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The Role of Turmeric (Curcuma longa) Powder in Improving Liver Function to Increase Vitellogenin Synthesis and Deposition in the Oocytes of Catfish (Pangasianodon hypophthalmus)

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Abstract

This experiment was designed to improve nutrients deposition in the oocytes of catfish by improving the liver functions through supplementation of turmeric powder in the feed and to evaluate the optimum dosage of turmeric powder supplementation in the feed of catfish. The experiment used 40 catfish with the average body weight of 3.75 kg. Forty experimental catfish were divided into 4 treatments, and each treatment used 10 catfish. The treatments were doses of turmeric supplementation in the diet consisting of 0 mg/100 g feed (T0 as a control), 120 mg/100 g feed (T1), 240 mg/100 g feed (T2), and 480 mg/100 g feed (T3). The experimental catfish were fed 2 times a day in the morning and in the afternoon at the level of 3% body weight for 8 weeks. The results of the experiment showed that turmeric powder supplementation at a dose of 480 mg/100 g ration could increase the absolute body weight of the catfish, vitellogenin deposition in the eggs, and gonad development. The results of the present experiment indicate that turmeric powder supplementation of the catfish can be used to improve reproduction performance of catfish and teleost fish.

Keywords: Turmeric powder, Catfish (Pangasianodon hypothalamus), Egg Vitellogenin, Gonad Development

1. Introduction

The production of catfish in Indonesia increases every year, but the production level has not reached the production target. The efforts to increase the production of catfish have been conducted; however, to reach the optimum production, it is required to improve and optimize the physiological functions of body organs of the catfish. Catfish farmers are facing limitations in breeding so that demands for larvae, juvenile, and fries production for catfish farmers have not been achieved optimally. In catfish farming and aquacultures, there are some problems and limitation in the availability of quality larvae, juvenile, and fries by qualities brood stocks. Therefore, to obtain good quality larvae the strategy can be started from improving the quality of eggs (Kjorsvik *et al*., 1990).

The quality of eggs can be improved by improving the feed quality of the brood stocks. To meet the expectations, one way is to increase reproduction by increasing the brood stocks quality through the feed. The feed is an important component in the process of vitellogenin synthesis. Basically, vitellogenesis is a process of nutrients accumulation in the oocyte so that the availability of egg yolk in the oocyte will determine the qualities of oocytes (Sequeira *et al.,* 2012). The feeding of fish with a quality feed is required for meeting the nutrient requirement during gonad growth and development in the brood catfish. The nutrients content of ration is an important factor in determining ration quality.

In order to support an optimum reproduction process of the brood catfish, it is required to add materials that contain certain nutrients. One of the materials that can be used is turmeric (*Curcuma longa*). Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae (Chan *et al.,* 2009). Turmeric powder contains a flavonoid that has phytoestrogen activity acting like estrogen in stimulating the liver to synthesize vitellogenin. Turmeric powder contains curcumin, beta carotene, fat 5.1%, carbohydrate 69.4%, protein 6.3%, vitamin B1, B2, B6, B12, and Vitamin E (Ravindran *et al*., 2007; Pari *et al*., 2008; Dono, 2013). Curcumin has a hepatoprotective activity that can prevent and cure the destruction of hepatocytes (Tung *et al.* 2017) and improve the hepatocyte function in synthesizing vitellogenin under the stimulation of estrogen (Saraswati *et al.,* 2013).

Our preliminary studies in catfish strongly confirm that turmeric supplementation improves liver function and increases total egg production (Dewi *et al.,* 2018). The present experiment was designed to investigate the use of curcumin in turmeric powder to improve vitellogenin deposition in the oocyte and gonad development of the experimental catfish.

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2. Materials and Methods

The experiment was conducted from May to August 2015. The experiment was conducted in the Babakan Experimental Pond, Faculty of Fishery and Marine Science, IPB University. The measurement of vitellogenin contents of the ovulating eggs was conducted in the Laboratory of Biochemistry of Inter-University Center, Bogor Agricultural University. Forty catfish (*Pangasionodon hypopthalmus*), cultured in local freshwater with the average body weight of 3.75 kg, were used as experimental models. The catfish were chosen due to their high fecundity. The experimental catfish were maintained in a 28 x 20 x 6m^3 maintenance pond. The optimum supplemental dose of turmeric powder for catfish (2.4 g/kg feed) was calculated according to the method of Laurence and Bacharach (1964) based on the optimum dose of turmeric powder in human. The experimental doses used were no turmeric supplementation, half of the optimum dose, the optimum dose, and twice the optimum dose. Turmeric (with 5.34% curcumin content) was mixed with commercial feed to produce the treatment doses of 0, 120, 240, and 480 mg/100 g feed.

2.1. Catfish Maintenance

The catfish were acclimated to the experimental conditions and the ponds for 1 month before treatment after being selected based on their gonad maturity (stage I). During the maturation process, the experimental catfish were kept in the maintenance pond, which was later partitioned into 4 smaller 7 x 5 x 1.5m^3 experimental net cages. Each net cage contained 10 catfish that were subjected to these conditions for 8 weeks. During the experiment, the catfish were fed commercial feed with a protein content of 38% two times a day (in the morning and in the afternoon). Feed was provided at 3% of the BW, and the nutrient composition of the feed is presented in Table 1.

Table 1. Proximate analyses of experimental rations supplemented with turmeric powder at doses of 0, 120, 240, and 480 mg/100 g commercial ration.

The dose of turmeric (mg/100 g ration)	Protein	Fat	Ash	Water	Carbohydrate	
					Crude Fiber	NFE
0 (T0)	32.99	7.61	9.23	8.06	4.45	37.66
120 (T1)	32.76	7.68	8.95	8.40	5.35	36.86
240 (T2)	31.60	7.09	8.92	8.69	4.63	39.07
480 (T3)	31.81	7.29	9.06	8.37	4.22	39.25

2.2. Samples Collections and Measurements

At the end of 8 weeks of turmeric supplementation, the experimental catfish were sacrificed to observe the gonad. Before being sacrificed, the experimental catfish were weighed for measuring body weight. Each group of experimental catfish was represented by 3 catfish to be sacrificed. The gonad of selected experimental catfish was evaluated by collecting the gonads of 3 selected experimental catfish. The weights of gonads were measured. The eggs vitellogenin concentrations were measured to determine the gonad maturity due to turmeric supplementation.

Vitellogenin concentrations of the eggs were measured in two steps. The first step was the isolation of the vitellogenin from the eggs of experimental catfish by using SDS polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad, Hercules, California, USA) (Walker, 2002). The second step was the quantification of the isolated vitellogenin using the Bradford method (Kruger, 2002).

In the isolation of vitellogenin, 1g of egg sample of the experimental catfish was mixed with 15 µL of sample buffer and the mixture was dissolved in distilled water with the final volume of 1 mL. The mixture was heated at 100°C for 5 minutes. Fifteen microliters (15 µL) of this mixture of sample preparation was used per well, so the weight of egg sample in each well was 15 µg. The electrophoresis was run at 60 mV and 20 mA for 4 hours. After completing the electrophoresis, the gel was processed for silver staining, and then the gel was put into a stop solution for 5 minutes. Then, the gel was washed with ddH2O for 5 minutes and scanned. The relative mobility (Rf) of the sample was measured, and the molecular weights of the proteins in the sample were calculated using the standard protein marker.

The molecular weight of 240 kDa for avian vitellogenin (Deeley *et al.*, 1975) was used as a criterion for selecting and isolating the vitellogenin. Therefore, in this method, the band with 240 kDa was cut and separated, ground with deionized distilled water (ddH_2O) and then centrifuged at 3360 g for 15 minutes. The supernatant was added with ddH_2O to make a final volume of 200 µL. Then, the supernatant was added to 2 mL of the Bradford solution and then vortexed, and the solution was allowed to sit for 3–15 minutes (stable for 1 hour). Then, 3.3333 µL of the mixture of supernatant and Bradford solution was used to measure the absorbance of the solution using a spectrophotometer at the wave length of 595 nm. The blank solution was made by mixing 200 µL of deionized distilled water with 2 mL of the Bradford solution. The absorbance of the blank solution was also measured. The corrected absorbance of the sample was calculated by reducing the sample absorbance with the blank absorbance.

Then, the standard curve was made by using bovine serum albumen (Sigma) as a standard protein at the ranges of 0, 0.02, 0.04, 0.06, 0.08 and 0.10 mg/mL, which equals the range of concentrations of 0, 20, 40, 60, 80 and 100 ppm. The concentration of sample protein (mg in 200 µL egg) can be calculated from the equation of the standard curve of the BSA. The final concentration of the vitellogenin concentrations of the egg (mg/mL) was calculated by correcting the dilution of the eggs sample in the analysis.

Further, the gonad organs were isolated and then stored in 10% of buffered neutral formalin for histological preparation. Qualitative observations of gonad tissues were determined from the description of histological observations of the gonad by using paraffin and Hematoxcylin-Eosin staining (Bancroft and Gamble, 2008).

2.3. Water Quality Parameters

The water quality parameters measured were temperature, pH, and dissolved oxygen. Temperature was measured by using thermometer. The water pH was

measured by using Ph-meter. The dissolve oxygen in the water was measured by DO meter.

2.4. Data Analyses

The collected data were analyzed with Analyses of Variance by using Microsoft Excel 2010 and SPSS version 16.0. When the effect of treatment was significant, the Duncan Test was conducted to test the difference between doses of turmeric supplementation with 95% confidential interval.

3. Results

3.1. Absolute Body Weight

The observation on the absolute body weight gain of experimental catfish fed with commercial ration supplemented with turmeric powder at doses of 0, 120, 240, and 480 mg/100 g ration is presented in Table 2. The statistical analyses showed that the supplementation of catfish with turmeric powder significantly increased absolute body weight gain ($P<0.05$). The Duncan test also manifested that catfish fed ration supplemented with turmeric powder at a dose of 480 mg/100 g ration showed the highest absolute body weight gain (4.17 kg) that was significantly different from the other doses of turmeric supplementation. Turmeric powder supplementation at doses of 120 and 240 mg/100 g ration did not significantly increase absolute body weight gains compared to control catfish without turmeric powder supplementation ($P>0.05$).

Table 2. Average absolute body weight gains of experimental catfish fed commercial ration supplemented with turmeric powder at doses 0 mg/100 g ration (T0), 120 mg/100 g ration (T1), 240 mg/100 g ration (T2), and 480 mg/100 g ration (T3) for 8 weeks.

The dose of turmeric powder supplementation (mg/100 g ration)	Absolute body weight gain (kg)
0	3.01 ± 0.86^{a}
120	2.94 ± 0.85^{a}
240	3.14 ± 0.16^{a}
480	4.17 ± 0.43^{b}

Different superscripts in the same column indicate significant differences ($P<0.05$).

3.2. Vitellogenin Concentration of the Egg

The vitellogenin concentrations of the eggs as an indicator of vitellogenin synthesis and deposition in the oocytes of the experimental catfish fed commercial ration supplemented with turmeric powder at doses 0, 120, 240, and 480 mg/100 g ration are presented in Table 3. The results of statistical analyses showed that turmeric powder supplementation at various doses significantly increased ($P<0.05$) vitellogenin synthesis and deposition in the oocyte with the final effect on the increased concentrations in the eggs. Further Duncan test showed that the highest vitellogenin deposition in the oocytes was found in the experimental catfish fed ration supplemented with turmeric powder at a dose of 480 mg/100 g ration i.e., 3.99 mg/g egg. The vitellogenin concentration in this experimental catfish fed ration supplemented with turmeric powder at a dose of 480 mg/100 g ration was significantly different ($P<0.05$) from the experimental catfish fed ration with turmeric powder at doses of 0, 120, and 240 mg/100 g ration.

Table 3. Mean vitellogenin concentrations in the eggs of experimental catfish fed ration supplemented with turmeric powder at doses of 0 mg/100 g ration (T0), 120 mg/100 g ration (T1), 240 mg/100 g ration (T2), and 480 mg/100 g ration (T3) for 8 weeks.

The dose of turmeric powder supplementation (mg/100 g ration)	Vitellogenin concentration in the eggs (mg/g)
0	0.78 ± 0.15^{a}
120	0.22 ± 0.24^{a}
240	2.06 ± 0.38^{b}
480	3.99 ± 1.29^{c}

Different superscripts in the same column indicate significantly

3.3. Gonad Development

Experimental catfish supplemented with different doses of turmeric powder had significantly different gonad maturities. This result indicates that different doses of turmeric supplementation affect the gonad development of experimental catfish that is the initial stage of effects on reproduction. Gonad development of catfish in this experiment was determined based on the observation of the catfish gonad and the level of gonad maturity in each experimental catfish. The level of gonad maturity of experimental catfish was determined morphologically based on the form, color, size, and the development of gonad contents. Figure 1 and Figure 2 present the photos of the level of gonad maturity of the experimental catfish at the end of the experiment.

From the observation of gonad maturity at the end of the experiment, it was clear that gonad maturity in the experimental catfish supplemented with turmeric at a dose of 480 mg/100 g ration was faster compared to the control catfish (Figures 1 and Figure 2). However, the catfish supplemented with turmeric at doses of 0 mg/100 g ration and 120 mg/100 g ration showed the early gonad maturity at the end of experiment while the experimental catfish supplemented with turmeric at a dose of 480 mg/100 g ration could maintain the gonad maturity until the end of experiment.It could be seen in Figure 2 that there were higher number of eggs in the gonad of experimental catfish.

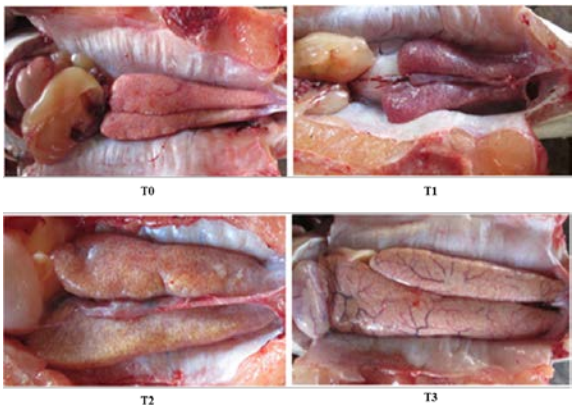

Figure 1. The conditions of ovaries of experimental catfish supplemented with turmeric powder at doses of 0 mg/100 g ration (T0), 120 mg/100 g ration (T1), 240 mg/100 g ration (T2), and 480 mg/100 g ration (T3) for 8 weeks.

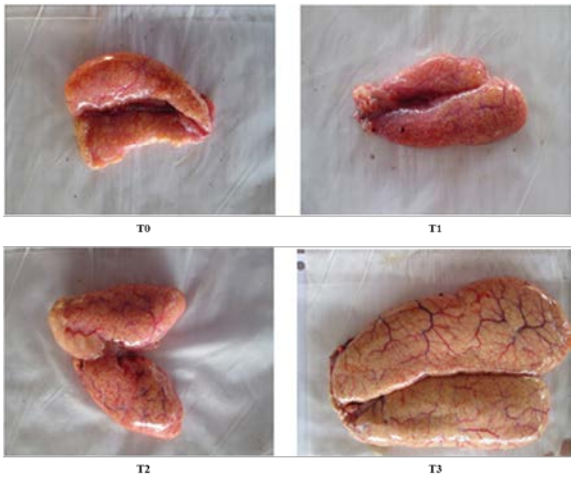

Figure 2. The gonads of experimental catfish supplemented with turmeric powder at doses of 0 mg/100 g ration (T0), 120 mg/100 g ration (T1), 240 mg/100 g ration (T2), and 480 mg/100 g ration (T3) for 8 weeks

3.4. Histology of Gonad

Histologically, catfish supplemented with turmeric powder at a dose of 480 mg/g feed showed a better gonad maturity and ready for ovulation and spawning. There were different stages of oocyte development in one ovary indicating that the experimental catfish have partial or asynchronous spawning. The most significant results from histological observation of gonads were the higher number and size of oocytes in catfish supplemented with turmeric powder at a dose of 480 mg/100 g feed.

3.5. Water Quality

The high quality of the water media was maintained under controlled conditions during the experiment. The water pH ranged from 6.0 to 7.5; the temperature ranged from 27.7 to 30°C; and the dissolved oxygen ranged from 3.5 to 5.42 mg/L. The standard water pH, temperature, and dissolved oxygen ranges were 6.85 to 7.50, 28.0 to 30.0, and 3.0 to 6.0 respectively.

4. Discussion

Supplementation of turmeric powder in the ration improved the growth of experimental catfish during 8 weeks of treatment as was indicated by the increased absolute body weight ($P<0.05$) (Table 2). At the end of 8 weeks of turmeric powder supplementation, the experimental catfish fed ration supplemented with turmeric powder at a dose of 480 mg/100 g ration had the highest absolute body weight gain compared to the other catfish supplemented with turmeric powder at doses of 0, 120, and 240 mg/100 g ration.

The increases in body weights were related to the increased feed intake as was reported by Rajput et al. (2012) that turmeric powder supplementation in broiler chickens increased appetite that further increased feed intake that eventually met the nutrient requirement of the animals. Supplementation of the ration with Nigella sativa or black cumin combined with turmeric at doses of 5-10 g/kg ration for 60 days could increase the daily growth rate of Lates calcarifer fingerlings (Abdelwahab and El Bahr, 2012). Mahmoud et al. (2014) concluded that 0.5% turmeric supplementation improved growth performance of Nile tilapia. The similar result was also observed by Mukherjee et al. (2009) investigating the effect of turmeric powder on growth performance and body color of guppy. In this study, 0.03, 0.06, 0.09, 0.10, and 0.20 percent of turmeric powder were added to the basal diet, and the results revealed that fish fed with diet contained 0.09% turmeric powder had a better growth performance compared to the other groups. Turmeric powder supplementation at a dose of 7.5% ration in African catfish (Clarias gariepinus) could also increase body weight gains during 8 weeks of treatment in African catfish (Sodamola et al., 2016). The similar result was also observed in fish supplemented with turmeric powder at a dose of 0.3% ration increased body weight gain of Green terror (Andinocara rivulatus) (Mooraki et al., 2019).

Experimental catfish fed ration supplemented with turmeric powder at a dose of 480 mg/100 g ration had the highest vitellogenin concentrations in the eggs i.e., 3.99 mg/mL. This result could be related to the phytoestrogenic effects of flavonoid in the turmeric that could act like estrogen in stimulating the liver to synthesize vitellogenin. The liver is the main site of vitellogenin biosynthesis as a precursor of egg yolk (Turker and Bozcaarmutlu, 2009). Dewi et al. (2018) reported that turmeric powder supplementation at a dose of 480 mg/100 g ration in catfish could increase the plasma vitellogenin concentrations i.e., 5.375 mg/mL. Turmeric powder supplementation dramatically increased the capacity of hepatocytes to synthesize vitellogenin in catfish that eventually increased vitellogenin deposition in the developing oocytes during gonad maturity that was confirmed by the increased fecundity and with the higher diameters. These results in catfish were similar to the results reported by Rawung et al. (2019) that turmeric powder supplementation at a dose of 0.5% ration and thyroxine at a dose of 0.1 mg in african catfish (Clarias gariepinus) could increase the vitellogenin concentrations in the eggs i.e., 8.17 mg/mL. Turmeric powder supplementation prior to gonad maturity would stimulate earlier cell growth and receptors that were indicated by the increased synthesis of vitellogenin that eventually increased egg production. The vitellogenin concentration in the eggs found in the present experiment indicated that turmeric powder could increase vitellogenin synthesis and further deposition in the eggs. Turmeric powder supplementation could improve liver capacity and functions to produce the precursor of egg yolk that eventually stimulates the follicle growth during gonad maturity. During the process of vitellogenesis, the number and granules of egg yolk increase so that the volume of oocytes will increase. During the deposition of egg yolk, curcumin as a bioactive component of turmeric is assumed to be deposited in the developing oocytes (Kasiyati et al., 2016a).

The turmeric powder supplementation could increase the diameter of the eggs in the experimental catfish. The increased egg diameter is the results of the increased synthesis, secretion, and deposition of egg yolk precursors in the present experiment. Egg diameter indicates the number of materials and energy deposited in the eggs that will be further used for embryonic growth and development. The egg diameter will increase with the increased gonad development. This increase is related to the increased deposition of nutrients in the oocytes during

gonad maturation that will increase with the increased size of the oocytes. The egg diameter is determined by the amount of vitellogenin deposited in the egg during oocyte growth and development. The increased diameter of the egg is caused by the growth and development of oocytes and the growth and development of the oocytes are related to the deposition of egg yolk. The development of egg during the uptake of vitellogenin will be stopped when the oocyte has reached the maximum size. Dewi et al. (2018) reported that turmeric powder supplementation at doses of 240-480 mg/100 g ration could increase the egg diameter in catfish. Curcumin supplementation in the ration could increase the diameter of F1 follicles in Magelang ducks (Kasiyati et al., 2016b).

The diameters of sample eggs of catfish showed different sizes. In the control catfish, the diameter of the egg was lower compared to those supplemented with turmeric powder at a dose of 480 mg/100 g ration. Lee & Young (2002) stated that the egg size also played a role and determined the survival of the fish developed from this egg. The egg size also correlated with the size of the larvae. Larvae with larger size will survive better during starvation compared to smaller larvae hatched from the egg with the smaller size (Kamler, 1992). The positive relation between the larvae size and egg size was reported in salary Salmon, Oncorhynchus mykiss, and turbot (Scopththalmus maximus. L) (Kjorsvik et al., 1990). Some researchers showed that eggs with larger sizes produced larvae and fish with better survivals. Kamler (1992) proposed an equation of survival for sea pelagic fish that mortality rate of egg and larvae had a negative correlation with the egg size. If there was no external feed, the larger larvae hatched from a larger egg could survive longer compared to larvae hatched from a smaller egg.

The gonad maturity in fish fed ration supplemented with turmeric powder at doses of 240-480 mg/100 g ration was faster compared to control catfish (Figure 2). The acceleration of gonad maturity is assumed to be related to the role of turmeric powder in improving liver function to synthesize vitellogenin and the vitellogenin will be further transported and deposited in the developing oocytes for the growth and development of follicle hierarchy. The higher the number of developing follicles, the faster the follicle is to be ovulated. Dewi et al. (2018) reported that turmeric powder supplementation at doses of 240-480 mg/100 g ration could produce 100% catfish reached gonad maturity with gonad maturity stage IV (final vitellogenesis) during 8 weeks of treatment. The similar result was also observed in catfish treated with hormone (PMSG+ anti-dopamine, each with doses of 0.25 mL dan 0.1 mg) and turmeric at a dose of 480 mg/100 g in feed could produce 100% gonad maturity in catfish (Pangasionodon hypophthalmus) (Arfah et al., 2018).

The differences in histological profiles of gonads in each experimental catfish can be seen in Figure 3. In control catfish, the development of gonad at week 8 is still in the early stage of development; oogonia are very small with a round form and a very large nucleus compared to the cytoplasm, and oogonia are found in group even though some are single. However, the catfish supplemented with turmeric powder at a dose of 480 mg/g feed showed the oocyte that were in the last stage of maturation with a larger size; the number of egg yolk that covered the cytoplasm was higher. Figure 3 shows that the oocytes of catfish in the same gonad maturity have different and various development stages. The difference in developmental stages of gonad is an indication of the experimental catfish with the partial or asynchronous spawning type. Partial or asynchronous spawning is the development of oocytes when the ovary contains all stadia of oocytes.

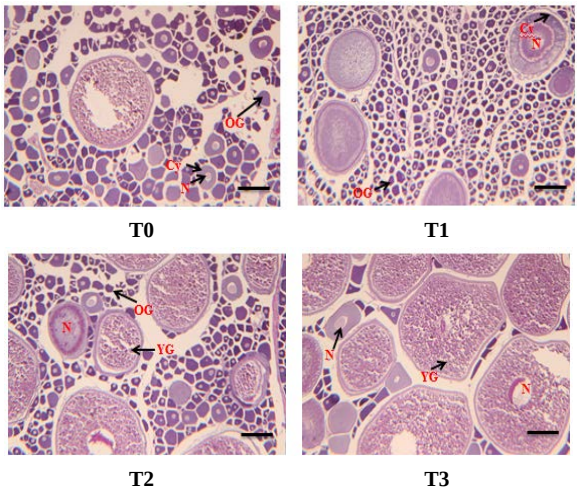

Figure 3. Histology of gonad of experimental catfish supplemented with turmeric powder at doses of 0 mg/100 feed (T0), 120 mg/ 100 g feed (T1), 240 mg/ 100 g feed (T2), and 480 mg/ 100 g feed (T3). Nucleus (N), Cytoplasm (Cy), *Yolk Globule* (YG), Oogonium (OG). Staining with H&E, Scale line represented 50 µm. Enlargement 40 x 10 times.

The water quality during catfish maintenance showed the condition that is able to support the maintenance of experimental catfish. The degree of water acidity (pH) during experiment ranged from 6.0-7.5, and this pH range was in the range of optimum pH for catfish as Hossain et al. (2006) stated that the optimum pH to support the life of catfish ranges from 6.85–7.5. The water temperature during the experiment ranged from 27.7-30.0ºC, and the temperature was in the normal range. According to Slembrouck et al. (2009), in general, catfish can survive to live in the range of water temperature of 28-30ºC. Dissolved oxygen (DO) during experiment ranged from 3.5-5.42 mg/L, and this range was suitable for catfish maintenance to support the optimum catfish growth that ranged 3.0–6.0 mg/L (Rahman et al., 2006).

5. Conclusions

Turmeric powder supplementation can improve absolute body weight gain, vitellogenin synthesis and deposition in the eggs as well as the stage of gonad development of experimental catfish. The improved absolute body weight gain, vitellogenin deposition in the eggs, and gonad development are the initial indicators that curcumin supplementation in the ration has a great prospect to be used in improving reproductive performances of catfish and teleost fish.

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