International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.07 pp 435-442, 2016

The Effectiveness of Gonad Extract of Yellowfin Tuna Fish *(Thunnus albacares)* on Increase of Reproduction Factor of Nilem Carp (*O. hasselti*)

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Abstract : Nilem carp *(Osteoschilus hasselti)*one of tropical fish in Indonesian fresh water which is have a high economical value. The production of nilem carp seed depends on the availability of parent stock of mature gonad in great level of quantity and quality. One alternative way to accelerate gonad maturity is through hormonal theurapy. This study employs four different treatments in terms of the dosage of induction of the extract and three repetitions. The main parameter being observed is estradiol 17β (pg/ml), egg diameter (mm), Gonado Somatic Index (%), Hepato Somatic Index (%), and Gonad Histology, while the supporting parameter consists of temperature, pH value, and Dissolved Oxygen (DO) value. The best result is obtained through Treatment B (dosage of 0.7 ml/kg of nilem carp weight) with increase of estradiol 17β up to 107.49 pg/ml, growth of diameter up to 0.95-1.25 mm, GSI value of 24.1%, and HSI value of 0.88%. The water quality during the study is normal with the temperature ranging from 28.00-31.00^{0}C, pH value between 7.6-8.2, and DO level between 5.40-6.20 mg/L.
Keywords: estradiol 17-β, egg diameter, gonado somatic index, hepato somatic index, gonad histology, Osteochilus hasselti, Thunnus albacores.

Introduction

Background of the Study

Freshwater fish farming belongs to one potential business sector to establish in Indonesia. With this farming activity, rural community empowerment and a chance to create new jobs are enabled through utilization of land and water as the place for such fish farming activity[1]. One type of freshwater fish potential to develop for its high economical value is nilem carp[2]. In recent time, the increasing demand of nilem carp seed affects its stock for the sake of fulfilling the needs of the society.

The production of nilem carp depends heavily on the great number of availability of parent stock with somatic gonad in terms of its quantity and quality. However, climate change may reduce the availability of the seed as its production relies on the current season. Thus, an alternative way is urgely needed in order to ensure constant availability of nilem carp parent stock with somatic gonad[3]. Once it is established, the attempt to provide high quality and quantity seeds can be achieved since quality is the fundamental requirement for the sustainability and success of aquacultural production[4,5].

The alternatif way proposed in this study is hormonal therapy. Until recently, gonad waste of tuna fish has not been well utilized, while inside the gonad is estradiol 17β hormon. Estradiol 17β is mostly used as an alternatif tool to stimulate the biosynthesis of vitelogenin which plays a role in the development of fish gonad[6].

The selection of gonad waste of yellowfin tuna is in accordance with Andamari et al.'s proposal[7]who suggest that tuna fish is placed as Indonesia's top export commodity. The number of yellowfin tuna fish caught in Indonesia is more abundant than the other species under the tuna fish family, so that its availability is guaranteed. Therefore, the mentioned problem is hoped to overcome with the induction of gonad extract from yellowfin tuna fish (*T.albacares*) in order to stimulate the biosynthesis of vitelogenin which acts as a biostimulation for gonad maturity.

Objectives of the Study

The main objectives of the study are (1) to investigate the effectiveness of the estradiol 17β hormon of yellowfin tuna fish on accelerating the development of nilem carp egg, (2) to determine the estradiol 17β hormon dosage of yellowfin tuna fish in order to accelerate maturity gonad of nilem carp, and (3) to study the histological oocyte of nilem carp after induced with estradiol 17β hormon from gonad of yellowfin tuna fish.

Significance of the Study

This study is expected to provide basic knowledge on the effectiveness of induction of gonad of yellowfin tuna fish in order to increase the accumulation of the egg yolk and to accelerate the development of egg diameter of nilem carp by utilizing gonad waste of yellowfin tuna fish. Moreover, the study is hoped to support the cultivation of nilem carp in order to provide a great amount of somatic gonad in a sustainable manner.

Research Location and Time

The study was conducted between February to April 2016 at the Fish Reproduction Laboratory, Universitas Brawijaya, Indonesia. The extraction of tuna fish gonad was carried out at the Chemistry Laboratorium of Gondol Main Station of Mariculture Research and Development, Bali Province, Indonesia. The blood centrifuge of nilem carp was observed at Molecular Biology Laboratory, Universitas Islam Malang, Indonesia. Finally, the estradiol 17β in nilem carp blood was observed at Central Laboratory of Saiful Anwar Hospital, Malang City, Indonesia.

Research Methodology

Method

This study is by nature an experimental, the type of research method which aims to manipulate and control) the natural occurrence into an artificial one with regard to the objective of the study[8].

Research Design

The design of the experiment was the Completely Randomized Design (CDR). It is mostly employed for experiments with similar media or place of research.

The treatments involved the induction of gonad extract of yellowfin tuna fish in different dosages. Each treatment was repeated 3 times, so that 12 experimental results were obtained. The dosages were 0.5 ml/kg BB of tested fish, 0.7 ml/kg BB of tested fish, and 0.9 ml/kg BB of tested and control fish.

Results and Discussion

Gonad Extract of Yellowfin Tuna Fish

From the research, it is revealed that the gonad extract of yellowfin tuna fish contains estradiol and progresteron hormones (see Table 1).

Table 1. Test Results of Estradiol 17β and Progresteron Hormones of Gonad of Yellowfin Tuna Fish

No.	Hormone	Amount
1	Estradiol 17β	574,30 pg/mL
2	Progesteron	2,53 ng/mL

According to Table 1, it is found out that the tuna gonad used in the study has the amount of estradiol 574.30 pg/mL and the amount of progesteron 2.53 ng/mL. This fact indicates that the tuna gonad is able to stimulate the liver in order to synthesize and release vitolegenin which accelerates the gonad maturity process as the value of estradiol 17β and progesteron is considered high.

Estradiol-17β

The observation on estradiol 17β of nilem carp was carried out by sample media in the form of blood serum. The value of estradiol 17β in the nilem carp blood before and after the treatment can be shown in Figure 1.

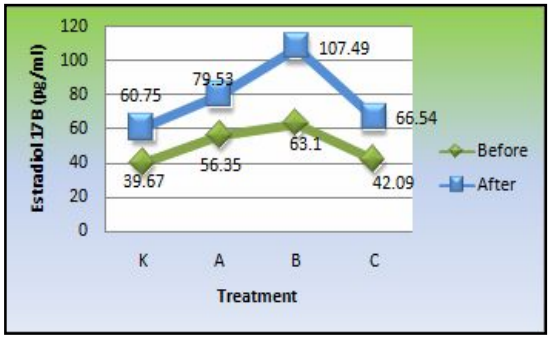

Figure1. Increase of nilem carp estradiol

Figure 1 indicates increase of estradiol 17β in the nilem carp blood in that the highest level of increase is seen on Treatment B (the induction of gonad extract of yellowfin tuna fish with 0.7 ml/kg dosage of the tested fish) with the average of 107.49 pg/ml. It is then followed by Treatment A (with 0.5 ml/kg dosage of the tested fish) with the average of 79.53 pg/ml. Treatment C (with 0.9 ml/kg of the tested fish) comes at the next place with the average of 66.54 pg/ml. The lowest increase is shown in Treatment K (without treatment/controlling treatment) with the average of 60.75 pg/ml.

The nilem carp estradiol gradually increases during the vitelogenesis phase in all treatments. This finding is supported a previous study[9] that shows that the increase of estradiol 17β will increase the vitelogenin concentrate in blood. In other words, change of estradiol 17β concentrate in the fish body is in line with the change of vitolegenin in blood.

Vitolegenesis is defined as a process of induction and synthesis of vitolegenin in liver as a response to estradiol 17β hormone[10]. After that, the vitolegenin produced by the liver is released into the bloodstream system and, then, absorbed by oocyte to be collected as future egg yolk. Furthermore, an increase of estradiol concentrate in blood will stimulate the liver to carry out vitolegenesis which later will accelerate the gonad maturity process[11].

Egg Diameter

The egg diameter, produced among different species or individuals within the same species, varies[12]. Measurement of egg diameter is one way to find out if a fish is ready to be bred. The distribution of the egg diameter affects the maturity level of fish gonad. The more equally distributed the egg diameter is, the more ready a fish is to be bred. This study employs cannulation in order to obtain egg samples which will be used to learn the distribution of their diameter. The distribution of nilem carp's egg diameter can be seen in Table 2.

Table 2. Growth of Nilem Carp (O.hassetti) Egg Diameter

Size D = (mm)	A		B		C		K	
	Before	After	Before	After	Before	After	Before	After
0.775							1	
0.825			5		1			
0.85	5 3		1		3		1	
0.875	5 5 5 1		5 5 5 4		5 5 5 2		5 4	
0.9	5 2		5 5 3		5 5		5 3	1
0.925	5 3		5 5 2		5 5		5 4	5 4
0.95	5 5 3	4	5 2	2	5 5 4	4	5 5 2	5
0.975	5 3	5 4	5 1	5	5 4	5 4	5 5 3	2
1	5 5 3	5 5 5 4	5 5 3	5 2	5 5 2	5 5 5	5 5 5 5 4	5 5 5 3
1.025	2	5 5 5	1	5 3	5 4	5 5 1	5 2	5 5 4
1.05	1	5 3		5 5 4	3	5 5 2	5	5 5 2
1.075	5 4	5 5 5 5 4		5 5 3	3	5 5 2	3	5 1
1.1	4	5 3	3	5 5		5 5 1		5 5
1.125		5 3	1	5	1	5		5 4
1.15	1		1	5 5 1		2		3
1.175		4		5		1		
1.2		1		1		2		4
1.225				4		4		

Explanation :

1 : 1 grain 2 : 4 grain
4 : 2 grain 5 : 5 grain
3 : 3 grain

A = induction of gonad extract of tuna (dosage of 0.5 ml/kg of tested fish weight)
B = induction of gonad extract of tuna (dosage of 0.7 ml/kg of tested fish weight)
C = induction of gonad extract of tuna (dosage of 0.9 ml/kg of tested fish weight)
K = controlling

During the vitelogenesis process, the yolk granules increase in terms of both their number and size, so that the occyte volume also increases[13]. The bigger the size of the egg diameter filled with vitelogenin during the vitelogenesis process, the bigger the gonad weight to every induced fish species.

This fact proves that the induction of gonad extract of yellowfin tuna fish influences the gonad maturity as observed from the development of the nilem carp's egg diameter. One of gonad maturity attributes is how develop the average diameter of the egg as well as the distribution pattern of the egg size[14]. Prior to ovulation, the egg diameter will increase due to the mass of homogenous egg yolk after increase of estrogen and vitelogenin concentrates[15]. The higher the level of gonad maturity, the bigger the egg diameter in the gonad.

Gonad Histology

The production of secondary oocyte is greate following the high level of gonad maturity, while the production of primary oocyte in ovarium is more abundant following the low level of gonad maturity of parent nilem carp. The result of observation on the gonad histology can be seen in Figure 2.

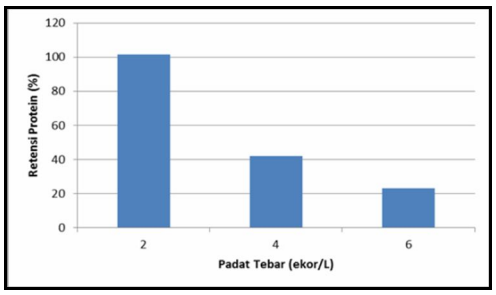

Figure 2. Gonad histology of female parent nilem carp: Primary Oocyte (PO) and Secondary Oocyte (SO)

Apart from the number of primary and secondary oocytes, gonad maturity is also determined by the size of the secondary oocyte. The bigger the size of secondary oocyte, the higher the level of egg maturity in ovarium.

Gonado Somatic Index (GSI)

The overall gonado somatic index (GSI) is between 17.71% to 24.1% (see Figure 3). The highest average of GSI is on the nilem carp under Treatment B (with the dosage of 0.7 ml/kg), that is, 24.1%, followed by Treatment A (induction dosage of 0.5 ml/kg), that is, 19.52%. Coming at the next place is Treatment C (dosage of 0.9 ml/kg) with GSI reaching 18.76%. The lowest GSI is shown by Treatment K (controlling treatment/no treatment), which is 17.71%.

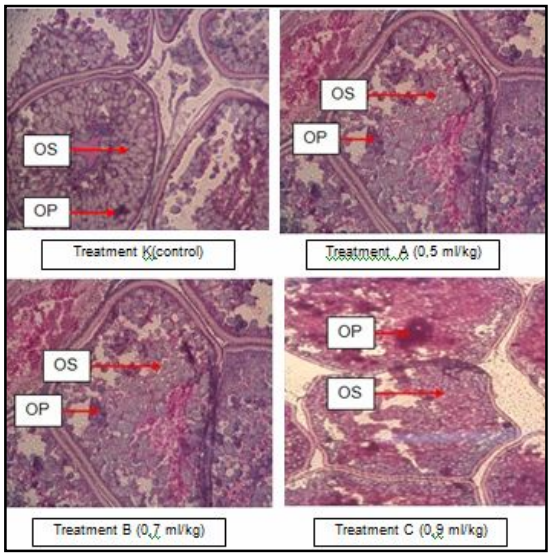

Figure3. Gonado Somatic Index (GSI) of nilem carp

The increase of GSI in the study is influenced by the development of oocyte due to massive collection of egg yolk where GSI is closely related to vitelogenesis taking place in the liver[16]. Egg yolk is the main component of the growing oocyte[13,14]. During the vitelogenesis, the egg yolk granule increases in terms of quantity and size, so that the oocyte volume increases. During that process, most result of metabolism is headed toward the development of gonad. This causes the gonad to experience a change in terms of increase of volume and weight.

Furthermore, following the gonad development, the GSI of the fish increases and will reach the maximum level when it is about to breed[17,18]. Most often the increase of gonad weight takes place along with the increase of body weight which leads to increase of GSI level.

Hepato Somatic Index (HSI)

The overall Hepato Somatic Index (HSI) is between 0.65% to 0.88% (Figure 4). The highest average of HSI is on the nilem carp given Treatment B (dosage of 0.7 ml/kg), that is, 0.88%, followed by Treatment A (dosage of 0.5 ml/kg), that is, 0.78%. Treatment C (dosage of 0.9 ml/kg) comes at the next order with the HSI of 0.71%, followed by the lowest HSI index, which is Treatment K (controlling treatment/no treatment) with HSI of 0.65%.

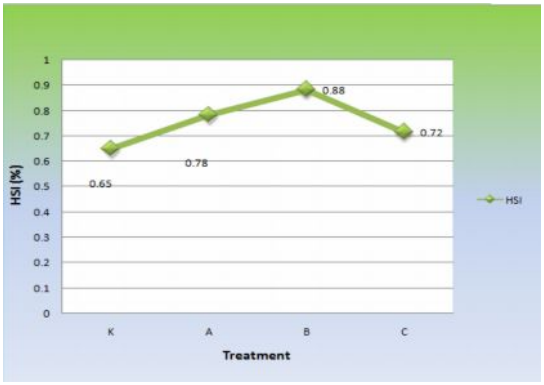

Figure 4. Hepato Somatic Indeks (HSI) of nilem carp

The best induction treatment of gonad extract of yellowfin tuna fish on the increase of nilem carp's HSI is Treatment B and followed by Treatment A, C, and K. The treatments result in increase of the HSI of between 0.65% to 0.88%. The liver (particularly of female fish) acts as the main source during the vitelogenesis process. Fish being in the process of vitelogenin production has by nature higher rate of liver synthesis[22]. With the increase of liver activity, the percentage of HSI will also increase.

This statement is supported by Ishibashi et al.[19]who conclude that the HSI of female parent fish increases prior to vitelogenesis. The high value of HSI is closely related to the accumulation of estradiol 17β steroid in the liver which functions to synthesize vitelogenin during the vitelogenesis process[14]. In a similar tone, Indriastuti[9] asserts that vitelogenesis causes the HSI and GSI values increase.

Parameter of Water Quality

The quality of water is a vital parameter to measure as it serves as the habitat of nilem carp. The quality can be measured in terms of its temperature, pH level, and dissolved oxygen (DO) level.

The temperature to keep nilem carp belongs to "good tolerance" category, ranging from 28.00 to 28.44^{0}C in the morning and from 30.12 to 31.00^{0}C in the afternoon. The temperature during the study did not indicate high fluctuation. This is in line with Budiharjo (2002) who states that fresh water fish such as gourami, parrot fish, goldfish, and baby fish generally live in water temperature of between 26-30^{0}C. Mulyasari[20]also believes that the optimum temperature for nilem carp to live is between 18-28^{0}C.

The pH value of water for keeping nilem carp during the study is between 7.6-7.9 in the morning and 7.9-8.2 in the afternoon. Based on the measurement, it can be said that the pH value is normal. This is in line with Budiharjo[21]stating that the normal pH value for several species of fish is between 7 to 8, while Mulyasari[20]asserts that the optimum pH value for nilem carp is between 6 to 8.

The level of DO during the study is between 5.60-6.20 mg/L in the morning and 5.40-6.10 mg/L in the afternoon. From this finding, it can be said that the level of DO of the water is in good tolerance as the ideal DO level for fish is > 5 mg/L. Furthermore, Mulyasari[20]states that the optimum DO level for nilem carp is between 5-8 mg/L.

Conclusion and Suggestions

Conclusion

From the study of the effectiveness of yellowfin tuna fish gonad towards increase of reproduction factor of nilem carp, some points can be concluded as follows.

- The induction of gonad extract of yellowfin tuna fish provides significant influence on the growth of egg diameter, particularly with the dosage of 0.7 ml/kg of the nilem carp weight, which is around 0.95-1.25 mm.

- The gonad extract dosage of 0.7 ml/kg of the nilem carp weight is the exact dosage to stimulate gonad growth as seen from the growth of its egg diameter which is around 0.95-1.25 mm, with GSI value of 24.1% and HSI value of 0.88%.
- The histology of nilem carp gonad with the dosage of 0.7 ml/kg indicates that the secondary oocyte production is higher than the primary one.

Suggestions

The findings and discussion of the study may provide some suggestions as follows:

- A periodical induction of gonad extract of yellowfin tuna fish is need to accelerate ovulation and spawning processes.
- Further study shoud be conducted on various saltwater fish.

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