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Research Article

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Reproductive Performances and Egg Qualities in African Catfish (*Clarias gariepinus)* Broodstocks Supplemented with Curcumin and Thyroxine Hormone

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ABSTRACT

An experiment was designed to study the effects of curcumin and thyroxine hormone supplementation in the diet to improve the reproductive performance of catfish broodstock by improving egg quality that eventually increase the production of seedlings. Catfish used in this experiment were supplemented with curcumin and thyroxine hormone through their feeds for 12 weeks. The results showed that there was no significant difference ($p > 0.05$) in the hepatosomatic index (HSI), gonadosomatic index (GSI), percentage of gonad maturity, total cholesterol concentration in the spawned eggs, high density lipoprotein (HDL) concentration in the spawned eggs, fertilization rate of spawned eggs, and hatching rate of fertilized eggs. However, there were significant differences ($p < 0.05$) in the concentration of vitellogenin in the spawned eggs, egg diameter of the spawned eggs, and the triglycerides contents of spawned eggs. It was concluded that curcumin and thyroxine supplementations of African catfish increased vitellogenin concentrations and diameters of spawned eggs that have great potential to improve the reproductive performance.

***Keywords**: African catfish, curcumin, egg quality, reproductive performance, thyroxine*

ABSTRAK

Percobaan dirancang untuk mempelajari efek suplementasi curcumin dan hormon tiroksin dalam makanan untuk meningkatkan kinerja reproduksi induk ikan lele dengan meningkatkan kualitas telur yang pada akhirnya meningkatkan produksi bibit. Ikan lele yang digunakan dalam percobaan ini dilengkapi dengan hormon curcumin dan tiroksin melalui pakan mereka selama 12 minggu. Hasil penelitian menunjukkan bahwa tidak ada perbedaan yang signifikan ($p> 0,05$) dalam indeks hepatosomatik (HSI), indeks gonadosomatik (GSI), persentase kematangan gonad, konsentrasi total kolesterol dalam telur yang bertelur, konsentrasi lipoprotein (HDL) kepadatan tinggi dalam telur bertelur, tingkat pembuahan telur bertelur, dan tingkat penetasan telur yang dibuahi. Disisi lain, ada perbedaan yang signifikan ($p <0,05$) dalam konsentrasi vitellogenin dalam telur yang bertelur, diameter telur dari telur yang bertelur, dan kandungan trigliserida dari telur yang bertelur. Kita dapat menyimpulkan bahwa suplementasi curcumin dan tiroksin dari lele dumbo meningkatkan konsentrasi vitellogenin dan diameter telur yang bertelur yang memiliki potensi besar untuk meningkatkan kinerja reproduksi.

***Keywords**: African catfish, curcumin, egg quality, reproductive performance, thyroxine*

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1. Introduction

The productivity of freshwater fish can be increased by improving the reproductive process of the brood stocks. Various studies have been conducted to improve the quality of seedling fish such as improvement of feed quality (Luquet et al. 1986; Emata et al., 2001; Dewi et al., 2018a) and hormonal supplementation (Islami et al., 2017; Syano et al., 1993; Ayson and Lam, 1993; Mylonas et al., 2010) showing positive influences on the adequacy of nutrition and good physiological conditions to support fish productivity. African catfish is one of the popular fishes that has long been known and favored for consumption in Indonesia. To fulfill the increased demands for African catfish, the availability of the African catfish must be increased by improving the reproductivity and productivity of the brood stocks.

The growth and development of embryos and larva is determined by the availabilities of nutrients and materials in the egg to support the optimum growth and development of embryos and larvae. Therefore, survivals of fishes in their earlier lifes are strongly influenced by the egg quality. Good eggs will produce good individuals. Nutritional deficiencies in eggs can result in the inhibition or cessation of embryogenesis activities that can cause deaths in the new organisms before being hatched or the occurrence of abnormal growth of the larvae produced. Eggs with good qualities can be determined by evaluating the ability of the eggs to be fertilized and the capacity of the fertilized eggs to be hatched (Bobe, 2015). The availability of fish seeds is determined by the number of eggs that can be fertilized and hatched. The higher the percentage of fertilization and hatching, the higher the number of larvae that can be produced.

Egg quality is determined by the internal and external factors. Internal factor is related to the condition of brood stock that produces the eggs. The adequacy of nutrition and good health of brood stock during the process of vitellogenesis will affect the quality of eggs produced (Izquierdo et al., 2001). Adequacy of nutrition and good physical and physiological conditions can increase the capacity of brood-stock to produce enough materials of egg yolk precursor to fulfill the requirement of each developing oocyte. Estradiol is hormone that stimulate the expression of vitellogenin genes in hepatocytes to begin the synthesis of vitellogenin and then the synthesized vitellogenin is released into the blood circulation and further transported to the oocytes. The im.proved vitellogenin synthesis and deposition as a precursor of egg yolk in each developing oocyte can prevent the nutritional deficiency during the embryogenesis process that eventually will support the optimum growth and development and gene expression of embryo.

Research conducted by Dewi et al. (2018a, b) in Siam catfish showed that turmeric powder supplementation during gonad maturity improved reproductive performance with an increase in vitellogenin production by the hepatocytes, increased deposition of vitellogenin in the eggs, increased gonadosomatic index value and egg diameter. Turmeric powder supplementation in feed can increase the gonadosomatic index, egg diameter, and the percentage of mature sex of redfin shark in the non-spawning season (Islami et al., 2017). Curcumin is a phenol group compound which has various bioactivities such as hepatoprotective (Anand et al., 2008) and phytoestrogens (Bachmeier et al., 2010). Curcumin can prevent hepatic fibrosis (Mu-En Wang et al., 2012) because curcumin is able to increase the expression of antioxidant genes in the hepatocytes (Zhao et al., 2011). In oviparous animals such as fish, the liver is an important organ during the process of vitellogenesis. The protection of the liver cell by the hepatoprotective activity of curcumin increase vitellogenin synthesis in Siam catfish so that it can produce higher amounts of vitellogenin that will be deposited in each developing oocyte (Dewi et al., 2018a, b).

Thyroid hormone itself has several essential roles in supporting reproductive activity in fish, including the availability of intracellular energy needed in vitellogenesis activities. In fish reproduction process, thyroid hormones can stimulate the vitellogenesis activities (Lam and Loy 1985). The research conducted by Syano et al. (1993) showed that thyroid hormones can increase the synthesis of estradiol by granulose cells. Thyroid hormone in the plasma can be transported and deposited into the eggs (Babin, 1992) and thyroid hormone also supports the absorption and deposition of vitellogenin in the oocytes (Shibata et al., 1993). The thyroxine hormone besides being transferred into the oocyte also plays a role in the transport of vitellogenin into the oocyte (Monteverdi and Di Giulio, 2000). This study was designed to evaluate the effect of curcumin and thyroxine supplementations on the reproductive performance of the brood-stock and the quality of the eggs produced of African catfish.

2. Material and Methods

2.1 Location and time of study

The experiment was conducted in August-December 2018. The catfish maintenance was conducted in National Freshwater Aquaculture Center, Sukabumi, West Java, Indonesia. The analyses of lipid concentration were conducted in the Laboratory of Physiology, Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine, IPB University. Analysis of vitellogenin concentrations in the eggs and estradiol concentration in the serum were conducted in the Primate Animal Study Center, IPB University.

2.2 Experimental design

The experimental design used was a completely randomized design with a 2x2 factorial arrangement with four replications and each replication consisted of eight African catfish. The first factor was dose of curcumin supplementation consisted of two levels i.e., 0 and 0.5%.kg^{-1} feed. The second factor was dose of thyroxine supplementation consisted of two level i.e., 0 and 0.1 mg.kg^{-1} feed).

2.3 Experimental procedure

A total of 128 female African catfish were reared in 16 nets each with the size of 2x1x1 m^3 and each net contained eight catfish. The experimental African catfish used in this study were African catfish with an initial body weight ranged of 250-350 g. The experimental feed was given in the form of the commercial ration with 42.70 % protein content and then mixed with curcumin and thyroxine hormone according to the dose of treatment. The curcumin used in this experiment was produced by Plamed Green Science Limited with 93.71 % concentration of curcumin. The thyroxine hormone used was a tablet of Levothyroxine sodium/Euthyrox (Merck). The process of feed coating was started with the addition of carboxymethyl cellulose (CMC) powder as a binder to the commercial feed. The level of CMC addition was 10% in the commercial feed used. Further, the curcumin and/or thyroxine powder was added into the commercial feed mixed with CMC. Feeding treatment was carried out for 12 weeks of fish rearing.

2.4 Sample collection

Every three weeks, one catfish was taken randomly from each replication in all treatments. Before blood sampling and surgery, the catfish were anesthetized using clove oil at a concentration of 0.04 ml/l water. The blood were collected and put into a polyethylene tube and centrifuged at 3000 rpm for 10 minutes at 4 °C to obtain serum. The serum was used to analyze the concentration of estradiol hormone. Measurement of fish body weight was carried out before surgery. The gonads and liver obtained from surgery were weighed. Egg diameter was measured by taking 200 eggs and grouped by size to determine the distribution of egg diameter at each sampling time for 12 weeks of treatment. On the 12th week of treatment, the eggs were collected and used further for measurement of vitellogenin concentration in the egg. After 12 weeks of rearing, the fish was selected to determine the percentage of the gonad maturity. One fish that was ready to be spawned was taken from each replication of all treatments. Spawning was conducted artificially by injecting 0.2 ml of ovaprim/kg body weight of experimental African fish. The ovulated eggs were calculated to determine the relative fecundity. As much as 100 of ovulated eggs were taken to measure the egg diameter. Before conducting the artificial fertilization, the sperm was collected first. The male broodstocks that have mature gonads were sacrificed to pick up the sperm sac. The sperm sac was cleaved and sperms were collected in a container containing 0.9 % NaCl solution, then artificial fertilization was conducted.

2.5 Parameters Measurement

- Estradiol Assay
  Estradiol concentrations in the serum were determined with an enzyme linked immunosorbent assay (ELISA) method by using the kit estradiol (DRG Instruments GmbH, Germany)

Table 1. Treatment of combinations of thyroxine and curcumin supplementation.

Thyroxine (mg.kg^{-1} feed)	Curcumin (%.kg^{-1} feed)	
	0	0.5
0	A (0 % + 0 mg)/Control	B (0.5 % + 0 mg)
0.1	C (0 % + 0.1 mg)	D (0.5 % + 0.1 mg)

A= 0 %.kg^{-1}feed curcumin and 0 mg.kg^{-1}feed thyroxine; B= 0.5 %.kg^{-1}feed curcumin and 0 mg.kg^{-1}feed thyroxine; C= 0 %.kg^{-1} feed curcumin and 0.1 mg.kg^{-1}feed thyroxine; D= 0.5 % kg^{-1}feed curcumin and 0.1 mg.kg^{-1}feed thyroxine

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- Hepatosomatic Index (HSI)
  Hepatosomatic index was measured by using the following formula:

  $$HSI = \frac{\text{liver weight}}{\text{body weight}} \times 100$$

- Gonadosomatic Index (GSI)
  Gonadosomatic index was measured by using the following formula:

  $$IGS = \frac{\text{liver weight}}{\text{body weight}} \times 100$$

- Eggs diameter
  Eggs diameter were measured using Zeiss microscopy then grouped according to the size of those egg diameter.

- Coefficient of variation (CV)
  Coefficient of variation was measured by using the following formula :

  $$CV = \frac{\text{Standard deviation}}{\text{mean}} \times 100$$

- Concentration of vitellogenin in the eggs
  Egg vitellogenin concentration was measured in eggs obtained from catfish in the 12th week of treatment. Determination of vitellogenin concentration in the egg was done by ELISA method using Vitellogenin Fish Kit (Korain Biotech Co.Ltd, China).
- The concentrations of the lipids in the ovulated eggs
  The lipids contents measured in the ovulated eggs were cholesterol (determined by the cholesterol oxidation-phenol-4-aminoantipyrine-peroxidase (CHOD-PAP) method); triglyceride (determined by the method of glycerol phosphate oxidase-p-aminophenozone (GPO-PAP)); and the high-density lipoprotein (HDL) (determined by the CHOD-PAP method).
- Determination of relative fecundity
  Total number of ovulated eggs were divided by a total weight of broodstock gave the ratio of egg produced per gram body weight. The formula is given below:

  $$\text{Relative fecundity} = \frac{Total\ number\ of\ ovulated\ eggs}{\text{Weight of Broodstock}}$$

- Determination of fertilization rate
  Fertile eggs have a transparent shell and unfertile eggs are pale in color. The formula used to calculate the degree of fertilization is:

  $$\text{Fertilization rate} = \frac{Number\ of\ fertilized\ eggs}{\text{Total number of eggs}} \times 100$$

- Determination of hatching rate
  The formula used to calculate the hatching rate is:

  $$\text{Hatching rate} = \frac{Number\ of\ hatching\ eggs}{\text{Total number of fertilized eggs}} \times 100$$

2.6 Statistical analyses

The data obtained were analyzed by using analysis of variance (ANOVA) on MINITAB version 16 program. The differences between the means of the treatment were tested by using Tukey simultaneous test. All results of significantly different were expressed with $p<0.05$.

3. Results

3.1 Concentrations of estradiol in the serum of broodstocks supplemented with curcumin and thyroxine hormone for 12 weeks

The concentrations of estradiol in the serum of catfish supplemented with curcumin and thyroxine are presented in Table 2. In the initial week of observation, the average serum estradiol concentration in the experimental African catfish was 565.52±376.37 pg.mL^{-1}. The serum concentration of estradiol in catfish after three weeks of treatment showed no significant difference ($p > 0.05$) between treatments. In the sixth week of treatment, the statistical analysis showed a significant difference ($p < 0.05$) in serum estradiol concentration among groups of experimental catfish. Control catfish without curcumin and thyroxine supplementations (Group A) showed the highest estradiol concentration (1588±551.64 pg.mL^{-1}) followed by the catfish supplemented with curcumin and thyroxine (Group D; 948.9±162.89 pg.mL^{-1}), catfish supplemented with curcumin without thyroxine supplementation (Group B; 923.4 ±145.85 pg.mL^{-1}), and catfish supplemented with thyroxine without curcumin supplementation (Group C; 658.54±410.58 pg.mL^{-1}), respectively. The Tukey test showed that catfish without curcumin and thyroxine supplementations (Group A) had similar serum estradiol concentrations ($p > 0.05$) with catfish supplemented with curcumin and thyroxine (Group D) and catfish supplemented with curcumin without thyroxine supplementation (Group B), but differ ($p < 0.05$) from catfish

supplemented with thyroxine without curcumin supplementation (Group C).

In the ninth week of treatment, the serum estradiol concentration of catfish supplemented with curcumin and thyroxine (Group D), catfish supplemented with thyroxine without curcumin supplementation (Group C), and catfish supplemented with curcumin without thyroxine supplementation (Group B) had increase in serum estradiol concentrations. Conversely, catfish that were not supplemented with curcumin and thyroxine (Group A) had decrease in serum estradiol concentrations. However, the effect of curcumin and thyroxine supplementations did not show a significant difference ($p > 0.05$) among groups. In the 12th weeks of treatment, the catfish supplemented with curcumin (Group B and D) showed the higher values of serum estradiol concentration compared to groups that were not supplemented with curcumin (Groups A and C). Catfish supplemented with curcumin without thyroxine supplementation (Group B) showed the highest serum estradiol concentrations, while catfish without curcumin and thyroxine supplementations (Group A) showed the lowest serum estradiol concentrations. However, there was no significant difference ($p > 0.05$) between treatments for serum estradiol concentrations

3.2 Hepatosomatic index of broodstock catfish supplemented with curcumin and thyroxine hormone for 12 weeks

The hepatosomatic indexes (HSI) of African catfish supplemented with curcumin and thyroxine for 12 weeks are presented in Table 3. The average of HSI of the experimental catfish at the beginning of the experiment was 1.35±0.25 %. In the third week after supplementations of curcumin and thyroxine, the statistical analysis of HSI in all groups did not show a significant difference ($p > 0.05$). The same pattern was observed in the sixth, ninth, and 12th weeks of treatment. In the third week after supplementation of curcumin and thyroxine, there was an increase in HSI in all groups of experimental African catfish. In the sixth to ninth week after supplementation of curcumin and thyroxine, the HSI decreased, then increased again in week 12th.

Table 2. Serum estradiol concentrations ($pg.mL^{-1}$) in experimental catfish supplemented with curcumin and thyroxine for 12 weeks

Week of treatment	Thyroxine hormone ($mg.kg^{-1}$ feed)	Curcumin ($\%.kg^{-1}$ feed)	
		0	0.5
Three weeks	0	1558.57±895,46^{a}	1267.08±722.62^{a}
	0.1	955.88±299.66^{a}	1101.80±717.58^{a}
Six weeks	0	1588±551.64^{a}	923.4±145.85^{ab}
	0.1	658.54±410.58^{b}	948.9±162,89^{ab}
Nine weeks	0	1099.5±301.56^{a}	1017.33±316.42^{a}
	0.1	1219.3±523.84^{a}	1298.23±543.57^{a}
Twelve weeks	0	1202.80±427.34^{a}	1762.58±804.43^{a}
	0.1	1582.63±565.76^{a}	1713±523.20^{a}

Different superscripts in the same row and column indicate a significant difference

Table 3. Hepatosomatic index (HSI) of African catfish supplemented with curcumin and thyroxine for 12 weeks

Week of treatment	Thyroxine hormone ($mg.kg^{-1}$ feed)	Curcumin ($\%.kg^{-1}$ feed)	
		0	0.5
Three weeks	0	1.73±0.38^{a}	1.80±0.44^{a}
	0.1	2.01±0.22^{a}	1.75±0.19^{a}
Six weeks	0	1.27±0.23^{a}	1.44±0.24^{a}
	0.1	1.23±0.27^{a}	1.60±0.40^{a}
Nine weeks	0	1.26±0.08^{a}	1.10±0.10^{a}
	0.1	0.97±0.28^{a}	1.00±0.15^{a}
Twelve weeks	0	1.33±0.15^{a}	1.19±0.15^{a}
	0.1	1.15±0.05^{a}	1.36±0.38^{a}

Different superscripts in the same row and column indicate a significant difference

3.3 Gonadosomatic index (GSI) of broodstock that supplemented with curcumin and thyroxine hormone for 12 weeks

The gonadosomatic indexes of experimental African catfish supplemented with curcumin and thyroxine hormone for 12 weeks are presented in Table 4. The results of the statistical analysis of the gonadosomatic index (GSI) after curcumin and thyroxine supplementations in the third week showed no difference ($p > 0.05$) between groups. The same pattern was also observed in the sixth, ninth, and 12th weeks of treatment. The average of GSI in the initial week before treatment was 1.49±0.28 %. In the third week after curcumin and thyroxine supplementations, catfish supplemented with thyroxine (Groups C and D) tended to have a higher GSI compared to the catfish group without thyroxine supplementation (Groups A and B). In the sixth week of treatment, the groups that were not supplemented with curcumin (Groups A and C) showed the higher GSI compared to groups supplemented with curcumin (Groups B and D). In the ninth week after the treatment, catfish supplemented with curcumin (Group B and D) showed the higher GSI compared to the groups without curcumin supplementation (Groups A and C). At the end of the observation, in the 12th weeks after treatment the groups supplemented with curcumin and thyroxine (Group B, C, and D) showed a gonadosomatic indexes that tended to be higher than the control (Group A).

3.4 Diameter, diversity, and distribution of eggs in the catfish broodstock supplemented with curcumin and thyroxine for 12 weeks

The averages of egg diameter in African catfish during 12 weeks of curcumin and thyroxine supplementation are presented in Table 5. It was found that increase in the time of fish rearing was associated with the increase in the average of eggs diameter in all groups. In the third week after supplementation with curcumin and thyroxine, a statistical analysis of egg diameters showed a significant difference ($p < 0.05$) between groups. Catfish supplemented with curcumin and thyroxine (Group D) showed the highest average of egg diameter (0.97±0.07 mm) followed by catfish supplemented with thyroxine without curcumin supplementation (Group C; 0.95±0.08 mm), catfish supplemented with curcumin without thyroxine supplementation (Group B; 0.90±0.02 mm), and catfish without curcumin and thyroxine supplementations (Group A; 0.85±0.07 mm), respectively. The Tukey test showed that catfish supplemented with curcumin and thyroxine (Group D) did not differ ($p > 0.05$) from catfish supplemented with thyroxine without curcumin supplementation (Group C) and catfish supplemented with curcumin without thyroxine supplementation (Group B), but significantly different ($p < 0.05$) from catfish without curcumin and thyroxine supplementation (Group A/control). In the sixth week after curcumin and thyroxine supplementation, the statistical analysis showed that the averages of egg diameter did not differ ($p > 0.05$) between groups. The same pattern also appeared in the ninth week and 12th weeks after treatment.

Table 4. Gonadosomatic index of experimental catfish supplemented with curcumin and thyroxine for 12 weeks

Week of treatment	Thyroxine hormone (mg.kg^{-1} feed)	Curcumin (%.kg^{-1} feed)	
		0	0.5
Three weeks	0	4.97±3.34^{a}	3.08±1.63^{a}
	0.1	5.50±3.11^{a}	6.25±1.77^{a}
Six weeks	0	11.96±4.62^{a}	6.60±3.00^{a}
	0.1	9.88±3.45^{a}	6.69±2.86^{a}
Nine weeks	0	12.23±3.58^{a}	15.76±3.01^{a}
	0.1	13.33±2.68^{a}	13.84±5.61^{a}
Twelve weeks	0	15.17±1.65^{a}	16.96±2.18^{a}
	0.1	18.02±3.54^{a}	17.00±3.76^{a}

Different superscripts in the same row and column indicate a significant difference

Table 5. Eggs diameter (mm) of experimental catfish supplemented with curcumin and thyroxine for 12 weeks

Week of treatment	Thyroxine hormone (mg.kg^{-1} feed)	Curcumin (%.kg^{-1} feed)	
		0	0.5
Three weeks	0	0.85±0.07^{b}	0.90±0.02^{a}
	0.1	0.95±0.08^{a}	0.97±0.07^{a}
Six weeks	0	0.99±0.05^{a}	0.96±0.11^{a}
	0.1	1.06±0.02^{a}	1.06±0.03^{a}
Nine weeks	0	1.18±0.06^{a}	1.20±0.02^{a}
	0.1	1.22±0.05^{a}	1.22±0.07^{a}
Twelve weeks	0	1.21±0.05^{a}	1.26±0.03^{a}
	0.1	1.24±0.05^{a}	1.24±0.03^{a}

Different superscripts in the same row and column indicate a significant difference

Table 6. The coefficient of variation in egg diameter (%) of experimental catfish supplemented with curcumin and thyroxine for 12 weeks

Week of treatment	Thyroxine hormone (mg.kg^{-1} feed)	Curcumin (%.kg^{-1} feed)	
		0	0.5
Three weeks	0	31.72±1.53^{a}	32.34±4.15^{a}
	0.1	27.52±11.69^{a}	31.55±1.69^{a}
Six weeks	0	31.96±6.04^{a}	31.24±4.86^{ab}
	0.1	22.34±3.56^{b}	23.70±0.92^{ab}
Nine weeks	0	18.44±2.73^{a}	15.15±1.99^{ab}
	0.1	14.34±0.94^{b}	14.55±1.16^{b}
Twelve weeks	0	17.37±4.56^{a}	12.16±2.24^{ab}
	0.1	9.59±2.26^{b}	9.64±1.02^{b}

Different superscripts in the same row and column indicate a significant difference

The variances of eggs diameter in catfish supplemented with curcumin and thyroxine are presented in Table 6. The results of this study indicated that the variance in egg diameter decreases with the development of gonads. In the third week after the treatment, there was no difference ($p > 0.05$) in the variance of egg diameters among groups. Catfish supplemented with thyroxine (Group C and D) tended to have a lower variance compared to the groups without thyroxine supplementation (Groups A and B). After the sixth week of curcumin and thyroxine supplementation, the statistical results of the variance in the egg diameters showed a significant difference ($p < 0.05$) among the groups. Catfish without curcumin and thyroxine supplementation (Group A/control) showed the highest variance in egg diameter (31.96±6.04 %) followed by catfish supplemented with curcumin without thyroxine supplementation (Group B; 31.24±4.86 %), catfish with thyroxine and curcumin supplementation (Group D; 23.70±0.92 %), and catfish supplemented with thyroxine without curcumin supplementation (Group C; 22.34±3.56 %), respectively. The Tukey test showed that catfish without curcumin and thyroxine supplementation (Group A) did not differ ($p > 0.05$) from catfish supplemented with curcumin without thyroxine supplementation (Group B) and catfish supplemented with curcumin and thyroxine (Group D), but significantly different ($p < 0.05$) from catfish supplemented with thyroxine without curcumin supplementation (Group C).

In the ninth week of treatment, the results of statistical analysis showed a significant difference ($p < 0.05$) in the variances of egg diameters among groups. Catfish that were not supplemented with curcumin and thyroxine (Group A) showed the highest variance in egg diameter (18.44±2.73 %) which were followed by the catfish supplemented with curcumin without thyroxine supplementation (Group B; 15.15±1.99 %), catfish supplemented with curcumin and thyroxine (Group D; 14.55±1.16 %), and catfish supplemented with thyroxine without curcumin supplementation (Group C; 14.34±0.94 %), respectively. The Tukey test showed that catfish without curcumin and

thyroxine supplementation (Group A) did not show a significant difference ($p > 0.05$) in the variance of egg diameter compared to catfish supplemented with curcumin without thyroxine supplementation (Group B), but significantly different ($p < 0.05$) from catfish supplemented with thyroxine without curcumin supplementation (Group C), and catfish supplemented with curcumin and thyroxine (Group D). During the 12 weeks of treatment, the results of statistical analysis of variance in egg diameters showed the significant differences ($p < 0.05$) between groups. Catfish without curcumin and thyroxine supplementation (Group A) showed the highest variance in egg diameter (17.37±4.56 %) which were followed by catfish supplemented with curcumin without thyroxine supplementation (Group B; 12.16±2.24 %), catfish supplemented with curcumin and thyroxine (Group D; 9.64±1.02 %), and catfish supplemented with thyroxine without curcumin supplementation (Group C; 9.59±2.26 %). The Tukey test showed that catfish without curcumin and thyroxine supplementation (Group A) did not have significant difference in egg diameter variance ($p > 0.05$) compared to catfish supplemented with curcumin without thyroxine supplementation (Group B), but significantly different ($p < 0.05$) from catfish supplemented with thyroxine without curcumin supplementation (Group C), and catfish supplemented with curcumin and thyroxine (Group D).

Figure 1. Distribution of eggs diameter during 12 weeks of curcumin and thyroxine hormone treatment. A: Control without curcumin and thyroxine supplementation); B: Curcumin supplementation without thyroxine supplementation; C: Thyroxine supplementation without curcumin supplementation; D: Supplemented with curcumin and thyroxine

Figure 1 shows the distributions of egg diameter from the initial week to twelve weeks of curcumin and thyroxine hormone supplementation. In line with the time of rearing there was an increase in egg diameters in all groups. In the initial week of treatment, the percentage of eggs with diameter ≥ 1.0 mm was 30 %. In the third week of treatment, the percentage of eggs with a diameter ≥ 1.0 mm in catfish without curcumin and thyroxine supplementation (Group A) was 35.18 %, catfish supplemented with curcumin without thyroxine supplementation (Group B) was 46.11%, catfish supplemented with thyroxine without curcumin supplementation (Group C) was 53.02.5 %, and catfish supplemented with curcumin and thyroxine (Group D) was 53.08 %. In the sixth week of treatment the percentage of eggs with diameter ≥ 1.0 mm in group A, B, C, and D were 66.63 %, 57.89 %, 73.67 %, and 73.50 %, respectively. In the ninth week of the treatment the percentage of eggs with a diameter ≥ 1.0 mm for group A, B, C, and D were 83.38 %, 87.05 %, 87.59 %, and 88.56 %, respectively. During the 12th week of treatment the percentage of eggs with a diameter ≥ 1.0 mm in group A, B, C, and D were 86.75 %, 92.74 %, 95.10 %, and 94.20 %, respectively.

3.5 Percentage of gonad maturity and the relative fecundity of female catfish broodstock supplemented with curcumin and thyroxine for 12 weeks

After 12 weeks of treatment, it was calculated the percentage of fish with gonad maturity marked with the large size of belly and with a soft texture when it was touched. Table 7 presents data on the percentage of gonad maturity and relative fecundity produced. The results of the statistical analysis showed no significant difference ($p > 0.05$) among groups. Nevertheless, the group supplemented with curcumin (Group B and D) tended to have a higher catfish with percentage of gonad maturity than the groups without curcumin supplementation (Groups A and C).

The results of the statistical analysis of the relative fecundity of experimental catfish showed a significant difference ($p > 0.05$) between groups. Catfish supplemented with curcumin without thyroxine supplementation (Group B) showed the highest relative fecundity (163,038±12,831 eggs.kg^{-1}BW) then followed by catfish supplemented with thyroxine without curcumin supplementation (Group C; 131,582±4,053 eggs.kg^{-1}BW), catfish supplemented with curcumin and thyroxine (Group D; 116,323±21,037 eggs.kg^{-1}BW), and catfish without curcumin and thyroxine supplementation (Group A; 111,685±5,850 eggs.kg^{-1}BW), respectively. The Tukey test showed that catfish supplemented with curcumin without thyroxine supplementation (Group B) were significantly different ($p < 0.05$) from catfish supplemented with thyroxine without curcumin supplementation (Group C), catfish supplemented with curcumin and thyroxine (Group D), and catfish without curcumin and thyroxine supplementation (Group A/control).

Table 7. Percentage of gonads maturity and the relative fecundity of female catfish broodstock supplemented with curcumin and thyroxine for 12 weeks

Parameter	Thyroxine hormone (mg.kg^{-1} feed)	Curcumin (%.kg^{-1} feed)	
		0	0.5
Percentage of fish with gonad maturity (%)	0	81.50±13.99^{a}	95.00±10.00^{a}
	0.1	82.60±11.90^{a}	90.00±20.00^{a}
Relative fecundity (numbers of eggs.kg^{-1}BW)	0	111,685±5,850^{b}	163,038±12,831^{a}
	0.1	131,582±4,053^{b}	116,323±21,037^{b}

Different superscripts in the same row and column indicate a significant difference

3.6 Egg vitellogenin concentration before ovulated, the diameter and variance in eggs diameter of ovulated eggs from catfish supplemented with curcumin and thyroxine for 12 weeks

The vitellogenin concentrations in the eggs at the end of 12 weeks of treatment are presented in Table 8. The results of the statistical analysis of egg vitellogenin concentration showed that there were significant differences ($p < 0.05$) among groups. Catfish supplemented with curcumin and thyroxine (Group D) produced the highest egg vitellogenin concentrations (8.17 ± 2.74 $\mu g.mL^{-1}$) followed by the catfish supplemented with curcumin without thyroxine supplementation (Group B; 6.65 ± 1.10 $\mu g.mL^{-1}$), catfish supplemented with thyroxine without curcumin supplementation (Group C; 6.41 ± 0.21 $\mu g.mL^{-1}$), and catfish without curcumin and thyroxine supplementation (Group A; 4.97 ± 0.82 $\mu g.mL^{-1}$). The Tukey test showed that the eggs vitellogenin concentration of catfish supplemented with curcumin and thyroxine (Group D) was not significantly different ($p > 0.05$) from catfish supplemented with curcumin without thyroxine supplementation (Group B) and catfish supplemented with thyroxine without curcumin supplementation (Group C), but different ($p < 0.05$) from catfish without curcumin and thyroxine supplementation (Group A). These results indicated that the catfish supplemented with curcumin (Group B and D) tended to have a higher egg vitellogenin concentrations than the group without curcumin supplementation (Groups A and C).

The fishes with gonad maturity were spawned artificially. The results of statistical analysis of ovulated egg diameter showed a significant difference ($p < 0.05$) among groups. Egg diameter from catfish supplemented with curcumin without thyroxine supplementation (Group B) showed the highest value (1.44 ± 0.01 mm) then followed by catfish supplemented with curcumin and thyroxine (Group D; 1.43 ± 0.00 mm), catfish supplemented with thyroxine without curcumin supplementation (Group C; 1.37 ± 0.03 mm), and catfish without curcumin and thyroxine supplementation (Group A; 1.36 ± 0.02 mm). The results of the Tukey test showed that the ovulated egg diameter from catfish supplemented with curcumin without thyroxine supplementation (Group B) did not differ ($p > 0.05$) from catfish supplemented with curcumin and thyroxine (Group D), but significantly different ($p < 0.05$) from catfish supplemented with thyroxine without curcumin supplementation (Group C) and catfish without curcumin and thyroxine supplementation (Group A). These results indicated that the group of catfish supplemented with curcumin (Group B and D) had a larger ovulated-egg diameter than groups without curcumin supplementation (Groups A and C).

Table 8. Egg vitellogenin concentration before ovulated, the diameter and coefficient of variation in eggs diameter of ovulated eggs from catfish supplemented with curcumin and thyroxine for 12 weeks.

Parameters	Thyroxine hormone ($mg.kg^{-1}$ feed)	Curcumin ($\%.kg^{-1}$ feed)	
		0	0.5
Vitellogenin concentration in eggs (µg/ml)	0	4.97 ± 0.82^{b}	6.65 ± 1.10^{ab}
	0.1	6.41 ± 0.21^{ab}	8.17 ± 2.74^{a}
Diameter of ovulated eggs (mm)	0	1.36 ± 0.02^{b}	1.44 ± 0.01^{a}
	0.1	1.37 ± 0.03^{b}	1.43 ± 0.00^{a}
Coefficient of variation in ovulated eggs diameter (%)	0	4.36 ± 0.31^{b}	4.65 ± 0.27^{ab}
	0.1	5.39 ± 0.57^{a}	4.94 ± 0.58^{ab}

Different superscripts in the same row and column indicate a significant difference

The variance in the diameters of ovulated eggs showed a significant difference ($p < 0.05$) between groups. Catfish supplemented with thyroxine without curcumin supplementation (Group C) had the highest value (5.39±0.57 %) of variance in the diameter of ovulated eggs then followed by catfish supplemented with curcumin and thyroxine (Group D; 4.94±0.58 %), catfish supplemented with curcumin without thyroxine supplementation (Group B; 4.65±0.27 %), and catfish without curcumin and thyroxine supplementation (Group A; 4.36±0.31 %). The results of the Tukey test showed that the variance in the diameter of ovulated eggs in catfish supplemented with thyroxine without curcumin supplementation (Group C) were not different ($p > 0.05$) from catfish supplemented with curcumin and thyroxine (Group D) and catfish supplemented with curcumin without thyroxine supplementation (Group B) but different ($p<0.05$) from catfish without curcumin and thyroxine supplementation (Group A).

3.7 The lipid concentrations of ovulated eggs in catfish supplemented with curcumin and thyroxine for 12 weeks

Lipid concentrations in the ovulated eggs in experimental African catfish are presented in Table 9. The results of statistical analysis showed that total cholesterol concentrations in the ovulated eggs did not significantly different ($p > 0.05$) between groups. Nevertheless, it appeared that the group supplemented with curcumin (Group B and D) tended to have higher egg cholesterol concentrations than those without curcumin supplementation (Groups A and C). The HDL concentration in eggs in all groups did not show any significant difference ($p>0.05$). Catfish supplemented with thyroxine without curcumin supplementation (Group C) tended to show a higher concentration of HDL in the eggs compared to the other groups, and catfish that were not supplemented with curcumin and thyroxine (Group A) tended to have a lower concentration of HDL in the eggs than the other treatment groups.

The results of statistical analysis of triglyceride concentrations in the ovulated eggs showed a significant difference ($p < 0.05$) among groups. Catfish supplemented with curcumin and thyroxine (Group D) had the highest concentration of egg triglyceride (4.89±0.53 $mg.g^{-1}$) then followed by the catfish supplemented with curcumin without thyroxine supplementation (Group B; 3.54±0.12 $mg.g^{-1}$), catfish supplemented with thyroxine without curcumin supplementation (Group C; 3.38±0.91 $mg.g^{-1}$), and catfish without curcumin and thyroxine supplementation (Group A; 3.11±0.81 $mg.g^{-1}$), respectively. The Tukey test showed that the concentrations of egg triglycerides in African catfish supplemented with curcumin and thyroxine (Group D) were not different ($p > 0.05$) from catfish supplemented with curcumin without thyroxine supplementation (Group B), but significantly different ($p<0.05$) from catfish supplemented with thyroxine without curcumin supplementation (Group C), and catfish without curcumin and thyroxine supplementation (Group A).

3.8 The fertilization and hatching rate of eggs ovulated by catfish supplemented with curcumin and thyroxine for 12 weeks

The fertilization rates of ovulated eggs and hatching rate of fertilized eggs in the experimental African catfish are presented in Table 10. The results of the statistical analysis showed that there was no significant difference ($p > 0.05$) in the fertilization rates of ovulated egg among groups. Nevertheless, the fertilization rate of ovulated eggs in catfish supplemented with curcumin (Groups B and D) tended to be higher than in catfish without curcumin supplementation (Groups A and C). The same pattern was also found in the hatching rate which showed that there was no difference ($p > 0.05$) in hatching rates of fertilized eggs among groups. The hatching rate of fertilized eggs in catfish supplemented with curcumin (Groups B and C) tended to be higher than in control catfish without supplementation of curcumin and thyroxine hormone (Groups A and C).

Table 9. The lipid concentrations of ovulated eggs in catfish supplemented with curcumin and thyroxine for 12 weeks.

Parameters	Thyroxine hormone (mg.kg^{-1} feed)	Curcumin (%.kg^{-1} feed)	
		0	0.5
Cholesterol (mg/g sample)	0	2.70±0.97^{a}	3.29±0.49^{a}
	0.1	3.12±0.52^{a}	3.19±0.74^{a}
HDL (mg/g.sample)	0	0.14±0.01^{a}	0.17±0.03^{a}
	0.1	0.18±0.04^{a}	0.16±0.05^{a}
Triglyceride (mg/g sample)	0	3.11±0.81^{b}	3.54±0.12^{ab}
	0.1	3.38±0.91^{b}	4.89±0.53^{a}

Different superscripts in the same row and column indicate a significant difference

Table 10. The fertilization of ovulated eggs and hatching rate of fertilized eggs in African catfish supplemented with curcumin and thyroxine for 12 weeks

Parameters	Thyroxine hormone (mg.kg^{-1} feed)	Curcumin (%.kg^{-1} feed)	
		0	0.5
Fertilization rate (%)	0	94.95±3.07^{a}	97.67±1.66^{a}
	0.1	96.69±5.11^{a}	97.57±2.92^{a}
Hatching rate (%)	0	86.13±7.26^{a}	89.70±3.55^{a}
	0.1	84.36±11.67^{a}	87.35±9.59^{a}

Different superscripts in the same row and column indicate a significant difference

4. Discussion

The results of the present experiment in African catfish confirm that curcumin supplementation increased vitellogenin synthesis and secretions into the circulation that eventually increased its deposition in the developing oocytes to support the growth and development of embryo and larvae. Curcumin has well-known activity as a hepatoprotective that improved the biological conditions of hepatocytes (Dewi et al., 2018b) to synthesize vitellogenin under the stimulation of estradiol. The stimulation of the hepatocytes to synthesize vitellogenin in started the binding of estradiol to its receptors in the hepatocyte cells of the liver. The binding between estradiol and its receptor in the hepatocyte immediately stimulates the cells to synthesize vitellogenin which is the precursor of egg yolk. The supplementation of thyroxine hormone during reproduction in catfish will stimulate ATP availabilities in the hepatocyte that will support vitellogenin synthesis. In addition, the thyroxine hormone supplemented in the feed of broodstocks will be deposited in the oocytes (Iromo et al., 2014) that will stimulate the production of ATP to support the synthesis of material and molecules required by the growing and developing embryos and larvae.

The presence of estradiol during the reproductive period will determine the activity of vitellogenin synthesis. The increased estradiol synthesis will stimulate vitellogenin synthesis for gonadal development (Lee and Yang, 2002). However, the physiological function of the liver plays a significant role in vitellogenin synthesis. Improved liver functions by curcumin supplementation dramatically increase the productivity of the liver to synthesize vitellogenin (Dewi et al., 2018b). This study showed that supplementation of curcumin and thyroxine in catfish did not directly increase the level of estradiol in the blood but increases the productivity of the liver thereby increasing the amount of vitellogenin produced which was reflected in a higher vitellogenin concentration in the eggs produced and the increased the recruitment of growing oocytes as was reflected in the increased number of ovulated eggs.

In addition to the presence of estradiol, the physiological state of the liver has a major influence on vitellogenin synthesis (Kasiyati et al., 2016b). However, the supplementation of curcumin not only protects the liver cells but also increases their bioactivities as phytoestrogen that binds to the estradiol receptor and further stimulates the synthesis of vitellogenin (Kasiyati et al., 2016a; Dewi et al., 2018b). Research conducted by Turker and Bozcaarmutlu (2009) showed that feed containing high levels of phytoestrogens could increase vitellogenin production in carp.

Nevertheless, the binding activity of curcumin as phytoestrogen to the estradiol receptors in the liver cells is very weak compared to estradiol itself (Bachmeier et al., 2010).

In the present study, it appears that thyroxine supplementation can increase the vitellogenin content in the eggs and increase the number of ovulated eggs even though it is still lower when compared to those supplemented with curcumin. This ability is thought to be due to the thyroxine activity that increases the protection of the liver through the mechanism of antioxidant activity (Salami et al., 2016; Baskol et al., 2007) and increases estradiol synthesis from granulosa cells (Syano et al., 1993). The research conducted by Iromo et al. (2014) show that thyroxine hormone is needed during the ovarian development. This condition is confirmed by the increased concentrations of these hormones in every developmental process of vitellogenesis in the crabs. According to Manalu et al. (1997) thyroid hormones are involved in the processes that affect metabolic activity through the provision of a stream of nutrients and minerals and in the provision of ATP during the process of ovarian maturity, especially in the process of assembling glucose, amino acids, fatty acids, and glycerol into glycogen, protein, and fat during vitellogenesis.

The increases in HSI at the beginning and the end of the observations indicate that liver cells undergo proliferation. According to Dewi et al. (2018a), an increase in liver weight was related to DNA concentration in the liver tissue. The increase in the number of liver cells detected at the beginning of the study was marked by the increase in the liver weight which further increased the HSI. Increase in the liver weight is thought to be a response to the stimulation of estradiol because the liver is the site of vitellogenin synthesis. Therefore, observations in the following week showed the HSI was decreased. The decrease in HSI is due to an increase in body weight that was not accompanied by the increase in liver weight. This condition indicates that cellular activity that occurs in hepatocyte cells is dominated by protein synthesis activities, in this case, vitellogenin synthesis.

In line with the time of rearing, the GSI also increases. Increase in the GSI is the reflection of the increased gonad weight as the end product of the vitellogenesis. Vitellogenin produced in the liver is then transported into the gonad and deposited in the developing oocytes. The increased gonadosomatic index observed in the present experiment is partly due to the increased deposition of vitellogenin in the developing oocytes as was reflected in the increase in the size of the egg diameter and mass which ultimately increases the size and mass of the gonads in fish (Yaron and Silvan, 2006). In addition, the increased vitellogenin synthesis will recruited a higher number of developing oocytes that eventually will increase the population of growing and developing oocytes in the ovary that will eventually increase gonad weight and GSI. After the egg yolk formation ends, the oocytes do not change shape for a while waiting for good environmental conditions (this stage is called the resting or dormant stage) (Lee and Yang, 2002). The results of this study indicated that supplementation of curcumin and thyroxine accelerated the time of gonad maturity with the percentage of eggs diameter $\geq$ 1 mm reaching more than 70% in the sixth week compared to controls. These results have impacts on the percentage of fish with gonad maturity in catfish supplemented with curcumin and thyroxine that tends to be higher compared to control catfish without curcumin and thyroxine hormone.

In this study, the supplementation of curcumin in broodstock significantly increased the relative fecundity by 45.98% compared to control. The increase in relative fecundity is related to the increase in vitellogenin synthesis which then increases the number of oocytes recruited to develop and further deposited with vitellogenin in sufficient quantities. The results of this study indicate that the African catfish supplemented with curcumin and thyroxine hormone had higher deposition of vitellogenin in the developing oocytes as is reflected in the higher vitellogenin concentrations in ovulated eggs. Deposition of vitellogenin in the eggs can be affected by the number of vitellogenin synthesized and then released into the blood circulation and transported into the gonad and deposited in into the developing oocytes by mechanism of pinocytosis. Vitellogenin concentrations in the ovulated eggs increased in catfish supplemented with curcumin. The results observed in the present experiment showed that the concentration of vitellogenin in eggs are more affected by the supplementation of curcumin than thyroxine supplementation. In previous studies, curcumin increased follicular development and increased follicular hierarchy in poultry (Radwan et al., 2008; Kasiyati et al., 2016b; Saraswati et al., 2013) and increased the fecundity in catfish (Dewi et al., 2018a). Fish eggs contain nutrients that are needed to support cellular growth and homeostatic during

the development of the embryo until the larvae begin to eat (Fyhn, 1989).

Curcumin supplementation showed the increased diameter of ovulated eggs in African catfish. African catfish supplemented with curcumin ovulated eggs with higher diameters than those without curcumin supplementation. Before ovulated or injection of ovaprim, catfish supplemented with curcumin without thyroxine supplementation tended to show larger egg diameters than the other groups. However, there is no significant difference in egg sizes between all groups. The variance of egg diameter of all groups was smaller at the time after ovulation than before ovulation or injection with ovaprim. Therefore, the egg diameter after ovulation is more uniform. This condition is related to the observation of some chemical changes during the growth and development of eggs up to spawning (Matsubara et al., 1999).

The contents of lipids in ovulated eggs tend to be higher in African catfish supplemented with curcumin and thyroxine. This high lipids contents could be one of the factors that influence the qualities of the oocytes so that the fertilization rate of ovulated oocytes and hatching rate of fertilized eggs tends to be higher. In the present study it was found that the fertilization rate of ovulated eggs and hatching rate of fertilized eggs in all groups of experimenta African catfish were quite high, ranging from 94.95-97.67% and 84.36-89.70%, respectively. The same thing is also reported by Sunarma (2016) on the broodstock of catfish *Clarias gariepinus* which are given 45% protein feed resulting in a high level of fertilization rate of ovulated eggs and hatching rate of fertilized eggs. According to Tong et al. (2017) during the process of embryogenesis to hatching, the dominant energy used is carbohydrate during the period before division, amino acids at the gastrula stage, and fatty acids until the second day after hatching. Fertilized eggs and larval egg sacs are rich in neutral lipids which decrease in number during the developmental process (Vazques et al., 1994).

The higher hatching rate in the African catfish supplemented with curcumin can be related to the energy and nutrients availability during embryogenesis up to hatching that is provided by the increased concentrations of vitellogenin in the ovulated eggs. The vitellogenin contains all nutrients and materials required by the growing and developing embryo up to the early development of larval life. The significant increase in fertilization rate of ovulated eggs and hatching rates of fertilized eggs as wells the survival rate of the larvae 8 days after hatching without feeding were reported in Siam catfish supplemented with turmeric powder (Dewi et al., 2019). These additional data confirm that improved liver functions of oviparous animals could improve vitellogenin synthesis and secretions and deposition into the ovulated eggs that will optimize the biological processes to support embryonic growth and development.

4. Conclusion

Supplementation of curcumin and thyroxine in catfish improves vitellogenin deposition in the ovulated eggs that have a great potential to improve the reproductive performance of female broodstock and the egg quality produced by increasing the relative fecundity, egg diameter, egg vitellogenin content, egg lipids concentration, fertilization rate, and hatching rate. The optimum environment during embryonic growth and development will optimize the genes expressions to produce superior offspring in aquaculture hatchery.

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