VITELLOGENESIS ACTIVITY OF *CHANNA MARULIOIDES, BLEEKER*, 1851, IMPACT OF FOLICLE STIMULATING HORMONE AS REPRODUCTIVE DOMESTICATION

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ABSTRACT

This study aims to determine the vitelogenesis activity by FSH hormone induction in the early stage of gonadal maturation. The treatament is a differences FSH dose with indicators of vitelogenesis activity were estradiol-17β levels in the blood, Hepatosomatic index (HSI) and Gonadosomatic Index (GSI). The study used a treatment A : Dose FSH at 1.0 ml/kg; B: Dose FSH at 1.25 ml/kg; C : Dose FSH at 1.5 ml/kg; control: nothing induction. The results showed that there was an effect of FSH dose on the estradiol 17β levels, HSI and GSI, there ongoing vitelogenesis activity. The trend of the GSI and HSI has a close correlation to levels of estradiol 17β, with the best dose of the hormone is 1.25 ml/kg resulting levels of esradiol 17β amounting to 63.35 pg / ml and the GSI and HSI are 3.45% and 1.48%. Furthermore, the best treatment successively resulted in increased levels of estradiol 17β, HSI and GSI in treatment C of 41.77 pg/ml, 1.48%, 3.24%; A : 28.01 pg / ml, 1.14 %, 3.42%;. FSH is suitable for reproductive domestication in *Channa marulioides*.

Keywords: Gonad Maturation, Estradiol 17β, Hepatosomatic Index, Gonadosomatik Index, Aquaculture.

كتدجين للجريب المنبه الهرمون تحريض تأثير ، 1851 ، بليكر ، *Channa marulioides* لـ الحيوية الخلايا تكوين نشاط تناسلي

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المستخلص

تهدف هذه الدراسة إلى تحديد نشاط التكون الحيوي عن طريق تحريض هرمون فش في المرحلة المبكرة من نضوج الغدد التناسلية العلاج هو جرعة فش الاختلافات مع مؤشرات النشاط فيتلوجينيسيس كانت مستويات استراديول 17β – في الدم مؤشر هيباتوسوماتيك) له (ومؤشر غونادوسوماتيك) غسي .(استخدمت الدراسة علاجا أ :جرعة فش عند 1.0 مل / كجم ؛ ب : جرعة فش عند 1.25 مل/كجم؛ ج : جرعة فش عند 1.5 مل/كجم ؛ التحكم :لا شيء تحريض .وأظهرت النتائج أن هناك تأثير جرعة فش على مستويات استراديول 17β، هسي و جيس، هناك نشاط فيتلوجينيسيس المستمر اتجاه غسي وهسي لديه علاقة وثيقة لمستويات استراديول 17β، مع أفضل جرعة من هرمون هو 1.25 مل/كغ الناتجة مستويات إسراديول 17ounting تصل إلى 63.35 بيكوغرام / مل و غسي و هسي هي 3.45 ٪ و1.48 ٪ .علاوة على ذلك، أدى أفضل علاج على التوالي إلى زيادة مستويات استراديول 17β ، هسي و غسي في العلاج ج من 41.77 بيكوغرام/مل ، 1.48 ٪، 3.24٪ ؛ أ 28.01 : بيكوغرام / مل ، 1.14 ٪ ،3.42 ٪؛ .فش هو مناسبة لتدجين الإنجابية في تشانا مارولييدس

كلمات مفتاحية: نضوج الغدد التناسلية، استراديول 17β، مؤشر الكبد، مؤشر الغدد التناسلية، تربية الأحياء المائية
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INTRODUCTION

The development of aquaculture is one of the efforts in maintaining biodiversity. Indonesia is one of the tropical countries with most of its territory is water. This has an impact on high aquatic biodiversity. Indonesia has the highest diversity of species compared to other countries in the Indian Ocean that is 3,215 species (24). *FishBas*e noted that the number of species in Indonesia ranges from 1193 species, with freshwater species diversity in Indonesia ranked third in the world (7). This species richness needs to be harmonized with comprehensive conservation efforts to support technical activities of aquaculture development with domestication of endemic fish species that have economic and aesthetic value. The fisheries sector is one of the vital sectors due to its economic and health importance (2). Fish farming has become one of the approved methods for developing and increasing the quantity and quality of fish production and has becomea major role in investment expansion in the fish sector (4). Conservation efforts with reproductive domestication within the scope of fish farming in *channa marulioides* has never been done in a structured manner. It is necessary to consider conservation efforts related to the development of the *chann*a clan fish trade (12). The future sustainability of fish farming depends on the ability to partially or completely control the life cycle on the scope of aquaculture for some new fish species (23). Different Vitelogenesis types seem to respond differs from estrogen in terms of dose response kinetics and maximum production rate, requires the need to consider the degree of multiplicity of vitelogenesis in the target species before undertaking development (20). One of the efforts to control part of the life cycle or the so-called rudimentary domestication that is relevant is the identification of hatchery techniques, especially the gonad maturation process in fish, one of which is *Channa marulioides*. The domestication of fish reproduction is carried out gradually starting from the identification of early gonadal maturation patterns through hormonal intervention. Hormones that affect the process of oogenesis is the FSH (Follicle Stimulating Hormone), which has a role in the maturation of follicles in the process of vitellogenesis (9). Referring to *Eschmeyer's Catalog* of fish, that the distribution area of *Channa marulioides* is in the freshwater waters of western Indonesia and Malaysia (8). Market demand for *Channa marulioides* continues to increase along with the needs of consumption as well as ornamental fish, but the majority of *Channa marulioides* demand is used for ornamental fish. The availability of this fish is currently declining in its natural habitat in Indonesian waters in the Kapuas River. Local researchers reviewed the availability of *Channa marulioides* in the Central Kapuas River in a "small" category, namely in one season catches less than five fish per day per person, while in the lower waters of the Kapuas River *Channa marulioides* catches were not found (1). The decrease in the availability this fish is allegedly a result of the absence of initial efforts in fish hatcheries that result in the availability of *Channa marulioides* currently obtained from natural catches. Early domestication efforts in the form of identification of reproduction through early stage gonad maturation needs to be done in order to support the availability of *Channa marulioides* seeds in a sustainable manner so as not to depend on natural catches. Early stage gonad maturation is one of the indicators in the response of fish to the physiological conditions of reproduction to become the development of eggs and subsequently hatch into larvae. Estradiol 17β levels in blood plasma affect the expression of the vitelogenin gene in the liver and increase granulosa in ovarian follicular cells in the early stages of gonadal maturation process (25). Increasing the concentration of estradiol in the blood of fish will spur the liver to carry out the process of vitelogenesis and will further accelerate the maturation process of the gonads. Estrogens stimulate the production of vitellogenin in the liver of teleost fish through estrogen receptors that support oocyte vitellogenesis (26). So The gonadosomatic index (GSI) is a bioindicator that provides structural information to determine the physiological function of health and the maturation status of the gonads (5). In females, the liver has a function in synthesizing vitelogenin in the process of maturation of the gonads in the reproductive

season. (17). So it will affect the value of hepatosomatic index (HSI) and Gonadosomatic Index (GSI). The strategy in the process of maturation of the gonads is by induction of the FSH (*Folicle Stimulating Hormone*). Induction of hormones containing FSH is effective in the maturation of gonads of *Channa* genus fish is that *Channa striata* (22). Assessing the potential of FSH hormone in gonad maturation, it is hoped that it can be applied to the development of *Channa marulioides* culture through reproductive domestication. This study aims to determine the vitelogenesis activity of *Channa marulioides* in the form of estradiol response 17 β in the blood, HSI and GSI values of female *Channa marulioides* induced hormone FSH as an early stage domestication efforts in the process of maturation of the gonad.

MATERIALS AND METHODS

The process of observation of the vitelogenenesis activity of emperor snakehead females was carried out within the scope of aquaculture. Before the observation of indicators of brood parent through the maintenance stages for 90 days as follows :

1- The process of adaptation emperor snakehead in aquaculture containers in the form of open concrete ponds for 72 hours after the transportation process.

2- Preparation of an aquarium container with a size 40x25x30 cm with kapuas River Water media that has been treated before stocking the parent fish with a risirculation aquaculture system for 48 hours with mechanical and biological filtration to remove solid wastes and carbon dioxide, oxidise ammonia and nitrites (13)

3- Selection of parent fish is done by choosing a female based on weight classification 550-600 gr, body length 30-40 cm with a percentage of uniformity of size >90%.

4- After the parent is selected according to the size of the stocking in the aquarium with the number of stocking 3 individual / Aquarium in accordance with the number of treatment.

5- *The female parent of Channa marulioides* before treatment in measuring the content of estradiol 17β by taking blood using a syringe 1 ml, then analyze the content of estradiol.

6- For culture are given feed with the same dose and amount in each treatment with feeding time morning, afternoon and evening. The feed given is dry maggot with a combination of dry shrimp with a dose of 3% of the weight.

Observation of estradiol levels 17β done with blood sampling before and after treatment and inserted into the micro tube. Furthermore, the blood plasma was tested for estradiol hormone levels using the Radio Immuno Assay method (16). Observation of Gonado somatic Index (GSI) and Hepatosomatic Index (HSI) were performed as supporting data at the end of culture by weighing gonadal weight and liver weight after treatment. The GSI value is determined by calculating the percentage ratio between gonadal weight and body weight. Similarly, hepatosomatic index (HSI) was calculated as: HSI (%) = Weight of liver (g) / Weight of fish (g) × 100 (15). The hormone given is the hormone PMSG (Pregnant mare serum gonadotropin) which contains a high concentration of FSH for early stage gonadal development (22). The hormone is injected in the intramuscular part with an inclination of 45^0. The treatment dose is divided into two injections with a latent time of 72 hours from the first induction. The study used a complete randomized design with 4 treatments and 3 repetitions, the treatment dose of hormones used was :

- Treatment A (P1): Induction of FSH hormone at a dose of 1.0 ml/Kg parent weight
- Treatment B (P2): Induction of FSH hormone at a dose of 1.25 ml/Kg parent weight
- Treatment C (P3): Induction of FSH hormone at a dose of 1.5 ml/Kg parent weight
- Control treatment (C): Without induction of FSH hormone

Statistical analysis data were processed using IBM SPSS Statistic 23 software to observe the effect of treatment on the response with a confidence level of 95%.

RESULTS AND DISCUSSION

Estradiol 17β: Observation of estradiol levels in the blood of *Channa marulioides* is done to determine the impact that occurs due to the induction of FSH hormone. The average result of estradiol levels in the blood is presented in Teble 1.

Table 1. Average Estradiol 17β Content

Treatment	Weight	Estradiol Before Induction (Pg/ml)	Estradiol After Induction (Pg/ml)	Δ Estradiol Level (pg/ml)
P1	580,16	26,09	54,1	28,01
P2	565,8	23,32	86,67	63,35
P3	589,66	27,06	68,83	41,77
C	591,26	27,09	28,18	1,09

This study result is a estradiol levels in the blood during the observation showed that the best dose of FSH hormone in P2 treatment with a dose of 1.25 ml/kg with an increased amount of estradiol levels of 63.35 pg/ml, then the treatment of P3, P1 and the lowest in the amount of increased levels of estradiol is a control treatment sebeesar 1.09 pg / ml. To find out the difference in each treatment, followed by statistical tests of real differences in Table 2.

Table 2. Test significantly different levels of Estradiol in the blood

Estradiol 17β					
Tukey HSD[a]					
Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
C	3	1.0933			
P1	3		28.0100		
P3	3			41.700	
P2	3				63.567
Sig.		1.000	1.000	1.000	1.000

Statistical results showed that the results of estradiol embeds in each treatment is significantly different. This suggests that there is a response of estradiol 17β to the induction of each dose of FSH. This study showed that there were significant differences in hormone administration treatment with treatment without horomon induction in female parent. Reviewing the data showed that there was an increase in estradiol levels after induced FSH 17β between P1 treatment with P2 treatment, then there was a decrease between P2 treatment with P3 treatment. Data from the observation of estradiol content 17β showed that the treatment of P1 and P2 is directly proportional to the addition of FSH dose given. The higher the dose of the FSH hormone given, the content of estradiol 17β in the blood will also increase. Straight comparison that occurs in treatment P1 and P2 does not apply to treatment P3. Although the number of doses administered continued to be increased from treatment B, the estradiol content of 17β did not increase. An increase in estradiol levels in each treatment is shown in Figure 1.

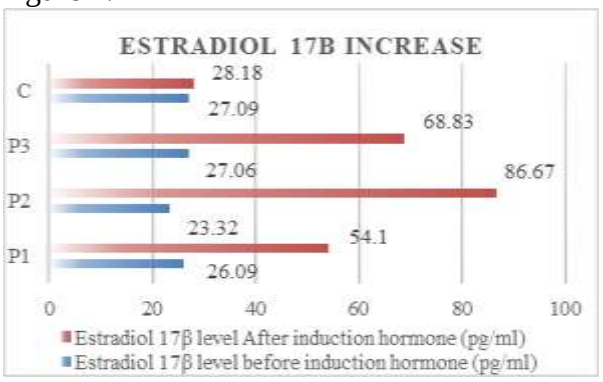

Figure 1. Graph of The Increase in Estradiol 17β Content.

The increase in estradiol content of 17β that occurred in P1 and P2 treatment did not occur in P3 treatment. Addition to the increased dose of hormones from P2 treatment, the content of estradiol 17β contained in fish in P3 treatment did not increase, but decreased. It is alleged that when in the body of is a high content of lipid FSH due to the induction of FSH hormones, lipid composition in animals influences sexual maturation (14). The high lipid FSH in boy fish can disrupt the balance of hormones in the body, it will happen feedback where the content of gonadotropin hormones that are too high will lead to the cessation of hypothalamic performance. In fish telestoi common negative feedback hormone estradiol (19), where the content of gonadotropin hormones containing FSH is high enough to suppress the work of endogenous LH. The reduced performance of endogenous LH suppresses the action of gonadotropin hormones to stop the synthesis of estradiol 17β. In addition to the negative feedback on the process of maturation of the early stages of gonads or GtH 1, it is possible induction of excess hormones in the body can disrupt the balance of the amount of hormones contained in the body of fish so that hypophysis reduces secretion at the stage of GtH 1. This can affect the development of the egg cell as a result of impaired vitellogenin synthesis. The decrease in hepatic synthesis of vitellogenin is due to a combination of a deficiency in circulating estradiol-17β levels

and a decrease in the sensitivity of the vitellogenin pathway to 17β-estradiol (6). Increased levels of estradiol 17β after induction of FSH hormone allegedly due to liver physiological methobilism in the process of vitellogenesis. The process of maturation of the gonads occurs due to the activity of vitelogenesis that will result in increasing the level of gonad maturity level over time. The process of vitellogenesis itself is the induction and synthesis of vitellogenin in the liver in response to the hormone estradiol 17β. Vitellogenin will then be released into the circulatory system and selectively absorbed by the developing oocyte to be hoarded into egg yolk. Increasing estradiol levels in the blood is expected to be one way in the process of maturation of the gonads to obtain reproductive patterns with artificial spawning techniques through hormonal engineering. Based on the results of estradiol content of 17β in the blood after being induced by the FSH hormone, the best results obtained of estradiol 17β content is in the treatment of P2. Recommendations that can be given as domestication efforts in gonad maturation is the induction of FSH hormone at a dose of 1.25 ml / kg in female *Channa marulioides*.

Gonadosomatic Index (GSI)

The percentage value of the gonadosomatic index (GSI) is one of the indicators in determining gonad maturity in fish. The increasing weight of the gonads is one of the causes of the increasing oosyt filled with vitelogen which means the maturity of the gonads is developing. Treatment B with a dose of 1.25 ml / kg had the greatest impact on the increase in gonadal weight of 3.45% of the parent body weight. Treatment C and A addressed the value of the next best GSI is 3.42% and 3.24%. The results of the differences in each treatment of GSI values are presented in Table 3.

Table 3. Test significantly different GSI each treatment

Gonadosomatic Index			
Tukey HSD[a]			
Treatment	N	Subset for alpha = 0.05	
		1	2
C	3	.9900	
P3	3		3.2433
P1	3		3.4200
P2	3		3.4533
Sig.		1.000	.621

Significantly different test results showed that there was no significant difference between treatment A, B and C. However, the induction treatment of FSH hormone has a real difference to the treatment without the administration of FSH hormone. The induction of FSH on gonad maturation process showed no significant difference, so it can be said that the intervention of different doses of FSH hormone on gonad maturation process was not significantly different. The increase in GSI levels during the study had the same trend towards an increase in estadiol 17β levels. It was observed that treatment B has a high GSI value of 3.45% which corresponds to an increase in estradiol levels in treatment B of 63.35. GSI value in *Channa* genus fish (*Channa striata*) at the stage of vitelogenesis process ranges from 3-6% (10). The correlation between the two parameters is presented in the following graph :

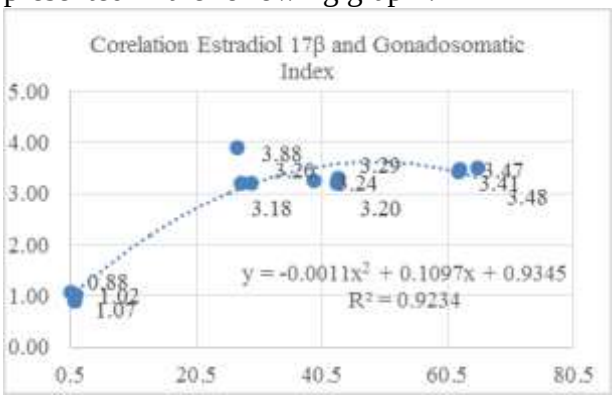

Figure 2. Correlation relationship of increased Estradiol levels with Gonadosomatic Index.

The addition of estradiol levels of 17β has a strong correlation to the addition of GSI values, it is seen in the graph with a high correlation value of R^2 which is equal to 0.92. The graph shows that the increasing levels of estadiol 17β in the blood plasma, the gonads will experience development with increasing weight indicated by a high percentage value of GSI. The GSI value of treatment with FSH induction is significantly different from treatment without FSH hormone inuksi, but the difference in hormone dose has no effect on the GSI value. The increase in gonadal weight in each treatment did not experience significant differences during the study, it shows the process of vitellogenisis is still ongoing along with the increase in gonadal weight gradually. The process of votellogenesis that occurs in emperor

snakehead females during the study aimed at gonadal maturation process focusing on the physiological metabolism of the liver in producing vitelogenin which has an impact on increasing estradiol 17β levels in blood plasma. In the process of maturation of the gonads, the increase in GSI values is not in line with the increase in HSI result, it is because the increase in liver performance will stop when the ovary in ganad has been filled completely vetelogen. In general, vitellogenin in the blood is absorbed by receptors on the plasma membrane of the oocyte and enters into the egg cell (11, 21), this results in the value of HSI decreases as gonad maturation increases. Estradiol levels in the process of vitellogenesia has a pattern associated with GSI values along with different stages of ovarian development (18), so that when the vitelogenesis process takes place, the GSI value will develop along with the increase in gonadal weight so that the GSI value in the vitelogenesis process can still increase.

Hepatosomatic index (HSI)

Observation of hepatosomatic index (HSI) value is an analysis of the impact of liver performance in the process of votelllogenesis as a result of increased levels of estradiol 17β in the blood. The mean results of the hepatomatic index showed that the increase in liver weight along with increased levels of estradiol 17β. Treatment with FSH dose of 1.25 ml / kg showed the highest percentage value of HSI compared to other treatments that is equal to 1.48%, then treatment C, treatment A and liver development showed no significant increase in control without induction of FSH hormone. Induction of FSH hormone has a different impact on each treatment, where the results are shown in Table 4.

Table 4. Test Significantly Different Hepatosomatic Index Of Each Treatment

Hepatosomatic Index					
Tukey HSD[a]					
Treatment	**N**	**Subset for alpha = 0.05**			
		1	**2**	**3**	**4**
C	**3**	**.3867**			
P1	**3**		**1.1433**		
P3	**3**			**1.2767**	
P2	**3**				**1.4767**
Sig.		**1.000**	**1.000**	**1.000**	**1.000**

The results of significantly different trials in each treatment showed that the difference in the dose of FSH hormone induced in emperor snakehead females showed significant differences. Analyzing from these results, the difference in the dose of FSH Horn given gives a hardening of the HSI value with the best treatment, namely the dose of FSH hormone 1.25 ml/kg. The increase in liver weight is allegedly due to the activity of liver metabolism in response to estradiol 17β in the blood, it is shown that there is a similar trend with the measurement of estradiol 17β levels in the blood. The relationship between these two parameters is presented in the following correlation graph in Figure 3:

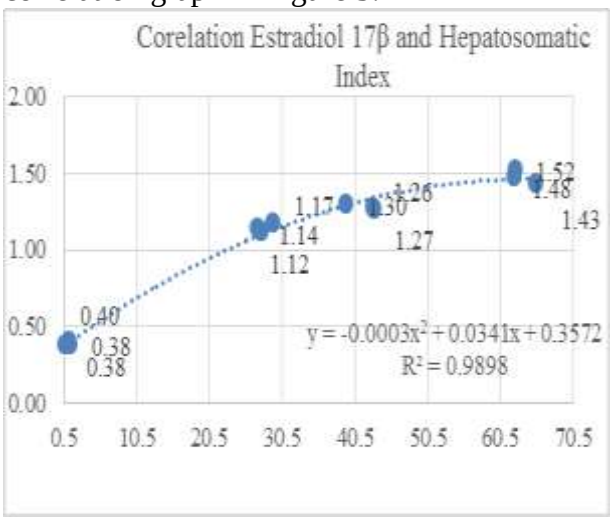

Figure 3. Correlation between Increased Estradiol Levels and Hepatosomatic Index

The graph of the relationship between estradiol 17β and the HSI value above shows that the correlation between these two parameters is very close, namely the value of R^2: 0.98. This study shows that along with the increasing levels of estradiol 17β in the blood, it will be followed by increasing the physiological metabolism of the liver in producing vitellogenin in the vitellogenesis process. An increase in liver weight as indicated by an increase in the HSI value indicates that gonadal maturation metabolism is taking place along with the increase in blood levels of estradiol 17β during the process of vitellogenesis. The vitellogenesis process at the maximum vitelgone production stage in the *Channa* genus that is *Channa striata*, showed a maximum HSI value of around 1.84% (12). The hepatosomatic index is related to the stage of ovarian development (17). Various stages of

reproduction as ovarium development, show differences in the concentration of FSH content in the body (3). The observation results show that the pattern of increasing HSI values is in line with the increase in GSI, where the influence of estradiol is very close to liver weight gain. GSI and HSI will develop along with increasing maturity of the parent which refers to body weight and length. During the reproductive phase vitellogenin is synthesized by estrogen stimulation and excreted into the bloodstream and blood plasma is absorbed by vitellogen receptors on the oocyte surface and cleaved into smaller yolk proteins (26). The observation results show that the vitellogensis process is ongoing as a result of the intervention of the FSH hormone during the early stages of gonadal maturation.

CONCLUSIONS

Reviewing the results of research on vitellogenesis activity due to the induction of the hormone FSH in emperor snakehead, several conclusions can be drawn as follows:

1- Giving FSH hormone has an impact on increasing levels of estradiol 17β in blood plasma of female parent emperor snakehead compared to without FSH hormone induction, with the best dose of 1.25 ml/kg.

2- Increased levels of 17β estradiol have an impact on increasing HSI and GSI values in the early stages of gonadal maturation process with the best dose of FSH hormone being 1.25 ml/kg resulting in an HSI value of 1.48% and an GSI value of 3.45%.

3- The FSH hormone at a dose of 1.25 ml/kg can accelerate the early gonadal maturation process as an effort to domesticate fish at the reproductive stage.

As an effort to domesticate *Channa marulioides*, it is suggested to be able to continue research related to hormone intervention in the final stage of gonad maturation until ovulation occurs with more comprehensive reproductive status monitoring parameters so that the availability of maru fish seeds can be supplied from hatcheries in a sustainable manner.

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