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EFFECTS OF 17ß-ESTRADIOL ON THE REPRODUCTION OF GREEN CATFISH (*Hemibagrus nemurus*, BAGRIDAE)

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Abstract- The aim the research is to increase the reproductive potential of *H.nemerus* female that is time matured gonads, somatic ovi index, fecundity, egg diameter, hatching rate and time to hatching in the control group (injection 0.9 NaCl) and exposed to 200 µg/kg, 400 µg/kg, 600 µg/kg body weight of 17ß-estradiol. Treatment of 17ß-estradiol levels were significant (p<0.05) different with respect to time ripe gonads, ovi somatic index, fecundity and egg diameter. Females exposed to 400 µg/kg body weight of 17ß-estradiol can increased time matured gonads 28 days, somatic ovi index 10.32%, absolute fecundity 63,724 egss/spaw, relatif fecundity 90 eggs/ g gonad, weight and egg diameter 1.22 mm. But hatching rate and time to hatching did not differ between the treatments (p>0,05).

Keywords- *Hemibagrus nemurus*, 17β- estradiol, reproduction, hatching rate, time to hatching.

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Introduction

Pekanbaru city of Riau Province is the center of government with a population of as many as 754,467 people in 2006 increased to 995,887 people in 2013 or during the last five years increased 241,420 people [1]. The population growth giving opportunity to restaurants to provides a menu of fish. Selecting a suitable fish species for culture and consumer tastes very important for the future development of the industry to meet the food needs of fish. Some species of the family Bagridae among others green catfish (*Hemibagrus nemurus*) in Riau Province found in Siak river and Indragiri river [2], Rangau river [3], Kampar Kanan river [4], Kampar Kiri river and Koto Panjang reservoir [5,6]. Food habits of *H.nemurus* in the wild are fish, shrimp, insects [7], but cultured in ponds and cages can accept artificial feed [8] can adapted to the environment and resistant to disease ([9,10], rapidly growth at high stocking density that is equal to 20.24 g/month [11].

H.nemurus is highly favored by consumers because the thick meat, little prickly and the delicious taste, weight can reach sizes from 750 to 1000 g / individual so as to have a high market value i.e. 60.000 to 75.000 IDR/kg [6]. At the present time in Riau Province has been cultured with intensively by farmers in cages river basin and ponds. But the rapid development of cultured *H. nemurus* have not balanced by the high production because not supported by larvae production with good quality and quantity. This is due, among other the difficulty of getting broodstock mature gonads. Moreover, the results showed that fecundity of *H.nemurus* still slightly ranged from 20,815 to 32, 000 eggs / kg body weight with hatching rate are hatching rate ~37% [12-14].

Demand of *H.nemurus* juvenile age 50 days for cultured in Pekanbaru City and Kampar Regency is currently as1,000,000 individuals every year, the majority of juvenile derived from wild fish catch and highly dependent on season and availability stocks. In order available at any time juveniles in quantity and quality for cultured to be supplied from hatcheries. The method has been conducted among others enrichment of feed with vitamin E [9], LHRHa implantation [12], fed a commercial diet [10], enrichment of feed with vitamin E and C (15]. Such methods have not been able to produce larval be mass of *H.nemurus*. Some researchers have reported this decade has been using hormone 17ß-estradiol to increased fish reproduction, growth, sex reversal and gonadal structure,

performance spawning, among others *Danio rerio* [16], *Oryzias javanicus* [17], *Oryzias latipes* [18], *Lepomis macrochirus* [19], *Kryptolebias marmoratus* [20]. According Pandian and Sheela [21] 17β-estradiol, a natural estrogen, has been shown to be an effective feminization hormone in Cyprinidae, Anabantidae, Poecilidae, Ictaluridae, Salmonidae and Cichlidae. Based on this important experiments 17ß-estradiol of green catfish (*H.nemurus*, Bagridae) in order to increase the reproductive potential so that can be produced larval quality and quantity.

Material and Methods

Broodstock

Adult female and male green catfish (*H. nemurus*) much as 24 individuals were obtained from a commercial fish farm in Sungai Paku Village, Kampar Regency, Riau Province and have been kept for >1.5 years in pond,

average 750±100 g/ individuals. *H. nemurus* has been given 17ß-estradiol were distributed into twelve cages (200×100×100 cm) each stocked with one individual. All cages are placed in pond (22×8×3.6 m) with an average water height of one meter. The pond water comes from Sungai Paku reservoirs to debit 0.2 m^3/second, pond water temperature ranged from 26 to 28°C. Feeding was done twice daily and fish were fed a predetermined ration of 10% body weight day^{-1}. The feed is fresh meat seashell freshwater (*Pilsbryoconcha exilis* ; Unionidae). Proximate composition are water content (% wet weight) 89.37%, crude protein 7.08%, lipid 0.82%, carbohydrate 0.27% and crude ash 0.29%.

Checking the Oocytes Maturation

All fish were individually marked using floy-tags and weighed. Oocytes sampled in vivo were taken from females using the method described by Syandri [22], and were placed in Serra's solution (6:3:1, 70% ethanol, 40% formaldehyde and 99.5% acetic acid). for clarification of the cytoplasm. After 5 min, the position of oocytes nucleus was determined using a four-stage scale:

Stage 1: germinal vesicle in central position

Stage 2: early migration of germinal vesicle (less than half of radius)

Stage 3: late migration of germinal vesicle (more than half of radius)

Stage 4: periphery germinal vesicle or germinal vesicle breakdown (GVBD)

Hormonal Treatment

Hormone pellet is a mixture of the 17ß-estradiol much as 1,000 µg, 70 mg cholesterol, 20 mg of cocoa butter, 1 ml etanol 70%. The dough molded into pellets much as ten grains and each pellet contains 100 µg of 17ß-estradiol. Experiments conducted on *H.nemurus* female gonad maturity stage one with four groups and three replications. Control group (injection from 0.9% NaCl) and three experimental ones. Group two, three and four given a single dose of 17ß-estradiol with 200 µg, 400 µg and 600 µg per kg body weight respectively. 17ß-estradiol intra muscular implanted under the dorsal fin. The spawning *H.nemurus* conducted with GnRHa stimulation with dopamine antagonist at a dose of 0.5 ml / kg body weight. Egg samples from each treatment and replications. Gilson is preserved with a solution consisting 100 ml of 60% alcohol, 880 ml of distilled water, 15 ml of nitric acid, 18 ml of glacial acetic acid and 20 grams of mercury chloride. Furthermore, the diameter of the eggs was measured with a microscope Olympus CX21 to 30 eggs of from each treatment and replications. Fertilized eggs were collected after ten hours and checked under a stereomicroscope. The eggs were randomly separated into four groups of aquarium (40x20x20 cm, eight liters of water volume), with three replicates for the control and three replicates for each exposure group. The total number of eggs in the control and the exposure groups was 200. The water temperature, dissolved oxygen (DO), and pH levels of the test chambers were regularly monitored.

Time mature gonadal calculated from the time the fish began to 17ß -estradiol granted until the fish reaches a mature gonadal [days]. Ovi somatic index (IOS) was determined using the expression: IOS = BTO/BW x100%; where BTO : ovulation egg weight and BW : Body weight. Absolute fecundity (AF) =OVA×GW; where OVA: oocyte number per ovary gram and GW: gonadal weight. Relative fecundity was estimated using the formula: RF = AF/BW; where AF: absolute fecundity; BW: Body weight.

Statistical Analyses

Data were analyzed by one-way analysis of variance (ANOVA) and then tested using Dunnett-s test. All statistical analyses were performed using SPSS Versi 13. In all tests, the level of significance used was 0.05.

Result

The Time Sexual Maturity and Percentage of Eggs Weight

The mean time to reach the mature gonadal of the control group was 55 days, whereas in 17ß-estradiol treatment group on average ranged from 26 to 35 days [Table-1]. There were no significant differences (p>0.05) between treatment groups 17ß-estradiol against time reaches mature gonads, but significantly different (p<0.05) with the control group. Average egg ovulation in the control group was 4.53%, whereas the treatment group ranged from 5.01% to 10.32% [Table-

1]. There were significant differences (p<0.05) between control and treatment groups 17ß-estradiol.

Table 1- Time matured the gonads and percentage of eggs weight

Level 17ß-estradiol (µg/kg body weight)	Time matured the gonadal (days)	Somatic Ovi Indeks (%)[a]
Control	55 ±14[a]	4,53 ± 0,71[a]
200	35 ±10[b]	5,01 ± 1,03[b]
400	28 ±13[b]	10,32 ± 0,76[c]
600	26 ±12[b]	7,94±2,25[d]

[a]Weight of eggs 100%/female weight.

[ab]Values with the different superscript in each column are significantly different from each other (p<0.05).

Fecundity and Eggs Diameter

The mean absolute fecundity *H.nemurus* of the control group was 35,950 eggs/spaw, while that of the exposure groups ranged from 40,043 to 63,457 eggs/spaw [Table-2]. Absolute fecundity tended to increase with increasing level of 17ß-estradiol, although the increases occurred at level of 200 and 400 mg / kg body weight. At a level of 600 µg/kg body weight has not increase fecundity when compared with a level of 400 mg/kg of body weight, but larger than the level of 200 µg/kg body weight. The absolute fecundity were significantly (p<0,05) among the each 17β-estradiol level.

Table 2- Fecundity and eggs diameter in the control and treatment groups exposure to various levels of 17ß-estradiol

Dose 17ß-estradiol (µg/kg body weight)	Average body weight (g)	Fecundity (number of eggs per spawn)	Relative fecundity (number of eggs per g gonadal weight)	Eggs diameter (mm)
Control	793±46	35,950±963[a]	37±2.0[a]	1.13±0,03[a] (n=50)
200	776±25	40,875±1,816[b]	47± 2.0[b]	1.17±0,03[b] (50)
400	790±36	63,724±1,163[c]	90±5.0[c]	1.22±0,01[c] (50)
600	810±50	52,500±2,920[d]	60±6.0[d]	1.16±0,02[d] (50)

Values with the different superscript in each column are significantly different from each other (p<0.05).

The mean relative fecundity (number of egss/ g gonadal weight) of four 17ß-estradiol exposure group [Table-2]. Fecundity relative on control group of 37 eggs / g gonadal weight. On the group two, three and four, respectively 47, 90 and 60 eggs/g gonadal weight. The relative fecundity was significant (p<0,05) differences between the control and exposure groups.

There were significant changes (p<0.05) in egg diameter among the 17ß-estradiol levels for all four spawning [Table-2], The eggs diameter on the control group was 1.13 mm, whereas in group 17ßestradiol imposed ranged from 1.16 to 1.22 mm.

The mean hatching rate of the control group was 64.16% while that of the exposure groups ranged from 65.83% to 67.83% [Table-3]. The mean hatching time in the control group was 31.10 hours while in the exposure groups it each one is 30.4, 29.0 and 29.66 hours [Table-3]. No significant (p>0.05) differences were found between hatching rate and mean hatching time in the control and exposure groups.

Table 3- Hatching rate and time to hatching of embryos in the control and treatment groups exposure to various levels of 17ßestradiol

Dose 17ß-estradiol (µg/kg body weight)	Hatching rate[b] (%)	Time to hatching (hours)
Control	64.16±5.13[a] (n = 200)	31±1.0[a] (n= 128)
200	66.50±2.29[a] (200)	30.33±1.52[a] (133)
400	67.83±2.25[a] (200)	29.0±1,0[a] (135)
600	65.83±5.20[a] (200)	29.66±1.52[a] (140)

[a]Values with the same superscript in each column are not significantly different from each other (p>0.05).

Discussion

The *H. nemurus* females group 17ß-estradiol exposed can reaches the gonadal mature more rapidly, increased of the index ovi somatic, absolute fecundity and relative fecundity, when compared to with the control group, but had not effective on hacthing rate and time to hatching. The best level 17ß-estradiol to rapidly of mature gonadal and increased fecundity was 400 µg/kg body weight. while the exposed to levels of 200 µg / kg and 600 µg/kg longer time matured gonads and less fecundity. Imai et al., [17] reported that Java medeka (*Oryzias javanicus*) exposed to 68 ng/l of

17ßestradiol can increased number of eggs, whereas exposed to 159 ng/l and 243 ng/l decreased number of eggs. The 17ß-estradiol plays an important role in the reproductive physiology of fish, especially in the process of vitellogenesis [23,24]. However every female fish species require 17ß-estadiol level optimal for increase reproductive [19]. Research of *H.nemurus* by Utiah [30] with 17ßestradiol at the level of 400 µg / kg and in combination with thyroxine 10 mg / kg body weight time reached a mature gonads was 79 days. In this study exposed to 400 µg/kg of the 17ßestradiol can reached the gonads a mature was 28 days. The 17ßestradiol able to accelerate the biosynthesis vitelogenin and gonadal development so the fish gonadal a mature rapidly [16]. Vitelogenin which is synthesized in the liver and with the stimulation of 17ß-estradiol secreted into the bloodstream selectively and is absorbed by the follicle oocytes [26]. Vitelogenin absorption by the oocyte supported by gonadotropin and thyroxine hormone. Thyroxine is also serves to stimulate the development embryo [27] and increased are larval survival [28].

The *H. nemerus* in natural spawning in September to December namely when the river floods and the time it was widely available natural food for larvae and juveniles [13,29]. The research was conducted from April to July 2012 are outside the spawning season, so that the effect of 17ß-estradiol visible to reproductive of *H.nemurus* females. According Indriastuti [30] that are 17ß-estradiol implantation is not effective when given to the fish spawning season. Thus it can be stated rapidly mature gonadal, increased fecundity and eggs diameter of *H. nemurus* is due to 17ß-estradiol. Sinjal et al., [31] stated that of catfish (*Clarias gariepinus*) exposed to 250 µg/kg body weight of 17ß-estradiol be combined to 1200 mg/ kg *ascorbyl phosphate magnesium* / kg weight of feed time matured gonads average of 39 days, whereas in the control group during the 95 days.

In general the exposed to 200 to 600 µg/kg body weight of 17ßestradiol can increased ovi somatic index of *H. nemurus* ranged between 5.01% to 10.32%, whereas in the control group was 4,53%. According Aryani et al., [12] that of *H. nemurus* exposed to 400 µg/kg body weight to produced somatic ovi index 8.08%, whereas of *Channa pleurothalmus* are 8.07% [32]. The fish reproduction is influenced by the quality of feed [33], hormone levels [32] and environmental factors [34]. In this research the same type of feed given fresh meat that is fresh water seashell (*Pilsbryoconcha exilis* ; Unionidae) and maintained in the same environment with indicators of water quality parameters is important that is water temperatures ranged from 24 to 27°C, pH ranged from 7 to 8 and dissolved oxygen ranged from 6 to 8 mg.l^{-1}.

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Conflicts of Interest: None declared.

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