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Production of *Clarias gariepinus* seeds through accelerated female broodstock rematuration

Prama Hartami[1,*], Mahdaliana Mahdaliana[1], Eva Ayuzar[1]

[1]*Aquaculture Study Program, Faculty of Agriculture, Malikussaleh University, North Aceh, Indonesia.*

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ABSTRACT

This study aims to determine the effectiveness of Oodev and Turmeric flour on feed to accelerate the rematuration of female broodstock catfish for increasing seed production. Oodev hormone is believed to accelerate the maturation process of broodstock, while turmeric flour with its active ingredients can improve liver performance in the vitellogenesis process. The method was a non-factorial completely randomized design with 4 treatments and 3 replications. The treatments are Control, (A) Oodev 0.3 ml/kg of feed, (B) Turmeric flour 5 gr/kg of feed, and (C) Combination of Oodev + Turmeric flour. The results showed that C treatment was the best treatment which produced a GSI value of 14.57 ±2.53%, period of rematuration was 32.33 ±4.16 days, egg diameter was 1.27 ±0.12 mm, fecundity was 22,939.97 ±880.38 eggs/kg of broodstock weight, fertilization rate was 80.18 ±9.75%, hatching rate was 79.80 ±2.88%, and larval survival reached 83.17 ±9.69%. Meanwhile, the specific growth of seed length in all treatments had no significant effect between treatments. The results of this study will certainly provide useful information for increasing catfish seed production both in Aceh and on a Nationally. This acceleration of maturation also has a positive impact in maximizing the available broodstock without having to increase the number of catfish broodstock to produce seeds of superior quality and benefit the cultivators.

Introduction

The success of fish spawning activities is primarily determined by the quality of the broodstock used to produce superior and quality seeds. Meanwhile, the continuity of seed production is primarily determined by the number of existing and the speed of the broodstock to recover and produce eggs again (rematuration) after the previous spawning. Therefore, the length of time for rematuration from brood fish is a limiting factor in the availability of seeds to supply the most important link in rearing fish or other aquaculture organisms. In general, catfish take about 2 months or more depending on the availability and quality of broodstock feed and environmental conditions of the rearing pond (Ath-thar and Prakoso, 2015; Iswanto *et al.*, 2016). Thus, brood fish rematuration efforts must be considered carefully so that the availability of a supply of seeds remains available. Broodstock feed enriched by adding hormones, Turmeric flour, or a combination thereof based on several kinds of literature such as Jufri *et al.* (2019), Pamungkas *et al.* (2019), Samara *et al.* (2019), Sudrajat and Rasid (2020), Lestari *et al.* (2020) and Gustiano *et al.* (2020) are very effective in accelerating rematuration and increasing the reproductive performance of broodstock both during and outside the spawning season in producing seeds.

This approach to solving the problem of catfish rematuration through enrichment of feed using Oodev hormone and Turmeric flour has been widely used and can be used as a solution to accelerate rematuration. Oodev hormone contains PMSG (pregnant mare serum gonadotropin) which

* Corresponding author.
Email address: prama.hartami@unimal.ac.id

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works to accelerate the gonadal maturation process (Sudrajat and Rasid, 2020). According to Dufour *et al.* (2010) and Bryant *et al.* (2016) PMSG also contains antidopamine to stop the performance of the hormone dopamine, this dopamine is a neurotransmitter that plays a role in inhibiting gonadal maturation. The inhibited dopamine activity will stimulate the synthesis and release of the lutein hormone which is initiated by GnRH in the pituitary gland. Furthermore, the hormone lutein and follicle hormone will enter the target organ, namely the gonads through the blood. Turmeric flour contains flavonoid compounds that are phytoestrogens and hepatoprotection which are useful for optimizing liver performance in producing vitellogenin (Arfah *et al.*, 2018; Sudrajat and Rasid, 2020). Provision of phytoestrogens derived from Turmeric flour in feed can increase the hormone estrogen in the body (17 -estradiol) carried to the liver to synthesize vitellogenin which will then be distributed to follicles that can stimulate oocyte growth (Reading and Sullivan 2011). Vitellogenin is the raw material for the formation of egg yolk in fish reproduction. Based on this, we are very interested in testing it on catfish broodstock and hope that the problem of the limited number of broodstock will no longer be an obstacle in producing seeds.

This study aims to determine the effectiveness of using Oodev hormone and Turmeric flour mixed into feed to accelerate the rematuration of catfish broodstock. The hypothesis that we propose in this study is that the presence of the Oodev hormone and Turmeric flour as feed enrichment material for broodstock can accelerate the process of gonadal maturation and rematuration of catfish. Assuming that the broodstock catfish that became the test animal in this study was a superior broodstock so that the treatment given gave optimal results. The scope of the research to be carried out is limited to the parameters of the success of the reproductive development of the female broodstock because it has a longer maturation time than the male. This study's results can also trigger small-scale catfish seed producers to be optimistic again in producing seeds even though the number of broodstocks is few.

Materials and Methods

Location and time of research

This research was conducted for 4 months starting from August 1st – November 29th, 2020 in Fish Hatchery and Aquaculture Technology Laboratory, Agricultural Faculty, Malikussaleh University, Indonesia.

Research materials

Some of the main materials used in this study include Oodev produced by the Laboratory of Fish Breeding and Genetics FPIK IPB, Desaku turmeric powder, Ovaprim produced by Syndel Laboratories Ltd., CMC flour as a feed adhesive, and Prima Feed PF-128 as broodstocks feed. This feed has a grain size of 9.5 - 10.9 x 5.2 - 6.2 mm, contains a minimum of 38% protein, a minimum of 5% fat, a maximum of 6% fiber, a maximum ash content of 12%, and maximum water content of 11%. The equipment used was a sprayer, stirrer, large basin, digital scale, syringe with capacities of 1 ml and 5 ml, and tarpaulin to dry the enriched feed.

Research design

This study was used a non-factorial completely randomized experiment design, using 4 (four) treatments including control with 3 (three) replications for each treatment. These treatments include: (A) Control, (B) Oodev hormone 0.3 ml/kg feed, (C) Turmeric flour 5 gr/kg feed, (D) Combination of Oodev hormone 0.3 ml + Turmeric flour 5 gr/kg feed.

This treatment is a modification of Sudrajat and Rasid (2020), where the previously used Oodev hormone was 0.5 ml/kg of feed and Turmeric flour as much as 3 g/kg of feed. The decrease in the dose of Oodev and the increase in the amount of Turmeric flour was carried out because the broodstock used was broodstock that had been completely ovulated so that the gonad maturity status of the test brood was in the same condition. Thus, each brood does not require high doses of Oodev hormone but requires a higher hepatoprotection due to post-spawning sourced from Turmeric flour.

Feed enrichment

The first thing to do is to weigh the broodstock feed that will be used, measure the amount of Oodev hormone needed, weigh Turmeric flour and CMC (1% per kg of feed for the second and fourth treatments) according to the treatment. Specifically for the fourth treatment (combination of Oodev + Turmeric flour), add CMC to the feed and stir evenly as much as 1% per kg of feed, spray the Oodev hormone which has previously been mixed with water as much as 10% of the amount of feed as much as parts. Next, add turmeric powder according to the dose in the feed and stir evenly, then spray the remaining Oodev in the sprayer until it runs out. The feed which has been mixed with Oodev and Turmeric flour is dried for 2 hours and

then stored in a container and stored in a dry place. In the second treatment, add and stir evenly CMC as much as 1% per kg of feed and sprayed with Oodev which has been mixed with water as in the fourth treatment while stirring evenly and dried and stored. While in the third treatment, only mix the feed with Turmeric flour as much as the required dose and stir until evenly distributed and stored in a container. While the control treatment is feeding the broodstock without the addition of enrichment substances.

Rematuration application treatment

Broodstock catfish weighing 0.9 - 1 kg as many as 3 individuals for each treatment was first artificially spawned to uniform the status of gonad maturity before rematuration testing was carried out. The ovulated broodstock was placed in a concrete tank measuring 1 x 1.5 x 1 m with a water level of 0.8 m located in a semi-outdoor room. The enriched feed according to the treatment was given to the test fish as much as 3% of the total body weight with a frequency of 2 times a day at 09.00 a.m and 17.00 p.m (Western Indonesian Time). The length of time for each treatment was until the gonads were mature and ready to be spawned again. Checking the level of maturity of the gonads is carried out every 15 days by looking at the size of the abdomen and the color of the female broodstock's genitals. If during the period of checking for gonad maturity, it is found that the test brood is ready to be spawned, then immediately stimulated spawning using Ovaprim hormone at a dose of 0.3 ml/kg female broodstock and 0.1 ml/kg male broodstock, spawning is also done artificially. Seeds resulting from rematuration treatment are reared until they reach a length of 2 inches or for 2 months.

Data collection

To measure the success rate of rematuration treatment of catfish broodstock, several parameters were measured including:

a. Gonado somatic index (GSI)

The GSI calculation was carried out at the end of the study in each treatment by comparing the weight of the gonads at the time of stripping with the bodyweight of the broodstock of the treatment fish. The GSI formula refers to Tyor and Pahwa (2017) as follows.

$$GSI = \frac{Gonad\ weight\ (gr)}{Body\ weight\ (gr)}\ x\ 100$$

b. Length of time for rematuration

This was done by calculating the length of time required by each broodstock in each treatment until the research period ended (Sudrajat and Rasid, 2020).

c. Measurement of egg diameter

Measurements were carried out using a caliper by taking a sample of eggs from each catfish broodstock that managed to spawn as many as 50 eggs (Sudrajat and Rasid, 2020).

d. Broodstock fecundity (F)

Calculation of broodstock fecundity was carried out to determine the total number of eggs produced at spawning at the end of the study. The fecundity formula refers to (Mylonas *et al.* 2010) as follows.

$$F = \frac{Gonad\ weight\ (gr)}{Gonad\ sample\ (gr)}\ x\ Number\ of\ egg\ samples$$

e. Fertilization rate (FR)

The fertilization rate is carried out after 8 hours of fertilization by observing the physical characteristics of the eggs, fertilized eggs are marked with a clear color, while unfertilized eggs are marked with milky white color. The formula for calculating the fertilization rate refers to Yustina and Darmawati (2003) as follows.

$$FR = \frac{Number\ of\ fertile\ rate\ (grain)}{Number\ of\ eggs\ sample\ (grain)}\ x\ 100$$

f. Hatching rate (HR)

The hatching rate of catfish eggs was carried out after 24 hours of incubation. The formula for calculating the fertilization rate refers to Yustina and Darmawati (2003) as follows.

$$HR = \frac{Number\ of\ hatching\ eggs\ (grain)}{Number\ of\ fertile\ eggs\ (grain)}\ x\ 100$$

g. Specific growth rate (SGR)

Measurement of specific length growth in larvae or seeds was carried out every 15 days until they reached a size of 2 inches or for 2 months of larvae rearing. The formula used refers to De Silva and Anderson (1995) as follows.

$$SGR = \frac{\ln final\ length - \ln initial\ length}{rearing\ period}\ x\ 100$$

h. Survival rate (SR)

The survival rate of catfish seeds was calculated at the end of rearing for each treatment. The formula for calculating the fertilization rate refers to Yustina and Darmawati (2003) as follows.

$$SR = \frac{Number\ of\ live\ fish\ at\ the\ end\ of\ rearing}{Number\ of\ hatching\ eggs\ (individual)}\ x\ 100$$

Table 1. Measurement of reproductive parameters of catfish broodstock from rematuration treatment.

Treatments	GSI (%)	Rematuration Period (day)	Egg Diameters (mm)	Fecundity (egg/kg)
Control	10.77±0.38[a]	62.67±3.79[a]	1.15±0.05[a]	17,662.00±1,494.19[a]
A	11.57±0.61[a]	54.67±2.08[b]	1.13±0.15[a]	20,236.81±1,609.42[b]
B	10.91±0.62[a]	61.67±5.69[a]	1.12±0.03[a]	17,673.25±2,577.29[a]
C	14.57±2.53[b]	32.33±4.16[c]	1.27±0.12[b]	22,939.97±880.38[c]

Note: Different superscript letters in the same column indicate significant differences between treatments ($P < 0.05$).

Table 2. Measurement of spawning parameters and growth of catfish seeds resulting from rematuration treatment.

Treatments	Fertilization Rate (%)	Hatching Rate (%)	Length SGR (% per day)
Control	52.00±9.79[a]	57.73±6.69[a]	7.85±0.64[a]
A	65.20±14.35[b]	69.47±5.87[b]	7.69±0.36[a]
B	54.87±5.84[a]	60.13±6.11[a]	7.58±0.70[a]
C	80.18±9.75[c]	79.80±2.88[c]	7.99±0.40[a]

Note: Different superscript letters in the same column indicate significant differences between treatments ($P < 0.05$).

Data analysis

The data obtained during the study were tabulated and statistically analyzed using the ANOVA test with a 95% confidence level. Data on reproduction performance and growth of catfish seeds were analyzed using SPSS 16.0 software. The analysis in question includes any data obtained in the Data collection Sub-Chapter. The water quality parameter data including temperature, pH, and availability of dissolved oxygen used as supporting data were analyzed descriptively.

Results

Reproduction performance of female catfish broodstock

The measurements of several reproductive parameters of catfish that have been treated with rematuration including GSI parameters, length of time for rematuration, measurement of egg diameter, and broodstock fecundity are presented in Table 1.

The female catfish who received enrichment treatment using Oodev hormone and Turmeric flour (C) during the rematuration period had the best reproductive performance parameter values compared to other treatments. The GSI value was 14.57±2.53%, the length of time for rematuration was 32.33±4.16 days, egg diameter was 1.27±0.12 mm and egg fecundity was 22,939.97±880.38 eggs/kg brood weight. If the percentage increase in reproductive performance between treatment C compared with control will be obtained a value of 35.28% for GSI, 48.41% shortens the re-maturation period, 10% increases in egg diameter and 29.88% produces more eggs (fecundity). Statistical analysis also showed that administration of a combination of Oodev hormone and turmeric powder had a significant effect ($P < 0.05$) on all test parameters. The enrichment of feed by adding the hormone Oodev (A) to the feed is only able to have an effect on the length of time for rematuration and increase egg fecundity. While the enrichment treatment by adding Turmeric flour (B) the results were relatively the same as the control treatment in all parameters of the female catfish reproductive performance test.

Spawning and seed growth

The other main parameters to see the effect that can be obtained from the enrichment treatment of broodstock are the percentage of fertilization rate, egg hatching, and length growth. These parameters are important in every activity of spawning and observing the growth of cultured fish seeds. The measurement results obtained during the study of feed enrichment for catfish are presented in Table 2.

Table 2 shows that the diet enriched with the combination of Oodev hormone and Turmeric flour (C) also had a significant effect ($P < 0.05$) on the fertilization rate of 80.18±9.75% and the percentage of hatching eggs 79.80± 2.88%. As for the daily length growth of seeds, there was no difference in all treatments. While the data on seed weight is not the main thing in this study to be measured, because every fish production is in a state of the seed, weight is not the thing that determines the selling price.

Table 3. Measurement of survival rate of catfish seeds resulting from rematuration treatment.

Treatments	Survival Rate (%)
Control	64.03±6.76[a]
A	76.50±6.22[b]
B	62.87±17.21[a]
C	83.17±9.69[c]

Note: Different superscript letters in the same column indicate significant differences between treatments ($P < 0.05$).

Catfish seed production

Seed production data is the main thing in fish hatchery activities because it becomes a parameter of success whether the activity can produce a high number of seeds according to needs with uniform sizes. The production referred to in this study is limited to how many seeds live to a size that has a selling value. The survival data can be seen in Table 3.

The results of this rematuration test showed the level of seed production reached 83.17±9.69% (C) or there was an increase of 19.14% compared to the control treatment. This is a good result for catfish seed producers to apply rematuration through a combination of Oodev hormone and Turmeric flour.

Discussion

According to Iswanto *et al.* (2016), in general, the GSI value of pearl catfish is between 10-20% with a fecundity of 50,000 eggs/kg brood weight, a length of time for rematuration of up to 1.5 months, and an egg diameter of 1.20-1.54 mm, depending on the age and size of female broodstock. So that more larger the size of the broodstock, the value of GSI, fecundity, and egg diameter also increases. Meanwhile, Jufri *et al.* (2019), who combined Oodev with astaxanthin into the feed were able to reduce the rematuration period up to 2 – 3 weeks with a larger Oodev dose method due to the reference to the weight of the broodstock used coupled with the antioxidant content of astaxanthin which was higher than with antioxidants in Turmeric flour. In this study, the length of the rematuration period was 1-2 weeks longer with the use of a lower dose of Oodev and also influenced by the time of the study conducted at the end of the dry season. This is following the opinion of Sudrajat and Rasid (2020), which states that catfish do not reproduce in the dry season and only reproduce in the rainy season. The length of time for rematuration of catfish broodstock in this study is the same as that obtained in the study of Sudrajat and Rasid (2020), who also researched outside the catfish spawning season.

The diameter of the eggs in each treatment showed various values, where the diameter of the eggs ranged from 1.12 mm to a larger size of 1.27 mm. The results of observations during the study, overall each treatment had egg diameters that varied in size. Turmeric contains phytosterols, carotenes, vitamin E, and curcumin which are similar to phytoestrogens and hepatoprotection from 27 groups of flavonoids capable of acting as estrogens that stimulate the liver to synthesize vitellogenin. This is following Lestari (2016) statement, that vitamins are organic compounds that are important for the growth, reproduction, and health of fish and as a metabolic booster in the fish body. So that the feed given to catfish broodstock that already contains turmeric will affect reproduction in fish.

Several studies using hormonal applications or combinations with other ingredients to accelerate rematuration have shown a high success rate, including Prihardianto *et al.* (2015), using an egg stimulant of 1g/kg of betok fish feed; Fani *et al.* (2015), which induces the FSH hormone at a dose of 0.4 ml/kg broodfish; Fadhillah (2017), combined the addition of Oodev 1 ml/kg fish, rGH 0.1 mg/kg fish, fish oil 30 g/kg nilem fish feed; Anwar *et al.* (2018), injected 0.5 ml/kg of Oodev broodfish; Fitriatin *et al.* (2018), tested the Oodev hormone by injection on nilem fish at a dose of 1 mg/kg broodstock; Pamungkas *et al.* (2019), injected catfish at a dose of 20 IU/kg broodstock; Samara *et al.* (2019), injecting catfish with a combination of PMSG hormones 10 IU/kg and HCG 5 IU/kg and Lestari *et al.* (2020), orally induces 1.16 ml/kg Oodev hormone for betta fish. Specifically, the rematuration experiment on catfish resulted in the index of HSI, GSI, egg diameter, the shortest rematuration, and the highest percentage of maturity as was done by Manurung (2011), combining the GtH hormone 5 IU/kg/week with 2% spirulina flour per week; Nainggolan *et al.* (2014), enriched feed with 15 IU Oodev and 3% spirulina flour; Mariyama (2019), using Oodev at a dose of 5 IU/kg; Jufri *et al.* (2019), gave a combination treatment of adding 1 ml/kg of Oodev and 100 mg/kg of Turmeric flour and Sudrajat and Rasid (2020), enriching the feed with 0.5 ml/kg of Oodev and 3% Turmeric flour per kilogram of feed weight.

Specific reproductive cycles in female fish are separate from the gametogenesis process and the

maturation phase is controlled by reproductive hormones found in the brain, pituitary, and gonads (Tyor and Pahwa, 2017). However, the gonad developmental phase in cultured fish is in dire need of external hormonal induction, especially to accelerate egg maturity and ovulation (Mylonas *et al.*, 2010). Hormone induction to accelerate gonadal maturation aims to secrete FSH hormone by the pituitary to stimulate theca cells and granulosa cells in the follicles to secrete steroid hormones in the form of estradiol-17β (E2) to produce egg proliferation and vitellogenesis, and progestogens to stimulate meiosis of the ovum and mature follicles and stimulate ovulation (Reading and Sullivan, 2011). Furthermore, hormone injection is useful for inhibiting the hormone dopamine from releasing the hormone GRIF (Gonadotropin Release Inhibiting Factor) so that the FSH hormone is still secreted so that the gonadal maturation process takes place optimally (Bryant *et al.*, 2016). According to Dufour *et al.* (2010), combined treatment of GnRH (Gonadotropin-releasing Hormone) and Anti-Dopamine (AD) hormone induction is a new method for artificial spawning in the field of a fish hatchery.

The secretion of hormones, especially stimulating hormones that are induced from the outside, serves as a trigger for accelerating gonadal maturation. The application of this hormone induction is widely used as an effort to uniform the maturity level of the broodstock and assist the process of ovulation and spermiation in fish hatchery centers (Cabrita *et al.*, 2008). However, things that must be considered in the application of this hormone are the type of hormone, the species that is the recipient, and the time of application. This is following the results of research by Gustiano *et al.* (2020), on snakehead fish which concluded that hormone induction to improve reproductive performance is an effective alternative, especially during the spawning season, but has a small impact outside the reproductive season, and still produces better performance compared to the spawning season without hormonal induction.

Hormone induction to accelerate gonadal maturity has been widely applied to brood fish. One of them is the use of the Oodev hormone (oocyte developer) which is a synthetic hormone-containing PMSG (Pregnant Mare Serum Gonadotropin) and AD (Anti-Dopamine). The principle of application and function of this hormone is to stimulate an increase in GnRH secretion, which will then stimulate the pituitary gland to produce gonadotropin hormones (Sudrajat *et al.*, 2016). Accelerating the gonadal maturation process through the hormonal application or in combination with other ingredients is not always effective in fish as done by Davis *et al.* (2000), by mixing the hormone trenbolone acetate in channel catfish feed for 60 days which still seed size with a dose of up to 100 mg/kg of feed inhibits the growth and development of female gonads as adults. Moran *et al.* (1990), stated that the administration of the hormone trenbolone could delay puberty and inhibit gonadal development.

Nainggolan *et al.* (2015) and Farida *et al.* (2018), stated that hormonal induction did not affect the quality of eggs and larvae produced, but rather on the quality of nutrition obtained by the broodstock during gonadal development. The research of Izquierdo *et al.* (2001), also stated that the quality of eggs and the performance of the seeds produced were strongly influenced by the nutritional content contained in the broodstock feed, especially the fatty acid content. This can also be seen in the length growth data of catfish produced from broodstock treated with rematuration using Oodev hormone which did not show any difference in each treatment. In addition to nutrients in the feed that can improve egg quality, it turns out that the addition of antioxidants, both natural and synthetic, to the feed also has a significant effect on the rate of fertilization and hatching of fish eggs (Bakhareva and Grozesku, 2013). The presence of protein and fat in feed can affect the presence of steroid hormones in the gonads, especially estradiol 17-β to accelerate the vitellogenesis process (Prihardianto et al 2015), as well as the presence of carotenoids in feed (García-Chavarría and Lara-Flores, 2013). Turmeric flour contains flavonoid compounds that are phytoestrogens and hepatoprotection which are useful for optimizing liver performance in producing vitellogenin (Sudrajat and Rasid, 2020). In addition to using the Oodev hormone to accelerate rematuration, at the time of spawning the broodstock is still stimulated using the Ovaprim hormone to optimize the level of maturity of catfish eggs and sperm. This is also supported by the opinion of Azis and Kalesaran (2017), that the stimulation of spawning by applying hormonal induction in the form of Ovaprim can increase the rate of fertilization and hatching of eggs.

The activity of observing the growth performance of catfish resulting from broodstock by adding Oodev, Turmeric flour, and their combination to the feed is the first published observation compared to previous researchers with the same object. Previous researchers were limited

to observing the reproductive performance of the broodstock and the quality of the eggs produced, so the authors found it difficult to discuss and compare the results of this study with the results of previous studies. But in general, the discussion is related to a good catfish survival rate, which ranges from 80-100% (Fadhil, 2013; Nainggolan *et al*., 2015; Tjodi *et al*., 2016; Sitio *et al*., 2017; Azis and Kalesaran, 2017). The survival of catfish fry is strongly influenced by stocking density, thereby increasing food competition and triggering cannibalism (Santoso *et al*., 2018). Sadomov (2010), explained that both larvae and seeds are very susceptible to digestive disorders, respiratory systems, olfactory organs, visual systems, and organ damage in the mouth, thereby reducing their ability to survive to a certain size. The problem of seed survival is not only among small-scale hatcheries but is also faced by the hatchery industry (Romanova *et al*., 2019). Several efforts can be made to increase seed viability through the addition of vitamins to feed, because the essential components contained in it can increase body resistance and prevent negative effects of the environmental impact of the media on the seeds that are kept (Bakhareva and Grozesku, 2013).

Conclusion

The conclusion that can be drawn from the results of this study is that enrichment of catfish broodstock feed by adding a combination of Oodev hormone and Turmeric flour can improve reproductive performance, spawning, larval growth, and increase seed production. The suggestion that the author recommends is to do the same tests on other cultured broodstock and conduct tests during the peak spawning period so that the results obtained will be more optimal.

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