The potency of curcumin and the thyroxine hormone to support the antioxidant activity during reproduction in common carp (*Cyprinus carpio* L)

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**Abstract.** In the reproductive period, the female fish experiences an increase in metabolism due to the variety of reproductive activities, including the synthesis of vitellogenin. High metabolic activity with insufficient availability of endogenous antioxidants can increase free radical production. The measurement of free radical levels can be determined by calculating the concentration of malondialdehyde. This study was conducted to know the potency of curcumin and thyroxine hormone supplementation to support the antioxidant activity during the reproduction period. Our study used female broodstock of common carp as the experimental animal. In this study, we used forty female broodstock divided into 8 groups of treatment. The results showed that supplementation of curcumin and thyroxine hormone during the reproduction period has the potency to support the antioxidant activity.

1. **Introduction**

In oviparous animals especially fish when they enter the reproductive period, the metabolic activity will be higher with more energy requirements [1]. This energy requirement is aimed at ongoing reproductive activity especially in female fish for eggs production [1,2]. During this time, the activity of vitellogenesis is being stimulated. Vitellogenesis is a series of processes for the synthesis, transport, and deposit of vitellogenin into developing oocytes [3]. Vitellogenin itself is a precursor to egg yolk protein, which synthesized by hepatocyte cells [4]. When egg yolk protein is deficient, it can affect the quantity and even the quality of eggs produced [5].

The high metabolic activity that is taking place in the reproductive period has the potential to increase the free radical material produced. Naturally, activities of metabolism can produce free radicals [6]. The imbalance between endogenous antioxidants and free radicals produced can result in damage to cells and tissues [7], so it can reduce the activities of physiology. The liver is an organ that is very important during the reproductive process in oviparous animals [8-10]. This is because the synthesis of vitellogenin occurs in the hepatocytes cells of the liver [4]. The very high activity of liver cells during this reproductive period can reduce the physiological activity of the liver, so it will give a negative impact on the activity of vitellogenin synthesis [8]. This will be affect the availability of egg yolk protein, which in turn will affect the quality and quantity of eggs produced [5].

Malondialdehyde (MDA) is a marker of oxidative stress as an end product from the chain reaction of lipid peroxidation [11]. The presence of MDA can indicate damage to cells due to the inability of
endogenous antioxidants to scavenge free radicals that are formed [7,12]. In addition, the Serum Glutamic Pyruvic Transaminase (SGPT) enzyme levels can also be an indication of damage, especially to the liver [13]. This is because this enzyme is found mostly in the liver [14] and when there is damage to the liver, the SGPT enzyme will be released into the circulatory system [15].

Curcumin is a phenolic compound that has a lot of bioactivity including hepatoprotective, antioxidants, and phytoestrogens [16-18]. The activity of curcumin as a hepatoprotective and antioxidant has the potential to protect the liver so that it maintains the performance of liver cell's productivity [8,10]. Meanwhile, phytoestrogen activity from curcumin can stimulate the synthesis of vitellogenin [17]. When curcumin bind to estradiol hormone receptors on hepatocyte cells, it will stimulate the hepatocyte cells to start the synthesis of the vitellogenin. Thyroxine hormone plays a role in increasing the basal metabolic rate by increasing cellular energy production [19]. This cellular energy is very required during the reproductive period.

This study aims to determine the effect of curcumin supplementation and injection of the thyroxine hormone during the reproductive period in common carp on antioxidant activity by observing the parameters of MDA and SGPT.

2. Materials and methods

2.1. Experimental design

The experimental design used was a completely randomized design with a 2x4 factorial. The first factor was dose of curcumin supplementation consisted of four levels i.e., 0; 0.25; 0.5 and 1 % kg⁻¹ feed). The second factor was dose of thyroxine injection consisted of two level i.e., 0 and 0.1 µg.g⁻¹ Body weight). A total of 40 common carp were divided into eight groups, each group has five replication; the first group (A) was considered as control group without supplementation of curcumin and injection of thyroxine hormone; the second group (B) was supplied curcumin to feed by a dose of 0.25%.kg⁻¹ feed and without injection of thyroxine hormone; the third group (C) was supplied curcumin to feed by a dose of 0.5%.kg⁻¹ feed and without injection of thyroxine hormone; the fourth group (D) was supplied curcumin to feed by a dose of 1%.kg⁻¹ feed and without injection of thyroxine hormone; the fifth group (E) was not supplied by curcumin but with an injection of thyroxine hormone by a dose of 0.1 µg.g⁻¹ body weight (BW); the sixth group (F) was supplied curcumin to feed by a dose of 0.25%.kg⁻¹ feed and with injection of thyroxine hormone by a dose of 0.1 µg.g⁻¹ BW; the seventh group (G) was supplied curcumin to feed by a dose of 0.5%.kg⁻¹ feed and with injection of thyroxine hormone by a dose of 0.1 µg.g⁻¹ body weight (BW); and the eighth group (H) was supplied curcumin to feed by a dose of 1%.kg⁻¹ feed and with injection of thyroxine hormone by a dose of 0.1 µg.g⁻¹ body weight (BW).

2.2. Experimental procedure

The experimental animals used in this study were female common carp with an initial body weight 1.41±0.48 kg. The experimental fish were reared in the rearing pond in the 8 nets with the size of net was 2x2x1.5 m³ and each net contained five catfish. The common carp were reared for 16 weeks, and fed with commercial ration containing of 33% protein. As a treatment, the curcumin were mixed with the commercial ration according to the dose of curcumin. The thyroxine hormone used was a tablet of levothyroxine sodium/ euthyrox (MERCK). The tablet of the hormone was dissolved in the physiologist solution (NaCL, 0.9%) for injection preparation. In rearing period, fishes were fed daily as much as 3% of their body weight. The thyroxine hormone was injected twice on day 14 and 21.

2.3. Sample collection

At the end of the rearing, the blood was collected from all the fishes. Before sampling, the fish was anesthetized using clove oil with a concentration of 0.04 ml/l water. The blood was collected using a 3 ml syringe from the caudal vein. The collected blood was put into a 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 keep in -20°C until analyses for MDA and SGPT.
2.4. Parameters measurements

2.4.1. Malondialdehyde (MDA) assay. The MDA concentration was measured by using the TBA method [20]. TEP (1.1.3.3-Tetraethoxy-propab, ≥96%) MW 220.31 (ALDRICH, USA) was used as a standard for MDA, and was read using a spectrophotometer at 532 nm of wavelength.

2.4.2 SGPT assay. Kinetic method was used for the determinations of SGPT (ALAT) activities according to the recommendations of the Expert Panel of the IFCC (International Federation of Clinical Chemistry) without pyridoxal phosphate activation. The concentrations of SGPT measured by using the kit of GPT (ALAT) (HUMAN, GERMANY) and was read using a spectrophotometer at 340 nm of wavelength.

2.5. Statistical analyses
The data obtained were analyzed by using analysis of variance (ANOVA). The whole data analysis were 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 of significantly different were expressed with p<0.05.

3. Results and discussion

3.1. Concentration of Malondialdehyde (MDA) in serum
The MDA concentrations of common carp that supplementation with curcumin and injection of thyroxine hormone showed in Figure 1. At the end of rearing the highest concentration of MDA was showed by Group A/control ((16.5±0.91 mmol/L) and followed by Group F (14.17±0.25 mmol/L); Group H (10.95±0.57 mmol/L); Group E (8.69±0.7 mmol/L); Group D (8.2±0.69 mmol/L); Group C (7.8±1.09 mmol/L); Group G (7.79±1.04 mmol/L); and Group B (7.3±1.48 mmol/L), respectively. Tukey test showed that the group of fish without supplementation of curcumin and injection of thyroxin hormone (Group A/control) has no difference with Group F (p>0.05). While, there were significant differences (p<0.05) between Group A and F to Group B, C, D, E, G, and H.

The results showed that curcumin supplementation can reduce MDA levels in the serum of common carp. However, when given additional injections of the hormone thyroxine, the MDA levels in serum increased. Provision of 0.25% curcumin supplementation (Group B) showed more optimal results in reducing MDA levels in the serum of common carp. The MDA concentration of group F did not differ from Group A. It was possible because the fish in group F experienced stress during rearing, which increased levels of MDA. Meanwhile, the increase in MDA concentrations that occurred in the group injected with the thyroxine hormone thought due to an increase in the basal metabolic rate as a form of activity of the thyroxine hormone. This increase in metabolic rate can affect the formation of free radicals. Insufficient antioxidants to neutralize free radicals that formed can lead to cell damage caused by lipid peroxide indicated by an increase in MDA levels [7,11,12]. Meanwhile, the injection of the thyroxine hormone combined with curcumin supplementation 0.5% (Group G) showed lower MDA values compared to other combinations. It showed that the combination can optimize antioxidant performance as indicated by low levels of MDA in serum.
3.2. Concentration of Serum Glutamic Pyruvic Transaminase (SGPT) in serum

The SGPT concentrations of common carp that supplementation with curcumin and injection of thyroxine hormone showed in Figure 2. At the end of rearing, the highest concentration of MDA was showed by Group B (31.42±6.6 unit/L) and followed by Group A/control (31.1±2.54 unit/L); Group D (27.61±6.6 unit/L); Group H (25.7±4.76 unit/L); Group G (22.85±1.9 unit/L); Group C (22.67±0.18 unit/L); Group E (22.1±2.77 unit/L); and Group F (16.18±4.7 unit/L), respectively. Tukey test showed that the concentration of SGPT in common carp of Group A and B were significantly different to common carp in Group F (p<0.05), but not different to Group C, D, E, G, and H (p>0.05).

The results showed that the combination of curcumin supplementation and the injection of thyroxine hormone can reduce the SGPT concentration in common carp serum. It was showed that supplementation of 0.25% curcumin combined with the injection of the thyroxine hormone can optimize liver performance because of the low damage to the liver as indicated by the lower SGPT value in the
serum of common carp. These results suggest that curcumin and thyroxine supplementation have the potential to protect the liver during the reproduction period in common carp females.

The effect of curcumin and thyroxine supplementation on MDA and SGPT concentrations did not appear to have the same pattern. It happens because the release of MDA may not only come from the liver [21], while the release of SGPT tends to mostly come from the liver [13,14]. When curcumin enters the circulatory system, it will not only be brought down to the liver but also to other organs. Research conducted by Manju et al showed that supplementation of curcumin in Anabas testudineus fish can protect the liver and kidneys from oxidative stress [22]. The results showed that the supplementation of curcumin and the thyroxine hormone can support the antioxidant activity and protect the liver. Research conducted on groups of catfish that were supplemented with curcumin and the thyroxine hormone in the feed also showed a decrease in serum concentrations of SGPT and liver MDA during the reproductive period [5,8].

4. Conclusion
Supplementation of curcumin and thyroxine hormone in female broodstock of common carp can support the activity of antioxidant during reproduction period and can protect liver during reproduction period. The supplementation of curcumin with dose 0.25% .kg⁻¹ feed showed more optimal results in supporting antioxidant activity as indicated by decreasing MDA levels in common carp serum. Meanwhile, the supplementation of curcumin with a dose of 0.25% .kg⁻¹ of feed combined with the injection of the hormone thyroxine provided optimal protection against the liver as indicated by a lower SGPT concentration.

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