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The effect of periodical estradiol-17 β injections with different doses on Java barb (*Puntius javanicus*) gonadal development

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Abstract. The aim of research was to determine the effect and the best dosage of estradiol-17 β hormone on gonadal development of Java barb (*Puntius javanicus*). The subjects of this study were 10 months old 48 female (\varnothing) Java barb weighing ± 200 gram. This research was done experimentally by applying a randomised design (CRD) with 4 treatments and 3 replications. Dosage treatments used were 0, 100, 200, 300 $\mu\text{g}/\text{kg}$ body weight. The data observed include body weight, body length, gonado somatic Index (GSI), Gonado Maturation Stage (GMS), hepato somatic index (HSI), total protein plasma (TPP) and water quality. The results showed that the highest body weight was obtained at treatment C 21.42 \pm 1.23 g. The highest body length was on treatment C 1.17 \pm 0.18 cm. The highest percentage GSI was at treatment C 14.84 \pm 0.79%. Meanwhile, the highest GMS were treatment C and D as all samples reached GMS IV on the 30th day. The highest percentage of HSI was at treatment C 2.25 \pm 0.36%. Treatment TTP A ranged 4.2-4.6 gr/dL, B 4.4 - 5.4 gr/dL, C 4.4-5.7 gr/dL, and D 4.5-5.8 gr/dL. The water quality of cultivation media was in the proper range at the temperature 26.5-28 $^{\circ}\text{C}$, DO 3-4.1, and pH 7-8. This research concluded that estradiol-17 β injection had a significant effect ($P < 0.05$) on body weight gain, GSI, HSI, yet it has not significant effect on body length. The best treatment is treatment C dose of 200 $\mu\text{g}/\text{kg}$ body weight.

1. Introduction

Java barb (*Puntius javanicus*) is Indonesian freshwater aquacultured fish. This fish usually lives in rivers and is commonly found in Sumatra and Java islands. Java barb spawns seasonally, especially at the beginning of the rainy season of which it reaches the spawning peak in September to October [1]. The current erratic season change in Indonesian regions makes Java barb's spawning activities especially for Java barb fish farmers become hampered and uncertain. In addition, it is well understood by the farmers that optimal handling of the brooder fish is a



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significant factor in producing intensive seeds both in terms of quantity and sustainability which is one of the keys to successful fish cultivation.

The availability of mature gonad brooders in a short time can increase the sustainability of seeds supply. Moreover, the farmers can also work efficiently. Optimal handling on Java barb brooders can be conducted internally by accelerating gonad maturity through hormone injection. One of such hormones is estradiol 17 β hormone. Estradiol-17 β hormone existence in female brooder body can help accelerate gonad maturation [2]. Estradiol-17 β injection is able to improve the liver performance to synthesize vitellogenin. However, each female fish species requires an optimal level of Estradiol-17 β to increase its reproduction. Moreover, vitellogenin is synthesized in the liver and stimulated by estradiol-17 β which is selectively secreted into the bloodstream and absorbed by oocyte follicles. The increase of estradiol-17 β level is positively correlated with the increase of IGS value in fish. Estradiol-17 β stimulation in synthesizing vitellogenin in the liver increases deposition of yolk and lipid globules at each vitellogenic stage that it eventually leads to an increase of IGS values [3].

This research was conducted by estradiol-17 β injection dose at 0, 100, 200, 300 $\mu\text{g}/\text{kg}$ body weights at regular intervals since the gonads will develop if hormone given is continuously available. The use of estradiol-17 β hormone with injection dose of 200 $\mu\text{g}/\text{kg}$ body weight has been proven to accelerate the development of goldfish oocytes for 12 days [4] and a dose of 400 $\mu\text{g}/\text{kg}$ body weight has also been proven to accelerate the maturation of catfish gonads with 10.32% GSI value. 22

The purposes of this study were to determine the effect of periodical estradiol-17 β hormone injection and to obtain the best dose for the development of Java barb gonads. This research was conducted from January 7 to February 28, 2019 at the Workshop of Freshwater Fish Hatchery and Aquaculture (PBIAT- *Perbenihan dan Budidaya Ikan Air Tawar*) Ngrajek, Magelang Regency, Central Java Province, Indonesia.

2. Methodology

The test fish in the study were 48 female Java barb weighing ± 200 grams and 10 months old of age from PBIAT Ngrajek, Magelang regency. The maintenance containers used were hapa ponds of 1 m² in width containing 4 Java barb fish. The hormone used was estradiol -17 β hormone which was then diluted using olive oil with 1 mg of estradiol dissolved with 1 mg of olive oil ratio [2]. The feed used was commercial feed containing 30% protein. In addition, feeding method used was *ad satiation* with feeding frequency given at 8:00am and 15:00pm.

Injection treatment were periodically provided every 10 days as much as 4 times for 30 days and sampling process was conducted by measuring body weight, body length, gonad weight, liver weight and blood collection to determine total protein in plasma. Weight data collection was conducted surgically by dissecting the fish from the anus to the operculum using a *sectio* kit. Blood sampling was done drawing blood through the vein using a 1ml syringe and then inserted into EDTA vacutainer. Measurement of total blood plasma protein was done using HandRefractometer SPR-N (ATAGO) at Animal Hospital Prof. Soeparwi, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta.

Experimental method was used in this study by using a completely randomized design (CRD) with 4 treatments and 3 replications. The doses of estradiol-17 β hormone used in this study were treatment A (0 $\mu\text{g}/\text{kg}$ body weight), treatment B (100 $\mu\text{g}/\text{kg}$ body weight), treatment C (200 $\mu\text{g}/\text{kg}$ body weight) and treatment D (300 $\mu\text{g}/\text{kg}$ body weight).

2.1. Parameters

Data variables obtained included absolute weight, absolute length, Gonado Somatic Index (GSI), Gonad Maturation Stage (GMS), Hepato Somatic Index (HSI) and Total Plasma Protein (TPP). In addition, water quality measurement included temperature, pH and DO.

2.1.1. Absolute Weight

Absolute weight growth can be calculated by finding out the average final weight and the average initial weight. This can be calculated using formula as follows [5]:

$$W = W_t - W_0$$

2.1.2. Absolute Length

The following is a formula to measure absolute length [6]:

$$P = P_t - P_0$$

2.1.3. Gonado Somatic Index (GSI)

Data of gonad observation were retrieved by calculating the value of gonad weight comparison with the total body weight of fish. GSI values can be determined using the following formula [7]:

$$\text{Gonado Somatic Index (GSI)} = \frac{\text{Gonad Weight}}{\text{Total Body Weight}} \times 100 \%$$

2.1.4. Hepato Somatic Index (HSI)

Hepato Somatic Index (HSI) is a percentage value between fish liver weight and total body weight. The formula used to calculate HSI was as follows [6]:

$$\text{IHS} = \frac{\text{Liver Weight}}{\text{Body Weight}} \times 100 \%$$

2.1.5. Gonad Maturation Stage (GMS)

Gonad Maturation Stage can be determined by observing the fish morphologically and histologically. Morphological observation was conducted by observing gonad shape, gonad color, gonad weight and gonad length [6].

2.1.6. Total Plasma Protein (TPP)

Total protein in blood plasma was measured by using HandRefractometer SPR-N (ATAGO). Furthermore, total blood plasma protein was recorded by reading the scale on the back part of the tool. The scale can be read in a well lighted room [8].

2.1.7. Water Quality

Water quality parameters measured during the study were water temperature, pH and DO. Water temperature and DO parameters were calculated using DO meters, while pH was measured pH indicator and universal pH. The measurements were conducted once in every 10 days in the morning, afternoon and evening.

2.2. Data Analysis

The data obtained included body weight, body length, GSI, GMS and HSI. Moreover, the data were tested with normality diversity, homogeneity and additives. Then, the data were statistically analyzed using Variance Analysis (ANOVA) with 95% significance level. If the treatment is proven to have a significant effect, Duncan test is then performed to determine the difference between median values of the treatment and to determine the best treatment [9]. In addition, data of GMS, total plasma protein and water quality were analyzed descriptively.

3. Results

The results showed the development of Java barb fish (*P. javanicus*) gonads after one month culture as proven by its growth in body weight, body length, Gonado Somatic Index (GSI), Hepato Somatic Index (HSI), Gonad Maturation Stage (GMS), and Total Plasma Protein (TPP).

3.1. Absolute weight growth

The figure 1 presents data of absolute weight growth calculation.

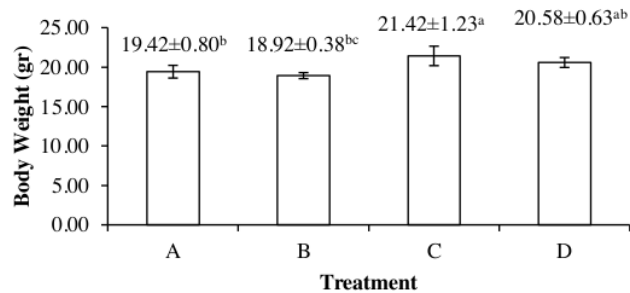


Figure 1. Results of Java barb (*P. javanicus*) Absolute Weight Growth

The results of data variance analysis showed that providing estradiol-17 β hormone has significantly affected absolute weight growth of Java barb ($P < 0.05$). Duncan double regions test showed that treatment C was not significantly different from treatment D (20.58±0.63 g), but it was significantly different from treatment A (19.42±0.80 g) and treatment B (18.92±0.38 g). Treatment D (20.58±0.63g) showed not significantly different result from treatment A and B (18.92±0.38 g).

3.2. Absolute length

Based on the calculation of Java barb absolute length growth, the data present in Figure 2.

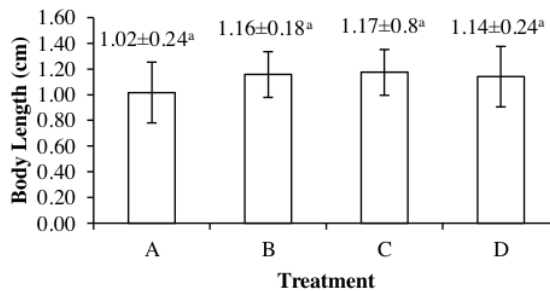
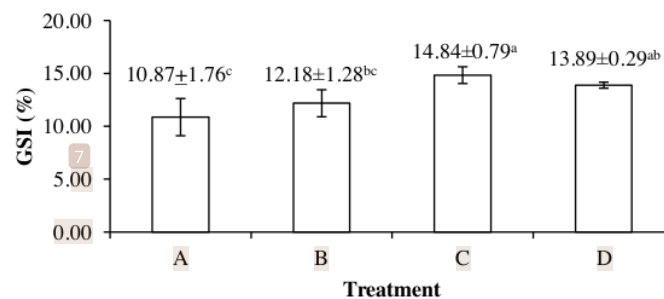


Figure 2. Results of Java barb (*P. javanicus*) Absolute Length Growth

Based on data variance analysis of Java barb absolute length, it showed that injecting estradiol-17 β hormones with different dosages periodically has no significant effect ($P > 0.05$) on absolute length of Java barb because F count $< F$ table.

3.3. Gonado somatic index (GSI)

The following figure 3 presents a GSI values calculation result of Java barb.

Figure 3. Graph of Java barb (*P. javanicus*) during the Study

The results of data variance analysis concluded that the injection of estradiol showed significantly different results ($P < 0.05$) on Java barb with F count > F table. In addition, Duncan region test results showed that treatment C (200 $\mu\text{g}/\text{kg}$ body weight) was not significantly different from treatment D (13.89±0.29%), but it was significantly different from treatment A and B (12.18±1.28%). Moreover, treatment D (13.89±0.29%) on treatment B (12.18±1.28%) showed no significant difference, while treatment B (12.18±1.28%) showed not significantly different results with treatment A (10.87±1.76%).

3.4. Gonado maturation stage (GMS)



The results of observation on gonad maturity level of Java barb fish from GMS III to GMS IV present in Table 1 and Table 2.

Table 1. Development of Java barb fish (*P. Javanicus*) during study

Treatment	Replication	Gonado Maturation Stage			
		D0	D10	D20	D30
A	1	III	III	III	III
	2	III	III	III	III
	3	III	III	III	III
B	1	III	III	III	III
	2	III	III	IV	IV
	3	III	III	III	IV
C	1	III	III	IV	IV
	2	III	III	III	IV
	3	III	IV	IV	IV
D	1	III	IV	IV	IV
	2	III	III	III	IV
	3	III	III	IV	IV

Observation results showed that gonad maturity level of Java barb fish at the beginning of culture was in the same condition, i.e. GMS III. Furthermore, treatment A showed that there was not any increase until the 30th day, treatment B could reach GMS IV on the 20th day at the second replication and on the 30th day at the second and third replication. Treatment C and D could reach GMS IV on the 10th day at each replication and all replications could reach GMS IV on the 30th day. Morphological gonad development of Java barb present in Table 2.

Table 2. Gonado maturation stage of Java barb (*P. javanicus*) during study

Gonad Morphology	GMS	Description
	III	Gonad was green, filling 1/3 of the body cavity, the egg granules were not yet clearly visible
	IV	Gonad was yellowish green, filling 2/3 of the body cavity, egg granules could be visible by bare eyes

Note: Arrows indicate gonad of Java barb (*P. javanicus*)

3.5. Hepato somatic index (HSI)

The result of observation conducted on HSI is presented this following figure 4.

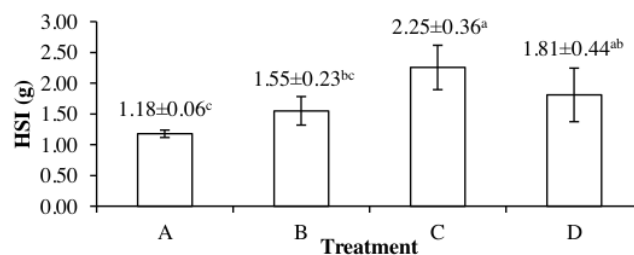


Figure 4. Graph of Java barb (*P. javanicus*) HSI Results during the Study

The results of data variance analysis obtained showed that giving estradiol led to significantly different results ($P < 0.05$) on Java barb HSI, with F count > F table. Moreover, Duncan region test result showed that treatment C (200 µg/kg body weight) was not significantly different from treatment D (1.81±0.44%), but significantly different from treatment A and B (1.55±0.23%). In addition, treatment D (1.81±0.44%) showed were not significantly different result to treatment B (1.55±0.23%), whereas treatment B showed the result which was not significantly different from treatment A (1.18±0.06%).

3.6. Total plasma protein

The result of Java barb blood sample test on TPP present in Figure 5.

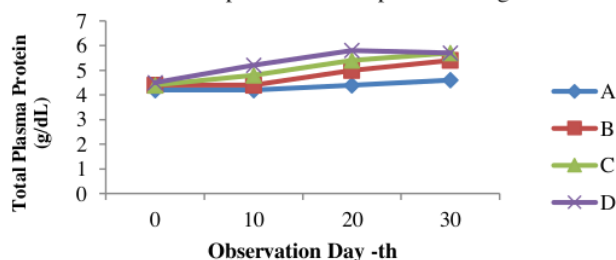


Figure 5. Results of Total Plasma Protein (TPP) values of Java barb (*P. javanicus*) during observation

Based on the observation result of Total Plasma Protein, it could be concluded that TPP value in treatment A showed a non significant increase from observations of 0th, 10th, 20th, and 30th day, i.e. 4.2 g/dL; 4.2 g/dL; 4.4 g/dL; and 4.6 gr/dL respectively. Treatment B did not show an increase in TPP value on day 0 (4.4 g/dL) until the 10th day, but there was an increase on day 20 by 5 g/dL and day 30 (5.4 g/dL). Treatment C showed an increase from day 0, 10, 20, and 30, i.e. 4.4 g/dL; 4.8 g/dL; 5.4 g/dL; and 5.7 g/dL. Treatment D showed an increase from 0th, 10th, and 20th day by 4.5 g/dL; 5.2 g/dL; and 5.8 g/dL but it decreased on the 30th day to 5.7 g/dL.

3.7. Water quality

Based on the results of water quality measurement carried out once every 10 days during observation, it was found that the water was still properly suitable to be used as a medium for Java barb culture. The range of water quality values obtained present in Table 3.

Table 3. The result of water quality measurement on Java barb (*P. javanicus*) during study

Water Quality Parameters	Range of Water Quality Parameter Value	References (Feasibility)
Temperature (°C)	26.5-28.0	25 – 30 [10]
pH	7-8	6.5 – 8.0 [5]
DO (mg/l)	3.0 – 4.1	>3 ppm [11]

4. Discussion

4.1. Absolute weight

The results of variance analysis showed that the administration of estradiol-17β hormone could make a significant effect ($P < 0.05$) on the growth of Java barb absolute weight. Body weight growth in fish is influenced by several factors, one of which is gonad weight. Estradiol-17β hormone injection will stimulate the fish liver to synthesize vitellogenin which will become egg yolk. Moreover, vitellogenin will then be circulated to the gonad through blood vessels. Furthermore, Vitellogenin accumulation in the gonad will make the gonad weight increase. This fish gonad weight increase can reach 10-25% of the body weight during gonadal development until it becomes mature [12].

Treatment C (200 μg/kg body weight) showed the highest absolute weight value (21.42±1.23 g), but it was not significantly different from treatment D (300 μg/kg body weight) and treatment D decreased to (20.58±0.63 g). Too high and too low doses were assumed to cause ineffective hormones performance in Java barb fish body. In addition, treatment B at a dose of 100 μg/kg body weight was not significantly different from treatment A (19.42±0.80 g) without injecting estradiol-17β. Treatment B resulted in the lowest value (18.92±0.38 g). Then, it can be concluded that gonadal development in adult female fish is linear to the increase in body weight.

Treatment C with 200 μg/kg estradiol dose was not significantly different from treatment A without injection. Thus, It was assumed that treatment A had greater somatic (meat) growth since the test fish in treatment A could utilize the feed given for somatic growth causing not significantly different result. Gonadal growth can occur if the available energy meets the needs for fish body maintenance and somatic growth [13].

4.2. Absolute length

The results on the development of Java barb gonads treated by periodically injecting estradiol-17β at different doses showed that the injection of estradiol-17β hormone treatment did not significantly affect the absolute length growth as seen in the analysis of variance or ANOVA

test ($F_{count} > Table (0.05)$). The result of absolute length growth measurement of Java barb from the highest to the lowest respectively was found in treatment C at a dose of 200 $\mu\text{g}/\text{kg}$ (1.17 ± 0.18), treatment B at a dose of 100 $\mu\text{g}/\text{kg}$ (1.61 ± 0.18), treatment D at a dose of 300 $\mu\text{g}/\text{kg}$ (1.14 ± 0.24), and treatment A at a dose of 0 $\mu\text{g}/\text{kg}$ (1.02 ± 0.24). Based on previous study [14], the concentration of estradiol-17 β at a dose of $<550\text{ng}/\text{l}$ has no effect on the increase in fish length and width.

This result showed that the injection of estradiol-17 β hormone with different doses did not affect the growth of Java barb absolute length during 30 days culture. The increase of the adult fish length cannot be as fast as its weight gain since the amount of energy obtained will be used for meat and gonad growth. As mentioned by Yenni *et al* [15], there are two factors which can affect fish growth, i.e. internal and external factors. Internal factors include heredity, sex and age of the fish; while the external factors which can significantly influence are feed and the environment.

4.3. Gonado somatic index (GSI)

The results of variance analysis showed that estradiol-17 β hormone injection had a significant effect ($P < 0.05$) on the Java barb GSI values. The highest gonad maturity index value was obtained in treatment C, $14.84 \pm 0.79\%$, followed by treatment D $13.89 \pm 0.29\%$, treatment B $12.18 \pm 1.28\%$, and the lowest was in treatment A $10.87 \pm 1.76\%$. High or low value of GSI is related to vitellogenesis process. According to earlier report [16], an increase in the concentration of estradiol-17 β in fish blood can increase vitellogenin concentration which will be distributed by blood to gonad. Gonad size gets bigger due to the presence of vitellogenesis. Meanwhile, the increase in GSI is due to the development of oocytes containing vitellogenin. Vitellogenin or future egg yolk will increase in number and size so that the volume of oocytes becomes larger.

Differences in gonad maturity index values in each treatment showed that the injection of estradiol hormone dose gave a different effect on oocyte development. Treatment C with a dose of 200 ($14.84 \pm 0.79\%$) did not show significantly different result from treatment D at a dose of 300 $\mu\text{g}/\text{kg}$ body weight ($13.89 \pm 0.29\%$). This was probably because the administration of 200 $\mu\text{g}/\text{kg}$ body weight dose was sufficient to stimulate vitellogenesis process which caused gonadal weight to increase. In contrast, treatment D showed a decrease by $13.89 \pm 0.29\%$ which may be caused by excessive hormone concentration that it could inhibit the performance of the targeted organs and provide feedback to the hypothalamus due to high estradiol content in fish body. Nagahama and Yamashita [17] mentioned the possibility of negative feedback mechanism so that a quite high FSH content can increase the work of endogenous LH which then will suppress gonadotropin to stop estradiol-17 β synthesis. Excessive hormone induction can disrupt the balance of hormones in fish body. Consequently, excess hormones are released by fish body through its excretion system. Meanwhile, treatment B did not show significantly different results to treatment A because the dose given was insufficient to fulfill the need for estradiol hormone in increasing the development of Java barb gonad.

4.4. Gonado maturation stage (GMS)

The results of the study on the development of Java barb gonad showed that the maturity level of Java barb gonad at the beginning of the study (day 0) in GMS III was at maturation stage and the gonad looked morphologically small filling up 1/3 of abdominal cavity, had green color and the eggs could already be visibly distinguished by bare eyes, whereas in GMS IV the eggs looked yellow filling 1/2 or 2/3 of the abdominal cavity. The GMS III is gonad maturation stage with the following characteristics: the ovaries are yellow, the eggs morphologically begin to show their granules which can be seen by bare eyes, the surface of the testes looks jagged, the colors get whiter and the size gets bigger [6].

Based on the four treatments experimented, treatments C and D on the third and first replication resulted in GMS IV on the 10th day after estradiol injection. This result was much faster than other treatments. On the 30th day, all replications had reached GMS IV. Treatment B showed a change from GMS III to GMS IV on the 20th and 30th day, whereas treatment A showed no change in gonad maturity level until the end of cultivation. These results indicate that different

doses lead to different time to reach gonad maturity stage and estradiol hormone injected to Java barb can accelerate gonad maturity. In conclusion, gonadal development shows a positive response from fish to the treatment. The increase of estradiol concentration causes a strong stimulus to vitellogenesis process which affects gonadal development [18]. In addition, increasing estradiol content also gives positive feedback to the hypothalamus and pituitary to synthesize LH hormone which functions for gonad maturation. Thus, the increase of estradiol is followed by the increase of LH concentration.

4.5. Hepato somatic index (HSI)

Based on the result of variance analysis in this study, it can be stated that periodical injection of estradiol-17 β hormone to Java barb showed that estradiol-17 β hormone had a significant effect ($P < 0.05$) on Java barb HSI value (*P. javanicus*). The highest HSI value was found in treatment C with 200 $\mu\text{g}/\text{kg}$ body weight dose of $2.25 \pm 0.36\%$, and followed by treatment D with 300 $\mu\text{g}/\text{kg}$ body weight dose of $1.81 \pm 0.44\%$, treatment B with 100 $\mu\text{g}/\text{kg}$ body weight dose of $1.55 \pm 0.23\%$, and the lowest value was in treatment A with 0 $\mu\text{g}/\text{kg}$ body weight dose of $1.18 \pm 0.06\%$. Estradiol hormone can affect Java barb HSI value because the high level of estradiol-17 β in the liver will improve liver performance in synthesizing vitellogenin.

Treatment C, 2.25 \pm 0.36% dose, showed not significantly different result from treatment D 1.81 \pm 0.44% dose, but showed significantly different results from treatment B as much as 1.55 \pm 0.23% and so it was to treatment A. Treatment A without giving estradiol dose 1.18 \pm 0.06% showed not significantly different result from treatment B and D. Meanwhile, treatment B showed significantly different results to treatment C. Hepato Somatic Index (HSI) value became higher along with the increasing dose of estradiol hormone injected and it reached the peak in the treatment of estradiol-17 β 200 $\mu\text{g}/\text{kg}$ injection. Then, it decreased along with the increasing dose of the hormone given. Excessive hormone dose will decrease the effectiveness of the hormone work performance on the target organ that it will cause negative feedback. The greater oocyte growth would lead to the higher availability levels of estradiol-17 β in the body [19]. Thus, it causes negative feedback to the hypothalamus to suppress FSH and then results in positive feedback in stimulating the pituitary to release LH. Moreover, the increasing levels of LH in the fish body can stimulate the activity of 20 β -hydroxysteroid dehydrogenase (20 β -HSD) to produce 17, 20 β dihydroxyprogesterone so that oocyte maturation occurs which is followed by ovulation. The HSI also decreases with maturity in fish ovaries; this decrease occurs as the energy stored in the liver is used for gonad development [20].

4.6. Total plasma protein (TPP)

Based on the results of the research concerning the development of Java barb by periodically injecting estradiol hormone at different doses, it showed that the range of total plasma protein (TPP) in Java barb at each treatment, i.e. TPP of treatment A was at 4.2 - 4.6 g/dL, treatment B at 4.4-5.4 g/dL, treatment C 4.4-5.7 g/dL, and treatment D 4.5 - 5.8 g/dL. Total value of plasma protein among treatments has increased along with the increasing dose. Protein content in blood is one of the important components in fish reproductive system. This has been proven and supported by earlier study [21] which reporting that protein is used as a basic material for hormone and vitellogenin synthesis in liver.

Treatment A showed a non-significant increase in observation day 0, 10, 20, and 30, i.e. 4.2 g/dL; 4.2 g/dL; 4.4 g/dL; and 4.6 g/dL respectively. Treatment B did not show any increase on day 0 and day 10 (4.4 g/dL) but it increased on the 20th day by 5 g/dL and on the 30th day by 5.4 g/dL. Treatment C showed a continuous increase from the 0th day by 4.4 g/dL until the 30th day by 5.7 g/dL. Treatment D has increased in TPP values from the 0th day to the 20th day; however, it decreased on the 30th day to 5.7 g/dL. The increase in TPP value presumably occurs due to an increase in the results of lipoprotein or vitellogenin secreted by liver through the bloodstream because of the existing response from estradiol. According to previous report [22], vitellogenin is produced from vitellin, a combination of lipoprotein and contains 48% fat, 50% protein and 2% carbohydrate.

Total plasma protein during the cultivation period continuously increased along with the increase estradiol dose given. Nevertheless, there was a decrease in TPP value on the 30th day D treatment which was presumably due to the high dose of estradiol-17 β resulting a negative feedback to the hypothalamus to suppress FSH and increase LH performance. Value of protein in blood plasma is generally used as an index of vitellogenin level and TPP value increases gradually during vitellogenesis [23]. This happens due to an increase of lipoproteins secreted by liver through the bloodstream caused by an existing response from estradiol. The increase in protein is probably in order to make the formation of steroid hormones (especially estrogen) is sufficient during vitellogenesis process.

4.7. Water quality

Water is the most important component in conducting fish culture. In addition, the level of fish survival is also influenced by water quality. When the water quality in aquaculture pond is good, the cultivated fish will live longer. Water quality is a factor limiting the type of biota which can be cultivated in waters. Measurement on water quality parameters in research media includes temperature, pH, and dissolved oxygen.

Water quality measurements in this study were carried out every week and the variables observed included temperature, dissolved oxygen and pH. The temperature during Java barb culture ranged between 26.5 - 28°C. This temperature range still fits with the culture feasibility of Java barb cultivation. The feasibility range of the water temperature for Java barb is 18 - 30°C since Java barb at 10°C will stop eating and their growth will stunt when the temperature reaches 5°C [24].

The content of dissolved oxygen found in study showed DO values of Java ranged 3 – 4.1. Moreover, the value of dissolved oxygen during cultivation was still feasible for Java barb. The speed of oxygen consumption by fish varies depending on the type, size, activity, temperature, food status and oxygen content in water. This Java barb fish or sometimes called as Silver Barb fish favor swift waters condition, i.e. 30-50 m/sec [25]. Based on earlier study [26], DO values ranging from 4-7 mg/l are feasibly good for the life of aquatic biota while 0.3 – 1.01 mg/l oxygen level can kill fish if it lasts long enough.

The pH value measured in Java barb culture media was 7-8. pH influences the process and rate of chemical reactions in water and biochemical reactions in the tissues of Java barb body. Moreover, the pH value is still in feasible range to support the life and development of Java barb. According to Mahendra [24], the optimum pH value for Java barb brooders cultivation ranges from 6.5 to 8.6.

5. Conclusion

The results of the study lead to these following conclusions:

1. Periodic injections of estradiol-17 β hormone with different doses have a significant effect (Fcount > Ftab) on absolute weight, Gonado Somatic Index (GSI), Hepato Somatic Index (HSI), and Gonad Maturation Stage (GMS) but it has no significant effect on absolute length.
2. The best treatment of estradiol-17 β hormone injection is found in treatment C with a dose of 200 μ g/kg body weight resulting Gonado Somatic Index (14.84 \pm 0.79%) and (2.25 \pm 0.36%) HSI value.

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