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The Blood Chemistry Profile of African Catfish *(Clarias gariepinus)* Broodstock that Supplemented with Curcumin and Thyroxine Hormone during Vitellogenesis

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Abstract

The biochemical analysis provides valuable information in monitoring fish health and condition. Fish reproduction is one of the factors that strongly influence the organism's internal environment. This study was designed to study the biochemical profile of the blood from female broodstock catfish supplemented with curcumin and thyroxine during vitellogenesis. The study was conducted using a completely randomized design (CRD) 2x2 factorial pattern and each treatment consisted of four replications. The first factor is the thyroxine hormone which consists of two doses, i.e. 0 and 0.1 mg/kg of feed. The second factor is the dose of curcumin in feed which consists of two levels, i.e. 0 % and 0.5 %/kg of feed.

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Results showed that there were significant differences ($p < 0.05$) in the concentration of glucose, triglyceride and high-density lipoprotein (HDL) in serum, but was not significant differences ($p > 0.05$) in concentration of protein and cholesterol in serum. The conclusion of this research are the blood chemistry profile of experimental catfish is strongly influenced by the ongoing reproductive activity, and the supplementation of curcumin in catfish broodstock showed a better ability to provide nutrients needed for the synthesis of vitellogenin.

Keywords: African catfish; Curcumin; Blood chemistry; Thyroxine hormone; Vitellogenesis.

1. Introduction

Blood analysis can show the condition of nutrition, physiology, and environment of fish. For example in response to stress, pollution, and nutrition as well as ecological and physiological conditions in which affect the profile of the blood. The main changes that usually occur in the composition of blood in response to it are fluctuations in protein, glucose, cholesterol, and other blood components [1]. The biochemical analysis provides valuable information in monitoring the fish health and condition. Changes in biochemical index depend on fish species, age, sexual maturity cycle, and fish health condition [1,2]. Fish reproduction is one of the factors that strongly influence the organism's internal environment. Adequate nutrition and good physiological conditions during the reproductive process in fish not only improve the quality of eggs and sperm, but also the seeds produced [3]. During the reproductive period, nutrient mobilization occurs in developing oocytes. Nutrient variations in serum can be caused by gonad maturation and the transport of nutrient from several organs to the gonads through the circulatory system [4]. The sufficient availability of chemical compounds that needed as basic material and source of energy during the gonad development process can optimize the reproductive development of fish. Especially during the process of vitelogenesis, nutritional requirements are very high in fulfilling the formation of vitelogenin which is the main precursor of egg yolk. The constituent components of vitellogenin in fish shows that the main constituents of vitellogenin are proteins (79%), lipids (19%), carbohydrates (0.3%), and other compounds such as phosphorus protein (0.7%) and calcium (0.7%) [5]. Therefore the availability of basic ingredients for the synthesis of vitellogenin such as glucose, protein and lipids must be fulfilled. Research conducted on catfish (*Panasianodon hypophthalmus)* shows that the addition of turmeric flour to the broodstock feed can increase the content of vitelogenin in serum and fish eggs [6]. The same thing also appears in ducks given curcumin supplementation where there is an increase in vitelogenin levels [7]. This shows that there may be an effect of curcumin supplementation on the process of providing nutrients as raw material for the synthesis of vitellogenin. However, the chemical parameters of blood such as protein, glucose, triglycerides, cholesterol, and high-density lipoproteins (HDL) in serum from female brood fish supplemented with curcumin are not yet known. Therefore, this study was designed to study the biochemical profile of the blood such as protein, glucose, lipid (triglycerides, cholesterol and HDL) in serum from female broodstock catfish supplemented with curcumin and thyroxine during vitelogenesis.

2. Methodology

This research was conducted from August 2018 until January 2019. The treatment fish was reared at the Center for Freshwater Aquaculture, Sukabumi, West Java, Indonesia. The study was conducted using a completely

randomized design (CRD) 2x2 factorial pattern and each treatment consisted of four replications. The first factor is the thyroxine hormone which consists of two doses, i.e. 0 and 0.1 mg/kg of feed. The second factor is the dose of curcumin in feed which consists of two levels, i.e. 0 % and 0.5 %/kg of feed. The experimental group consisted of: Group A (Control: 0% curcumin / kg. Feed and 0 mg. Thyroxine / kg. Feed); Group B (0.5% curcumin / kg. Feed and 0 mg. Thyroxine / kg. Feed); Group C (0% curcumin / kg. Feed and 0.1 mg. Thyroxine / kg. Feed); and Group D (0.5% curcumin / kg. feed and 0.1 mg. Thyroxine / kg. feed). The experimental animals used in this study were female catfish with initial body weights between 250-350 g. The catfishes were reared in the net. The Catfishes were reared for 12 weeks and the experimental feed was given in the form of commercial feed with 42.70% of the protein that added with curcumin and thyroxine. The curcumin concentration was used i.e. 0.5% / kg of feed and the thyroxine i.e. 0.1 mg/kg of feed. The feed was given to fish as much as 2% of body mass and is carried out twice a day in the morning and evening. As much as one fish every three weeks was taken randomly in each replicate in all treatments for blood drawn. The obtained blood was put into a polyethylene tube and centrifuged at 3000 rpm for 10 minutes at 4 °C to obtain serum. Furthermore, the serum was used for blood chemistry observations. The parameters observed were blood glucose levels determined by *glucose oxidase-peroxidase aminoantypirin* (GOD-PAP) method [8]; Serum protein concentration was determined using the biuret method [8]; Triglyceride concentrations were determined by *gliserol phosphate oxidase-p-aminophenozone* (GPO-PAP) method [9]; Blood cholesterol concentration was determined by *cholesterol oxidation-phenol-4-aminoantipyrine-peroxidase* (CHOD-PAP) method [10]; The concentration of high density lipoprotein (HDL) blood was determined by the CHOD-PAP method [10]. Then all data collected was analyzed using Analysis of Variance (ANOVA) on MINITAB (version 16). Differences between the treatment mean values were tested using the Tukey Test.

3. Results

3.1 Concentration of blood sugar in the serum of catfish given curcumin and thyroxine supplementation for 12 weeks

The catfish blood glucose concentrations for 12 weeks of treatment are presented in Table 1. The Concentration of glucose in serum at the beginning of the observation before treatment was 139.21 $\pm$ 39.69 mg/dL. After the three weeks of curcumin and thyroxine supplementation treatment in catfish there was a significant difference ($p < 0.05$) between treatments in blood glucose concentration. Catfish supplemented with curcumin and thyroxine (Group D) showed the highest blood glucose concentration (133.56 $\pm$ 13.56 mg/dL) and followed by catfish supplemented with thyroxine without curcumin (Group C; 114.36 $\pm$ 9.4 mg/dL), catfish supplemented with curcumin without thyroxine (Group B; 100.96 $\pm$ 5.43 mg/dL), and catfish which were not supplemented with curcumin and thyroxine (Group A/control; 89.56 $\pm$ 6.72 mg/dL). Tukey test results showed that catfish supplemented with curcumin and thyroxine (Group D) were no different ($p > 0.05$) from catfish supplemented with thyroxine without curcumin (Group C), but there was a significant difference ($p < 0.05$) compared to catfish supplemented with curcumin without thyroxine (Group B) and catfish that are not supplemented with curcumin and thyroxine (Group A).

Table 1: Concentration of blood glucose (mg/dL) of catfish supplemented with curcumin and thyroxine hormone for 12 weeks

Week of treatment	Groups of treatment			
	A	B	C	D
Three weeks	89.56 ± 6.72^{c}	100.96 ± 5.43^{bc}	114.36 ± 9.40^{ab}	133.56 ± 13.56^{a}
Six weeks	149.10 ± 16.11^{a}	143.29 ± 17.55^{a}	131.74 ± 15.45^{a}	121.02 ± 17.55^{a}
Nine weeks	132.28 ± 15.91^{a}	92.44 ± 19.54^{b}	97.03 ± 19.48^{ab}	98.91 ± 11.37^{ab}
Twelve weeks	94.88 ± 10.29^{a}	95.39 ± 15.16^{a}	90.73 ± 10.63^{a}	95.11 ± 17.60^{a}

Means ± standard deviation with different superscripts indicate a significant difference ($p<0.05$).

At the sixth week after treatment, there was no significant difference ($p> 0.05$) between treatments in blood glucose concentration. At the ninth week after treatment there was a significant difference ($p <0.05$) between treatments. Control, catfish which were not supplemented with curcumin and thyroxine (Group A) showed the highest blood glucose concentration (132.28 ± 15.91 mg/dL) which were followed by catfish supplemented with curcumin and thyroxine (Group D; 98.91 ± 11.37 mg/dL), catfish supplemented with thyroxine without curcumin (Group C; 97.03 ± 19.48 mg/dL), and catfish supplemented with curcumin without thyroxine (Group B; 92.44 ± 19.54 mg/dL). Tukey's test showed that catfish which were not supplemented with curcumin and thyroxine (Group A) were no different ($p> 0.05$) compared to catfish supplemented with curcumin and thyroxine (Group D) and catfish supplemented with thyroxine without curcumin (Group C), but there were catfish supplemented with curcumin and thyroxine (Group D) and catfish supplemented with thyroxine without curcumin (Group C), significant difference ($p <0.05$) compared to catfish supplemented with curcumin without thyroxine (Group B).At the 12th weeks after treatment there was no significant difference ($p> 0.05$) in blood glucose concentration between treatments. The results of this study indicated that there was a fluctuation in blood glucose concentrations in all treatment groups for 12 weeks of treatment. This fluctuation can be influenced by the physiological condition of experimental catfish that are undergoing a process of reproduction. The analysis showed that there was no interaction ($p> 0.05$) between the addition of curcumin and the thyroxine hormone in the concentration of glucose in the serum in the third, sixth, and 12th weeks. At the ninth week ($p <0.05$) it appears that there is an interaction between the addition of curcumin and the thyroxine hormone to the concentration of glucose in the serum.

3.2 Concentration of protein in serum of catfish supplemented with curcumin and thyroxine for 12 weeks

The concentration of protein in the serum of catfish for 12 weeks of treatment is presented in Table 2. The concentration of protein in the serum at the beginning of the observation before being given treatment was 2.64 ± 1.25 g/dL. At the third week of treatment, the statistical analysis of serum protein concentrations in all treatments did not show a significant difference ($p> 0.05$). The same pattern was observed in serum cholesterol concentrations at the sixth, ninth, and 12th weeks. These results indicate that the supplementation of curcumin and thyroxine in catfish experiments did not affect the concentration of protein in the serum. Increased serum protein concentrations occur in line with the rearing time. The results showed that control, catfish which were

not supplemented with curcumin and thyroxine (Group A) had protein concentrations in serum which tended to be higher compared to treatments added with curcumin and thyroxine, whereas fish given thyroxine without curcumin (Group C) seemed to have protein concentrations in serum which tends to be lower compared to other treatments.

Table 2: Concentration of serum protein (g / dL) catfish supplemented with curcumin and thyroxine hormone for 12 weeks

Week of treatment	Groups of treatment			
	A	B	C	D
Three weeks	3.55 ± 0.97^{a}	3.44 ± 0.95^{a}	3.23 ± 0.49^{a}	3.45 ± 0.60^{a}
Six weeks	4.90 ± 0.48^{a}	4.52 ± 0.27^{a}	4.28 ± 0.42^{a}	4.80 ± 0.45^{a}
Nine weeks	4.97 ± 0.23^{a}	4.97 ± 0.33^{a}	5.16 ± 0.27^{a}	5.07 ± 0.20^{a}
Twelve weeks	5.64 ± 0.32^{a}	5.79 ± 0.10^{a}	5.33 ± 0.20^{a}	5.40 ± 0.40^{a}

Means ± standard deviation with different superscripts indicate a significant difference ($p<0.05$).

The analysis showed that there was no interaction ($p> 0.05$) between the addition of curcumin and the thyroxine hormone to the concentration of protein in serum in the third, ninth, and 12th weeks. At the sixth week, the analysis showed an interaction ($p <0.05$) between the addition of curcumin and the thyroxine hormone in the serum protein concentration.

3.3 Concentrations of triglycerides in the serum of catfish supplemented with curcumin and thyroxine for 12 weeks

Triglyceride concentrations in catfish serum for 12 weeks of treatment are presented in Table 3. Triglyceride concentrations in serum at the beginning of the observation before treatment was 167.89 ± 40.76 mg/dL.

Table 3: Serum triglyceride concentration (mg/dL) of catfish supplemented with curcumin and thyroxine hormone for 12 weeks

Week of treatment	Groups of treatment			
	A	B	C	D
Three weeks	200.20 ± 52.12^{b}	192.06 ± 27.62^{b}	337.54 ± 19.01^{a}	267.77 ± 94.12^{ab}
Six weeks	198.92 ± 17.90^{ab}	220.80 ± 34.65^{a}	166.99 ± 25.75^{b}	205.85 ± 20.90^{ab}
Nine weeks	150.28 ± 10.16^{a}	153.62 ± 13.36^{a}	151.53 ± 2.27^{a}	147.77 ± 5.03^{a}
Twelve weeks	154.89 ± 10.93^{b}	146.14 ± 9.36^{b}	146.48 ± 6.88^{b}	171.52 ± 7.10^{a}

Means ± standard deviation with different superscripts indicate a significant difference ($p<0.05$).

At week 3, curcumin and thyroxine supplementation treatments showed a significant difference ($p <0.05$) between treatment groups in the concentration of triglycerides in serum. Catfish supplemented with thyroxine

without curcumin (Group C) had the highest triglyceride concentration (337.54 ± 19.01 mg/dL), and followed by catfish supplemented with curcumin and thyroxine (Group D; 267.77 ± 94.12 mg/dL), catfish did not supplemented with curcumin and thyroxine (Group A / control; 200.20 ± 52.12 mg/dL), and catfish supplemented with curcumin without thyroxine (Group B; 192.06 ± 27.62 mg/dL). Tukey test showed that catfish supplemented with thyroxine without curcumin (Group C) were significantly different ($p < 0.05$) compared to catfish which were not supplemented with curcumin and thyroxine (Group A) and catfish supplemented with curcumin without thyroxine (Group B), but not different ($p > 0.05$) compared to catfish supplemented with curcumin and thyroxine (Group D). At the sixth weeks after the treatment of curcumin and thyroxine supplementation there was a significant difference ($p < 0.05$) between treatments in the concentration of triglycerides in serum. Catfish supplemented with curcumin without thyroxine (Group B) showed the highest triglyceride concentration (220.80 ± 34.65 mg/dL), then followed by catfish supplemented with curcumin and thyroxine (Group D; 205.85 ± 20.90 mg dL), catfish was not supplemented with curcumin and thyroxine (Group A; 198.92 ± 17.90 mg/dL), and catfish supplemented with thyroxine without curcumin (group C; 166.99 ± 25.75 mg/dL). Tukey test results showed that catfish supplemented with curcumin without thyroxine (Group B) were no different ($p > 0.05$) compared to catfish supplemented with curcumin and thyroxine (Group D) and catfish that were not supplemented with curcumin and thyroxine (Group A), but significantly different ($p < 0.05$) compared to catfish supplemented with thyroxine without curcumin (Group C). At nine weeks after administration of curcumin and thyroxine supplementation there was no significant difference ($p > 0.05$) between treatments for the concentrations of serum triglyceride. At the 12th weeks after treatment, supplementation of curcumin and thyroxine showed a significant difference ($p < 0.05$) between treatment groups in triglyceride concentrations in serum. Catfish supplemented with curcumin and thyroxine (Group D) showed the highest triglyceride concentrations (171.52 ± 7.10 mg/dL), which were then followed by catfish which were not supplemented with curcumin and thyroxine (Group A; 154.89 ± 10.93 mg/dL), catfish supplemented with thyroxine without curcumin (Group C; 146.48 ± 6.88 mg/dL), and catfish supplemented with curcumin without thyroxine (Group B; 146.14 ± 9.36 mg/dL). Tukey's test showed that catfish supplemented with curcumin and thyroxine (Group D) were significantly different ($p < 0.05$) compared to catfish that were not supplemented with curcumin and thyroxine (Group A), catfish supplemented with curcumin without thyroxine (Group B), and catfish supplemented with thyroxine without curcumin (Group C). The analysis showed that there was no interaction ($p > 0.05$) between the addition of curcumin and thyroxine to the concentration of triglycerides in serum in the third, sixth, and ninth weeks. At the 12th week, the analysis showed an interaction ($p < 0.05$) between the addition of curcumin and the thyroxine hormone to the concentration of triglycerides in serum.

3.4 Concentration of cholesterol in serum of catfish supplemented with curcumin and thyroxine for 12 weeks

The concentration of cholesterol in the serum of catfish for 12 weeks of treatment is presented in Table 4. The concentration of cholesterol in serum at the beginning of the observation before being given treatment was 129.33 ± 23.35 mg/dL. At the third week of treatment, statistical analysis of serum cholesterol concentrations in all treatments showed no significant difference ($p > 0.05$). The same pattern was observed in serum cholesterol concentrations at the sixth, ninth, and 12th weeks. These results indicate that supplementation of curcumin and thyroxine in catfish experiments does not affect serum cholesterol concentrations. The analysis showed that there was no interaction ($p > 0.05$) between the addition of curcumin and the thyroxine hormone to the

concentration of cholesterol in serum.

Table 4: The total of serum cholesterol concentration (mg/dL) from catfish that supplemented with curcumin and thyroxine hormone for 12 weeks

Week of treatment	Groups of treatment			
	A	B	C	D
Three weeks	141.86±18.83[a]	123.81±7.47[a]	141.07±15.22[a]	124.08±18.97[a]
Six weeks	123.87±32.52[a]	121.10±23.72[a]	132.16±25.74[a]	135.70±24.26[a]
Nine weeks	147.93±26.16[a]	122.49±20.83[a]	147.93±11.87[a]	135.50±23.76[a]
Twelve weeks	122.97±16.58[a]	126.65±17.98[a]	118.22±18.36[a]	136.56±14.67[a]

Means ± standard deviation with different superscripts indicate a significant difference ($p<0.05$).

3.5 Concentration of HDL-cholesterol in serum of catfish supplemented with curcumin and thyroxine for 12 weeks

The concentration of HDL in serum catfish during the 12-weeks of treatment is presented in Table 15. At the beginning of the observation, the average HDL concentration was 108.75 ± 18.91 mg/dL. In the third, sixth, and ninth weeks of curcumin and thyroxine supplementation there were no differences between treatments for HDL concentrations in serum ($p> 0.05$).

Table 5: The serum cholesterol-HDL concentration (mg/dL) of catfish supplemented with curcumin and thyroxine hormone for 12 weeks

Week of treatment	Groups of treatment			
	A	B	C	D
Three weeks	89.66±14.31[a]	67.45±18.03[a]	73.34±9.21[a]	78.91±16.67[a]
Six weeks	80.03±14.65[a]	72.13±13.31[a]	76.08±21.18[a]	92.91±22.01[a]
Nine weeks	88.15±12.17[a]	104.06±19.04[a]	96.97±6.11[a]	93.83±13.29[a]
Twelve weeks	107.65±11.14[ab]	108.49±10.81[ab]	92.54±7.46[b]	137.90±38.90[a]

Means ± standard deviation with different superscripts indicate a significant difference ($p<0.05$).

At 12 weeks after supplementation with curcumin and thyroxine, statistical analysis of HDL concentrations in serum showed a significant difference ($p <0.05$) between treatments. Catfish supplemented with curcumin and thyroxine (Group D) showed the highest serum HDL concentrations (137.90 ± 38.90 mg/dL) which were followed by catfish supplemented with curcumin without thyroxine (Group B; 108.49 ± 10.81 mg/dL), catfish which not supplemented with curcumin and thyroxine (Group A; 107.65 ± 11.14 mg/dL), and catfish supplemented with thyroxine without curcumin (Group C; 92.54 ± 7.46 mg/dL). Tukey test showed that catfish supplemented with curcumin and thyroxine (Group D) were no different ($p> 0.05$) compared to catfish supplemented with curcumin without thyroxine (Group B) and catfish that were not supplemented with

curcumin and thyroxine (Group A), but there was a significant difference ($p < 0.05$) compared to catfish supplemented with thyroxine without curcumin (Group C). The results of this study indicate that an increase in HDL concentrations in plasma is in line with the rearing time. The analysis showed that there was no interaction ($p > 0.05$) between administration of curcumin and the thyroxine hormone in HDL concentrations in serum.

4. Discussions

The concentration of glucose serum in this study showed an increase at the beginning of the treatment then fluctuated and then decreased at the end of the treatment. Fluctuations in glucose concentrations that occur during the rearing due to the activity of taking and transporting glucose as a source of energy for body cells which were also influenced by the physiology of experimental catfish that are at the stage of gonad maturation. The results of this study showed that starting from the third week, serum glucose levels in the catfish group that treated with the addition of curcumin and thyroxine increased compared to controls. An increase in serum glucose levels can be associated with a temporary period of gonad maturation. In the period of gonad maturation an increase in the percentage of glucose in the blood [11,12]. Glucose was used as energy for the synthesis of vitellogenin that needed by each egg that is developing simultaneously, and the glucose itself also one of the constituent ingredients of vitellogenin [5]. It caused the high use of glucose in the period of gonad maturation After nine weeks of treatment, the addition of curcumin and the hormone thyroxine decreased glucose levels in serum compared with controls who experienced a decrease in serum glucose levels after 12 weeks of treatment. This shows that the catfish given curcumin and thyroxine supplementation experienced faster gonad maturation compared to controls. Protein in blood serum plays an important role in the transport of various types of endogenous and exogenous chemicals, the defense of organisms against infections, parasites, and xenobiotics as well as various other functions. The composition of serum proteins and the level of concentration of each component depend on fish species, age, life cycle, and gonad maturity, feed, health, and environmental factors [13]. In this study, the concentration of protein in the serum continued to increase in line with rearing time. The increase in protein concentration in this study occurred due to the process of vitellogenesis that was occurring in catfish experiments which was indicated by an increase in the gonadosomatic index. The condition of catfish experiments that are still in the stage of growth and development which is characterized by the increase in length and body weight are also the factors that influence the increase in serum protein levels because protein is needed for the formation of tissues in the body. In addition, high protein availability in feed is also one of the factors that influenced the increase in serum protein concentration. Research conducted on Cyprinus carpio carp showed that an increase in protein concentration was followed by an increase in the gonadosomatic index (GSI) value [14]. High protein concentration in serum during the process of vitellogenesis was due to the transport of vitellogenin to oocytes through the circulatory system, where the main constituent components of vitellogenin are proteins [5]. The higher increase in protein content and metabolic rate will affect the appearance of reproduction [15]. Conversely, a decrease in protein during gonad development will decrease body protein and decrease the appearance of the embryo, which can specifically reduce the availability of nutrients, especially essential amino acids, which are essential for growth. In general, in all groups there occurred the fluctuation of lipid concentration such as triglycerides, cholesterol, and HDL in fish blood serum. Fluctuation that occurred during this study was thought to be the response of each individual catfish experiment to the reproductive activity. The results of this study showed that catfish supplemented with a combination of curcumin and

thyroxine hormone had higher triglyceride concentration value. Higher triglyceride concentrations were detected in female fish entering the stage of vitellogenesis. The process of vitellogenesis requires very high energy and fat tends to be an energy source [16].

The concentration of cholesterol in the serum of experiment catfish without curcumin supplementation showed a pattern of fluctuation, whereas in the catfish group supplemented with curcumin showed a constant pattern. This shows that besides the effect of reproductive activity that is happening in experimental catfish, there is a special mechanism of curcumin supplementation that affects serum cholesterol concentrations. At the end of the observation, groups of catfish supplemented with curcumin showed an increase in cholesterol concentration, whereas catfish that were not supplemented with curcumin showed a decrease in serum cholesterol concentration at the end of the observation. A decrease in cholesterol concentration is possible because of the need for cholesterol as part of the cell membrane and endogenous structure of the egg during the reproductive process [12]. The results of this study indicate an increase in HDL concentrations during the process of vitellogenesis. This is thought to be related to the function of HDL besides being able to act as an alternative carrier of the transport of fatty acids in the blood it also plays a role in the metabolism of low density lippoprotein (LDL) [17]. LDL itself plays a role in the transport of fatty acids to the gonads which then these fatty acids will be deposited in each developing oocyte. During the process of vitellogenesis there is an increase in the transport of fatty acids to the oocytes which can cause an increase in serum LDL concentrations. That is possible that increase in LDL then allows an increase in HDL which plays a role in LDL metabolism. In this study, there was an increase in HDL values in the serum of catfish supplemented with curcumin. This shows the role of curcumin in increasing serum HDL levels [18].

5. Conclusion

The blood chemistry profile of experimental catfish is strongly influenced by the ongoing reproductive activity and supplementation of curcumin in catfish broodstock showed a better ability to provide nutrients needed for the synthesis of vitellogenin.

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