REPRODUCTIVE RESPONSE OF NILE TILAPIA *(Oreochromis niloticus)* AFTER INDUCED THE GONADAL SUPERNATANT OF YELLOW FIN TUNA *(Thunnus albacares)*

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Abstract

The aim of this study was to examine the reproductive response of nile tilapia after induced the gonadal supernatant of yellow fin tuna and determine its effect. The research methods were used experimental and descriptive method. The results showed that the lowest GMS (Gonad Maturity Stages) scoring for the dose of 0.7 mL/Kg with a value of 2.67 ± 0.44 and the highest in the control fish with a value of 4±0.44. The lowest of GSI (Gonado Somatic Index) value at 0.7 mL/Kg dose with a value of 0.87±0.75 and the highest value found in the control fish with a value of 2.86±0.75. The lowest of HSI value at 0.9 mL/Kg dose with a value of 3.19±0.43 and the highest value contained in a dose of 0.7 mL/Kg with a value of 4.24±0.43. Oocyte diameter size of 19.8 to 93.28 µm and a diameter of vitelogenin (Vtg) ranged from 5.72 to 25.52 µm. Overall value of some parameters resulted in the above studies were not significant, but the visual difference was the reproduction response of tilapia (*O. niloticus*) after induced the gonadal supernatant of yellow fin tuna (*T. albacares*).

Keywords: Nile Tilapia, supernatant, GMS

1. Introduction

Tilapia (*O. niloticus*) is one of the food ingredients that have prospects and demand will continue to increase. The production of cultivated tilapias has increased exponentially during the last 20 years (de Lapeyre, 2007), in East Java at 23,211 tons (DJPB, 2011). Market demand will need more, so it needs to be tried by applying the technology that is being developed.

Engineering reproductive technology in the fisheries sector includes the use of male or female monosex seeds from the application of hybridization (Tamamdusturi and Basuki, 2012), cutting of tail fin to increase gonadal maturity (Solang, 2010), steroid hormone administration through feed (Darwisito *et al.*, 2008) and the use of laser light (laserpuncture) as a biostimulation that has been used to produce desired broodstock and seeds (Rustidja, 2011). Broodstock and good seeds are obtained through cultivation stages in the hatchery sector. Hatcheries with adequate quality and quantity of seed production are fundamental to the success and sustainability of aquaculture production activities (Setiyanto *et al.*, 2013).

Hatchery activities must be carried out in a controlled manner, one of which was by accelerating gonadal maturity by using hormones. Hormones in fish consist of steroids, thyroxine, proteins and catecholamines produced by the pituitary gland, thyroid, gonads, kidneys and uropsis (Fujaya, 2008). Many types of hormones have been used to stimulate gonadal development such as the pituitary gland, Human Chorionic Gonadotropin (HCG), Luetinizing Hormone Releasing Hormone (LHRH), Gonadotropin Releasing Hormone salmon (GnRH-s) (Filho and Barbosa, 2008). The use of gonadotropin hormone from donor animals had been widely used. However, in this case, the utilization of gonad waste organs from

yellow fin tuna *(T. albacares)* containing reproductive hormones will be studied.

During this time the yellow fin tuna *(T. albacares)* gonad was not utilized and was a fishery waste from fishing activities at sea. Tuna fish caught and then immediately cleaned the digestive organs and gonads so that the fish do not rot quickly. The wasted gonads will be used as biostimulants because there are naturally occurring hormones that can be used as an alternative to stimulate the maturation of the gonads of tilapia *(O. niloticus)*. Therefore, research on the use of gonadal supernatant of yellow fin tuna *(T. albacares)* needs to be done.

2. Materials And Methods

The tools were used digital scales, pH meters, DO meters, micropipets, centrifuge devices, sectional sets, microscopes, rotary microtomes, water heaters and trays. The ingredients used are tilapia, gonad of yellow fin tuna, aquadest, 10% formalin, hematoxylin-eosin. The research method used the experimental method, namely the method carried out by experimental activities. This method aims to investigate whether there was a causal relationship between existing variables by manipulating and controlling natural situations into artificial, as well as providing treatment and control as a comparison in the study (Narbuko and Ahmadi, 2007). The next method that will be used was descriptive method. Data retrieval with descriptive method was carried out by direct observation in the field. This method aims to explain the conclusion (Subandi, 2011).

Preparation and Maintenance of test fish

The test fish used in this study were female tilapia *(O. niloticus)*. The selected fish was then acclimatized for 2 weeks then put into a bulkhead pond. Separated between fish with each other to facilitate observation of different treatments.

Making Supernatan Gonad

Gonad of yellow fin tuna *(T. albacares)* was mashed by grinding using mortar and pestle. After a fine sample, weighed as much as 10 g, then added with distilled water as much as 10 mL (1: 1), put into a test tube then centrifuged for 10 minutes at a speed of 8000 rpm at 4^{o}C. The results of the centrifuge was taken from the liquid at the top using a micropipette and inserted into the tube. The preparation of the supernatant was carried out at the Chemical Laboratory of Gondol, Singaraja-Bali. The supernatant samples were tested in the Hospital Central Laboratory to determine the levels of estradiol 17β and progesterone hormones contained in the gonad of yellow fin tuna *(T. albacares)*.

Supernatant Injection

Supernatant injection of the gonadal yellow fin tuna *(T. albacares)* in accordance with the treatment dose as much as one injection due to the quantity of doses that are too little to be injected into the muscles of the tilapia. In addition, one injection was carried out to find out how the response of tilapia by measuring these parameters. Blood collection was carried out 24 h after injection. Blood samples was taken at the caudal or base of the tail (Hosseinzade, 2012).

Surgery Proccess

Female tilapia *(O. niloticus)* after treatment and control. Surgery began from the anal to the vertical lateral line then dissected to the pectoral section horizontally, slashed towards the ventral direction. Surgery was done carefully so that it was not to damage the internal organs, then observe the gonad and liver conditions, has taken and weighed gonads and hearts to obtain GMS scoring, GSI and HSI scores.

Research Parameters

Observations in this study to determine the reproductive status of fish, including:

GMS *(Gonad Maturity Stages)*

The level of gonadal maturity in tilapia was carried out visually according to Kesteven (1960) in Bagenal (1968) and histologically which measured oocyte diameter according to stadiance.

GSI *(Gonado Somatic Index)*

GSI was a comparison percentage between gonad weight and fish body weight. According to Sadekarpawar and Parikh (2013), GSI measurements can use the formula:

$$GSI\ (\%) = \frac{Wg}{Wb} \times 100$$

Where :

GSI = Gonado Somatic Index (%)
Wg = Weight of gonad (g)
Wb = Weight of body (g)

HSI *(Hepato Somatic Index)*

The value of HSI was the percentage comparison between liver weight and body weight of fish (Ighwela et al., 2014; Odedeyi et al., 2014). HSI measurements can be formulated as follows:

$$HSI\ (\%) = \frac{Wl}{Wb} \times 100$$

Where :

HSI = Hepato Somatic Index (%)
Wl = Weight of liver (g)
Wb = Weight of body (g)

Size of Oocytes and Vitelogenin

The measurement of oocytes and vitelogenin includes the diameter, circumference and extent of one oocyte and vitelogenin individual with a sample of 5 through histological preparations. This measurement was done because tilapia eggs cannot be cannulated, so that the size of the oocytes and vitelogenin in the treatment was compared with the size conditions in the control. This oocyte and vitelogenin diameter size was used as a supporting parameter in the reproduction response of tilapia. According to Unus and Syarifuddin (2010), egg diameter was related to nutrient availability. One parameter to determine reproductive potential was by knowing the variation in egg diameter in the ovaries.

Water Quality Parameters

Observation of water quality was very important because water was a living medium for fish. According to Karmila et al. (2012), water quality parameters, including temperature, pH and DO

3. Discussion and Result

The results of the preliminary study regarding the production of female gonadal supernatant of yellow fin tuna *(T. albacares)* and the level of reproductive hormones contained there in. Reproductive hormones regulated by the hypothalamus would be playing an important role in the process of gonadal development. Therefore, the content of reproductive hormones was produced and accumulates in the theca and granulosa cells as a constituent of follicles in the gonadal tissue. According to Anwar (2005), the synthesis of sex steroid hormones was produced mainly by gonads which are regulated by two types of gonadotropin hormone produced by adenohipofisa namely FSH (Follicle Stimulating Hormone) and LH (Luetinizing Hormone).

The following levels of estrogen (estradiol 17β) and progesterone contained in the gonads of yellow fin tuna can be seen in Table 1.

Table 1. The Gonadal Hormone Levels of Yellow Fin Tuna (*T. albacares*)

Hormones	Gender	Levels
Estradiol 17β	Female	574,30 pg/Ml
Progesterone	Female	2,53 ng/mL

The following results from several research parameters can be seen in Table 2.

Table 2. The main value of research parameters

Supernatant Dose Treatments (mL/Kg)	The Main Parameters		
	GMS Scoring	GSI (%)	HSI (%)
Kontrol	4[a]±0.44	2,86[a]±0.75	3,59[a]±0.43
0,1	3,33[a]±0.44	1,83[a]±0.75	3,33 [a]±0.43
0,3	3[a]±0.44	1,15[a]±0.75	3,96[a]±0.43
0,5	3,33[a]±0.44	1.72[a]±0.75	3,25[a]±0.43
0,7	2,67[a]±0.44	0,87[a]±0.75	4,24 [a]±0.43
0,9	3,33[a]±0.44	2.24[a]±0.75	3,19 [a]±0.43

Note: Different notations showed the significant test results

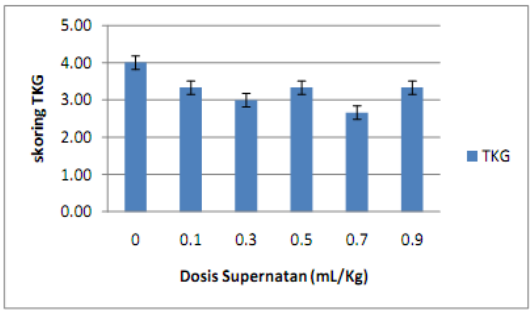

Figure 1. GMS Scoring

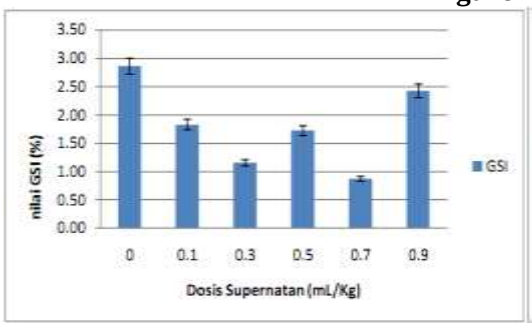

Figure 2. GSI's average

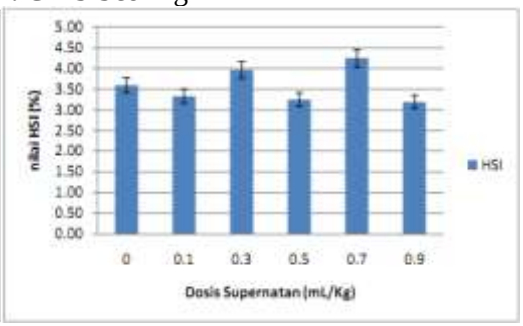

Figure 3. HSI's average

GMS Scoring

The mean GMS scoring (Fig. 1) showed that the lowest value occurs at a dose of 0.7 mL / Kg with a value of 2.67 ± 0.44 followed by a dose of 0.3 mL / Kg with a value of 3 ± 0.44. Furthermore, there was the same mean at 0.1 mL / Kg, 0.5 mL / Kg and 0.9 mL / Kg with a value of 3.33 ± 0.44. The highest value was in the control of 4 ± 0.44. Gonad conditions in control fish are greater than those treated. This showed the response of tilapia to induction compared to control fish that did not experience a drastic change in GMS. A response received by a fish can be either positive or negative because the condition of the fish greatly influence the income from the induction. Smaller GMS scoring showed that individual oocytes in the ovary are also smaller in size. This can occur atresia in the ovary because of its negative response to supernatant induction. Lubzens et al. (2010), several factors that cause atresia follicles are nutritional deficiency, stress and hormone treatment. Atresia often occurs in the vitelogenic oocyte phase and sometimes can also occur in the previtelogenic oocyte phase. Miranda et al (1999) added that stated from several studies also explained that morphologically atresia follicles were follicular cells would become

phagocytocytes and at the end of atresia would experience a decrease in the number of theca cells due to lytic enzymes in the yolk digestion process in the oocytes. Based on this statement, it can be explained that the condition of the ovaries that experience atresia will be smaller because of the absorption of protein in the oocyte. Dadzi and Wangila (1980) in Rustidja (2001) in GMS 3 were characterized by enlarged ovaries, dark yellow and there were oocytes starting to contain egg yolks, while in GMS 4 it was characterized by large, brown ovaries, microscopically oocytes easily differentiated and separated (oocytes are ready to be emulated).

GSI value

The average GSI value (Fig. 2) in the graph above shows that the lowest GSI value occurs at a dose of 0.7 mL/Kg with a value of 0.87 ± 0.75 followed by a dose of 0.3 mL/Kg with a value of 1.15 ± 0.75. The highest GSI value was found in control fish with a value of 2.86 ± 0.75. This was inseparable from the role of hormones on the hypothalamus, which was caused by a decrease in the level of the hormone estradiol 17β in the body of tilapia in the average dose during observation. Muslim (2007) states that the condition of hormones contained in the fish's body will affect the work of the endocrine glands associated with the reproductive process, thus affecting the maturity of fish gonads.

GSI value was proportional to the value of the GMS which explained that the maturity of the gonad can be seen from visual observations or in the form of numbers (values). The magnitude of the GSI value indicates an increase in the size of the egg diameter. Dewanti et al. (2012), the increase in the GSI value of female fish was due to the vitellogenesis process, which the process of deposition of egg yolk in each individual egg.

HSI value

The average of HSI value (Fig. 3) in the graph above showed that the lowest HSI value occurs at a dose of 0.9 mL/Kg with a value of 3.19 ± 0.43 followed by a dose of 0.5 mL/Kg with a value of 3.25 ± 0,43; dose of 0.1 mL/Kg 3.33 ± 0.43; the control was 3.59 ± 0.43 and the dose was 0.3 mL/Kg 3.96 ± 0.43 while the highest HSI value was at a dose of 0.7 mL/Kg with a value of 4.24 ± 0.43. The magnitude of the HSI value was related to the process of vitelogenesis that occurs in the liver. Yusuf (2005), this hormone was produced by the granulosa layer in follicles that are secreted into the bloodstream. Some of these hormones would lead to the liver played a role in the formation of vitelogenin which was the main component of egg yolk and some will provide stimulation back to the hypothalamus. According to Anwar (2005), circulation of estradiol was rapidly converted to estron in the liver with the help of 17-hydroxysteroid dehydrogenase.

Supernatan gonad which was induced in tilapia had a response that the weight of liver in the fish given supernatant induction had a heavier weight compared to gonadal organs. In contrast, in control fish, gonad weight was greater than that of liver weight. This was due to the response to the supernatant induction stimulation so that the occurrence of vitelogenesis process occurs in the liver. Katsu et al., (1999), the influence of hormones on oogenesis was not direct, but was mediated directly by steroid hormones produced by ovarian follicle cells. These steroid hormones were estradiol 17β and 17α, 20β dihydroxy-4-pregnen-3-one. Chattoraj et al. (2005), these cells act as steroid producers and played an important role in the synthesis of lipoproteins from the liver to the egg. Fujaya (2008), vitelogenesis was characterized by increasing the volume of egg yolks. During this process there was an increase the thickness in the radiata zone, granulose and theca cells. Theca cells were responsible for the synthesis of testosterone which granulose cells were converted to estradiol 17β.

Histology

A clearer observation can be seen from the cross section of gonadal organ tissue. Tilapia oocytes can be observed in their parts (Fig. 4) and the size of the oocytes (Fig. 5) and their vitelogenin (Fig. 6). The tilapia oocytes above are divided into parts, there are core, YVD, YGD and radiata zones that make up the follicles. Chattoraj et al. (2005) added that the oocyte was surrounded by two main cell layers, the outer layer (theca cell layer) and the inner layer (granulosa layer) separated by a membrane. The theca cell layer consists of collagen tissue, fibroblasts and blood capillaries. According to Nagahama (1983) in Pireira et al. (2016), the oocyte stage can be characterized by the volume of the cytoplasm, the appearance of the nucleus and nucleoli, and the presence of egg yolk.

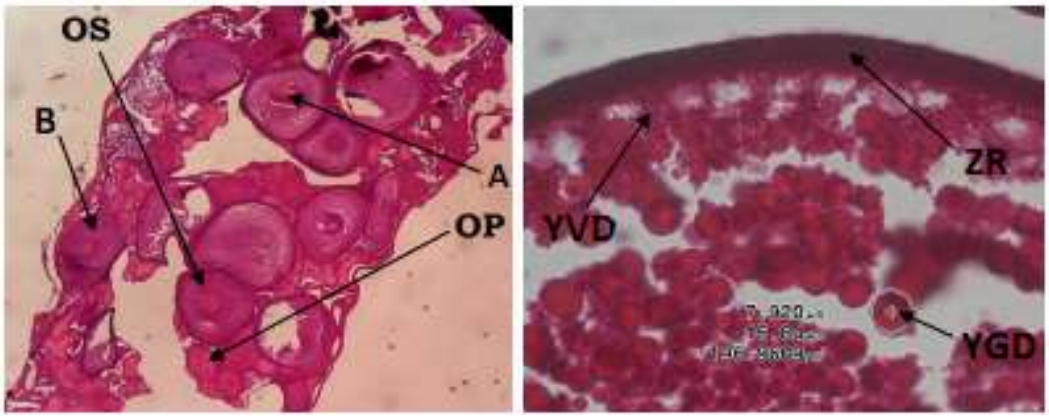

Figure 4. Gonad cross section

Where:

A	= Core	YVD	= *Yolk Vesicle Deposition*
B	= Atresia	YGD	= *Yolk Granule Deposition*
OP	= Primer Oocyte	ZR	= Radiata Zone
OS	= Secondary Oocyte		

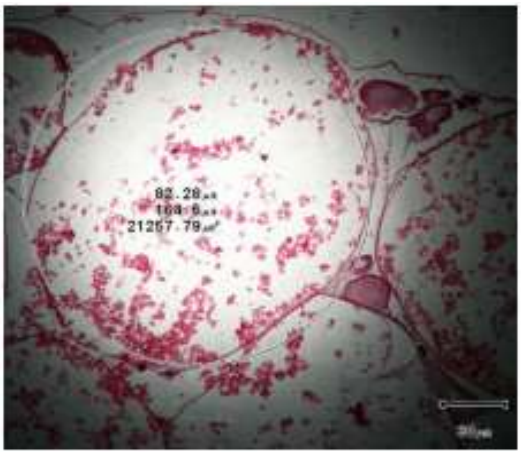

Figure 5. Oocyte size

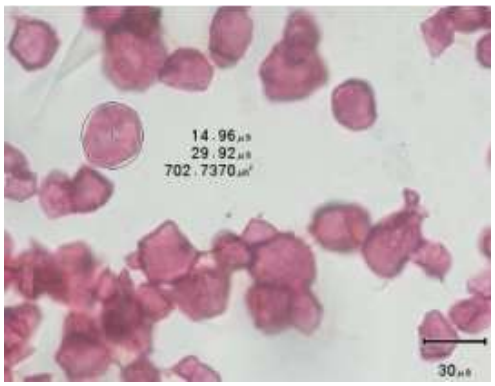

Figure 6. Vitelogenin Size

Oocyte diameter size ranged between 19.8-93.28µm. The diameter size of vitelogenin was also obtained that its size varies, ranging from 5.72-25.52µm.

Water Quality Parameters

Observation of water quality was carried out to determine the optimal range for fish life during the study because water quality played an important role and was one of the factors that influence the condition of fish. The results of water quality during the study were obtained at a temperature of 25-26oC, pH 7.2-8.2 and DO 5.8-6.8ppm. According to Hardy et al. (2011), good range value of water quality parameters, including temperature 28-29ºC, pH 5-6 and dissolved oxygen 4.8-5 ppm. Sutisna and Sutarmanto (1995) added that fish life parameters were in the temperature range of 25-26ºC, pH 6.7-8.6 and DO> 5ppm.

4. Conclusion

- Reproductive response of tilapia *(O. niloticus)* after induced the gonadal supernatant of yellow fin tuna *(T. albacares)* on average decreased its reproductive status. This was characterized by decreased estradiol levels, visual GMS scoring, GSI values and HSI values.
- Effect of gonadal supernatant on yellowfin tuna *(T. albacares)* on gonadal development of tilapia *(O. niloticus)* which was no significant.

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