Therefore, increasing the fry's growth rate is necessary, shortening production time. Shorter production times can lower production costs. The growth rate of fish can be increased by applying the thyroxine hormone to fish (Efrizal et al 2023).

Thyroxine hormone is known to speed up metabolic processes and stimulate active fish to respond well to food (Setiaji & Johan 2012). Protein and fat metabolism will increase with increased lipase and protease activity in the digestive tract after applying thyroxine hormone (Manurung et al 2017). The effectiveness of thyroxine hormone is influenced by hormone dosage, fish size, and immersion time (Sudrajat et al 2013). The immersion time affects the hormone levels absorbed by the body. In fry, the technique

that can be applied is immersion. Dissolved thyroxine hormone will enter the gills and mouth and be absorbed through the skin (Setiaji & Johan 2012).

The application of thyroxine hormone has been carried out in various species of fish. Immersion of snakehead (*Channa striata*) larvae (Muslim et al 2019; Pasaribu et al 2019), kissing gourami (*Helostoma temmincki*) larvae (Septerisno et al 2015), and white tilapia (*Oreochromis niloticus*) larvae (Andriawan et al 2020) in treatments with the same dose of thyroxine hormone, 0.1 mg L^{-1}, produced the best growth and survival at 24 h of immersion. The administration of thyroxine hormone in the fry phase has been carried out by Setiadi et al (2016) with the best dose of 0.75 mg L^{-1} for gourami fry, while Dayuni et al (2019), with a dose of 0.25 mg L^{-1} was able to provide a significant increase in weight with a 24 h immersion time for Asian redtail catfish (*Hemibagrus nemurus*) juveniles. Longer immersion time is likely to give better results. As for koi fry, the best immersion time using the thyroxine hormone has yet to be discovered. Therefore, this study aimed to determine the effect of immersion of koi fry in thyroxine hormone solution on their growth and survival and to determine the best immersion time.

Material and Method

Description of the study sites. Maintenance, treatment, and analysis of koi growth and survival parameters were performed at the Fish Production and Reproduction Laboratory, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram, Mataram, West Nusa Tenggara, Indonesia.

Vessel preparation. 5 L containers (for immersion treatment) and 45 L containers (for maintenance after immersion treatment) were cleaned using soap and rinsed with water. The containers were then dried. The smaller containers were arranged according to the layout of the experimental unit. Then a hose and an aeration stone were installed after the thyroxine hormone solution was placed into each 5 L container.

Fish. The koi fish used were fry with a total length between 4-5 cm from the Mataram City Fisheries Service. 150 koi fry were transported to the laboratory using a plastic container filled with water mixed with methylene blue (Merck; Indonesia) and oxygenated. Fry were transferred in the morning. Acclimatization was carried out by placing the fry in a plastic bag in a container filled with water for 20 minutes, to reach a balance of temperatures. The fry were reared for 2 days in a container installed with an aerator.

Ethical approval. All animal experimentation and rearing were handled according to the animal welfare procedures, under the national accreditation no. SNI 7775:2013 of the Republic of Indonesia.

Fish feeding. The feed used was commercial pellets Takari (Central Proteina Prima; Indonesia). Takari feed contains a minimum of 30% protein. The pellet had a 1 mm diameter, easy to be ingested by the koi fry. Feeding was conducted twice a day, in the morning and evening. The feeding dosage was based on the feeding rate of 5% of the total weight. The proximate content of the feed is presented in Table 1 (Setyogati 2021).

Experimental design. The experimental method was used to determine the effect of the length of immersion time of koi fry in the hormone thyroxine on their growth and survival. The research design used was a completely randomized design (CRD) using 1 control and 3 treatments with 3 replications for 12 experimental units. The treatments applied were as follows: T0 -no immersion in thyroxine hormone; T1 - immersion in thyroxine hormone for 24 h; T2 - immersion in thyroxine hormone for 48 h; T3 - immersion in thyroxine hormone for 72 h.

Table 1

Proximate composition of the feed

Proximate composition	*Content (%)*
Protein	30
Water	12
Fat	12
Ash	3
Fiber	4
Feed form	Floating
Feed size (mm)	1

Note: source: Setyogati (2021).

Thyroxine hormone. The thyroxine hormone used is synthetic thyroxine hormone with the brand name Euthyrox (Merck; Indonesia). One strip contains 25 thyroxine tablets containing 0.1 mg of thyroxine per tablet. 45 Euthyrox tablets were crushed using a grinder. The powder was mixed with 18 L of water to get a thyroxine solution at 0.25 mg L^{-1}. This solution was placed into a container, 2 L/container and then an aerator was attached. Furthermore, the fish were weighed and their total length was measured to obtain the initial research data. The koi fry were put into the containers (10 fish per container) and placed on a shelf in a position according to the randomization results. After reaching the specified time, the fish were transferred to a container containing 20 L of fresh water, with an aerator. The maintenance time was 45 days, including the treatment. Fish length and weight were measured once every 15 days by measuring all koi fry used (10 fish/treatment) to determine the growth.

Biological analysis. The parameters measured in this study were length growth, weight growth, specific growth rate (SGR), feed conversion ratio (FCR), survival rate, and water quality. Ten fry were randomly sampled from each replicate container on day 0, day 15, day 30, and day 45 for total length and weight. Fry length was measured using a scientific ruler. Fry weight was measured using an analytical scale to calculate the specific growth rate (SGR). In addition to growth performance values, feed conversion ratio, survival rate, and water quality were calculated as important objectives of this study.

Length growth. The length growth, according to Risky et al (2020), is calculated by the formula:

$$L=Lt-Lo$$

Where: L - length growth (cm); Lt - final fish length (cm); Lo - initial fish length (cm).

Weight growth. Weight growth, according to Asma et al (2016), is calculated by the formula:

$$W=Wt-Wo$$

Where: W - weight growth (g); Wt - final fish weight (g); Wo - initial fish weight (g).

Specific growth rate. The specific growth rate (SGR) was calculated using the following formula (Mulqan et al 2017):

$$SGR=[(lnWt-lnWo)]/t \times 100$$

Where: SGR - specific growth rate (% day^{-1}); Wt - final fish weight (g); Wo - initial fish weight (g); T - maintenance time (days).

Feed conversion ratio. The feed conversion ratio (FCR) was calculated using the following formula (Sulatika et al 2019):

FCR=F/[(Wt+D)-Wo]

Where: FCR - feed conversion ratio; Wt - total fish weight at the end of the experiment (g); Wo - total fish weight at the start of the experiment (g); D - total weight of fish that died during the experiment (g); F - total amount of feed given (g).

Survival rate. The survival rate (SR) was calculated using the following formula (Francisca & Muhsoni 2021):

SR=Nt/No x 100

Where: SR - survival rate (%); Nt - number of fish at the end of the experiment; No - number of fish at the start of the experiment.

Water quality. Water quality data is supporting data. Water quality control is very important because it affects the survival of fish. Water quality measurements were carried out at the beginning and the end of the maintenance. Water quality parameters measured were: temperature, dissolved oxygen (DO), and pH. Temperature was measured using an alcohol thermometer (Anugrah Putra Kencana; Indonesia). DO measurement was carried out using a DO meter (Lutron; China). The pH measurement was carried out using a pH meter (Lutron; China).

Statistical analysis. Growth and survival data were first normalized using an arcsine transformation before statistical analysis (Suantika et al 2021). Data on growth and survival parameters were analyzed using one-way ANOVA followed by Duncan post-hoc test, with a 95% confidence interval to evaluate the differences between treatment groups. All statistical analyses were performed using SPSS® Version 25.0 (SPSS; USA).

Results. The results of the 45-day experiment showed that there was a significant effect ($p<0.05$) of immersion in the thyroxine hormone solution on growth and survival of koi fry. The difference in the immersion time did not produce a significant effect ($p>0.05$) on growth and survival.

Length growth. The highest length growth value was obtained in T3, with an average of 3.07 cm, followed by T2 and T1, with averages of 3.03 and 2.97 cm, respectively. The lowest length growth value was obtained in T0, with an average value of 1.77 cm. The results of length growth are presented in Figure 1. The results of the analysis of variance (ANOVA) showed that there was a significant effect ($p<0.05$) of koi fry immersion in the solution of thyroxine hormone on length growth. The results of Duncan's test showed that there was a significant difference between the control and T1 (immersion for 24 h), T2 (immersion for 48 h), and T3 (immersion for 72 h). There were no significant differences between T1, T2, and T3.

Weight growth. The highest weight growth value was obtained in treatment T3 with an average of 5.43 g, followed by T2 and T1, with average values of 5.35 and 5.31 g, respectively. The lowest weight growth value was obtained in the control, with an average value of 3.16 g. The results of weight growth are presented in Figure 2. There was a significant effect ($p<0.05$) of koi fry immersion in a solution of thyroxine hormone on weight growth. The results of Duncan's test showed that there was a significant difference between the control and T1, T2, and T3. There were no significant differences between T1, T2, and T3.

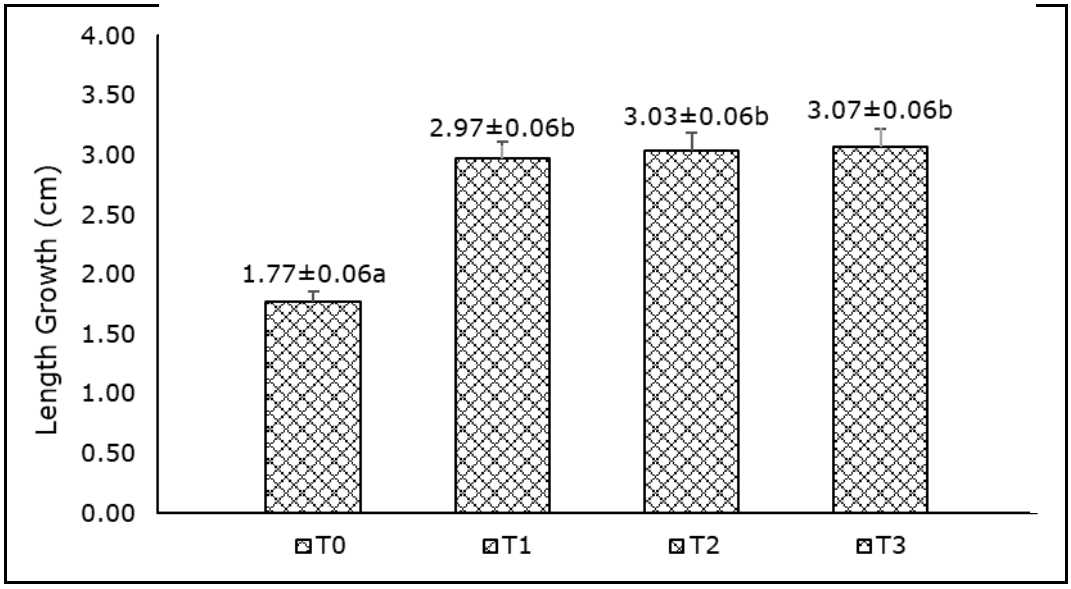

Figure 1. Length growth: T0 - without immersion in thyroxine hormone; T1 - immersion in thyroxine hormone for 24 h; T2 - immersion in thyroxine hormone for 48 h; T3 - immersion in thyroxine hormone for 72 h.

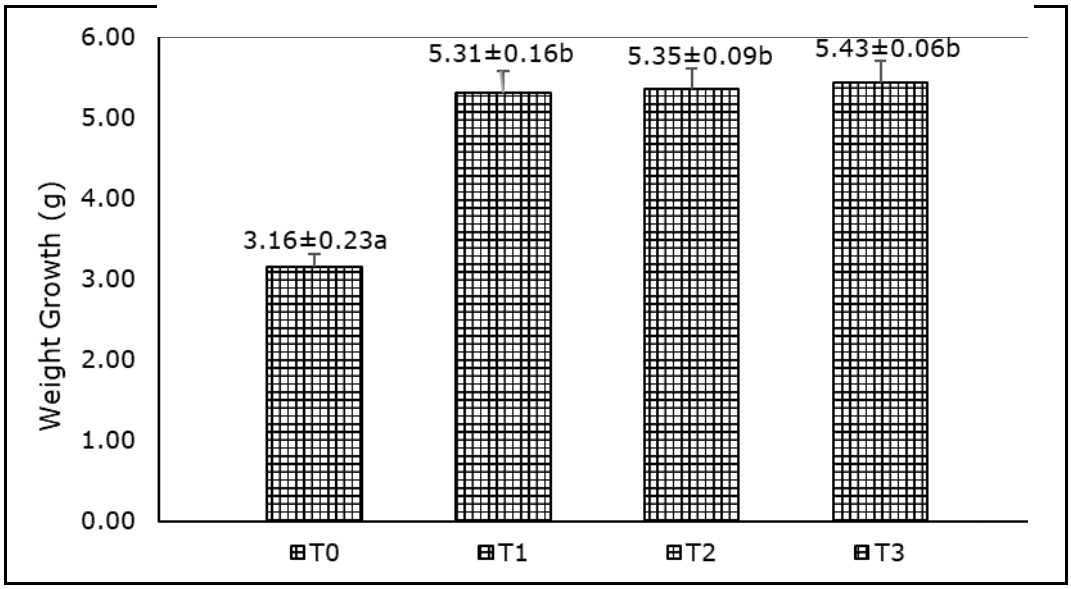

Figure 2. Weight growth: T0 - without immersion in thyroxine hormone; T1 - immersion in thyroxine hormone for 24 h; T2 - immersion in thyroxine hormone for 48 h; T3 - immersion in thyroxine hormone for 72 h.

Specific growth rate. The highest SGR was obtained in T3, with an average of 3.08% day^{-1}, followed by T2 and T1, with average values of 3.02 and 2.92% day^{-1}. The lowest SGR was obtained in T0, with an average value of 2.11 %/day. The SGR results are presented in Figure 3. There was a significant effect ($p<0.05$) of koi fry immersion in a solution of thyroxine hormone on SGR. The results of Duncan's test showed that there was a significant difference between the control and T1, T2, and T3. There were no significant differences between treatments T1, T2, and T3.

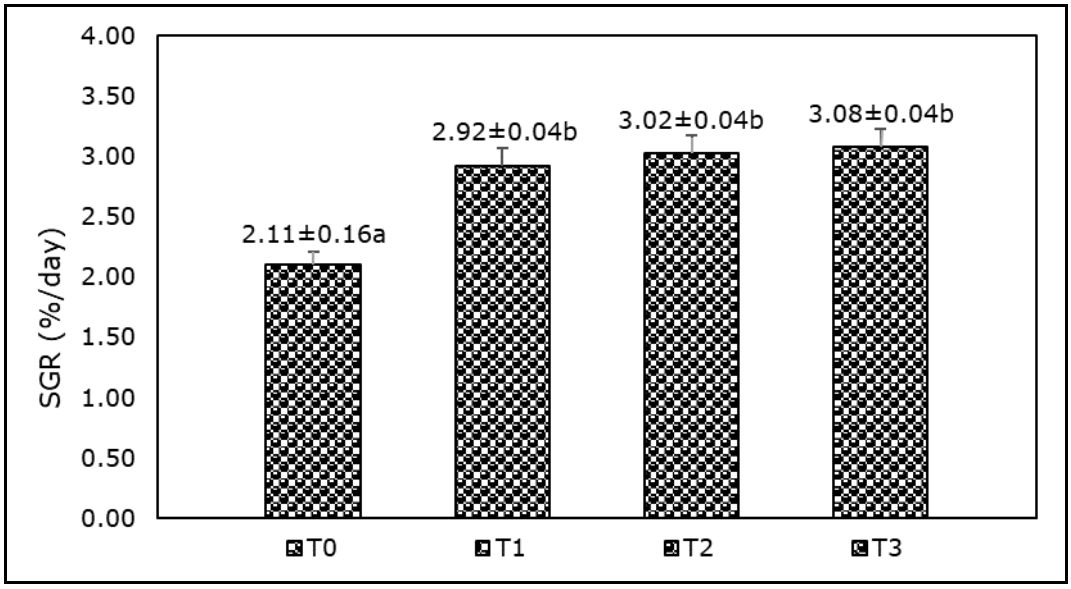

Figure 3. Specific growth rate; T0 - without immersion in thyroxine hormone; T1 - immersion in thyroxine hormone for 24 h; T2 - immersion in thyroxine hormone for 48 h; T3 - immersion in thyroxine hormone for 72 h.

Feed conversion ratio. The highest FCR value was obtained in T0, with an average of 2.26, followed by T1 and T2, with average values of 1.6 and 1.53, respectively. The lowest FCR value was obtained in T3, with an average of 1.47. The results of the FCR are presented in Figure 4.

There was a significant effect ($p<0.05$) of koi fry immersion in a solution of thyroxine hormone on the FCR. The results of Duncan's test showed that there was a significant difference between the control treatment and T1, T2, and T3. There were no significant differences between T1, T2, and T3.

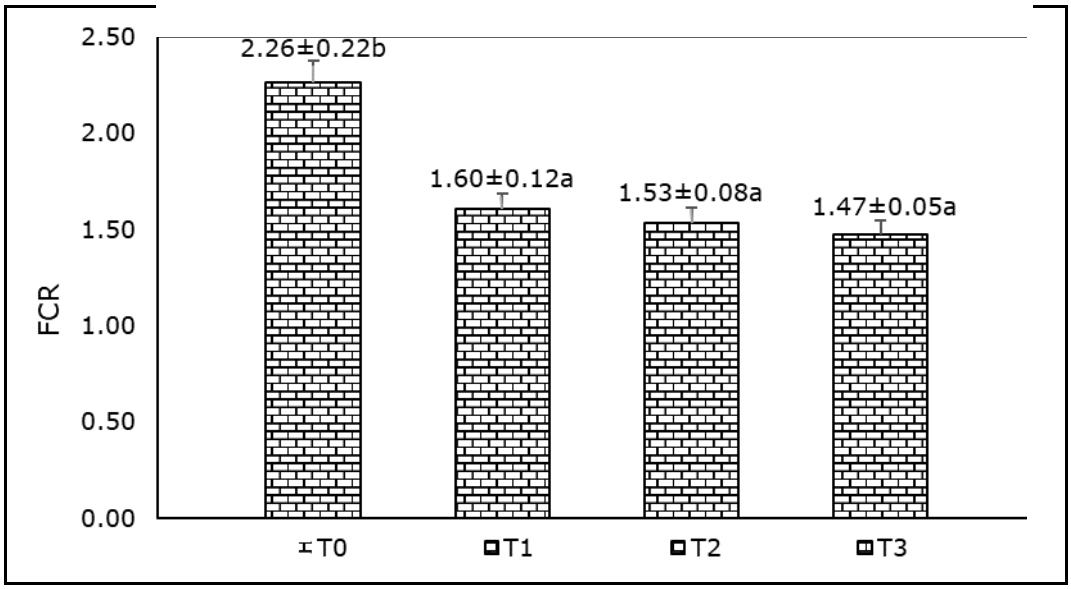

Figure 4. Feed conversion ratio; T0 - without immersion in thyroxine hormone; T1 - immersion in thyroxine hormone for 24 h; T2 - immersion in thyroxine hormone for 48 h; T3 - immersion in thyroxine hormone for 72 h.

Survival rate. The survival rate in this study was 100% in all treatments. During the immersion period and the maintenance period, no fish died.

Water quality. The results of water quality measurements during maintenance are presented in Table 2.

Table 2

Water quality parameters during the 45 days of experimental period

Parameter	*T0*	*T1*	*T2*	*T3*	*Tolerance range*
Temperature (°C)	28-29	27-29	27-29	27-28	26-30 (Nurhayati et al 2022)
DO (mg L^{-1})	4.4-4.9	4.2-4.9	4.3-4.8	4.3-4.6	4.8-6.0 (Lembang & Kuing 2021)
pH	6.9-7.6	7.4-8.3	7.2-8.4	7.4-8.2	6.5-8.5 (Rizky et al 2015)

Note: DO - dissolved oxygen; T0 - without immersion in thyroxine hormone; T1 - immersion in thyroxine hormone for 24 h; T2 - immersion in thyroxine hormone for 48 h; T3 - immersion in thyroxine hormone for 72 h.

Discussion. This study demonstrated a significant difference in length growth for koi fry immersed in a solution of thyroxine hormone. Koi fry immersion at 0.25 mg L^{-1} thyroxine hormone produced better fish length than the control. In a study conducted by Alang et al (2020), a dose of 1 mg L^{-1} thyroxine had a significant effect on weight growth, but no significant effect on length growth at 30 minutes of immersion in climbing perch (*Anabas testudineus*) larvae. Dayuni et al (2019) stated that a dose of thyroxine of 0.25 mg L^{-1} had a significant effect on weight growth, but no significant effect on length growth during 24 h immersion in Asian redtail catfish fry. Setiadi et al (2016) stated that the administration of thyroxine hormone could increase fish's metabolic rate and appetite. However, using too high a dose can result in slow and abnormal fish growth (Andriawan et al 2020).

The varying immersion times of koi fry in a 0.25 mg 0.25 mg L^{-1} thyroxine hormone solution did not yield significantly different results in length growth. It is presumed that by the 24-hour immersion time (T1), the fish had absorbed nearly all the thyroxine hormone, leaving minimal absorption after this period. As a result, the difference in length growth is very small. Andriawan et al (2020) state that the fish's body has a consistent ability to absorb thyroxine hormone from the environment, resulting in similar growth outcomes regardless of the immersion duration. According to Pasaribu et al (2019), the difference in the immersion time has a strong relationship with the increase in the length of snakehead larvae, with the best time immersion of 27.92-29.49 h. In this study, longer immersion times led to higher absorption of thyroxine hormone by the snakehead larvae. Consequently, after the optimal immersion duration, the absorbed hormone levels surpassed the amount that the larvae could effectively utilize. As a result, the value of larval growth is similar to the immersion time of 27.92-29.49 h. In the fry stage, the size of the fish is larger than in the larval stage. The dose of thyroxine hormone needed is also higher. In a study by Setiadi et al (2016), which immersed gourami (*Osphronemus gouramy*) fry in a thyroxine hormone solution, a dose of 0.75 mg L^{-1} produced higher growth and survival than lower doses. Abdollahpour et al (2019) added thyroxine hormone (1 mg kg^{-1} fish body weight) to sterlet (*Acipenser ruthenus*) in the adult phase, using the injection method and the results provided higher growth in length. This shows that the dose of hormone needed in the fry stage is higher than in the larval stage, which only requires an average dose of 0.1 mg L^{-1}. As for this study, a dose of 0.25 mg L^{-1} could be used properly for koi fry.

Koi fry immersion at 0.25 mg L^{-1} resulted in a better fish weight gain than the treatment without the thyroxine hormone. This dose is suspected to be proper for koi fry. According to Srivastava et al (2013), the use of thyroxine hormone must be proper; if it is excessive, it can inhibit the growth and produce the death of fish. Fish weight gain occurs when more energy is produced in metabolic processes than the energy needed for maintaining the body. A higher metabolic rate means that more energy is produced in a unit of time. This excess energy will be diverted to growth. The use of thyroxine hormone in fish is reported to be able to increase the metabolic rate. In research conducted by

Pratama et al (2022) on *Betta splendens* larvae, the best results for increasing fish weight were obtained by thyroxine hormone 24 h immersion time at a dose of 0.1 mg L^{-1}. Setiadi et al (2016) used gourami fry and the best dose discovered was 0.75 mg L^{-1}, whereas in the study of Dayuni et al (2019), who used Asian redtail catfish fry, a dose of 0.25 mg L^{-1} was able to provide a significant increase in weight with a 24 h immersion time. Thyroxine hormone enters the fish's mouth and then enters the blood circulation, from where it is distributed to the target cells. The hormone will bind to the hormone element receptors on the DNA sequence and promote energy metabolism in the mitochondria. This binding will activate cell transcription and trigger the production of RNA-messenger, causing cells to increase in number (Andriawan et al 2020). RNA-messenger that carries a copy of the genetic code will then go to the ribosome to undergo the translation process, namely the translation of the DNA code to produce polypeptide chains that make up proteins (Agirrezabala & Frank 2010). The resulting protein will be used as a constituent of enzymes, hormones, and antibodies. In addition, this protein is also used as a source of energy for the formation and repair of cells and tissues in the body.

The difference in the length of time for immersed koi fry in thyroxine hormone solution at a dose of 0.25 mg L^{-1} did not produce significantly different results on weight growth. This result is presumably because, at the 24 h immersion time (T1), almost all of the thyroxine hormone has been absorbed by the fish, so after 24 hours, very little of the hormone remains to be absorbed. In the research of Pasaribu et al (2019), the difference in the length of immersion time had a strong relationship with the increase in weight and length of snakehead larvae. The existence of this strong relationship indicates that there was a significant effect of the difference in immersion time on growth. Septerisno et al (2015) immersed kissing gourami larvae in a solution of thyroxine hormone for different lengths of time. The research shows that the best time for immersion is 24 h. After 24 hours, the weight gain was less than that achieved during the 24-hour immersion period. Heraedi et al (2018) also conducted a study of immersed pink zebrafish (*Brachydanio reiro*) larvae for 24 h in thyroxine hormone solution, and the best dose to increase body weight growth was 0.1 mg L^{-1}. The difference in the results of the three studies and this study is likely due to the different doses of hormones, species and age of fish.

This study showed significant differences in SGR of immersed koi fry in thyroxine hormone solution compared to the control. There were no significant differences in SGR based on immersion time. The SGR obtained in this study is better when compared to the SGR of Asian redtail catfish fry (Dayuni et al 2019) at the same dose and immersion time. This shows that administering thyroxine hormone to koi fry gives a better reaction than in Asian redtail catfish fry. The value of SGR in this study was also higher when compared to the SGR of mirror carp fry in the study of Rahman et al (2012), which was 1.71% day^{-1}. The administration of the thyroxine hormone orally gave a higher SGR than in the study of Sroyer et al (2020) on tilapia fry, which produced a SGR of 4.73% day^{-1} at a dose of 25 g kg^{-1} of feed and in the study of Sukendi et al (2021) on bronze featherback (*Notopterus notopterus*) juveniles, where it produced a SGR of 2.97% day^{-1} at a dose of 6 mg kg^{-1} of feed. According to Sutiana et al (2017), thyroxine hormone can accelerate the growth rate, increase appetite, and regulate metabolism in fish.

A lower FCR means lower feed costs. This study showed significant differences of FCR for immersed koi fry in a thyroxine hormone solution compared to the control, but no differences were observed depending on the time of immersion. This is presumably because the koi fry is more voracious in the treatment with thyroxine hormone, eating all the feed. Increased appetite is related to an increased metabolic rate due to the administration of the thyroxine hormone. The value of the FCR in this study (1.47) was better than the FCR in the study of Sutiana et al (2017), who used a combination of thyroxine and rGH hormones, which was 1.92 in the best treatment. In the study of Susanti et al (2016), the FCR for nilem fish (*Osteochillus hasselti*) was 1.44. Dedi et al (2018) reported that cantang grouper (*Epinephelus fuscoguttatus-lanceolatus*) fry had a FCR of 2.71. Rahman et al (2012) obtained a FCR of 4.31 for koi. The comparisons shows that the FCR value in this study is good.

The survival rate in this study was 100% in all treatments and control. In the study of Setiaji & Johan (2012), the survival rate of jambal siam (*Pangasius hypophthalmus*) fry under a thyroxine dose of 0.5 mg L^{-1} was 100%, the same as reported by Kurniawan et al (2014) for gourami fry immersed in thyroxine hormone. Zulkifle et al (2021) reported that *Parosphromenus tweediei* immersion in a thyroxine hormone solution resulted in a survival rate of 90%. Setiadi et al (2016), who immersed gourami fry in a thyroxine hormone solution of 0.75 mg L^{-1}, observed a high survival rate of 88%. This means that, at higher doses, the fry can survive because the required dose of thyroxine is also higher, unlike in the larval stage, which, on average, can only receive a dose of 0.1 mg L^{-1} (Sudrajat et al 2013).

The good survival rate in this study is suspected to occur because the dose used was in a good range for the survival of koi fry. The use of thyroxine hormone in excessive and inappropriate doses can cause death in fish.

Water quality influences the growth and survival of fish. Good and optimum water quality can optimize fish growth. Too high temperatures can cause stress to fish, reducing appetite (Sumantri et al 2017). The temperature obtained in this study was classified as good, between 27-29°C. According to Suryanto et al (2021), a good temperature for koi survival is 25-32°C. DO is needed in metabolic processes, so it must be available in the aquaculture media in proper levels. Lack of DO in the waters can cause death. The optimal DO value for koi cultivation is 4-8 mg L^{-1} (Andayani et al 2022). In this study, all experimental units had DO values between 4.2-4.9 mg L^{-1}, good for the survival of koi fry. In this study, the pH range was between 6.9-8.4. A good pH range for koi is 6.5-8.0 (Vipriyandhito et al 2022). The pH values classified as high or low can disrupt metabolic and respiration processes in fish (Priyono et al 2013).

Conclusions. Based on the results of data analysis in this study, it can be concluded that koi fry immersion in 0.25 mg L^{-1} thyroxine hormone had a significant effect ($p<0.05$) on growth and survival. The immersion time had no significant effect on the growth and survival of koi fry ($p>0.05$). Further research needs to be carried out using higher doses (more than 0.25 mg L^{-1}) and a longer time to determine the extent of the effect of the thyroxine hormone.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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