The Effect of Laserpuncture on Accelerate Gonadal Maturity of Female Striped Catfish (Pangasianodon hypophthalmus)

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Abstract. This study aimed to determine the effect of different laserpuncture doses on the gonadal maturity of female striped catfish. The fish 800 to 1000 g with 1 gonad maturity were used. This study was used a red diode soft-laser with doses of 0.2, 0.4, 0.6, and 0.8 Joule. The positive control was injected choric gonadotropin hormone (CGH) and ovaprim™ intramuscular and the negative control was not used any treatment. Red diode soft-laser was shot at the reproductive point every week for three weeks, then the CGH and ovaprim™ were injected in the final week. Gonad maturity level and gonadosomatic index (GSI) were measured in the last week. The result showed that laserpuncture had significant effect (P<0.05) on the gonadal maturity and GSI of female striped catfish. A dose of 0.4 Joule laserpuncture have the best gonadal maturity level in stage IV. Meanwhile, highest GSI were treated at dose 0.4 Joule laserpuncture, which was 3.4%. Induction of laserpuncture at the reproductive point once a week for three weeks accelerate gonadal maturity of female striped catfish.

1. Introduction

Spawning of Striped catfish is very dependent on the season and generally only occurs once a year in December - April during the rainy season [2]. This is because reproduction is controlled by the hypothalamus-pituitary-gonads and is influenced by several things such as the hormone system, environmental signals and reproductive organs (Zairin, 2003). The catfish gonads were successful in shooting maturity using hormones such as human chorionic gonadotropin hormone (HCG) and ovaprim [3] and helium neon (He-Ne) user technology [15]. The use of He-Ne lasers at the reproductive point of female catfish succeeded in increasing the level of gonad maturity, but low fecundity and small egg diameter [14]. Fecundity and egg diameter on laserpuncture shooting can be increased by increasing the response of production follicle stimulating hormone (FSH) through metabotropic and ionotropic receptors by firing at reproductive acupuncture points (governor vessel) which is 2/3 of the distance between the anus and the pectoral fins [10].

Low-level laser therapy requires light with a suitable wavelength and the right dose in order to stimulate reproductive nerve cells that are sensitive to light and wave interactions (photosensitizers) so that the gonadal maturation process occurs faster. The gonadal maturation process is an important phase in the reproductive cycle [15]. In addition, sperm and egg cells with good quality and the maximum level of gonad maturity are prerequisites for the success of aquaculture production[24] [17]. Laserpuncture shooting is proven to accelerate the process of growth, development and maturation, spawning of fish gonads. In addition, several studies have shown that laser puncture can improve the endocrine, vascular
and various other body systems[9]. Based on the various benefits of laser puncture shooting on fish reproduction, this study aims to see the effect of different laser puncture doses on the gonadal maturity of female striped catfish to provide mass and continuous production of Striped catfish broodstock.

2. Material and methods
Female striped catfish were obtained from the Aquaculture Installation (IPB) Mojokerto. The weight of broodstocks are 800-1000 grams/head and not having matured gonads (TKG I) a total of 24 fish consisting of 4 negative controls, 4 positive controls, and 16 as treatment. Detection of reproductive points using an electroacupuncture device was carried out on the ventral 2/3 of the Striped catfish. After finding the right point, it was shot using a red light laser with a wavelength of 650 nm. The laser beam was fired once at the reproductive point of female Striped catfish at a dose of 0.2 J/cm², 0.4 J/cm², 0.6 J/cm², 0.8 J/cm² once a week in three weeks. In the last weeks ovaprim and HCG hormones were injected into the positive control treatment. Observations of gonadal maturity level were carried out morphologically [25]. calculated Gonado Somatic Index and Hepato Somatic Index values [7].

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GSI = \frac{B_g}{B_t} \times 100\%
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Description:
GSI: Maturity Index
Bg: Weight (grams)
Bt: Total fish weight (grams)

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HSI = \frac{B_h}{B_t} \times 100\%
\]

Description:
HSI: Liver
Bh: Index: Liver weight (grams)
Bt: Total fish weight (g)

Data were analyzed using Analysis of Variance (ANOVA) to determine the effect of a given treatment. If it shows significant results, then the calculation is continued with Duncan's Multiple Range Test to determine the difference between treatments using a confidence level of 0.05.

3. Results and Discussion
3.1 Gonad Maturity Level
The gonad maturity level of female Striped catfish that was fired by red diode laser at different doses for three weeks was as follows (Table 1). The gonads of female Striped catfish without laser implantation treatment (negative control) did not develop, 100% TKG I. Positive control treatment with Ovaprim and HCG hormone injection resulted in gonad development reaching 25%. On the P1 75% gonad development on the III level, P2 gonad maturity level IV 100% , P3 gonad maturity level II 75% and P4 gonad maturity level I 100%

| Treatment                | Gonad Maturity Level (%) |
|--------------------------|--------------------------|
|                          | I | II | III | IV |
| K- (without treatment)   | 100 | 0 | 0 | 0 |
| K+ (ovaprim and HCG)    | 75 | 25 | 0 | 0 |
| P1 (0.2 Joule dose)      | 0 | 25 | 75 | 0 |
3.2. Gonado Somatic Index

Gonado Somatic Index (GSI) of female Striped catfish that were shot by red diode laser at different doses for three weeks were as follows (3)

| Treatment          | Gonado Somatic Index (%) ±SD |
|--------------------|------------------------------|
| K- (without treatment) | 0.41676 ± 0.0629              |
| K+ (ovaprim and HCG) | 0.43836 ± 0.08826             |
| P1 (dose 0.2 Joule)  | 2.20337 ± 0.37673             |
| P2 (0.4 Joule dose)  | 3.42355 ± 0.48519             |
| P3 (0.6 Joule dose)  | 1.47392 ± 0.45783             |
| P4 (0.8 Joule dose)  | 0.93294 ± 0.42156             |

Description: Superscript letter (a,b different, c,d) in the same column shows that there is a significant difference (p<0.05).

The results of the Analysis of Variance (ANOVA) test showed that firing a red diode laser with different doses at the reproductive point (ovrenor vessel) had a significantly different effect on the Gonado Somatic Index (GSI) value of female Striped catfish (p<0.05). Duncan test results show the highest mean Gonado Somatic Index (GSI) value at a dose of 0.4 Joule (P2) of 3.42355 ± 0.48519 while the lowest Gonado Somatic Index value is found in the negative control (K-) treatment of 0.41676 ± 0.0629 which is not significantly different from treatment, positive control (K+) and red diode laser firing at a dose of 0.8 Joules (P3).

3.3 Hepato Somatic Index

Hepato Somatic Index (HSI) of female Striped catfish that was shot with a red diode laser at different doses for three weeks (Table 3).

| Treatment          | Hepato Somatic Index (%) ±SD |
|--------------------|-------------------------------|
| K- (without treatment) | 0.52733 ± 0.06198             |
| K+ (ovaprim and HCG) | 0.65673 ± 0.08112             |
| P1 (dose 0.2 Joule)  | 1.06538 ± 0.10505             |

Note
Gonad maturity level 1= Immature, Gonad maturity level 2= Immature, Gonad maturity level 3= Late Mature, Gonad maturity level 4= Mature
The increase in the hormone GtH stimulates the reproductive meridian points to increase A development of gonadal stage IV TKG. This shows that laser induction with a dose of 0.4 Joules can stimulate the release of gonadotropin hormones (GtH) and GtH production of a balance of energy in the organs. This is because the increase in photons causes an increase in ATP and stimulates the transfer of Reactive Oxygen Species (ROS).

Increased ATP causes cellular homeostatic stimulation to produce cyclic adenosine monophosphate (cAMP) which stimulates the opening of membrane vesicles of channels Ca²⁺ that regulate almost every process in the human body (muscle contraction, blood clotting, signal transfer in nerves, gene expression). An increase in Ca²⁺ will stimulate the formation of protein kinase C (PKC). Resulting in the release of neurotransmitters through exocytosis into the gaps in the synapses and continued to the brain thereby stimulating GABAergic neurons to synthesize Gama Amino Butyric Acid (GABA) which will stimulate the release of gonadotropin hormones (GtH-I and GtH-II) (Mueller et al., 2008; Lado et al., 2014; Watanabe et al., 2014). The increase in the hormone GtH-I stimulates theca cells to produce the hormone 17β-estradiol which stimulates the synthesis of vitellogenin in the liver and will stimulate granulosa cells which play a role in the final oocyte maturation and stimulates ovulation and spawning.

Red diode laser shooting at the reproductive point of Striped catfish dose of 0.4 Joule resulted in the development of gonadal stage IV TKG. This shows that laser induction with a dose of 0.4 Joules can stimulate the reproductive meridian points to increase ATP and optimize the number of ROS which act as second messengers so that gonadal maturation occurs faster. While the red diode laser shooting at a dose of 0.2 Joule, a dose of 0.6 Joule and a dose of 0.8 resulted in maturity levels of gonads III, II, and I. Red diode laser shooting at a dose of 0.2 Joule was less effective because of energy (ATP) and ROS. The yield is too low so that the target organ photoreceptors cannot absorb optimally and the chemical change process runs more slowly and cannot stimulate reproductive active cells. Meanwhile, a dose of 0.8 Joule is the initial dose of sedation which results in a decrease in photoreceptor cells resulting in a balance of energy in the organs. This is because the increase in photons causes an increase in the production of reactive oxygen species (ROS) which can damage cells and interfere with the signaling process so that the gonads do not develop (Farivar, 2014).

Gonad development of female Striped catfish affects the Gonado Somatic Index (GSI) and Hepato Somatic Index (HSI) values. The highest GSI and HSI values were at a dose of 0.4 Joule at 3.42% and 1.26%, respectively. The average GSI and HSI values of Striped catfish induced by laserpuncture were
3.18% and 1.29%. [15]. The value of GSI and HSI is influenced by the hormone GtH-I which plays a role in increasing the production of estrogen which is transported to the liver by diffusion and stimulates the process of vitellogenesis [19]. The process of vitellogenesis increases liver weight because it stores fat and synthesizes vitellogenin [21], so that the amount of yolk increases the volume and weight of the oocytes [23].

4. Conclusion
Laserpuncture shooting on the reproductive acupoint of female striped catfish dose 0.4 joule can accelerate gonad maturity level on the IV level.

5. References
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