E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(4): 418-424

www.fisheriesjournal.com
Received: 03-05-2017
Accepted: 04-06-2017

Muhammad Faiz Islami
Department of Aquaculture, Bogor Agricultural University, Bogor, Indonesia, Jl. Agatis Kampus Dramaga, Bogor, Indonesia

Agus Oman Sudrajat
Department of Aquaculture, Bogor Agricultural University, Bogor, Indonesia, Jl. Agatis Kampus Dramaga, Bogor, Indonesia

Odang Carman
Department of Aquaculture, Bogor Agricultural University, Bogor, Indonesia, Jl. Agatis Kampus Dramaga, Bogor, Indonesia

Correspondence
Muhammad Faiz Islami
Department of Aquaculture, Bogor Agricultural University, Bogor, Indonesia, Jl. Agatis Kampus Dramaga, Bogor, Indonesia

Induction of maturation and ovulation of Red Fin Shark fish *Epalzeorhynchos frenatus* in non-spawning season

Muhammad Faiz Islami, Agus Oman Sudrajat and Odang Carman

Abstract
There are constraints on the production of red fin shark *Epalzeorhynchos frenatus* due to the lack of matured broodstocks in Non-spawning season and has a dependence on hormone induction during spawning process, because it can't spawn naturally in cultivation environments. Gonad maturation can be stimulated by the addition of hormones and potential plant-based materials to feed. Thus, the present study attempted alternative hormonal combination to induce both ovulation and spawning in order to provide farmers with alternative choices for reproduction. This research was divided into two sub-research (maturation and ovulation) in one main title. The first section of the study dealt with maturational induction and it used Completely Randomized Design with 5 treatments. Ten broodstocks were used in each treatment which was as follows: *Oodev* (0.25 and 0.5 mL. kg^{-1} fish) addition to feed, turmeric powder (250 and 500 mg 100 g^{-1} feed), and commercial feed (at least 30 % protein) as the control. Broodstocks with 20.61±0.57 g $fish^{-1}$ of weight were maintained for 56 days. Spawning was done by using ready-spawning broodstock with a ratio of male : female (3:1), which consisted of 4 treatments, namely Spawnprim (0.5 and 1.0 mL. kg^{-1} fish), 0.5 mL Ovaprim kg^{-1} fish and 0.9 % NaCl solution as positive and negative control. The addition of 0.5 mL Oodev kg^{-1} fish and 500 mg 100 g^{-1} turmerics respectively was able to produce matured broodstocks amounted to 50 % and 20 % of population, whereas matured broodstocks were not found in control. Spawnprim induction achieved 100% spawning rate, the same result was found on Ovaprim induction (P>0.05). The results of this study were expected to be a reference that mature broodstock should be available throughout the year and it is required to provide an alternative spawning hormone.

Keywords: gonad maturation, ovulation, spawning, hormone, turmeric, red fin shark

1. Introduction
Hobby on ornamental fish continues to increase. The indicator is the number of communities of ornamental fish emerging in Indonesia. Ornamental fish in the aquarium can be a relaxation medium of busy daily activities or just as as an activity done in the spare-time. The economic value of aquarium ornamental fish in the world is worth 15-30 billion USD (Penning *et al* 2009) [15], consisting of 5,300 freshwater fish and 1,802 marine fish. Around 90% of the traded ornamental fish are tropical freshwater ornamental fish, yet only 10 % of them are from cultivation activities, and the rest are from fish catch in nature (Olivier 2001) [13].

If the production of ornamental fish continues to rely on the capture of nature, there will be a decrease in the ornamental fish population in its original habitat. Thus, the production of aquaculture-based ornamental fish can be counted on to compensate the catching activities in nature and gives positive effect for sustainable ornamental fish in nature while supplying the demand of ornamental fish market. One of the freshwater tropical ornamental fish which quite draws interest in Indonesia is red fin shark *Epalzeorhynchos frenatus*. This fish is unique to the red-colored fins, the price is affordable (± Rp 3,500/fish for size of 3"- up) and easy to maintain. According to Vidthayanon (2016) [23] this fish originated from mainland Southeast Asia (Laos, Cambodia, Thailand, Vietnam). This fish can grow to a length of 15 cm, living on pH conditions of 6.2-7.5, hardness of 2-15 dH, and temperature of 23-26°C (Sedjati 2002) [19]. Based on the morphological characteristics, male fish have black marks on the anal fin and slimmer body shape, whereas females do not have black marks on anal fins with wider body shape than male fish.

However, there are constraints on the production of red fin shark namely the unavailable fish seed throughout the year due to the lack of matured broodstock during the dry season and has a dependence on hormone induction used during spawning. The administration of hormones The administration of hormones such as Oodev containing Pregnant Mare Serum gonadotropin (PMSG) and Antidopamine (AD) and the addition of plant-based material, that is turmeric, to the feed are able to solve the constraints on gonadal maturation. Moreover, Spawnprim containing Aromatase Inhibitor (AI), Oxytocin, Prostaglandin F2α (PGF2α), LHRH-a, and AD can be used to stimulating spawning on fish (Dhewantara 2013) [4].

PMSG contains many elements of follicle stimulating hormone (FSH), which plays a role in gonad maturation process (Moore and Ward 1980) [10]. Antidopamine is a chemical that can stop dopamine work so that stimulates gonadotropin secretion and increases spawning response (Vidal *et al.* 2004) [22]. According to Rachman (2013) [16], post-spawning sutchi catfish Pangasianodon hypopthalmus is capable of induced rematuration through the use of PMSG and AD hormone combination with a dose of PMSG 10 IU. kg^{-1} fish weight + AD 0.01 mg. fish weight $^{-1}$. Turmeric has the main content of curcumin that is phytoestrogens and hepatoprotectors that are able to stimulate the liver to synthesize vitellogenin which will be used for gonadal maturation. According to Dewi (2015) [3], turmeric with a dose of 480 mg. 100 g^{-1} of feed can accelerate gonad maturation and egg diameter development, and increase GSI, HSI, and produce high fish fecundity in Sutchi catfish (*Pangasianodon hypophthalmus*).

According to Abdullah (2007) [1], LHRH-a stimulates the secretion of gonadotropin hormones and pituitary gland that can stimulate the occurrence of ovulation and spawning. Furthermore, the added AD will inhibit the activity of dopamine. Dopamine should be inhibited because it inhibits gonadotropin secretion. The addition of AI hormone is expected to inhibit the action of aromatase that can stop the production of estradiol-17ß, so the pituitary will receive a signal to immediately produce luteinizing hormone (LH) that play a role in the final maturation process. According to (Broach 2009) [2], LH collaborates with PGF2α on ovulation process. PGF2α in fish plays a role in stimulating the mature oocytes release from the reproductive tract (ovulation).

The result of such cooperation increases the activity of the proteolytic enzyme in the follicle thus stimulating the egg nucleus moving from the middle to the edge of the cell and subsequently fusing toward the anima pole until the egg is ready to be ovulated. After that, the oxytocin hormone acts on the oxytocin receptor which results in ovarian contraction in pregnancy. The activity of oxytocin hormone will increase at the time of ovulation and plays an important role in the spawning process (Haraldsen *et al*. 2002) [7]. Research showed that the use of Spawnprim was able to trigger a semi-natural spawning on pangasius (Dewanthara 2013) [4].

This study was conducted with the aim to evaluate the effectiveness of the administration of Oodev and turmeric flour on gonad maturation, and Spawnprim for ovulation and spawning in order to increase the production of red fin shark.

2. Materials and Methods

2.1 Gonad maturation

2.1.1 Broodstock Selection

Female broodstocks of red fin shark were from Bogor, Indonesia. Fish were acclimatized for 20 days in 2 aquariums with a size of 80x40x40 cm^3 and water volume of 80 liter. For the first 10 days, fish were fed with the silkworm *Tubifex sp*. and 10 days later fish were fed with 30 % commercial protein. Then the broodstocks were weighed and the initial weight obtained was 20.61±0.57 g. $fish^{-1}$, the minimum age was 8 months, and the minimum length was 10 cm, with the condition of gonad maturation previtellogenic phase. Subsequently, each 10 broodstocks were transferred into aquariums test of 65x50x50 cm^3 with a water volume of 90 liters.

2.1.2 Production of feed mix and maintenance of experimental fish

Oodev hormone was obtained from Laboratory of Fish Reproduction and Genetic, Department of Aquaculture, Bogor Agricultural University. Turmeric flour used was commercial turmeric flour produced by PT. GANESHA ABADITAMA Jakarta, Indonesia. The ingredients were mixed with 30 % commercial protein feed according to the treatment dose. Egg white was used as a binder referring to the method conducted by Fadhillah (2016) [5]. Feed were given 2 times a day namely in the morning and afternoon with 3 % FR of broodstock biomass of each treatment. The stock feed was made for every 14 days of feeding. Maintenance was conducted for 56 days (May 23 - July 17, 2016) in 60x50x50 cm^3 aquarium with a volume of 90 liters of water, temperature of 27.5-29.1 °C, DO of 4.9-5.8 mg. L^{-1}, and pH 6.42-7.39 during maintenance.

2.1.3 Sampling of gonad and liver

Sampling of gonad and liver was done on day 0 and day 56 for analysis of gonadosomatic index (GSI), hepatosomatic index (HSI), and histology of fish gonad. Gonad and liver were obtained from fish broodstock dissection, on day 0, 5 broodstocks were dissected to represent the condition of gonad at the beginning of maintenance for all treatments. Subsequently, on day 56, 3 fish were dissected from each treatment. Gonads and livers were weighed with digital scales then preserved in containers of a sera solution (formalin, alcohol, and acetate with a ratio of 6:3:1). The preparate of gonad histology was performed in the Laboratory of Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University. Gonad were observed under a microscope with 10x10 magnification.

2.1.4 Blood Sampling

Blood sampling was performed on days 0, 28, 42, and 56. Before the blood was taken, the fish were anesthetized using an anesthetic stabilizer with a dose of 1 mL for 3 liters of water. Blood was taken from the dorsal aorta through the base of the tail as much as 0.8 to 1 mL (pulling from 10 broodstocks), using a 1 mL syringe without anticoagulants. Blood was put into microtube and directly inserted into ice box, then centrifugated with 10,000 rpm for 5 minutes. Separated blood plasma was inserted into a new micro tube and then stored in the -20 °C freezer before analysis. The measurement of estradiol concentration was done by enzyme-linked immunosorbent assay (ELISA) method using commercial kit (DRG international Inc; Catalog No. EIA 2693 and EIA 1559). The procedure was in accordance with product manual conducted at Research and Development Center for Ornamental Fish, Depok, Indonesia.

2.1.5 Percentage of pregnancy and egg diameter

Pregnancy examination was performed by cannulation

method using catheter by census every 7 days. Pregnant broodstock was a broodstock that had eggs which were visible at the cannulation and had a uniform egg size. Some eggs from pregnant broodstocks were taken to observe the egg diameter. Eggs were observed (n=100) under a microscope with 40x10 magnification. The egg diameter was determined by reference of micrometer (100-micrometer scale) through ImageJ software (National Institute of Health, USA).

2.2 Ovulation and Spawning

2.2.1 Broodstock selection and injection

Broodstocks used were broodstocks with ripe gonad which are ready for spawning with a ratio of female:male (3:1). Broodstocks originated from Bogor, Indonesia. Prior to the injection, the blood was taken from female ready spawning broodstock using 1 mL syringe then pulled (accumulation) from 5 broodstocks of each treatment to observe the concentration of estradiol before spawning. Subsequently, the broodstocks were rested for 7 days and fasted for 1 day before the injection and then injected on the intermuscular using 1 mL syringe according to the treatment dose. The injection was done once. Spawning method applied was a semi-natural spawning method. The success and length of time until ovulation began to be observed after 6 hours post-injection, then monitored every 30 minutes until the eggs came out. After 4 hours post-spawning, fish blood was taken to observe the concentrations of estradiol after spawning. Spawned eggs were calculated by sampling using a 300 mL container, with 10 replications of sampling. Furthermore, as many as 100 eggs were separated in 20x20x20 cm^3 aquarium with a volume of 6 liters of water then observed as a sample for the parameter of fertilization rate, hatching rate and larval survival for 7 days.

2.3 Data analysis

Data were analyzed using analysis of variance (ANOVA) at 95 % confidence interval. If treatment was significantly different, Tukey test was performed with α=0.05. The analysis was done using SPSS ver 22.0 and Microsoft Excel 2016 programs.

3. Results

3.1 Maturation Induction

3.1.1 Concentration of estradiol hormone

Concentration of estradiol (**Figure 1**) in Oodev treatment 0.5 and 0.25 on day 56 decreased compared with day 42 (693.062 pg. mL^{-1} and 687.843 pg. mL^{-1}). It was expected that the peak of vitellogenesis process occurred on day 42; thus, vitellogenin has been absorbed by oocytes on day 56 resulted in decreased concentration of 17-β estradiol (458.237 pg. mL^{-1} and 488.537 pg. mL^{-1}).

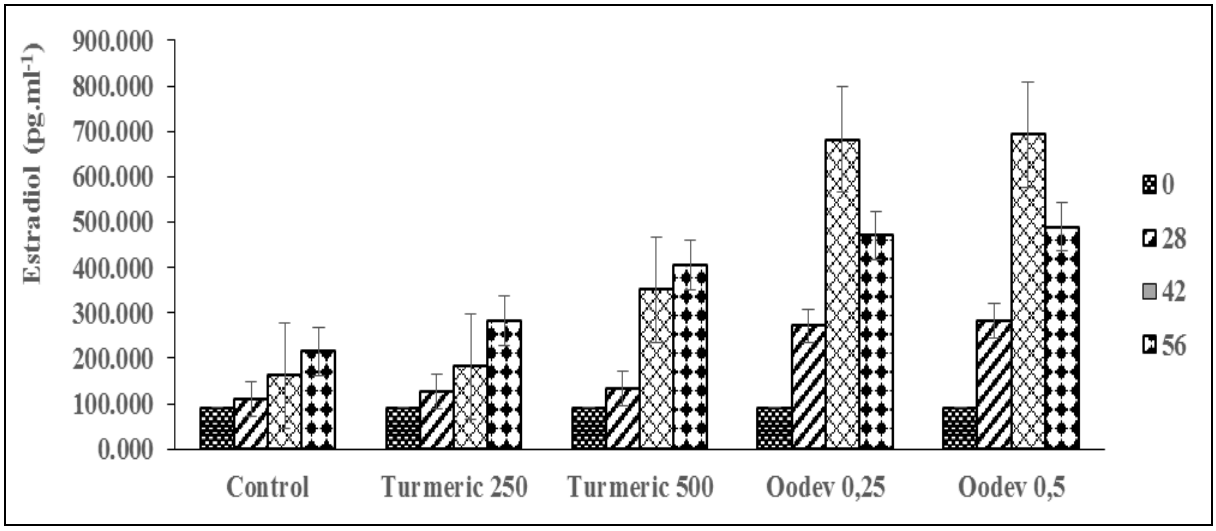

Fig 1: Concentration of estradiol hormone on day-0, 28, 42, and 56

3.1.2 Parameter of HSI, GSI and final egg diameter

There was an increase in GSI and HSI value on day 56 with the highest score of GSI parameter was found in Oodev 0.5 of 13.87±3.14 %, while the highest HSI values of 0.46±0.07 % and 0.46±0.06 % were obtained in Oodev 0.5 and turmeric 500, respectively. Subsequently, the highest final egg diameter was found in Oodev 0.5 treatment namely 0.9081±0.01 mm, whereas egg was not found in control treatment. The values of HSI, GSI and the final egg diameter are presented in Table 1.

Table 1: Value of HSI, GSI and egg diameter of red fin shark eggs

Treatments	HSI (%)	GSI (%)	Egg Diameter (%)
Initial	0.25±0.02	1.24±0.04	-
Oodev 0.5	0.46±0.07a	13.87±3.14a	0.9081±0.01a
Oodev 0.25	0.41±0.03ab	12.36±1.96a	0.8774±0.01a
Turmeric 500	0.46±0.06a	12.58±2.8a	0.8515±0.01a
Turmeric 250	0.38±0.06ab	5.48±1.26b	-
Control	0.27±0.05b	361±2.13b	-

Description: (a, b); Different letters on the same column show real differences at treatment (P<0.05)

3.1.3 Gonad Histology

Data of gonad histology were used to observe the results of hormonal treatment on the development of the broodstock egg oocyte of red fin shark. The data are presented in Figure 2. Treatments of Oodev 0.5 and 0.25, as well as turmeric 500 had entered mature phase, yet the treatment of turmeric 500 was still visible in late-vitellogenic phase. The treatments of turmeric 250 and control were in early-vitellogenic phase. While at the beginning of histology, oocytes still showed at the previtellogenic stage.

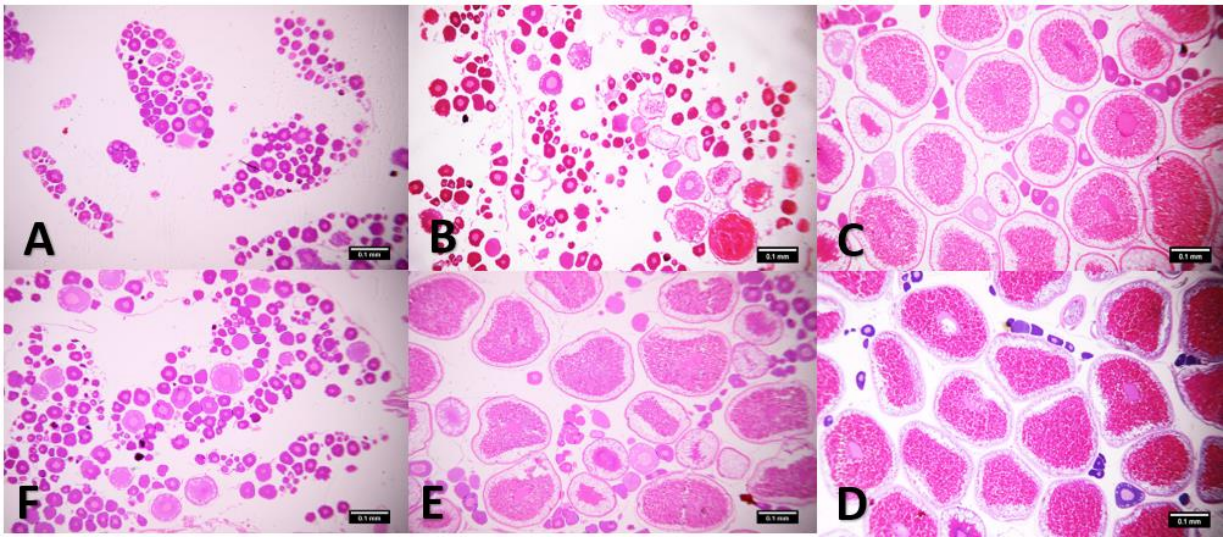

Fig 2: Gonad histology of redfin shark: A (Initial), B (Turmeric 250 mg. 100 g^{-1} feed), C (Oodev 0.25 mL. kg^{-1}), D (Oodev 0.5 mL. kg^{-1}), E (Turmeric 500 mg. 100 g^{-1} feed), F (Control). Bar scale represents 0.1 mm

3.1.4 Percentage of mature broodstocks

The best treatment with the number of mature broodstocks reached 50 % of individual population within 56 days was found in the treatment of Oodev 0.5. However, there was no broodstock with mature gonad in control. The percentage of accumulation and time of ripe broodstock during the study is presented in Table 2.

Table 2: Percentage of accumulation and time of ripe red fin shark broodstock of post-treatment on day 0 and 56

Treatment (n =10)	Mature broodstocks (%)								
	0	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**
Oodev 0,5	0	0	0	0	20	20	40	40	50
Oodev 0,25	0	0	0	0	20	20	30	40	40
Turmeric 500	0	0	0	0	0	0	0	0	20
Turmeric 250	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0

3.2 Induction of ovulation

3.2.1 Concentration of estradiol hormone

Concentrations of estradiol hormone before and after spawning are presented in Figure 3. In general, estradiol concentration in hormone-induced broodstocks decreased after spawning occurred. However, it was not shown in 0.9% NaCl treatment since spawning did not occur.

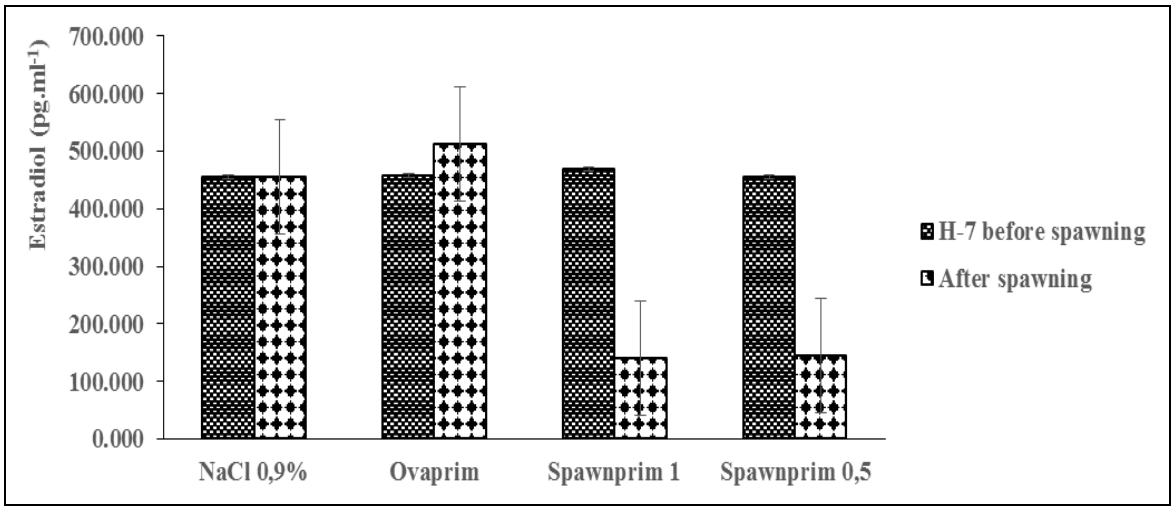

Fig 3: Concentrations of estradiol hormones before and after spawning, (*) not spawning.

3.2.2 The success of spawning and Length of Time to Ovulation (latency period)

The success rates and length of time until ovulation of post-injection hormones are presented in Table 3. The succes of spawning obtained was 100% and ovulation latent time was not significantly different (P>0.05) with ovaprim treatment.

Table 3: Success rates and length of time untill ovulation in redfin shark

Treatment n=5	Spawning success rate (%)	Average time (minutes)	Spawning Method
Spawnprim 0,5	100	564±57a	Semi-natural
Spawnprim 1	100	590±81a	Semi-natural
Ovaprim	100	510±54a	Semi-natural
0.9% NaCl	0	*	Semi-natural

Description: The same letter on the same column shows no significant difference (P> 0.05), (*) Not spawning

3.2.3 Gamete quality and viability of larvae

In general, values obtained in parameter of spawned eggs, fertilization rate, hatching rate, and larval survival rate did not show significantly different results in the treatment administrated with Spawnprim or ovaprim induction (P>0.05). The results are presented in Table 4.

Table 4: Gamete quality and viability of larvae

Parameter	Spawnprim 0.5 ml/kg fish	Spawnprim 1 ml/kg fish	Ovaprim 0.5 ml/kg fish	0.9 % NaCl
Spawned eggs (Egg)	3,361±636[a]	3,365±629[a]	3,928±744[a]	-
Fertilization rate (%)	94±0.02[a]	93±0.02[a]	95±0.01[a]	-
Hatching rate (%)	89±0.03[a]	87±0.03[a]	90±0.02[a]	-
Sulvival rate (%)	79±0.03[a]	77±0.04[a]	81±0.04[a]	-

Description: The same letter on the same line shows no significant difference in treatment (P>0.05), (-) Not spawning.

4. Discussion

4.1 Maturation induction

In this study, it was expected that the peak of vitellogenesis process occurred on day 42; thus, vitellogenin has been absorbed by oocytes on day 56 resulted in decreased concentration of 17-β estradiol. Estradiol concentration in fish naturally decreased after gonad maturation (Rottmann *et al.* 1991) [18]. In this study, the turmeric treatment of 500 also showed a higher concentration of 17-β estradiol when compared with control. This is because the main content of turmeric is curcumin with characteristics of phytoestrogen and hepatoprotector that can act as estrogen (Ravindran *et al.* 2007) [17]. HSI parameter increased associated with estradiol levels during vitellogenesis and oocyte growth. According to Siregar (1999) [20], the fish liver volume will increase during vitellogenesis. Treatments of Oodev 0.5 and turmeric 500 had higher HSI value compared with other treatments, especially controls. Oodev 0.5 had a high HSI value because of the high supply of 17-β estradiol in the blood due to hormonal administration. Considering turmeric 500, it was expected that curcumin material found in turmeric was capable of playing a role as estrogen so that 17-β estradiol was also much found in the body. Moreover, curcumin also functions as a hepatoprotector that made the liver remained in optimum condition when vitellogenesis occurred.

Increasing HSI values correlated with GSI values because vitellogenin produced from the liver will be carried by the bloodstream to the gonads, then absorbed and stored in oocytes. Continuous absorption leads to an increase in oocyte size and the amount of egg yolk during the vitellogenesis phase, which causes the GSI value to increase at the end of maintenance (Yaron and Silvan 2006) [23]. Egg diameter size of Oodev 0.5 was 0.9081±0.01 mm. This result was higher than the egg diameter of red fin shark administrated with egg stimulant of 0.767 mm (Murtejo 2008) [11]. Hence, the treatment of gonadal maturation acceleration did not decrease the quality of eggs produced.

Treatments of Oodev 0.5 and 0.25, as well as turmeric 500 had entered mature phase, yet the treatment of turmeric 500 was still visible in late-vitellogenic phase. The treatments of turmeric 250 and control were in early-vitellogenic phase. While at the beginning of histology, oocytes still showed at the previtellogenic stage. Gonads in the treatment of Oodev 0.5 and 0.25 had reached the mature phase. In this condition, the number of egg yolks has filled all ooplasm except under chorion, then the nuclear membrane begins to shrink, and the egg core spreads to the edge, waiting for Final Oocyte Maturation (FOM) (Genten *et al*. 2009) [6]. Gonad maturation of broodstock occurs due to the increasing number of eggs in the gonad and its diameter approach to the mature phase (Millan 2007) [9]. The percentage of broodstock with mature gonad in Oodev 0.5 accounted for 50 % of the total treated individuals followed by Oodev 0.25 (40%), and turmeric 500 (20%), while broodstocks with mature gonad were not found in turmeric 250 and control. During 56 days Oodev treatments showed higher percentage of matured broodstock compared to other treatments, it is about 2 times more than turmeric treatments. But it can't be denied if turmeric treatments has potential to stimulate gonad maturation in fish.

4.1.1 Ovulation and Spawning induction

In general, estradiol concentration decreased after spawning occurred. However, it was not shown in 0.9% NaCl treatment since spawning did not occur. After reaching post-vitellogenesis phase, 17-β estradiol production and aromatase activity will decrease. The decrease of aromatase activity on the treatment was also due to the AI content in Spawnprim treatment of 0.5 and 1. Decreased production of 17-β estradiol and aromatase activity was followed by an increase of 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP) so that the oocyte develops Germinal Vesicle Break Down (GVBD) and ends in ovulation (Nagahama *et al* 1995) [12]. The succes of spawning obtained was 100% and ovulation latent time was not significantly different (P>0.05) with ovaprim treatment. This indicates that spawnprim use was as effective as ovaprim use.

There are sGnRH-a and AD in ovaprim which play a role in stimulating pituitary to release gonadotropin (Lam 1985) [8]

thus stimulating ovulation in fish. The role of sGnRH-a and AD can be substituted by LHRH-a and AD on Spawnprim. LHRH-a stimulates the secretion of gonadotropin hormones and pituitary gland that can stimulate the occurrence of ovulation and spawning. Subsequently, the added AD will inhibit the work of dopamine. Dopamine should be inhibited since it inhibits gonadotropin secretion. Semi-natural Spawning can occur due to the role of oxytocin hormone which is able to stimulate the smooth muscle, results in contraction so that fish can perform semi-natural spawning. According to Haraldsen *et al*. (2002), activity of oxytocin hormone increases during ovulation and plays an important role in the spawning process. In addition, spawning also occurs because of the role of PGF2α that can stimulate the rupture of the follicle and release mature oocytes (Stacey and Goethz 1982) [21].

The most spawned eggs were found in ovaprim treatment of 3,928±744 eggs, whereas the lowest spawned eggs was found in the treatment of Spawnprim 1 of 3,365±629 eggs. However, these numbers did not show a significant difference ($P>0.05$). The number of spawned eggs was in line with the results of research by Murtejo (2008) [11] which ranged from 3,306-8,268 eggs. The highest fertilization rate, hatching rate and survival rate was found in ovaprim treatment but this result was not significantly different from Spawnprim ($P>0.05$). The value of fertilization rate, hatching rate and survival rate in the best treatment of Spawnprim (0.5) were 94 %, 89 %, and 79 %, respectively. The value of fertilization rate was influenced by the condition of mature eggs as well as the quality of sperm in the male broodstocks during spawning. The hatching rate was affected by the number of fertilized eggs, as well as environmental factors such as temperature, DO and pH (Oyen *et al*., 1991) [14]. Survival rate of larvae was high due to the amount of egg yolk during fish hatching and could also be affected by the environmental conditions of larval maintenance containers. Based on this reason, it can be concluded that the use of Spawnprim resulted in a performance which was as good as the use of ovaprim for ovulation induction and did not reduce the quality of fertilization and hatching on spawned eggs as well as did not reduce the quality of larvae obtained.

5. Conclusion

Oodev of 0.5 mL. kg^{-1} fish was the best dose to stimulate the maturation of gonad until ready for spawning on broodstock which reached 50 % of population, and turmeric with a dose of 500 mg 100 g^{-1} feed could be used as a hormone alternative because it was able to stimulate gonad maturation until ready for spawning which reached 20% of the population within 56 days out of the spawning season. The use of Spawnprim hormone effectively capable of producing larvae with gamete quality and viability of larvae as well as Ovaprim(R), therefore Spawnprim hormone can be an alternative spawning hormone.

6. References

1. Abdullah N. Efektivitas Pemberian Ovaprim Secara Topikal Pada Proses Ovulasi Dan Pemijahan Induk Ikan Mas Koki *Carassius auratus*. Thesis. Institut Pertanian Bogor, Bogor, 2007.
2. Broach J. Effects of steroid and prostaglandin injections on hybridization success between female channel catfish and male blue catfish. Thesis. The Graduate Faculty of Auburn University, Auburn, 2009.
3. Dewi CD. Khasiat Tepung Kunyit *Curcuma longa* dalam Pakan untuk Meningkatkan Performan Reproduksi Ikan Patin Siam *Pangasianodon hypophthalmus*. Thesis. Institut Pertanian Bogor, Bogor, 2015.
4. Dhewantara L. Induksi Ovulasi dan Pemijahan pada Ikan Patin Siam *Pangasianodon hypophthalmus* dengan Manipulasi Hormonal. Thesis. Institut Pertanian Bogor, Bogor, 2013.
5. Fadhillah R. Peningkatan produksi telur ikan nilem *Osteochilus hasselti* sebagai sumber kaviar melalui kombinasi Oodev, rGH, dan minyak ikan pada pakan. Thesis. Institut Pertanian Bogor, Bogor, 2016.
6. Genten F, Terwinghe E, Danguy A. Atlas of Fish Histologi. Science Publishers, Brussles, 2009.
7. Haraldsen L, Veronica SL, Goran EN. Oxytocin stimulates cerebral blood flow in rainbow trout *Oncorhynchus mykiss* through a nitric oxide dependent mechanism. Brain Research. 2002; 929(1):10-14.
8. Lam TJ. Induced Spawning in Fish. Reproduction and Culture of Milkfish, Oceanic Institute and Tungkang Marine Laboratory, Taiwan, 1985.
9. Millan Mc DB. Fish histology female reproductive system. Departemen of Biology, University of Western Ontario, Canada, 2007.
10. Moore Jr WT, Ward DN. Pregnant Mare Serum Gonadotropin: Rapid chromatographic procedures for the purification of intact hormone and isolation of subunits. Biology Chemistry. 1980; 255:6923-6929.
11. Murtejo, HE. Effektivitas Egg Stimulant dalam Pakan terhadap Pematangan Gonad dan Produktifitas Ikan Red Fin Shark *Epalzeorhynchos frenatus*. Essay. Institut Pertanian Bogor, Bogor, 2008.
12. Nagahama Y, Yoshikuni M, Yamashita M, Tokumoto T, Katsu Y. Regulation of oocyte growth and maturation in fish. Developmental Biology. 1995; 30:103-145.
13. Olivier K. The Ornamental Fish Market. FAO/Globefish Research Programme, United Nations Food and Agriculture Organization, Rome, 2001, 67.
14. Oyen FGF, LEC Campr and ESW Bongo. Effects of Acid Stress on the Embryonic Development of the Common Carp, *Cyprinus carpio*. Aquatic Toxicology. 1991; 19:1-12.
15. Penning M, Reid G McG, Koldewey H, Dick G, Andrews B, Arai K *et al.* Turning the Tide: A Global Aquarium Strategy for Conservation and Sustainability. World Association of Zoos and Aquariums, Bern, 2009.
16. Rachman B. Manipulasi Hormonal Pada Pematangan Gonad Ikan Patin Siam *Pangasianodon hypophthalmus*. Thesis. Institut Pertanian Bogor, Bogor, 2013.
17. Ravindran PN, Babu KN, Sivaraman K. Turmeric: The Genus Curcuma. Vol 45, CRC Press, Florida, 2007.
18. Rottmann RW, Shireman JV, Chapman FA. Hormonal control of reproduction in fish for Induced spawning. SRAC Publication. 1991, 424.
19. Sedjati IF. Embriogenesis dan Perkembangan Larva Ikan Red Fin Shark *Epalzeorhynchos frenatus*. Essay. Institut Pertanian Bogor, Bogor, 2002.
20. Siregar M. Stimulasi Pematangan Gonad Bakal Induk Betina Ikan Jambal Siam *Pangasius Hypophthalmus* dengan Hormon HCG. Thesis. Institut Pertanian Bogor, Bogor, 1999.
21. Stacey NE, Goethz FW. Role of Prostaglandins in Fish Reproduction. Canadian Journal of Fisheries and Aquatic Science. 1982; 39:92-98.
22. Vidal B, Catherine P, Nadine LB, Holland MCH, Sbaihi

M, Vernier P *et al*. Dopamine Inhibits Luteinizing Hormone Synthesis and Release in the Juvenile European Eel: A Neuroendocrine Lock for the Onset of Puberty. Biology of Reproduction. 2004; 71**:**1491-1500.
23. Vidthayanon C. *Epalzeorhynchos frenatus*. The IUCN Red List of Threatened Species. http://www.iucnredlist.org/details/181093/0. 15 February, 2016.
24. Yaron Z, Silvan B. The physiology of fishes. Edn 3, CRC Press, Florida. 2006, 343-386.