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17β-Estradiol Level of Nile Tilapia *(Oreochromis Niloticus)* after Induced with Supernatant of Yellowfin Tuna *(Thunnus Albacares)* Gonadal Female

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Abstract : Nile Tilapia (O. niloticus) is one commodity of freshwater which has high economical prospects as well as demand for this commodities to increase. Engineering reproductive technology has been applied. This research regarding the provision of gonadal supernatant biostimulan yellowfin tuna (T. albacares) for the reproduction of nile tilapia. The aim was assessed hormone 17β-estradiol level as its reproductive response. The method used was experimental and descriptive. The results showed that the value of 17β-estradiol in 24 h time series average decreased by the difference value in 52,8pg/ml to 4.139,3pg/ml. Time series in 72 h showed an increased value 117,21pg/ml to more than 4.300pg/ml although no impairment at the highest dose of 236pg/ml to 2.810pg/ml. Time series in 144 h showed the average value of 17β-estradiol that was too high at more than 4.300pg/ml.
Keywords: nile tilapia supernatant, estradiol 17β.

Introduction

Cultured of nile tilapia has increased about 20 years[1] and in Jawa Timur Provinceup to 23,211 tonnes[2]. Engineering reproductive technology in the fishery include using of seeds monosex male or female that result of the application hybridization[3], cutting the tail fin to increase the gonadal maturity[4],and administration of steroid hormones through the feed[5] as well as using of a laser beam (Laserpunktur) as biostimulator which has been used to produce the desired broodstock and seed[6]. Quality of broodstock and seed obtained through the stages of cultivation in the hatchery sector. Hatchery operations with quality and sufficient quantity of seed production was fundamental sustainability of aquaculture production[7].

Hatchery must be done in a controlled manner, either by accelerating the maturity of gonadal by using hormones. Hormones in fish consists of steroids, thyroxine, protein and catecholamines produced by the pituitary gland, thyroid, gonadal, kidneys and uropsis[8].

Many kind of hormones that have been used to stimulate the pituitary gonadal development such as Human Chorionic Gonadotropin (HCG), Luetinizing Hormone Releasing Hormone (LHRH), salmon Gonadotropin Releasing Hormone (GnRH-s)[9].

Utilization gonadothropin hormone derived from animals donor have been done. However, in this case will be studied organ utilization of the waste of the yellowfin tuna (T. albacares) gonadal female that contains the reproductive hormones.

During this time, yellowfin tuna (T. albacares) gonadal female was not used and it was a waste of fisheries in marine fishing activities. Tuna fish are caught and then immediately cleaned parts of the digestive organs and their gonads, so that the fish did not quickly decay. The gonadal was the waste has separated and then used as biostimulan because it has the natural hormone which can be used as an alternative to stimulate gonadal maturation in nile tilapia. Therefore, the research on the use supernatant of yellowfin tuna gonadal female was needs to be done.

The aim research was assessed the 17β-estradiol level as reproductive responses of nile tilapia after induced supernatan of yellowfin tuna gonadal female.

Materials and Method

This research was conducted at the Breeding and Reproduction of Fish Laboratory, Biosciences Laboratory, Central Laboratory of Saiful Anwar Hospital in Malang, Indonesia. Chemistry Laboratory of BBPPBL Gondol and Harbour of Benoa Bali, Denpasar, Bali Province, Indonesia, from March until May 2016.

The tools used were centrifuges, digital scales, micro pipettes, syringes dispossible, centrifuge tube, mortar pestle, sectio set and refrigerator. The materials used were nile tilapia, tuna fish gonadal, distilled water and physiological saline. The method used was descriptive method.

The study consisted of three phases: the first phase of preliminary research covering the supernatant preparation of gonadyellowfin tuna (T. albacares) used as a trigger biostimulan gonadal maturation in nile tilapia. Made the supernatant was conducted by the method of separation, first gonadal was cut in half and then crushed in a mortar. After that, taken partly refined gonadalthen weighed as much as 10 g on digital scales (accuracy 10^{-2}).Put in tubes of 20 ml capacity with the addition of distilled water 10 mL (1:1). Tubes were centrifuged at a speed of 8000 rpm and 4°C for 15 minutes. That supernatant has been formed then placed in a new tube and stored in the refrigerator (4°C).

The second phase was supernatants were injected in the nile tilapia. However, prior to the first injection of nile tilapia was prepared and measured weight and body length. Research firstly measured the 17β-estradiol levels early by taking blood before and after treatment using a 1 mL syringe, blood that had taken placed on appendorf.Blood samples were centrifuged at a speed of 3500 rpm and 4°C for 15 minutes. Serum was taken and stored in refrigerator (4°C). Measurement used ECLIA. Serum was analyzed levels were measured with a kit COAT - acount artificial estradiol 17β Los Angles Diagnostic Product Corporation, USA. Furthermore, the injection to the spine (intramuscular) with various doses namely A_1 = 0.1 mL/kg , A_2 = 0.3 mL/kg, A_3 = 0.5 mL/kg , A_4 = 0.7 mL/kg, A_5 = 0.9 mL/kg and the control (no treatment). Each induction treatment was performed at various time series difference in the range of 24 h, 72 h and 144 h.

The third phase was observed 17β-estradiol blood serum end of nile tilapia. Proceed with the surgery, nile tilapia dissected from the anus vertically toward linea lateralis then head towards the pectoral fins (pectoral fin) horizontally and ending surgery on the pelvic fins (ventral fin).

Result and Discussion

Results showed that the content the supernatant of yellowfin tuna gonadal female, include 17β-estradiol at 574.30 pg/mL and progesterone level at 2.53 ng/mL. The content has high value for a review used as biostimulan to stimulate reproduction. The synthesis of sex steroid hormone produced mainly by the gonadal. Its regulated by gonadotropin hormone[10].They are operated by FSH (Follicle Stimulating Hormone) and LH (Luetinizing Hormone).

The discussion examined from the findings of research reproductive response of nile tilapia.

Table 1. Data During Treatment

Time Series	Dose	L (cm)	Wb (g)	Wg (g)	Wh (g)	GSI (%)	HSI (%)	17β-estradiollevel (pg/mL)		
								BTL	ATL	DL
24 h	Kontrol	18.9	112.5	2.68	1.76	2.38	1.56	947.6	170.5	-777.1
	0,1 ml/kg	20.8	158.72	2.35	2.37	1.48	1.49	877.6	48.21	-829.39
	0,3 ml/kg	21.7	159.06	2.98	3.03	1.87	1.90	4300	160.7	-4139.3
	0,5 ml/kg	22.1	175.78	1.33	2.85	0.76	1.62	1824	5	-1819
	0,7 ml/kg	21.4	152.08	0.63	3.38	0.41	2.22	98.4	45.6	-52.8
	0,9 ml/kg	22.9	197.08	1.91	3.84	0.97	1.95	448	32.13	-415.87
72 h	Kontrol	20.8	141.89	7.31	4.8	5.15	3.38	2969	4300	+1331
	0,1 ml/kg	21.2	145.06	4.8	7.22	3.31	4.98	57.09	174.3	+117.21
	0,3 ml/kg	21.7	170.58	2.14	7.2	1.25	4.22	4300	4300	Over
	0,5 ml/kg	22.6	198.02	6.94	8.91	3.50	4.50	3231	4300	+1069
	0,7 ml/kg	22.7	197.34	2.79	9.79	1.41	4.96	134	370	-236
	0,9 ml/kg	20.9	166.69	2.71	6.89	1.63	4.13	4300	1490	-2810
144 h	Kontrol	20	147.12	1.53	8.59	1.04	5.84	4300	4300	Over
	0,1 ml/kg	21.4	159.73	1.11	5.62	0.69	3.52	4300	294.8	-4005.2
	0,3 ml/kg	20.2	133.6	0.44	7.69	0.33	5.76	4300	4300	Over
	0,5 ml/kg	21.4	150.39	1.35	5.45	0.90	3.62	4300	4300	Over
	0,7 ml/kg	20	131.2	1.04	7.27	0.79	5.54	4300	528	-3772
	0,9 ml/kg	24.6	292.2	13.66	10.17	4.67	3.48	4300	4300	Over

Where :

(+)17β-estradiol level increased
(-)17β-estradiol level decreased
(over) very high value, no detected
(BT) Before Treatment Level
(AT) After Treatment Level
(D) Difference level

(L) Length
(Wb) Weight of body
(Wg) Weight of gonadal
(Wh) Weight of hepato
(GSI) Gonado Somatic Index
(HSI) Hepato Somatic Index

Time Series 24h

17β-estradiol levels on time series 24 h can be seen in Figure 1.

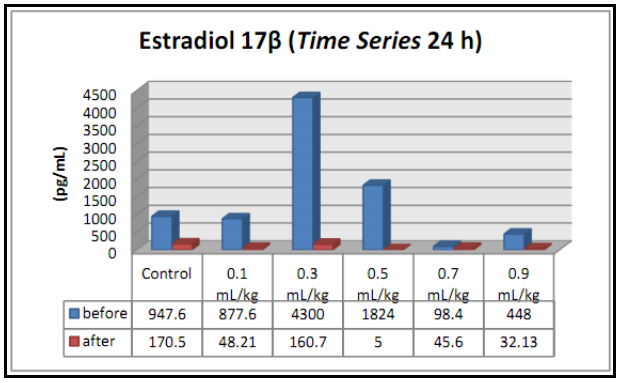

Figure 1. 17β-estradiol levels on time series 24 h

The results of the research on time series 24h final observation 17β-estradiol levels of blood sampling performed after 24 h from the time of injection, the result was showed the difference in value very high levels of 17β-estradiol on test fish that had been treated. The fifth dose decreased of 17β-estradiollevels. The treatments were given decreasing doses, respectively for 829,39pg/mL, 4.139,3pg/ mL, 1.819pg/mL , 52,8pg/mL and 415,87pg/mL.

The decline in 17β-estradiol levels was due to the feedback after the supernatant induced form of estrogen that may affect FSH stimulates the formation of follicles in the ovaries.In order to see the functions of FSH secretion of hormones that were released by the hypothalamus and physiological feedback mechanism of the targeted organ, the level were influenced by steroids, progesterone, estrogen and testosterone[10].

Time Series 72 h

17β-estradiollevels on time series 72 h can be seen Figure 2.

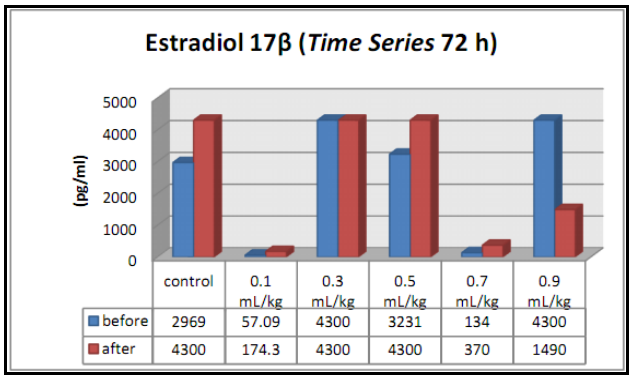

Figure 2. 17β-estradiol levels on time series 72 h

Results of research on the observation 72 h time series after injection showed that the levels of 17β-estradiol at a dose of 0,1mL/kg and 0.5 mL/kg increased by 117,21pg/mL and 1069pg/mL, but some were declining at a dose of 0,7ml/kg and 0.9ml/kg ie., 236pg/mL and 2.810pg/mL. Decreased levels of 17β-estradiol at high doses cause negative feedback so as to provide a response drop in estrogen levels in the blood. Fujaya (2008) feedback inhibition occurs by hormones that can be inhibitedpituitary hormones production.

Time Series144 h

17β-estradiol levels on time series 144 h can be seen in Figure 3.

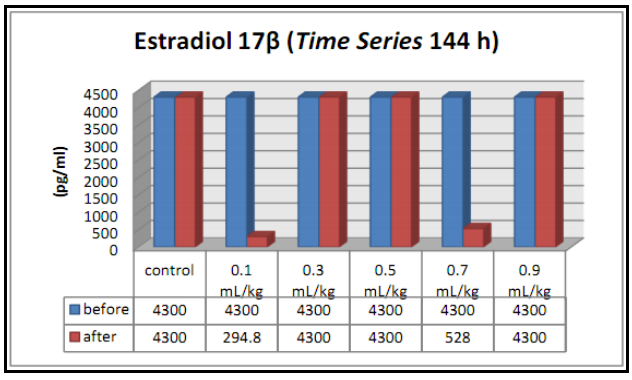

Figure 3. 17β-estradiol levels on time series 144 h

The results showed that levels of 17β-estradiol at a dose of 0,3mL/kg, 0,5mL/kg and 0,9mL/kg was too high so that its value can not be detected by the unit (pg/ml). Dose 0,1mL/kg and 0,7mL/kg decreased levels of 17β-estradiol was 4.005,2pg/mL and 3.772pg/mL. This decrease was due to reduced production of hormones in the body during the process of reproduction. According Anwar (2005), estrogen therapy in the long term can be increased the FSH and LH level.

Water Quality Level

Monitoring of water quality was conducted to determine the optimal range for fish survived during treatment because of water quality. An important role and it was one of the factors that affect the condition of

the fish. Water quality parameters measured in the study include: pH (acidity), temperature and dissolved oxygen. Results of water quality measurements during the study was still in the normal range for the maintenance of tilapia as temperature 25-26^{o}C, pH 7.2-8.2 and DO 5,8-6,8ppm.

Based on the above results, it can be concluded that 17β-estradiol level in nile tilapia reproduction response after induction the supernatant gonadal female of yellowfin tuna from multiple dose treatment the average was declined.

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