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Levels of 17β-estradiol, Vitellogenin, and Egg Diameters During the Reproductive Cycle of Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Estradiol, vitellogenin levels, and egg diameter are indicators of oocyte quality in aquaculture. This study evaluated the performance of 17β-estradiol (E2) and vitellogenin (Vtg) concentrations in eggs and oocyte diameter during the reproductive period of *Oreochromis niloticus* at the Fish Breeding Research Center, Sukamandi, Subang, West Java. Six-month-old female fish were fed a diet supplemented with varying doses of curcumin analog in the morning and evening for six weeks. The study used a completely randomized design with seven treatments and three replicates. E2 and Vtg concentrations in eggs were analyzed using the enzyme-linked immunosorbent assay (ELISA) Fish kit. The results indicated that the supplementation of curcumin analog in female broodfish of red tilapia increased the concentration of E2, which in turn stimulated Vtg synthesis in the liver, subsequently stored in developing follicles during gonadal maturation. The group supplemented with 2.4 mg of curcumin analog per 100 g of feed exhibited the highest concentrations of E2 (114.46 ng/mL) and egg Vtg (55.63 mg/mL). This study provides valuable insights for enhancing reproductive performance by incorporating curcumin analogs into the feed for commercial tilapia farming.
Keywords: Curcumin analog, fecundity, liver, *Oreochromis niloticus*, reproduction

ABSTRAK

Tingkat sirkulasi estradiol, vitellogenin dan diameter telur bisa menjadi referensi kualitas oosit dalam bidang akuakultur. Penelitian ini mengevaluasi kinerja konsentrasi estradiol-17β (E2), vitellogenin (Vtg) telur, dan diameter oosit selama periode reproduksi *Oreochromis niloticus* di Balai Riset Pemuliaan Ikan, Sukamandi, Subang, Jawa Barat. Ikan betina berumur 6 bulan diberi pakan dengan suplementasi analog kurkumin dosis berbeda pada pagi dan sore hari selama 6 minggu. Penelitian ini menggunakan rancangan acak lengkap dengan tujuh perlakuan dan tiga kali ulangan. Penentuan konsentrasi estradiol-17β dan vitellogenin telur menggunakan metode analisis *enzyme linked immunosorbent assay* (ELISA) *Fish kit.* Hasil penelitian menunjukan pemberian analog kurkumin pada induk betina nila merah meningkatkan konsentrasi hormon estradiol-17β sehingga merangsang sintesis vitellogenin di hati yang akan disimpan dalam folikel yang sedang berkembang selama kematangan gonad. Konsentrasi hormon estradiol-17β dan vitellogenin telur tertinggi ditemukan pada kelompok ikan yang disuplementasi 2,4 mg analog kurkumin per 100 g pakan yaitu sebesar 114,46 ng/mL dan 55,63 mg/mL. Informasi yang dilaporkan dalam penelitian ini berguna untuk meningkatkan kinerja reproduksi melalui pemberian analog kurkumin pada pakan dalam budidaya komersial nila.
Kata kunci: analog kurkumin, estradiol, fekunditas, oosit, *Oreochromis niloticus*, vitellogenin

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is the second most farmed fish globally and can live in both freshwater and brackish water (Wang & Lu, 2016). Nile tilapia (*Oreochromis niloticus*) is a significant economic commodity in freshwater aquaculture, which is rapidly developing in Indonesia. It is part of the revitalization program, highlighting the need for developments in aquaculture technologies concerning the availability of high-quality broodstock. High-quality fingerlings in sufficient and continuous quantities are crucial for production in freshwater aquaculture activities. The success rate of seed production in aquaculture activities is highly dependent on the success of broodstock spawning. The number of fry obtained through spawning is influenced by egg quality. In contrast, egg quality is greatly influenced by broodstock health and the nutritional content of feed provided during the reproductive period (Dewi et al., 2020).

Aquaculture technology development is closely linked to reproductive capacity and the availability of quality broodstock, which includes the synthesis of vitellogenin (Hara *et al*., 2016). Vitellogenesis refers to the synthesis, transport, and absorption of vitellogenin, a precursor for egg yolk (Reading & Sullivan, 2017). Vitellogenin, synthesized in the liver, is released into the blood plasma and transported to the oocytes. It binds to receptors on the oocyte surface and is subsequently absorbed via endocytosis.

Curcumin analogues are synthetic compounds derived from lawang oil (*Cinnamomum cullilawan* Blume) that utilize the reactivity of the dioxolane ring in safrole. These analogues exhibit pharmacological properties comparable to or superior to those of the parent compound (Yang *et al*., 2013), and demonstrate high biological activity and bioavailability without increasing toxicity. The synthesis of curcumin analog compounds aims to enhance their stability, potency, and selectivity of biological activity (Rahmawati *et al*., 2018). It serves as a synthetic product designed to mimic the effects of curcumin. Curcumin exhibits phytoestrogenic properties and has hepatoprotective benefits, belonging to the flavonoid class that can act as estrogen, thereby stimulating the liver to synthesize vitellogenin (Saraswati *et al*., 2013).

Curcumin compounds also stimulate the gallbladder wall to secrete bile into the small intestine, enhancing the digestion of fats, proteins, carbohydrates, and nutrients. Incorporating phytoestrogens into feed can elevate levels of estrogen hormones, specifically 17β-estradiol (E2), which are subsequently transported to the liver. The liver is stimulated to synthesize vitellogenin (Vtg). It is then distributed to key follicles for deposition, thus facilitating oocyte growth (Dewi *et al.*, 2018a).

The supplementation of curcumin analogues in feed aimed to enhance the liver's vitellogenin biosynthesis. This study evaluated the effect of curcumin analogues in feed on vitellogenin synthesis and deposition in red tilapia eggs during the reproductive period.

MATERIALS AND METHOD

The study was conducted from May to August 2021 at the Fish Breeding Research Center (BRPI) in Sukamandi, Subang, West Java. Estradiol-17β and vitellogenin hormone concentrations were measured in ovulated eggs at the Integrated Laboratory of BRPI, Sukamandi, Subang.

Research Animals

The research animals comprised 105 red tilapia broodstock aged six months, with a body length of 14-20 cm and a weight of 200-350 g. The tilapia were kept in 21 outdoor ponds measuring 2x1x1 m3, with a density of five fish per pond. This study employed a completely randomized design with seven treatments and three replications. Each replication consisted of five fish.

The treatments administered were Control/P0 (no supplementation), P1 (2.4 mg curcumin analog/100 g feed), P2 (4.8 mg curcumin analog/100 g feed), P3 (25 mg turmeric powder/100 g feed), P4 (50 mg turmeric powder/100 g feed), P5 (2.4 mg commercial pure curcumin/100 g feed), and P6 (4.8 mg commercial pure curcumin/100 g feed).

The broodstock were adapted for one month prior to receiving supplemented feed. The feed treatment was administered during the six-week maintenance period. During maintenance, the fish were fed commercial feed with a protein content of 33% supplemented with curcumin analog, turmeric powder, and commercial pure curcumin according to the treatment dose. Curcumin analog was obtained from the synthesis of lawang oil (*Cinnamomum cullilawan* Blume), and turmeric powder was obtained from the Indonesian Institute of Sciences (LIPI) in Cimanggu, Bogor. The pure curcumin used was produced by Xi'an Day Natural Inc. The feed administered was 3% of body weight twice daily, in the morning and evening. The feed coating process used carboxymethyl cellulose (CMC) as a binder at 3% of the total feed administered. All procedures involving the handling and care of experimental animals were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, IPB University, No. 004/KEH/SKE/II/2021.

Sample Collection

One fish was randomly selected every two weeks from each treatment group. Before dissection and gonad sampling, the fish were anesthetized using tricaine methanesulfonate (MS-222) at 1 mL/liter of water. Blood samples were collected for measurement of 17-β estradiol concentration. The blood samples were collected in reaction tubes, stored on ice, and centrifuged at 3000 rpm for 20 minutes at 4°C. The fish were then dissected, and gonadal samples were collected for histological preparation of gonadal tissue.

The concentrations of estradiol-17β and vitellogenin in ovulated eggs were quantified using the enzyme-linked immunosorbent assay (ELISA) method, employing the Fish Estradiol Kit (E2 ELISA Kit) and the Fish Vitellogenin Kit (BT Lab E0020Fi) in accordance with the procedures outlined in the kit manuals. The weight of the fish was measured using a digital scale with a capacity of 500 grams and an accuracy of 0.01 grams. Ten ovulated eggs were collected and measured using an digital scale. Egg diameter was measured using a Zeiss microscope. Gonadal organs were isolated and stored in 10% neutral formalin buffer for histological preparation. Histological descriptions of gonadal tissue were conducted using hematoxylin-eosin staining (Bancroft & Gambel, 2008). The stained preparations were subsequently examined using a microscope.

Data Analysis

The data obtained were analyzed using Microsoft Excel 2010 and RStudio version 1.3.1093. Differences between treatment means were tested using Tukey's test. Significant differences were indicated by $P<0.05$.

RESULTS

The primary objective of this study was to evaluate the profile of estradiol-17ß, vitellogenin concentration, and diameter of ovulated eggs during the reproductive period of red tilapia (*Oreochromis niloticus*). This study evaluated the transformation of estradiol-17ß and vitellogenin during the gonadal maturation process. The development of gonadal maturity in tilapia is reflected in the diameter of the ovulated eggs. Estradiol-17β and vitellogenin concentrations in eggs are closely associated with the stage of ovarian maturity (Lubzens et al., 2010; Baumann et al., 2013).

Estradiol-17β is the primary steroid in the process of vitellogenesis and plays a role in inducing the liver to synthesize vitellogenin, an important precursor for oocyte formation, ovarian growth, and steroidogenesis (Amaral et al., 2019; Samaee et al., 2009; Shappell et al., 2010). Furthermore, elevated steroid hormone levels during maturation correlate with high gonadosomatic index values in adult female broodstock. Ghosh et al. (2016) found that plasma levels of estradiol-17β and egg weight consistently peaked during the pre-spawning period of vitellogenesis. Estradiol-17β is predominantly found in adult female broodstock, while it is generally absent in juvenile stages and male fish.

Concentration of Estradiol-17β in Plasma

Figure 1 illustrates the concentration of estradiol-17β hormone in the blood plasma of tilapia over a 6-week feeding treatment with curcumin analog supplementation. The concentration of estradiol-17β hormone in the plasma of tilapia increased weekly and decreased in the sixth week before spawning.

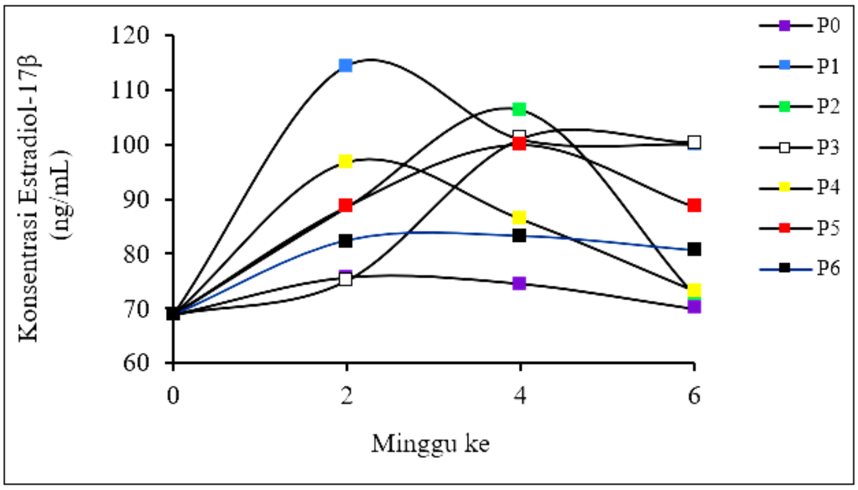

Figure 1. Concentration of estradiol-17β hormone in the blood plasma of red tilapia supplemented with curcumin analog using turmeric powder, and commercial pure curcumin as a control. Doses: 0.0 (P0), 2.4 (P1), 4.8 (P2), 25 (P3), 50 (P4), 2.4 (P5), 4.8 (P6) g/100 g feed.

Vitellogenin Concentration in Eggs

The vitellogenin content in ovulated eggs during 6 weeks of curcumin analog administration ranged from 32.15 to 55.63 mg/mL, as presented in Table 1.

Gonasomatic Index

Table 2 presents the gonadosomatic index values of red tilapia broodstock supplemented with curcumin analog for 6 weeks. The gonadosomatic index varied between 1.58% and 4.25%. Further Tukey tests on the gonadosomatic index following dietary supplementation with curcumin analog revealed that the highest gonadosomatic index (4.25%) was observed in the group of fish receiving 2.4 mg of curcumin analog per100 g feed (P1). This value was significantly different compared to the treatment with 25 mg of turmeric powder per 100 g feed (P3), 1.58%, and the control group without supplementation (P0), 2.91%.

Fecundity

The findings on the egg fertility of tilapia supplemented with various doses of curcumin analog over 6 weeks are presented in Table 3.

The study results indicate that the highest fecundity was observed in group P1, with 663.45 eggs per 100 g of broodstockstock. This was followed by group P2, P4 , P6, P5, and P3. The control treatment (P0), which did not involve superovulation, exhibited the lowest fecundity, with a production of 376.87 eggs per 100 g of broodstock.

Fertilization and Hatching Rates

Table 4 presents the fertilization and hatching rate of tilapia eggs fertilized after administration of different doses of a curcumin analog in feed.

Table 1 Vitellogenin content in tilapia eggs supplemented with curcumin analog, turmeric powder, and commercial pure curcumin for 6 weeks

Treatment Group	Egg vitellogenin (mg/ml)
P0	32.15±1.01^{c}
P1	55.63±7.09^{a}
P2	39.08±2.23^{bc}
P3	42.43±2.49^{b}
P4	35.91±1.19^{bc}
P5	39.44±1.27^{bc}
P6	37.89±0.59^{bc}

Note: Data represent the mean ± standard deviation. Different superscript letters in the same column indicate significant differences (P<0.05). (P0/Control = no supplementation, P1 2.4 = supplementation 2.4 mg curcumin analog/100 g feed, P2 4.8 = supplementation with 4.8 mg curcumin analog/100 g feed, P3 25 = supplementation with 25 mg turmeric powder/100 g feed, P4 50 = supplementation with 50 mg turmeric powder/100 g feed, P5 = supplementation with 2.4 mg commercial pure curcumin/100 g feed, and P6 4.8 = supplementation with 4.8 mg commercial pure curcumin/100 mg feed).

Table 2 Gonadosomatic index values of tilapia supplemented with curcumin analogues with turmeric powder and commercial pure curcumin as a control in feed for 6 weeks

Treatment Group	Gonadosomatic index (%)
P0	2.91±0.43^{b}
P1	4.25±0.40^{a}
P2	3.79±0.46^{ab}
P3	1.58±0.09^{c}
P4	3.51±0.48^{ab}
P5	3.15±0.76^{ab}
P6	3.61±0.36^{ab}

Note: Data represent the mean ± standard deviation. Different superscript letters in the same column indicate significant differences (P<0.05). (P0/Control = no supplementation, P1 2.4 = supplementation with 2.4 mg curcumin analog/100 g feed, P2 4.8 = supplementation with 4.8 mg curcumin analog/100 g feed, P3 25 = supplementation with 25 mg turmeric powder/100 g feed, P4 50 = supplementation with 50 mg turmeric powder/100 g feed, P5 = supplementation with 2.4 mg commercial pure curcumin/100 g feed, and P6 4.8 = supplementation with 4.8 mg commercial pure curcumin/100 mg feed).

Table 3 Egg fecundity of tilapia produced by broodstock supplemented with curcumin analog, turmeric powder, commercial pure curcumin, and control in feed for 6 weeks

Treatment Group	Fecundity (eggs/100 g female
Control	376.87±197.14^{a}
P0	663.45±58.90^{a}
P1	639.03±217.07^{a}
P2	483.38±60.14^{a}
P3	604.86±149.54^{a}
P4	513.13±146.45^{a}
P5	559.63±246.09^{a}

Notes:Data represent the mean ± standard deviation. Different superscript letters in the same column indicate significant differences (P<0.05). (P0 = without supplementation, P1 = supplementation with 2.4 mg curcumin analog/100 g feed, P2 = supplementation with 4.8 mg curcumin analog/100 g feed, P3 = supplementation with 25 mg turmeric powder/100 g feed, P4 = supplementation with 50 mg turmeric powder/100 g feed, P5 = supplementation with 2.4 mg commercial pure curcumin/100 g feed, and P6 = supplementation with 4.8 mg commercial pure curcumin/100 mg feed).

Table 4 fertilization rate of ovulated eggs and the hatching rates of eggs from dams supplemented with curcumin analog, turmeric powder, and commercial pure curcumin, used as a control, over a 6-week feeding period

Treatment Group	Parameters	
	Fertilization Rate (%)	Hatching rate of fertilized eggs (%)
P0	73.66±0.57^{a}	75.80±1.70^{ab}
P1	77.22±2.03^{a}	78.50±1.26^{a}
P2	75.44±1.50^{a}	77.11±0.99^{ab}
P3	76.66±1.52^{a}	75.96±1.49^{ab}
P4	75.89±2.00^{a}	75.08±0.52^{b}
P5	75.77±1.07^{a}	77.26±1.09^{ab}
P6	73.22±2.03^{a}	75.57±0.77^{ab}

Note: The data presented are mean values ± standard deviation. Different superscript letters in the same column indicate significant differences (P<0.05). (P0 = without supplementation, P1 = supplementation with 2.4 mg curcumin analog/100 g feed, P2 = supplementation with 4.8 mg curcumin analog/100 g feed, P3 = supplementation with 25 mg turmeric powder/100 g feed, P4 = supplementation with 50 mg turmeric powder/100 g feed, P5 = supplementation with 2.4 mg commercial pure curcumin/100 g feed, and P6 = supplementation with 4.8 mg commercial pure curcumin/100 mg feed).

Egg Diameter

Table 5 details the findings regarding the diameter and weight of tilapia eggs supplemented with varying doses of curcumin analogs in their feed over 6 weeks. The group P2 exhibited the largest egg diameter, measuring 2.40 mm. This value was significantly different from the groups P3 and P4. The results indicated that the group of fish receiving a supplement of 2.4 mg of curcumin analog per 100 g of feed (P1) exhibited the highest average egg weight, measuring 6.8 mg. The statistical analysis revealed a significant difference (P<0.05) when compared to the other treatment groups.

DISCUSSION

Concentration of Estradiol-17β in Plasma

The highest level of estradiol-17β hormone was found in the second week in the broodstock group receiving 2.4 mg of curcumin analog supplementation (P1), at 114.46 ng/mL. Meanwhile, the lowest level was found in the control group without supplementation (P0), at 75.66 ng/mL. During the fourth week, the broodstock group receiving 4.8 mg of curcumin analog supplementation (P2) exhibited the highest levels of estradiol-17β hormone, measuring 106.30

Table 5 Diameter and weight of red tilapia eggs ovulated with curcumin analog supplementation at different doses for 6 weeks

Treatments	Parameters	
	Diameter of ovulated eggs (mm)	Weight of ovulated eggs (mg)
P0	2.35±0.005[bc]	6±0.14[d]
P1	2.39±0.005[ab]	6.8±0.05[a]
P2	2.40±0.01[a]	6.3±0.05[c]
P3	2.34±0.03[c]	6.6±0.05[b]
P4	2.23±0.005[d]	6.5±0.05[b]
P5	2.38±0.005[ab]	6.4±0.05[bc]
P6	2.38±0.01[abc]	6.4±0.05[bc]

Notes: Data represent the mean ± standard deviation. Different superscript letters in the same column indicate significant differences ($P < 0.05$). (P0 = without supplementation, P1 = supplementation with 2.4 mg curcumin analog/100 g feed, P2 = supplementation with 4.8 mg curcumin analog/100 g feed, P3 = supplementation with 25 mg turmeric powder/100 g feed, P4 = supplementation with 50 mg turmeric powder/100 g feed, P5 = supplementation with 2.4 mg commercial pure curcumin/100 g feed, and P6 = supplementation with 4.8 mg commercial pure curcumin/100 mg feed).

ng/mL. Conversely, the control group without supplementation (P0) displayed the lowest levels at 75.50 ng/mL. At week 6, the highest estradiol-17β hormone levels were found in the broodstock group receiving 2.4 mg of curcumin analog supplementation (P1), at 100.06 ng/mL, and the lowest levels were found in the control group without supplementation (P0), at 69.94 ng/mL.

The supplementation of curcumin and turmeric powder in feed has been shown to elevate estradiol hormone levels in carp (Rawung & Jacson 2020) and catfish (Dewi *et al.,* 2018b). The estradiol-17β hormone stimulates the liver to synthesize vitellogenin. The concentrations of estradiol-17β and vitellogenin in blood plasma are associated with the stage of gonadal maturation (Chatakondi & Kelly 2013). Estradiol-17β is the primary steroid in vitellogenesis and plays a role in inducing liver cells to synthesize vitellogenin, an important precursor for oocyte formation, ovarian growth, and steroidogenesis. Ghosh *et al.* (2016) reported that estradiol-17β levels in plasma increased gradually during vitellogenesis, while vitellogenin levels increased consistently, peaking before spawning.

The maturation process of fish gonads is correlated with vitellogenin and estradiol-17β levels. Plasma steroid levels in the gonads can be used as an indicator of gonadal activity during the reproductive cycle (Nagahama & Yamashita 2008). Estradiol-17β concentrations in fish naturally decrease following gonadal maturation. The final stage of vitellogenin synthesis is followed by a decrease in estradiol-17β concentrations and typically occurs with the onset of full oocyte maturation (Zupa *et al.,* 2017). High concentrations of vitellogenin and estradiol-17β are associated with the stage of gonadal maturation. Estradiol-17β stimulates the synthesis of vitellogenin in the liver and its subsequent secretion, which is assimilated in oocyte development (Reading *et al.,* 2017).

Sullivan & Yilmaz (2018) reported that differences in vitellogenin levels during gonadal maturation stages correlate with fluctuations in estradiol-17β levels in sea bass (*Dicentrarchus labrax*). The ovaries produce estradiol-17β and release it into the blood plasma, where it reaches the hepatopancreas to stimulate vitellogenin synthesis—the activity of the aromatase hormone in fish increases and peaks following the vitellogenesis process. Estradiol hormone stimulates an increase in the capacity and activity of liver cells in synthesizing proteins. Additionally, estradiol is required to maintain the continuity of vitellogenin synthesis in liver cells, which synthesize proteins as the primary component of vitellogenin. Following the post-vitellogenesis period, the production of estradiol-17β decreases until the conclusion of the study.

Vitellogenin Concentration in Eggs

The Tukey test results for egg vitellogenin following feed treatment indicated that group P1 exhibited the highest concentration, measuring 55.63 mg/mL. The sequence proceeded with groups P3, P5, P2, P6, and P4. The control group without supplementation (P0) exhibited the lowest vitellogenin concentration, measured at 32.15 mg/mL.

The broodstock group receiving 2.4 mg of curcumin analog in their feed for 6 weeks exhibited a significant increase ($P<0.05$) in egg vitellogenin levels compared to the other treatment groups. The deposition of vitellogenin in eggs is determined by the synthesis of vitellogenin in liver cells. This protein is released into the bloodstream and transported to the gonads, where it is incorporated into developing oocytes via pinocytosis. Curcumin improves liver function in metabolizing nutrients utilized as components of egg yolk, thereby facilitating the development of follicular cells. During egg yolk deposition, the bioactive component curcumin is suspected to accumulate in the developing ovarian follicles (Mooraki *et al.,* 2019). Elevated vitellogenin levels in eggs from female broodstock receiving 2.4 mg of curcumin analog suggest that supplementation with curcumin analog influences vitellogenin concentration in eggs. According to Reading *et al.* (2018), fish eggs contain complex nutrients necessary to support embryonic and larval growth and cellular development.

Gonadosomatic Index

Supplementation of curcumin analog in feed significantly influenced the gonadosomatic index ($P<0.05$). The broodstock group treated with 2.4 mg of curcumin analog per 100 feed exhibited the highest gonadosomatic index, measuring 4.25%. This suggests that the tilapia in this study experienced gonadal maturation. A higher gonadosomatic index value indicates an increased likelihood of gonadal maturity in the brood fish and a proximity to the spawning period. Rawung & Jacson (2020) reported that increased vitellogenin concentrations after curcumin supplementation in feed also influenced the reproductive performance of catfish. According to Sang *et al.* (2019), changes in the concentration of estradiol-17β hormone in the plasma of female broodstock are associated with oocyte development and an increase in the gonadosomatic index value.

The gonadosomatic index value is related to the vitellogenesis process. Estradiol-17β stimulates liver cells to synthesize vitellogenin, the precursor of egg yolk. Vitellogenin synthesized in the liver is subsequently transported to the gonads through blood vessels, where it is absorbed and stored in the oocytes. This absorption occurs continuously as the size of the oocytes and the number of egg yolks increase. This leads to an increase in the gonadosomatic index value. Additionally, curcumin functions as a hepatoprotector, protecting the liver and aiding in liver function recovery by accelerating liver regeneration, thereby optimizing vitellogenin production and function.

Fecundity

The group of fish that received a supplement of 2.4 mg of curcumin analog exhibited greater fecundity compared to the control group. It is suspected that commercial feed supplemented with curcumin analog can stimulate egg production in tilapia. Rahayu *et al.* (2021) observed that supplementing curcumin in the feed enhanced gonadal maturity in female fish.

Gonadal maturation can be stimulated through feed by supplying nutrients that promote this process, including curcumin. Curcumin functions as a phytoestrogen and hepatoprotector, stimulating the liver to synthesize vitellogenin (Duke 2007; Saraswati *et al.,* 2013b). Supplementing feed with curcumin analogues can enhance follicle development and increase the number of follicle hierarchies, leading to greater egg production. Developing follicles are encouraged to ovulate promptly, which can influence the acceleration of the ovulation cycle (Saraswati *et al.,* 2013).

Egg Fertilization and Hatching Rates

Statistical analysis indicates that the administration of curcumin analogues did not significantly affect fertilization rates ($P>0.05$), but had a significant impact on hatching rates ($P<0.05$). The highest fertilization rate was observed in the broodstock group supplemented with 2.4 mg of curcumin analog per 100 g of feed (P1), at 77.22%. The lowest egg fertilization rate was observed in eggs produced by broodstock group supplemented with 4.8 mg of commercial pure curcumin (P6), at 73.22%. The highest egg hatching rate was observed in eggs produced by the broodstock group supplementated with 2.4 mg of curcumin analog per 100 g feed (P1), at 78.50%. Statistically, this value was significantly different ($P<0.05$) from the broodstock group supplementated with 50 mg of turmeric powder per 100 g feed (P4).

The results indicated that the average fertilization rate of ovulated eggs in the treatment group ranged from 73.22% to 77.22%, while the hatching rate of fertilized eggs ranged from 75.57% to 78.50%. Rawung *et al.* (2020) reported that the administration of curcumin to female broodstock catfish resulted in higher fertilization and hatching rates compared to other groups. The broodstock group treated with 2.4 mg of curcumin analog exhibited higher hatching and fertilization rates, which correlated with increased egg diameter and weight, attributed to elevated vitellogenin deposition in the eggs. Vitellogenin can function as an energy and nutrient source during the embryonic development period until egg hatching.

Huang *et al.* (2021) state that in fertilized fish eggs, lipids serve as energy reserves during embryonic development. The main components of embryos and larvae are carbohydrates, lipids, and proteins. Protein and lipid content decrease during the blastula stage, increase significantly during the segmentation stage, and then decrease thereafter. Carbohydrate content decreases significantly during development. Enzymatic activity shows a progressive increase from the embryo to the yolk sac larva stage. Pepsin and trypsin activity increase significantly during development. Amylase activity remains low before the blastula stage and increases rapidly afterward. Lipase shows a 'rising-stable-rising' trend. Changes in digestive enzyme activity indicate the dynamics of biochemical composition (Huang *et al.,* 2021). Therefore, supplementation of curcumin analogues into feed will affect the growth performance of tilapia until they reach adulthood (Mainassy *et al*., 2022).

Egg Diameter

The supplementation of curcumin analogs in feed influences both the diameter and weight of ovulated eggs. The broodstock group receiving 2.4 mg of curcumin analog supplementation exhibited greater egg diameter and weight values than the other groups. The diameter and weight of the ovulated eggs varied among the treatment groups. This variation is believed to be influenced by the egg maturation mechanism, which induces various chemical and physical changes in the egg prior to ovulation.

The increasing diameter of eggs results from the deposition of egg yolk and the distribution of oocytes that generate small particles utilized in embryonic development. The diameter of an egg reflects the energy stored within it, which is essential for embryonic development. The increase in egg diameter results from the deposition of hydrated egg yolk and the formation of oil droplets within the egg. The broodstock group receiving 2.4 mg of curcumin analog supplementation exhibited larger egg diameter and weight values, leading to increased fertilization rates, hatching rates, and larval survival rates compared to other treatment groups.

Large egg diameter and weight indicate high deposition of vitellogenin, a precursor to egg yolk, which provides essential nutrition for embryo and larval development. Vitellogenin is an endogenous food source before larvae can obtain and utilize food sources from the external environment.

Conclusion

Supplementation with a curcumin analog at a dose of 2.4 mg per 100 g of feed yielded optimal improvements in the reproductive quality and egg quality of red tilapia. This was evidenced by an increase in vitellogenin concentration in ovulated eggs, gonadosomatic index, fecundity, egg diameter and weight, fertilization rate, and hatching rate.

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