

Effectivity of Curcumin and Thyroxine Supplementations for Improving Liver Functions to Support Reproduction of African Catfish (*Clarias gariepinus*)

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Abstract

This experiment was designed to study the effect of curcumin and thyroxine supplementations on liver functions during the reproductive period of catfish. One hundred and twenty-eight African catfish (*Clarias gariepinus*) were assigned into a completely randomized design with a 2x2 factorial arrangement with four replications and each replication consisted of eight fish. The first factor was dose of curcumin supplementation which consisted of 2 levels i.e., 0 and 5 g kg-1 feed. The second factor was dose of thyroxine supplementation consisting of 2 levels i.e., 0 and 0.1 mg.kg-1 feed. The supplementation of curcumin and thyroxine hormone in catfish was given for 12 weeks. Results showed that the levels of serum vitellogenin concentrations, serum glutamic oxaloacetic transaminase (SGOT), and deoxyribonucleic acid (DNA) from liver tissue in all groups did not show a significant difference ($p > 0.05$). However, the concentration of malondialdehyde (MDA) in the liver tissue, the serum glutamic pyruvic transaminase (SGPT), and the ribonucleic acid (RNA) concentration in the liver tissue showed a significant difference ($p < 0.05$). The supplementation of curcumin and thyroxine protect the liver, and increase the productivity of catfish liver function during the reproductive period.

Keywords: curcumin; thyroxine; oxidative damage; Liver; vitellogenin

1. Introduction

In oviparous animals such as fish, the liver has a very important role in reproduction to synthesize and produce vitellogenin (Cerdeira *et al.* 1996; Tata, 1979). Vitellogenins are major precursors to the egg yolk protein, which provides essential nutrients and other materials required by the developing embryo (Sullivan and Yilmaz, 2018). During gonad maturity, the liver cells work harder to synthesize and produce a higher number of vitellogenin to fulfill the requirements of vitellogenin by thousands of developing oocytes at the same time (Arukwe and Goksoy, 2003).

High activities of hepatocytes during the gonad maturity process can cause the lowering functions and synthetic capacities of hepatocytes. The higher metabolic activities of hepatocytes can produce a higher number of intermediate products and free radicals (Watson, 2002; Kasiyati *et al.* 2016a). The normal process of metabolism in the body can produce free radicals as an intermediate product (Valko *et al.* 2007). According to Rahman (2007), if there is no balance between an endogenous antioxidant system and free radicals, or if there is excess of free radicals and lipid peroxidation it will cause oxidative damages to the organ or tissue. The occurrence of lipid

peroxidation is related to the high production of free radicals exceeding the availability of endogenous antioxidant (Rahman, 2007). Malondialdehyde (MDA) is the final product of lipid peroxidation; it can be used as an indicator of liver damage (Sewerynek *et al.* 1996). In the conditions of liver disorders, the serum glutamic pyruvic transaminase enzyme (SGPT) and serum glutamic oxaloacetic transaminase enzyme (SGOT) also increase in the plasma (Li *et al.* 2014).

Curcumin has an antioxidant activity, and it can inhibit the activity of inflammation enzymes and lipid peroxidation (Kohli *et al.* 2005; Akram *et al.* 2010; Abdulbaqi *et al.* 2018). Curcumin also has activity as a hepato-protector agent, and this compound can prevent liver damages and optimize the liver physiological function (Manju *et al.* 2012; Negi *et al.* 2007) to synthesize vitellogenin (Lubzens *et al.* 2010). Protection of hepatocyte cells is expected to optimize liver productivity in synthesizing the metabolites needed during the period of vitellogenesis.

One of the main functions of thyroxine hormone is to increase the amount and activity of mitochondria, which in turn increases the speed of formation of adenosine triphosphate (ATP) for cellular energy (Guyton and Hall, 2006). Researches on the process of vitellogenesis in fish show the involvement of thyroid hormone (Syano *et al.*

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1993; Nowell *et al.* 2001). Research conducted by Dewi (2018) showed that supplementation of thyroxine in combination either with turmeric or oodev can reduce oxidative stress in Siam catfish.

The present experiment was designed to study the effect of curcumin and thyroxine supplementations on the liver productivity, health, and physiological functions during the maturity of gonads in African catfish.

2. Materials and Methods

2.1. Location and time of study

The experiment was conducted during August-December 2018. The catfish were maintained in National Freshwater Aquaculture Center, Sukabumi, West Java, Indonesia. The concentration of malondialdehyde (MDA) concentrations in the liver tissue, SGPT, SGOT, liver DNA, and liver RNA concentrations were analyzed in the Laboratory of Physiology, Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine, Bogor Agriculture University. Vitellogenin concentrations were analyzed and estimated in the Primate Animal Study Center, Bogor Agriculture University.

2.2. Experimental design

The experimental design used was a completely randomized design with 2x2 factorial arrangement. The first factor was a dose of curcumin supplementation consisting of two levels i.e., 0 and 5 g kg⁻¹ feed. The second factor was a dose of thyroxine supplementation of two levels i.e., 0 and 0.1 mg. kg⁻¹ feed. A total of 128 catfish were divided into four groups, each group had four replicates (8 fish for each replication); the first group (A) consisted of catfish without curcumin and thyroxine supplementation as a control; the second group (B) consisted of catfish supplemented with curcumin at a dose of 5.0 g kg⁻¹ feed (Manju *et al.* 2012) without thyroxine supplementation, the third group (C) consisted of catfish without curcumin supplementation but with thyroxine supplementation at a dose of 0.1 mg.kg⁻¹ feed (Agusnimar and Rosyadi, 2015), and the fourth group (D) consisted of catfish supplemented with curcumin at a dose of 5.0 g kg⁻¹ feed and thyroxine at a dose of 0.1 mg kg⁻¹ feed.

2.3. Experimental procedure

The experimental animals used in this study were female catfish with an initial body weight ranging from 250-350 g. The experimental catfish were reared in the rearing pond in 16 nets each with the size of 2x1x1 m³ and each net contained eight catfish. The catfish were reared for 12 weeks and fed with commercial ration containing 42.70% protein. For the treatments, the required dose of curcumin and thyroxine were mixed with the commercial ration. The curcumin used in this experiment was produced by Plamed Green Science Limited (CHINA) 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. During the rearing period, the experimental catfishes were fed daily at the level of 2% body weight.

2.4. Sample collection

Every three weeks, one catfish was taken randomly from each replication of all the treatments and sacrificed. Prior to being sacrificed, the experimental catfishes were anesthetized using clove oil with a concentration of 0.04 ml l⁻¹ of water. Before being dissected, the blood was collected using a 3 ml syringe from the caudal vein. The collected blood was transferred to polyethylene tube and centrifuged at 3000 rpm for 10 minutes at 4°C to obtain serum. The serum was transferred into a new polyethylene tube and kept at -20°C until further analyses for SGPT, SGOT, and vitellogenin. The liver was divided into two parts: the first part was kept in sterile Eppendorf tube immediately immersed in liquid nitrogen and then stored at -20°C for Malondialdehyde determination (MDA), the second part of liver about 2g kept in Phosphate Buffer Saline (PBS) in sterile plastic tubes was stored at -20°C for DNA and RNA assays.

2.5. Parameters measurements

2.5.1. Vitellogenin assay

Vitellogenin concentration in the serum was determined with an enzyme linked immunosorbent assay (ELISA) using the Vitellogenin Fish Kit (Korain Biotech Co.Ltd, China).

2.5.2. Malondialdehyde (MDA) assay

As much as 1 g of catfish liver was finely chopped under cold condition and dissolved in 2 ml of PBS-KCl at a pH of 7.4. The mixture formed was centrifuged at 10,000 rpm for 20 minutes and then the supernatant was taken for further MDA assay. The MDA concentrations of the liver to determine the peroxidation activity of liver cell membranes were measured by using the thiobarbituric acid (TBA) method (Singh *et al.* 2002). TEP (1.1.3.3-Tetraethoxy-propabe, ≥96%) MW 220.31 (Aldrich, USA) was used as a standard for MDA.

2.5.3. SGPT and SGOT assays

Kinetic method was used for the determinations of SGPT and SGOT activities according to the recommendations of the Expert Panel of the IFCC (International Federation of Clinical Chemistry). The concentrations of SGPT and SGOT were measured by using the kit of GPT (ALAT) (Human, Germany) and kit of GOT (ASAT) (Human, Germany).

2.5.4. DNA and RNA assays

Analysis of DNA and RNA concentrations of liver tissues was made by isolating 2-3 g of liver, dried in an oven at 60°C for a day, the dried liver was crushed into a mash by using a mortar and then 10 mg of the liver tissues was weighed each for analysis of liver DNA and RNA. The DNA concentration of liver tissue was determined by DNA extraction method by using genomic DNA mini kit (Geneaid Biotech Ltd, Taiwan), while the concentration of RNA was determined by RNA extraction method by using total RNA mini kit (Geneaid Biotech Ltd, Taiwan).

2.6. Statistical analyses

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

3. Results and Discussion

3.1. Results

3.1.1. Concentrations of vitellogenin in serum of catfish

Concentrations of vitellogenin in the serum for 12 weeks of observation are shown in **Fig. 1**. Before treatment, the experimental catfish were in matured condition as it was indicated by the high serum vitellogenin concentration with an average value of 9.44 $\mu\text{g/ml}$. Serum vitellogenin concentrations as an indicator of gonad maturity slightly increased at 3 weeks of treatment and then decreased at 6 weeks of treatment, and then relatively stable at 9 and 12 weeks of treatment. There was no significant difference among the treatments ($p > 0.05$). Even though there was no significant difference in serum vitellogenin concentrations among treatments, catfish treated with different doses of curcumin and thyroxine supplementations showed the dynamic changes in serum vitellogenin concentrations. At 3 and 6 weeks of treatments, the highest serum vitellogenin concentrations were found in catfish fed ration supplemented with 0 g curcumin $\text{kg}^{-1}\text{feed}$ and 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$ (Group C), followed by catfish fed ration supplemented with 0 g curcumin $\text{kg}^{-1}\text{feed}$ and 0 mg thyroxine $\text{kg}^{-1}\text{feed}$ (Group A), catfish fed ration supplemented with 5.0 g curcumin $\text{kg}^{-1}\text{feed}$ and 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$ (Group D), and the lowest were found in catfish fed ration supplemented with 5.0 g curcumin $\text{kg}^{-1}\text{feed}$ and 0 mg thyroxine $\text{kg}^{-1}\text{feed}$ (Group B). At 9 and 12 weeks of treatments, the highest vitellogenin concentrations were found in catfish fed ration supplemented with 5.0 g curcumin $\text{kg}^{-1}\text{feed}$ and 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$ (Group D), followed by catfish fed ration supplemented with 0 g curcumin $\text{kg}^{-1}\text{feed}$ + 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$ (Group C), catfish fed ration supplemented with 5.0 g curcumin $\text{kg}^{-1}\text{feed}$ and 0 mg thyroxine $\text{kg}^{-1}\text{feed}$ (Group B), and the lowest were in control catfish fed ration supplemented with 0 g curcumin $\text{kg}^{-1}\text{feed}$ and 0 mg thyroxine $\text{kg}^{-1}\text{feed}$ (Group A). At 12 weeks of treatment, serum vitellogenin concentrations did not indicate any relationship with the curcumin or thyroxine treatment. However, 9th and 12 weeks of treatments, catfish treated with curcumin and thyroxine supplementations had consistently higher serum vitellogenin concentrations compared to control catfish without curcumin and thyroxine supplements.

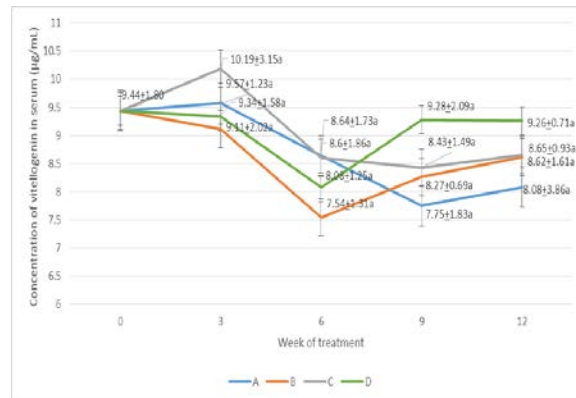


Figure 1. Concentrations of vitellogenin in serum of experimental African catfish for 12 weeks of treatment with various combinations of curcumin and thyroxine. A (0 g curcumin $\text{kg}^{-1}\text{feed}$ + 0 mg thyroxine $\text{kg}^{-1}\text{feed}$); B (5.0 g curcumin $\text{kg}^{-1}\text{feed}$ + 0 mg thyroxine $\text{kg}^{-1}\text{feed}$); C (0 g curcumin $\text{kg}^{-1}\text{feed}$ + 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$); D (5.0 g curcumin $\text{kg}^{-1}\text{feed}$ + 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$). Means \pm standard deviation with different superscripts indicate a significant difference ($p < 0.05$).

3.1.2. Concentrations of Malondialdehyde (MDA) in the liver tissues

The MDA concentrations in the livers of experimental catfish are presented in **Fig. 2**. Concentrations of MDA in the liver tissues were high before treatment, and decreased in three weeks of treatment, increasing during 6 weeks of treatment, and reduced at nine weeks of treatment, while increasing again at 12 weeks of treatment.

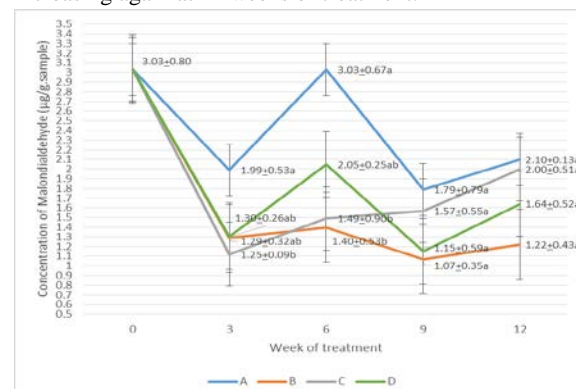


Figure 2. Concentrations of Malondialdehyde (MDA) in the liver tissues of experimental African catfish during 12 weeks of treatment with various combinations of curcumin and thyroxine. A (0 g curcumin $\text{kg}^{-1}\text{feed}$ + 0 mg thyroxine $\text{kg}^{-1}\text{feed}$); B (5.0 g curcumin $\text{kg}^{-1}\text{feed}$ + 0 mg thyroxine $\text{kg}^{-1}\text{feed}$); C (0 g curcumin $\text{kg}^{-1}\text{feed}$ + 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$); D (5.0 g curcumin $\text{kg}^{-1}\text{feed}$ + 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$). Means \pm standard deviation with different superscripts indicate a significant difference ($p < 0.05$).

Catfish without curcumin and thyroxine supplementations (Group A or control) had the highest liver MDA concentrations and catfish supplemented with 5.0 g curcumin $\text{kg}^{-1}\text{feed}$ without thyroxine supplementation (Group B) and catfish supplemented with 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$ without curcumin supplementation (Group C) had the lowest liver MDA concentrations and catfish supplemented with 5.0 g curcumin $\text{kg}^{-1}\text{feed}$ and 0.1 mg thyroxine $\text{kg}^{-1}\text{feed}$ (Group D) were moderate values (Fig.2). At three and six weeks of treatments, catfish of Group A had significantly the higher liver MDA concentrations compared to the other treatments ($p < 0.05$). At three weeks of treatment, catfish

of Group C had the lowest liver MDA concentration ($p < 0.05$). At six weeks of treatments, catfish of Group B and C had significantly lowest liver MDA concentrations ($p < 0.05$) when compared to Group A. However, at 9 and 12 weeks of treatment, there was no significant difference in liver MDA concentrations among the treatments ($p > 0.05$) even though catfish without curcumin supplementations (Group A and Group C) had the highest liver MDA concentrations, and catfish treated with curcumin (Group B and Group D) had the lowest liver MDA concentrations. The pattern of liver MDA concentrations during 12 weeks of treatment increased on weeks 6 and 12 of treatment. The highest liver MDA concentrations were found in control (Group A).

3.1.3. Concentrations of glutamic pyruvic transaminase (SGPT) in the serum of catfish for

Serum SGPT concentrations of experimental African catfish are presented in **Fig. 3**. The curve of serum SGPT concentrations was similar to those of liver MDA concentrations and serum SGOT concentrations. Observations in weeks 3 and 6 of treatments, serum SGPT concentrations in all groups did not differ significantly ($p > 0.05$). Further, in six weeks of treatment, serum SGPT concentrations increased compared to those at three weeks treatment. However, in weeks 9 and 12 of treatments, control catfish without curcumin and thyroxine treatment had significantly higher serum SGPT concentrations ($p < 0.05$). Even though they were not always statistically significant, the highest serum SGPT concentrations were found in control catfish (Group A). Catfish of Group B, C, and D had lower serum SGPT concentrations compared to Group A. Similar to the pattern of liver MDA concentrations, the curve of liver SGPT concentrations increased on weeks 6 and 12 of treatment and the highest liver MDA concentrations in these weeks of treatments were found in control.

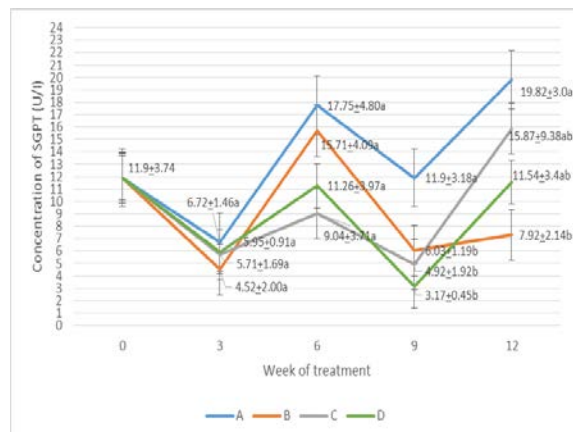


Figure 3. Concentrations of SGPT in the serum of African catfish during 12 weeks of treatment with various combinations of curcumin and thyroxine. **A** (0 g curcumin kg^{-1} feed + 0 mg thyroxine kg^{-1} feed); **B** (5.0 g curcumin kg^{-1} feed + 0 mg thyroxine kg^{-1} feed); **C** (0 g curcumin kg^{-1} feed + 0.1 mg thyroxine kg^{-1} feed); **D** (5.0 g curcumin kg^{-1} feed + 0.1 mg thyroxine kg^{-1} feed). Means \pm standard deviation with different superscripts indicate a significant difference ($p < 0.05$).

3.1.4. Concentrations of glutamic oxaloacetic transaminase (SGOT) in the serum of catfish

Serum SGOT concentrations of the experimental catfish during 12 weeks of treatment are presented in **Fig.**

4. Prior to the treatment, the serum SGOT concentrations were high and decreased at three weeks of treatment and maintained similar level until nine weeks of treatment and then increased after 12 weeks of treatment. Even though with dramatic changes after 12 weeks of treatment, serum SGOT concentrations in all experimental catfish groups were similar ($p > 0.05$). In the third week of treatment, serum SGOT concentrations did not differ significantly between the treatments ($p > 0.05$). The same patterns were observed in the serum SGOT concentrations sixth, ninth, and twelfth weeks of treatments. These results indicated that curcumin and thyroxine supplementations did not affect serum SGOT concentrations of catfish. However, at three weeks of treatment, catfish without curcumin and thyroxine supplementation (Group A) and catfish without curcumin supplementation but with 0.1 mg thyroxine kg^{-1} feed (Group C) had higher serum SGOT concentrations compared to catfish with 5.0 g curcumin supplementation kg^{-1} feed but without thyroxine supplementation (Group B) and catfish supplemented with 5.0 g curcumin kg^{-1} feed and 0.1 mg thyroxine kg^{-1} feed (Group D). There was a tendency that catfish without curcumin supplementation had higher SGOT compared to those supplemented with curcumin. Similar to the pattern of liver MDA and serum SGPT concentrations, the pattern of serum SGOT concentrations during 12 weeks of treatment increased after 6 and 12 weeks of treatment. However, it was not significant; the highest serum SGOT concentrations were found 6 week post treatment in catfish of Group B, followed by Group A, D and C. Even though it was not significant, the highest serum SGOT concentrations after 12 weeks of treatment were found in catfish of Group C, B, and D, followed by Group A. Similar to the curve of liver MDA concentrations, the curve of liver SGPT concentrations during 12 weeks of treatment increased after 6 and 12 weeks of treatment, and the highest liver MDA concentrations during the same period was found in catfish without curcumin and thyroxine supplements (control).

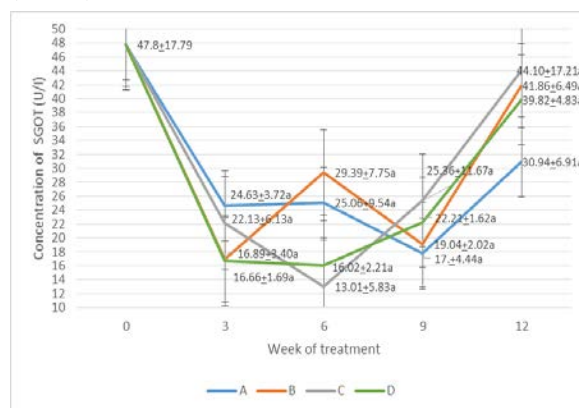


Figure 4. Concentrations of SGOT in the serum of experimental African catfish for 12 weeks of treatment with various combinations of curcumin and thyroxine. **A** (0 g curcumin kg^{-1} feed + 0 mg thyroxine kg^{-1} feed); **B** (5.0 g curcumin kg^{-1} feed + 0 mg thyroxine kg^{-1} feed); **C** (0 g curcumin kg^{-1} feed + 0.1 mg thyroxine kg^{-1} feed); **D** (5.0 g curcumin kg^{-1} feed + 0.1 mg thyroxine kg^{-1} feed). Means \pm standard deviation with different superscripts indicate a significant difference ($p < 0.05$).

3.1.5. Concentrations of DNA in the liver of catfish

Concentrations of DNA in the liver of experimental catfish during 12 weeks of treatment are presented in **Fig.**

5. Statistical analysis of liver DNA concentrations at three, six, nine, and twelve weeks of curcumin and thyroxine supplementations did not differ significantly ($p > 0.05$) among the treatments. These results indicated that the supplementation of experimental catfish with curcumin and thyroxine did not affect DNA concentration in the liver tissues. The average liver DNA concentrations of experimental catfish ranged from 12.15 -12.79 mg.g⁻¹. In general, there were the patterns of increase and decrease in the liver DNA concentrations. However, there was an irregular pattern of fluctuation within and between the treatments. Liver DNA concentrations increased after 3 and 9 weeks of treatments. After 9 weeks of treatment, liver DNA concentration was maximum in catfish fed ration supplemented with 5.0 g curcumin kg⁻¹ feed and 1.5 mg thyroxine kg⁻¹ feed (Group D) followed by the Groups A, B, and C respectively.

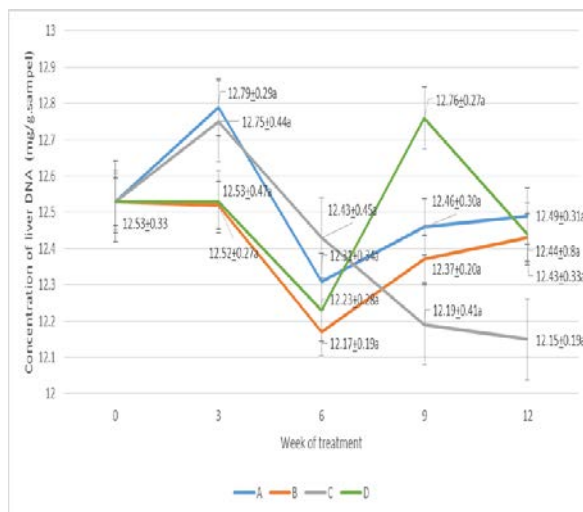


Figure 5. Concentrations of DNA in the liver tissues of experimental African catfish for 12 weeks of treatment, with various combinations of curcumin and thyroxine. **A** (0 g curcumin kg⁻¹ feed + 0 mg thyroxine kg⁻¹ feed); **B** (5.0 g curcumin kg⁻¹ feed + 0 mg thyroxine kg⁻¹ feed); **C** (0 g curcumin kg⁻¹ feed + 0.1 mg thyroxine kg⁻¹ feed); **D** (5.0 g curcumin kg⁻¹ feed + 0.1 mg thyroxine kg⁻¹ feed). Means \pm standard deviation with different superscripts indicate a significant difference ($p < 0.05$).

3.1.6. Concentrations of RNA in the liver of catfish

The RNA concentrations in the liver of experimental catfish are presented in **Fig. 6**. RNA concentration in the liver cells increased after the initiation of the curcumin thyroxine treatments and reached the peak after nine weeks of treatment and later decreased to the minimum level after 12 weeks of treatment. Statistically, liver RNA concentration did not affect after three, six, and nine weeks of curcumin and thyroxine supplementation ($p > 0.05$). However, at twelve weeks of curcumin and thyroxine supplementations, there was a difference ($p < 0.05$) between the treatments. The maximum RNA levels were found in catfish of Group B, followed by catfish of Group C, D, and A. Liver RNA concentrations increased after 3 and 6 weeks of treatment, and reached the peak concentration after 9th week. After 9 week of treatments, even though it was not statistically significant, the highest liver RNA concentrations were found in catfish of Group D followed by the other treatments with the order of catfish from Group C, B, and A.

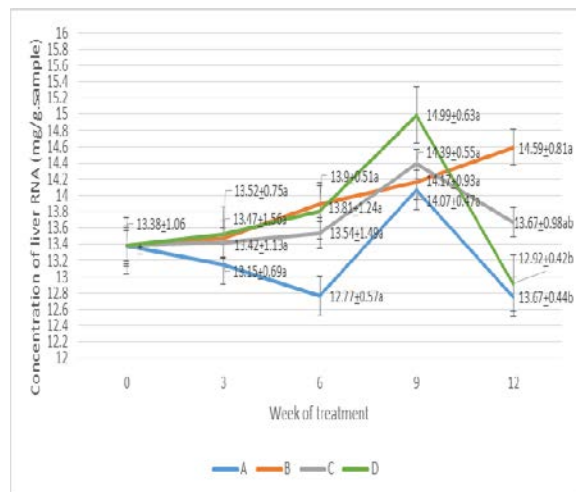


Figure 6. Concentrations of RNA in the liver tissues of experimental African catfish for 12 weeks of treatment with various combinations of curcumin and thyroxine. **A** (0 g curcumin kg⁻¹ feed + 0 mg thyroxine kg⁻¹ feed); **B** (5.0 g curcumin kg⁻¹ feed + 0 mg thyroxine kg⁻¹ feed); **C** (0 g curcumin kg⁻¹ feed + 0.1 mg thyroxine kg⁻¹ feed); **D** (5.0 g curcumin kg⁻¹ feed + 0.1 mg thyroxine kg⁻¹ feed). Means \pm standard deviation with different superscripts indicate a significant difference ($p < 0.05$).

4. Discussion

Liver plays an important role during reproduction of fish because as it functions as a main organ for synthesis of vitellogenin. Vitellogenin is an egg yolk precursor synthesized in the liver under the stimulation of estradiol-17 β . During the reproduction period, the liver will continuously synthesize the vitellogenin to fulfill the requirement of each growing oocytes (Cerdeira *et al.* 1996). During the vitellogenesis, liver tissues showed the formation of many vacuoles in the liver cells (Dewi, 2018). The vacuole contains fats, the component of vitellogenin, synthesized by liver cells (Kasiyati *et al.* 2016a). The vitellogenin will be released into the circulation system and transported to the gonads. In this study, the presence of vitellogenin in the circulation has been detected since the beginning of the observation (**Figure 1**). According to Dewi *et al.* (2018), supplementation of turmeric powder in Siam catfish increased vitellogenin synthesis.

In this study the concentration of vitellogenin in the circulation was almost the same for all groups. However, the group that supplemented by curcumin and thyroxine showed the increase of vitellogenin in the circulation. According to Cerdeira *et al.* (1996), the concentrations of vitellogenin in the plasma were basically constant throughout the reproductive cycle, except that there was more activity in taking vitellogenin by a growing population of eggs that decreased the level of vitellogenin in the plasma. According to Kasiyati *et al.* (2016b) increased in vitellogenin synthesis in ducks supplemented with curcumin was not associated with the high estradiol concentrations but with the capacity and function of hepatocytes to synthesize vitellogenin. Vitellogenin is not always stored in the liver cells, but secreted directly after being synthesized and then deposited into the developing oocyte. Therefore, the concentration of vitellogenin in the circulation is a result of the rate of vitellogenin synthesis and secretion into the circulation and the rate of

vitellogenin transport and deposits into the developing oocytes (Nath and Sundararaj, 1979; Kasiyati *et al.* 2016b).

The results of the present study on African catfish showed that supplementation of catfish with curcumin and thyroxine resulted in decreased MDA concentrations in the liver tissues (three and six weeks post treatments) and serum SGPT concentrations (after nine and twelve weeks) as indicators of the improved conditions and functions of liver cells. Catfish without curcumin and thyroxine supplementation had the highest MDA concentrations in the liver tissue (after three and six weeks) and highest serum SGPT concentrations (after nine and twelve weeks). This result indicated the liver protection of curcumin and thyroxine. Therefore, curcumin and thyroxine supplementations improved physiological conditions of the liver that could maintain the optimum cell number, synthetic capacity as it was indicated by the increased vitellogenin synthesis.

The role of liver as an organ for synthesis of various compounds needed by the body makes it very susceptible to the exposure to free radicals as intermediate products formed in various processes of reactions and metabolism. MDA is a final product of lipid peroxidation. The observation of catfish for 12 weeks of maintenance showed the development of fish gonads. Along with the development of the gonads, liver activity also increases in synthesizing vitellogenin as an egg yolk precursor. Observation of MDA concentrations of catfish liver for 12 weeks showed a significant effect indicated by curcumin and thyroxine supplementations decreased the MDA levels in the liver. The fluctuation of MDA occurred during this study could be related to the reproductive activities that were currently occurring in the experimental catfish. This study showed that supplementation of curcumin can reduce the MDA level in liver, it indicated that curcumin play a role as hepato-protector agent. Observation conducted by Manju *et al.* (2013) showed the increase in endogenous antioxidant in hepatocytes cells and glutathione concentrations in *Anabas testudineus* (Bloch) supplemented with curcumin in 40% protein ration. Curcumin directly plays a role in the scavenging of free radicals, stimulating the pathways of antioxidant enzymes and increasing the antioxidant level in the cells (Manju *et al.* 2012; Iqbal *et al.* 2003; Sharma *et al.* 2005). A study conducted by Dewi *et al.* (2018) showed that supplementation of turmeric powder decreased the MDA level of the Siam catfish liver. In this study, the addition of thyroxine showed a decrease in MDA concentrations of catfish liver at the third and sixth weeks of treatment. The hepatoprotective activity with thyroxine supplementation in this study occurred because of an increase in endogenous antioxidant. Research conducted by Baskol *et al.* (2007) showed that the addition of thyroxine reduced the serum MDA levels in patients with hypothyroidism. Meanwhile, Siam catfish supplemented with turmeric and thyroxine powder for eight weeks showed a significantly lower liver MDA concentration than controls (Dewi, 2018).

This study also showed that the addition of curcumin and thyroxine in the feed of catfish can reduce SGPT concentrations at ninth and twelfth weeks but did not affect SGOT concentrations. Park *et al.* (2000) reported that giving curcumin significantly reduced liver damage

and decreased SGOT and SGPT concentrations in the blood. The increased levels of SGPT and SGOT in the blood occurred along with the increasing age of fowl (Biswas *et al.* 2010). Chattopadhyay *et al.* (2004) suggested that curcumin acts as an antioxidant by activating macrophages to inhibit reactive oxygen species (ROS). Research conducted by Kasiyati *et al.* (2016a) showed that administration of curcumin protected hepatocytes from cell damage caused by free radicals characterized by the reduced levels of SGPT and SGOT in ducks supplemented with curcumin. Curcumin has been reported to induce nucleus translocation of NRF2 and increase the expression of a number of detoxifications of reactive oxygen species (ROS) and antioxidant genes in hepatocytes (Zhao *et al.* 2011). Therefore, supplementation of curcumin improved the status of cellular antioxidants, such as glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, and catalase, which is also accompanied by the increase in Phase II-metabolizing enzymes, namely glutathione s-transferase and quinone reductase in the liver and kidney (Iqbal *et al.* 2003), thereby reducing ROS (reactive oxygen species) activity (Sharma *et al.* 2005).

The observations on the liver DNA concentrations as an indicator of the number and concentration of hepatocytes in the liver tissue showed that treatment of curcumin and thyroxine did not significantly affect the number of liver cells. This result is similar with the study conducted by Dewi *et al.* (2018). The fluctuation of DNA during this study was not associated with the curcumin and thyroxine supplementation, but this fluctuation could be related to the gonadal maturation of the experimental catfish, it stimulated the hepatocyte growth.

In this study, supplementation of curcumin and thyroxine showed an increase of RNA concentrations in liver tissue. Increasing level of RNA in liver tissue indicated the increase capacities of liver to produce vitellogenin during the reproductive period. According to Kasiyati *et al.* (2016b), supplementation of curcumin in ducks improved the functions of liver cells, so it increased the biosynthesis of egg yolk/vitellogenin precursors as indicated by the increase in RNA concentrations of liver tissue. Our study showed a decrease in MDA in line with an increase in RNA concentration. The protection of liver is very important to support the optimization of their productivity. Meanwhile thyroxine plays a role in protein synthesis by increasing the activity of mitochondria, which in turn increases the speed of formation of adenosine triphosphate (ATP) for cellular energy (Guyton and Hall 2006). Increase in RNA concentrations in the liver of experimental catfish supplemented with curcumin and thyroxine (Group D) is thought to occur because of an increase in glutathione production caused by the addition of curcumin (Manju *et al.* 2012), where the glutathione is a co-factor of deiodinases which are responsible for the conversion of thyroxine (T4) to triiodothyronine (T3) (Mancini *et al.* 2016; Visser, 1980). On the other hand, supplemented thyroxine could increase the concentration of triiodothyronine because thyroxine will be converted to triiodothyronine (Mancini *et al.* 2016)

5. Conclusion

Our study shows that curcumin and thyroxine supplementation could inhibit the liver oxidative damage of catfish by reducing liver MDA levels and serum concentrations of SGPT, so it can protect the liver and increase vitellogenin synthesis as indicated by the increased RNA concentrations in the liver. In this study, the Group D (catfish fed ration supplemented with 5.0 g curcumin kg⁻¹ feed and 0.1 mg thyroxine kg⁻¹ feed) showed the optimum result for concentration of vitellogenin in serum and liver protection.

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