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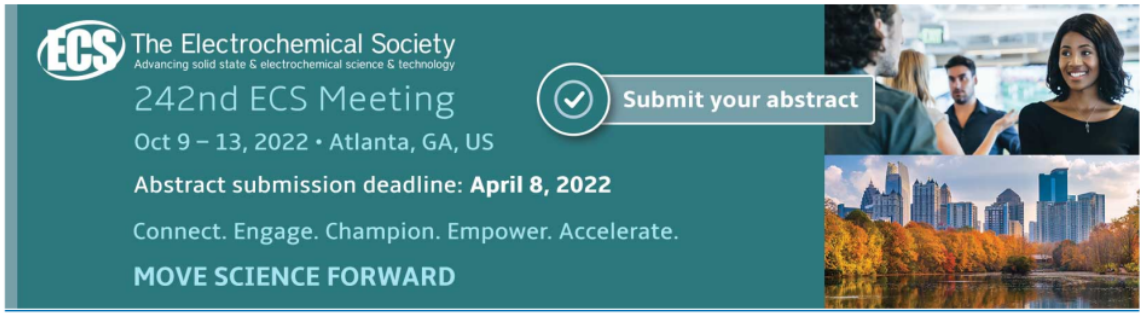

Use of periodically hCG hormones injection for the gonadal development of java barb (*Puntius javanicus*) as bioreproduction applied on aquaculture

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Abstract. Tawes fish or Java barb (*Puntius javanicus* Bleeker, 1855) as the one of the endogenous species in Indonesia has specific reproductive roles that its cycle still depends on local environment. It makes limited frequency of spawning in nature that has been decreasing seeds production either domestication or cultivation. As a seasonal spawner, it needs long time for gonadal development. Gonadal development of endogenous species broodstock by hormone is needed to improve bio-reproductive capacity and it will cause faster gonadal maturity of Java Barb. One of exogenous hormones that could trigger gonadotropin hormone (GtH) is the human chorionic gonadotropin (hCG) hormone and it will accelerate the maturity level of the gonad by periodically injection. The aim of research is to determine the effect and the best dosage of hCG hormone for the gonadal development of Java barb. This study used an experimental method with a completely randomized design, with 4 treatments and 3 repetitions in 30 days observed. 48 broodstock with 200 gram average weight were used and induced by hCG. The doses of hCG are 0, 100, 200 and 300 IU/kg. The parameters observed include the level of gonad maturity, gonado somatic index (GSI) and hepatosomatic index (HSI). After statistical analysis, result showed that dose of 200 IU could improve the best development of gonad in Java barb base on 3rd maturity level achieved, gonado somatic index (10.11 ± 0.74%) and the hepatosomatic index at (1.99 ± 0.06%) respectively. The observation which used hCG hormone can improve gonadal development and one step closer to reproduce domesticated and cultived of Java Barb.

1. Introduction

Tawes fish (*P. javanicus)* is one of the freshwater fish that has native habitat of various regions in Indonesia, so it has seasonal spawning properties. It stated that female tawes fish could be fertilized after the age of one year and usually mature mothers of gonad sized between 300-600 grams and for male tawes can already be fertilized at the age of 6-8 months with a size of 300-400 grams [1].

The maturity of the gonads in common fish is influenced by environment factors, i.e., water quality, the presence of the opposite sex and nutrition abundance, and internal factors, i.e., age maturity and hormone administration [2, 3]. Tawes as the one of an endogenous species in Indonesia has specific reproductive roles that its cycle still depends on the local environment. It makes limited frequency of spawning in nature that has been decreasing seeds production either domestication and cultivation. As a seasonal spawner, it also causes a long time for gonadal development and takes approximately two months [4]. Gonadal development of endogenous species broodstock by hormone is needed to improve

bio-reproductive capacity and causes faster gonadal maturity so it can improve the production of seeds in aquaculture.

The hormone that plays a role in the development of gonads is gonadotropin (GtH). Gonads will develop if GtH is available continuously. One of the trigger hormones of GtH is the hormone human chorionic gonadotropin (hCG), which will accelerate the maturity level of gonads of broodstock. The hormone hCG is a glycoprotein (peptide) hormone secreted by *syncytiotrophoblast cells* (placental formation cells) in pregnant women. This hormone activity was similar to *luteinising hormone* (LH) and *follicle stimulating hormone* (FSH) which is usually produced by the pituitary gland [2].

Injection of the hormone hCG has previously been performed on cork fish at a dose of 500IU/kg as much as three times which can accelerate the development of lyocyte diameter [3]. The hormone hCG has also been implanted in Kancera *fish (Tor soro)* with a dose of 500 IU/kg affected to increase of oosite size [5]. Implantation technique using the hormone hCG in nilem fish can increase the maturity of the broodstock [6]. The hormone hCG has also been used in siamese fish where periodic injection with hCG can stimulate the maturation of siamese gonads, weighting 500 gr at the best dose of 200 IU [7]. Therefore, the research of hCG hormone injection as bioreproductive manipulation and identifying of its optimum dosage is crucial to develop the production of Tawes seeds.

The purpose of this study is to find out the effect of the hormone hCG on the development of tawes gonads as endogenous species and determine t_{he} best dose of the hormone hCG for the development of gonads through intra muscular injection. The study will arrange the perspective roles of how far internal factor stimulated (by hormones injection) can be affected to endogenous species that highly affected by environment stimulant.

2. Methods

The tawes broodstock used are 48 fish at 9 months age with a weight of ± 200 gram. The density per happa as research site unit is 4 fish/m^2 in 30 days observed. The hCG hormone 5000 IU used by diluted first using the NaCl and injected every 10 days on all fish. The treatment used are A as control (0 IU/kg), treatment B (100 IU/kg), treatment C (200 IU/kg) and treatment D (300 IU/kg). The maturity and index of gonad; and index of hepatopancreas were observed at days of 0 and 30.

2.1. Maturity level of gonads

Determination of Gonad Maturity Level can be observed morphologically. Morphological observation by observing the shape of the gonad, the color of the gonad, the weight of the gonad and the length of the gonad.

2.2. Gonad maturity index

The Gonad Maturity Index is determined by using the following formula:

$$\text{Gonad Maturity Index} = \frac{\text{gonad weight (gram)}}{\text{body weight (gram)}} \text{ x } 100\%$$

2.3. Hepatosomatic index

Hepatomatic index is the ratio of liver weight to body weight formula:

$$\text{Hepatosomatic Index} = \frac{\text{Hepato weight (gram)}}{\text{Body weight (gram)}} \text{ x } 100\%$$

Data analysis is conducted through a series of statistical tests: normality, homogeneity and additives, then the data will be analyzed statistically using Variety Analysis (ANOVA) or F test to find out the effect of the treatment that has been done. The results of the test are then drawn conclusions and if the treatment proves to have a significant effect and determined of the best doses affected [8].

3. Results and discussion

The results showed that the development of tawes gonads for 30 days was evidenced by increased levels of gonad maturity, gonad maturity index (IKG) and hepatopathic index (IHS).

3.1. Maturity level of gonads.

The results showed that the maturity level of tawes gonads (*P. javanicus*) has improved by hCG hormones injected in various doses (see Figure 1).

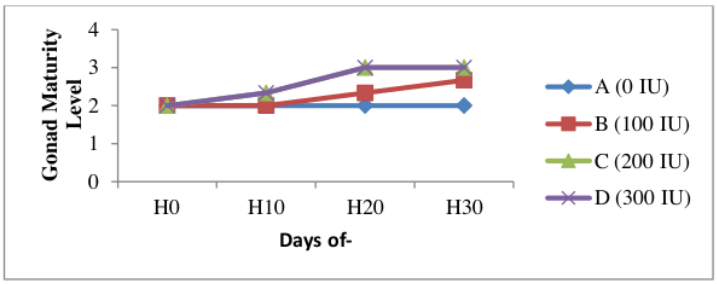
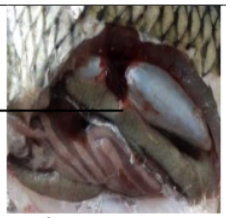
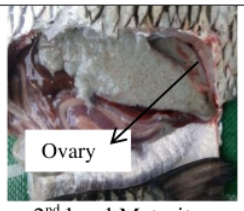
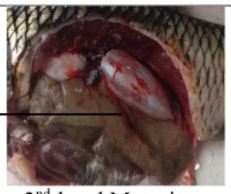
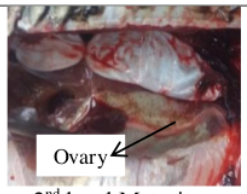


Figure 1. Maturity Level of Gonads Tawes Fish (*P. javanicus*) affected by various doses of HcG

The gonad maturity level before HcG injected is on 2nd level of all fish tested. In first 10th day observed, majority fish is still on 2nd level maturity but some fish in C and D treatment has entering 3rd level maturity. On 20th day observed, fish on treatment B showed elevated to 3rd level maturity and the C and D treatment all fish still at the same level. On day 30th, all of fish in treatment B through 3rd level maturity so did fish in treatment C and D. Characteristics of the maturity of tawes fish gonads (*P. javanicus)* given the hormone hCG presented in Figure 2.

Treatment	*Figure on H0*	*Description*	*Figure on H30*	*Description*
A Ovary ←	 2nd level Maturity	Small ovary, 1/3 body cavity, very green color, the eggs have not been visible clearly by eyes, and eggs have not been separated.	 2nd level Maturity	Ovary has not met stomach cavity, undesperate intestines, green colors, and invisibly visible eggs.
B Ovary ←	 2nd level Maturity	Small ovary, 1/3 cavity body, the egg has not been clearly visible by the eyes, the eggs are not recoverable.	 2nd level Maturity	Small ovary, 1/3 cavity of the body, yellowish-green color, eggs can not yet be clearly seen by the eyes, and the eggs have not been separated.

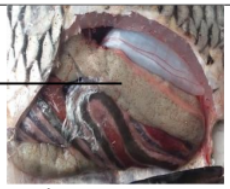
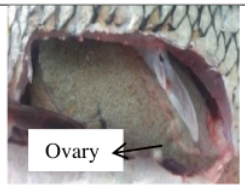
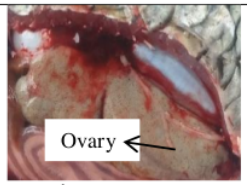
C Ovary	 2nd level Maturity	Small ovaries, 1/3 cavity body, the egg has not been clearly visible by the eyes, the eggs are not recoverable.	 3rd level Maturity	Ovari meets the cavity of the body, the intestines are pressed, the color is yellowish-green, the eggs can already be clearly visible to the eyes but can not yet be separated.
D Ovary	 2nd level Maturity	Small ovary, 1//3 cavity body, the egg has not been clearly seen by the eyes, the eggs have not been recoverable, the color is still green.	 3rd level Maturity	Ovary 2/3 fish body cavity, yellowish color, eggs can already be seen by the eyes, eggs can not be separated, there are blood vessels on the surface.

Figure 2. Maturity level of gonads

3.2. Gonad maturity index

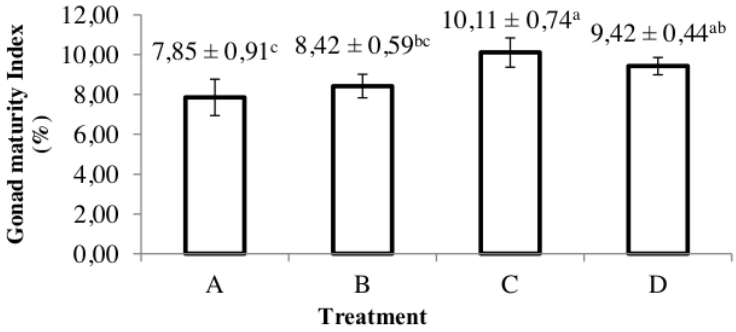


Figure 3. Gonad Maturity Index

3.3. Hepatomatic index

The results showed that both gonad maturity and hepatosomatic index of tawes have improved by hCG hormones, and doses 200 IU/kg significanally has better affect than doses 300, 100 IU/kg and control (see Figure 3 and 4) by F value on Anova analysis that showed 6,38 at gonad maturity and 6,74 at hepatosomatic index compared 4,07 as F-significant. The result shows that the development of gonads occurs in line with the increase in gonad weight which then affects the average value of the gonad maturity index, and will enter the spawning period. The hormone hCG is a hormone that can stimulate gonadotropin consisting of LH and FSH to increase [9].

The hormone hCG is *an exogenous* hormone that can increase gonadotropin which will enter the cell and stimulate testosterone production, in the absence of the help of the enzyme aromatase can convert testosterone into estradiol and stimulate the liver (hepar) to produce vitellogenin then carried by the

bloodstream to the gonads, inside the gonads it will selectively be absorbed by the exocite so that is called the process of egg development until maturation. Vitellogenin produced by the liver will be secreted to the gonad through the bloodstream and in the gonads, there will be the absorption of vitellogenin by oocytes so it will grow enlarged and result in an increase in the weight of the gonads. The hormone gonadotropin has a function to stimulate the process of egg development and gonadotropin hormone will enter the teca cell and stimulate testosterone production, then with the help of the enzyme aromatase convert it into the hormone estradiol will then stimulate the liver to produce vitellogenin [10, 11, 12, 13, 14].

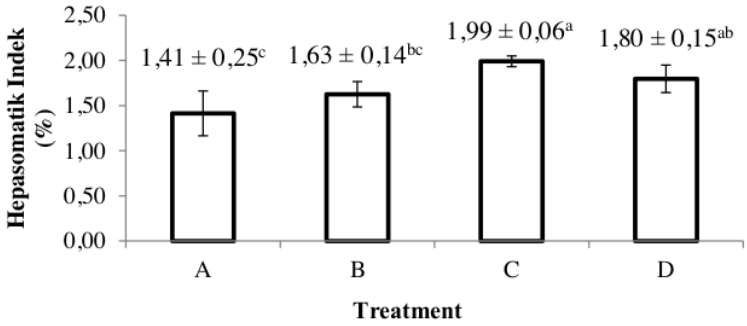


Figure 4. Hepatosomatic index

The decrease in gonad maturity index at a dose of 300 IU caused by inhibitation of hormonal mechanisms to be unbalanced and may inhibit the working activity of gonads. The process of absorption of vitellogenin i.e., lyocytes will grow enlarged until it stops when it has reached its maximum size or enters the dormant phase and the egg is waiting for the environmental signal to migrate [13].

Periodically injection of hCG hormone can maintain gonadotropin hormone if available continuously [2, 15, 16]. According to the result, bioreproductive manipulating can be applied to endogenous species in the limit of stimulating gonad maturity level and index that is usually only affected by environmental stimuli. The role of environmental stimuli could be replaced by artificial stimuli, so the frequency limitation of spawning in nature could be increased. It will conduct to maximize seeds production through domestication and cultivation in aquaculture in the future.

4. Conclusion

This research showed that hCG hormones have a significant effect to gain gonadal development of Tawes. The injection of the hormone hCG periodically can affect the maturity level and index of gonads, also hepatopathic indexes in tawes (*P. javanicus*). 200 IU is the best dose to increase: the development of gonad from 2nd to 3rd level maturity, optimized of index maturity of gonads to 10.11 ± 0.74% and hepatomatic index to 1.99 ± 0.06%. As endogenous species, tawes have been physiologically improving as fully domesticated fish in Aquaculture. This result showed that this domesticated process has been well-progress on gonadal development that can improve by induced-hormonal. The process physiology as hepatosomatic is clearly work as shown in this research. Those processes make a bioreproduction could be applied in off-season aquaculture that, one step closer, will increase a seed produce any time in the hatchery. The next research to fulfilled a domestication of this endogenous fish species are improving gametes-spawning, egg-hatching, and larva rearing so Tawes as endogenous species can be completely cultivated and produced.

Acknowledgements

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