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by Dyah Hariani

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FULL LENGTH ARTICLE

Utilization of laserpuncture induction as spawning stimulation in catfish (*Clarias* spp.) crossbreeding toward egg quality

Pungky S.W. Kusuma [a,*], **Ngadiani Ngadiani** [a], **Dyah Hariani** [b]

[a] *Department of Biology, Faculty of Mathematics and Natural Sciences, University of PGRI Adi Buana Surabaya, Surabaya, Indonesia*
[b] *Department of Biology, Faculty of Mathematics and Natural Sciences, Surabaya State of University, Surabaya, Indonesia*

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Abstract The induction of laserpuncture on the reproductive acupoint of catfish can accelerate gonadotropin hormone formation from the pituitary especially gonadotropin II (GTH-II) which has a role in the final stage of oocyte maturation, ovulation and spawning stimulation. The purpose of this study is to evaluate the effects of laserpuncture induction toward the egg quality from crossbreeding catfish male var. Paiton and female var. Sangkuriang. The egg quality was measured by the following parameters: fertilization rate (FR), egg hatching rate (HR), and larvae survival rate (SR). The research treatments were conducted using two levels along with eight repetitions. The results show that the crossbreed catfish using laserpuncture induction affected the parameters by increasing the mean value of fertilization rate, egg hatching rate and larvae survival rate significantly ($P < 0.05$) compared with mean value of fish without induction. This study concluded that laserpuncture induction on the crossbreeding between broodstock of male catfish var. Paiton and female var. Sangkuriang will increase FR, HR and SR.

Introduction

Catfish (*Clarias* sp.) has a promising potential in fish husbandry due to its ability to breed easily, relatively fast growth, be maintained at high density, and could be fed by various types of feed. If the broodstock is nurtured well and fed by nutritious feed, it might spawn throughout the year without any special requirements, but recently, the number of catfish in fishery production is decreasing and the management is unsustainable. The decrease in catfish production is the result of the bad quality of both broodstock and egg quality which are produced by fish farmers themselves and even the government of fish breeding centers. These are possibly due to the lack of occurrence inbreeding of the excellent catfish broodstock which exist in Indonesia, thereby if the productive capacity of catfish is reduced, it will lead to occurrence of very low population, small size, slow growth, and commercially less profit, so that the government target of 20.05 million tons catfish product in 2014 will be difficult to achieve. Otherwise, the catfish husbandry techniques are

* Corresponding author.
E-mail address: slametswk@yahoo.com (P.S.W. Kusuma).

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still carried out traditionally and have not reached optimal goals yet. Therefore, the supply of mature gonad broodstock in huge numbers simultaneously combined with laserpuncture technology is recommended , because it is the main key of success to fulfill the government target of catfish production.

Hybridization belongs to the important quality improvement program for fish as a cutting edge technology which is developed in order to improve and increase the genetic quality of produced fish for better offspring (Purdom, 1993; Rustidja, 1999). In some cases, hybrid fish has proven to have better growth rate than that of its broodstock (Purdom, 1993). Hybrid crossbreed fish between *Clarias batrachus* and *Clarias gariepinus* has proven well to produce offspring with better hatching capability, growth rate, and larvae survival rate than other similarly crossbreed offspring. Hybridization could produce excellent offspring which are sometimes sterile capable to produce new strains offspring (Rustidja, 1999).

In this study, crossbreed process of broodstock between male catfish var. Paiton and female var was performed. Sangkuriang has been done by laserpuncture technology. Laserpuncture is used to accelerate the provision of mature gonad and ready-to-spawn broodstock to produce good quality egg in huge numbers simultaneously.

Kert and Rose (1989) has proven that if an induction dose counts 0.5–1 J/cm^2 induced by low power laser of 5 mW with wavelength 632.8 nm improve the nerve regeneration ability located both in central and peripheral sides will be produced to increase the cellular activities, and the ability to produce hormones and enzymes. Our previous study showed that laserpuncture induction in reproduction acupoint of these broodstock catfish will accelerate the growth, development and maturation of gonad (Kusuma, 2013). It indicated that the induction of laserpuncture could increase the performances of the hormones activity which takes part in reproduction control system to accelerate the provision of growth, development, and gonad maturation of catfish. Therefore the desired objective result which is a crossbreed catfish between var. Paiton broodstock and var. Sangkuriang broodstock using laserpuncture will accelerate the provision of mature gonad broodstock and ready-spawning broodstock to produce good quality egg in huge numbers simultaneously.

Materials and methods

This study was conducted from January to March 2015 at Freshwater Aquaculture Management Unit (UPBAT), Kepanjen, Malang, Indonesia.

Laserpuncture

This study was using soft-fervency laser He–Ne which has 632.8 nm length, 0.2 cm^2 of light output area, and the 5 mW/cm^2 of output power of laser beam is equivalent to 0.375 J/cm^2 as the photobiomodulation laserpuncture. This laser has safe wavelength as photobiomodulation organ (Karu, 2000).

Table 1 Average mean FR (%) of crossbreed male catfish var. Paiton and female var. Sangkuriang.

Treatment	Crossbreed	*N*	Means FR (%) ± SD
Without laser	PT male vs SK female	8	96.21 ± 0.91
Laser	PT male vs SK female	8	98.22 ± 0.60

Description: PT = Paiton; SK = Sangkuriang.

Sample preparation

The 8–9 month old broodstock fish samples were collected from UPBAT Kepanjen, Malang. 16 male catfishes var. Paiton weighted 1010–1690 g and 16 female catfishes var. Sangkuriang which never had spawn with weighted 1140–1750 g.

Broodstock selection

The selected crossbreeds male catfish var. Paiton and female var. Sangkuriang were adapted in the separated cement pond sized 2 × 2 × 1 m for 14 days in order to avoid spawning before treatment. During the maintenance, the broodstock were given nutritious commercial food (Pokphan 781-3) in the morning and evening in order to allow the maturation of the broodstock gonads and added with 35% dose of protein produced by CP Prima as much as 5% of their body weight. Furthermore, the control treatments and laserpuncture inductions were executed on the selected broodstocks.

Laserpuncture induction

This study used experimental method using control and laserpuncture induction treatments. According to the previous study (Kusuma et al., 2012), the optimum dose to stimulate the reproduction acupoint laserpuncture induction was conducted at the reproduction acupoint exactly in 2/3 parts of ventral body for 15 s. The non-treatment control was conducted as a comparison for induction treatments. Both of non-treatment control and induction treatment used 8 pairs of mature gonad crossbreeds male catfish var. Paiton and female var. Sangkuriang. Afterward, the broodstocks were maintained in the grouped tarp pond sized 1.75 × 2 × 0.6 m which have been coded before and each section was filled by a pair of gonad-matured crossbreed male catfish var. Paiton and female var. Sangkuriang.

Spawning broodstock and the maintenance of the egg and larvae

The selected male and female broodstock have been induced by laserpuncture at the acupoint of reproduction for 15 s to stimulate spawning process. Then, the induced broodstocks were placed into a traditional *kakaban* sampling pond whose width is 10 × 10 cm as the attachment place of egg. After 8 h, the broodstock had already finished the spawning process, the *kakaban* were taken in order to calculate the fertilization manually. Then, the *kakaban* were put in the aerated aquarium filled by temperature and pH controlled water. The egg quality could be assessed from the fertilization rate (FR), hatching rate (HR) and larvae survival rate (SR).

Calculation of total egg and fertilization rate (FR)

The number of fertilized egg in the 10 × 10 cm sized *kakaban* sampling pond was manually counted and compared to the total number of eggs in *kakaban* the sampling pond. The

fertilization rate is equal to the percentage of fertilized egg from all ovulated eggs. The egg condition was observed for 8 h after fertilization. The color of fertilized egg was yellow–green while the non-fertilized egg was white–turbid. The fertilization rate also depends on the quality and quantity of sperm which was produced by the male broodstock then determined using the following Eq. (1) (Adebayo, 2006):

$$\text{FR } (\%) = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs counted}} \times 100 \quad (1)$$

Calculation of total egg and hatching rate (HR)

Hatching rate is equal to the percentage of hatched egg quantity in *kakaban* sampling pond from total number of fertilized eggs. Hatching rate observation was calculated manually from 3 days after fertilization process until no more hatching egg was detected. Hatching egg was indicated by the rotating movement of larvae in the bottom of aquarium, the color of non-hatched egg is yellow–turbid and attached to the *kakaban* sampling pond. Hatching rate could be determined using the following Eq. (2).

$$\text{HR } (\%) = \frac{a}{a+b+c} \times 100 \text{ (Adebayo, 2006)} \quad (2)$$

Description: a = Normal number of hatched egg, b = Abnormal number of hatced egg, c = Non-hatched egg.

Calculation of total hatched egg and larvae survival rate (SR)

The larvae survival rate from the crossbreed male catfish var. Paiton and female var. Sangkuriang is equal to the comparison number of egg at the final catfish preservation to the total eggs during 9 days after spawning process. Larvae survival rate could be determined using the following Eq. (3)

$$\text{SR } (\%) = \frac{\text{Nt}}{\text{No}} \times 100 \text{ (Adebayo, 2006)} \quad (3)$$

Description: SR = survival rate (%), Nt = Total egg in the final of preservation, No = Total egg in the first spawning.

Statistical analysis

The data were analyzed using software Statistical Package for Social Science (SPSS) 15.0 for windows. The treatment consisting of two levels along with eight repetitions was statistically done by *T*-test. The measured parameter is the comparison between control (non-treatment) group to the laserpuncture induction group involving FR, HR and larvae SR. The mean value of the statistical calculations from the each parameter was analyzed descriptively.

Result and discussion

The comparison between non-treatment spawn to laserpuncture induced egg quality from crossbreed male catfish var. Paiton and female var. Sangkuriang based on fertilization rate (FR)

The laserpuncture induction treatment results of crossbreed male catfish var. Paiton and female var. Sangkuriang showed significant effect on FR ($P < 0.00$) compared with the results of crossbreed catfish without laserpuncture induction treatment (Table 1).

This is possibly due to the nutrition of the feed which contains 35% of protein and had been treated with laserpuncture induction before conducting the crossbreeding on both male and female broodstock. Those previous treatments before crossbreeding have proven well in case to accelerate the gonad maturation and the spawning process of the broodstocks whereas, the gonad growth, development and maturation process in male catfish var. Paiton and female var. Sangkuriang without laserpuncture induction treatment occured but need longer time than the induced one.

The results show that 35% of protein in catfish feed would be used for spermatogenesis and oogenesis process by broodstock. If the protein as living basic required compounds is excessive in the fish body, thus the excess protein will be used for growth of the gonad, development and maturation of embryo. Laserpuncture induction treatment may increase the broodstock metabolism rapidly in order to generate the energy which is needed to fasten the gonad growth, development and maturation of embryo more than non-laser-induced treatment. Certainly, the crossbreed broodstock between male catfish var. Paiton and female var. Sangkuriang which have been induced with laserpuncture and fed with same feed obtained more fertilized egg FR.

The quality of crossbreed broodstock egg is a reflection of the quality of the yolk, which has been influenced by feed nutrient of broodstock and induction of the laserpuncture. Feed nutrition, especially protein, and laserpuncture induction are important factors that influences egg quality since the protein is used as base material to synthesize hormone and vitellogenin in the broodstock liver before spawning process.

The spawning catfish broodstock mechanism is started since the protein compounds had reached catfish digestive system through the feed. It will be digested from complex to simpler form by digestive enzymes which then is absorbed by intestinal epithelium cells Afterward, the protein will be taken into blood stream which flows directly to the liver, brain and gonad. Inside the liver, these proteins will be used for the base material in vitellogenin synthesis. Inside the brain, these proteins will be used to synthesize hormones. Inside the gonad, protein will be used for either ovum or sperm growth, development and maturation (Hariani, 2013).

Vitellogenin is a protein precursor in egg yolk of fish. Therefore, sufficiently high levels of proteins in feed will lead vitellogenin to the optimum synthesis because proteins have an important role in egg yolk formation (Nilsson et al., 2001; Ohkubo and Matsubara, 2002; Kapateh, 2009). Furthermore, the vitellogenin will affect the produced semen and ovum by supporting the gonad growth and development egg. This physiological activity will not run optimally if the broodstock fish has not been induced with laserpuncture.

The consumption of 35% protein fed to the broodstock before spawned, was confirmed as one of determining factors which affected the semen and ovum production. The optimal dosage of protein in feed can produce good quality semen and ovum based on high FR egg as an indicator (Madu et al., 2004; Ojutiku, 2008). High FR is a pre requirement that must be fulfilled for the fish breeders to sustain their catfish husbandry in order to avoid the economic loss.

In addition, egg FR which is produced from crossbreed broodstock is certainly inseparable with sperm quality for

fertilization process which is produced by male catfish broodstock. The results show that the fed treatment which contains 35% protein (and induced with laserpuncture) on male and female broodstock was proven to produce egg with high FR because the success of fertilization is influenced by the egg ovum and sperm quality of spawned broodstock.

The quality of egg and sperm determines the fertilization rate and the production of the egg. The results confirmed that protein's presence in feed and laserpuncture induction had increased the physiology activity of catfish since laserpuncture induction takes part by increasing the formation of gonadotropin (GtH-I and GtH-II) and estrogen hormones. (Kusuma et al., 2012; Hariani, 2013).

GtH-I plays a role in vitellogenin phase which had characterized based on gradually increased stage before oocyte was matured. Increased levels of vitellogenin will be used further in the physiology activity, for example, accumulation of egg-yolk activity for final oocyte maturation process. The yolk also plays a role in embryogenesis up to catfish larvae attaining 3 days of age after hatching. In addition, the proteins' presence in egg is required in the synthesis process of embryonic tissue and as an energy source metabolism for developing embryo (Sinjal, 2007).

The water quality is another factor which also plays a role in the spawning and egg hatching process besides laserpuncture induction and protein feed. The water quality measurements include temperature, pH, and dissolved oxygen should be in optimal condition. The temperature is between 27.3 and 27.6 °C, and pH 7. The optimal temperature for spawning ranged between 25 and 30 °C and optimal pH for hatching is 6.2–7.8 (Soetomo, 2000; Khairuman and Amri, 2002; Yustina and Darmawati, 2003). The dissolved oxygen is required for embryo metabolic process in the egg and during this study, the value of dissolved oxygen is 8 mg/L.

The results of the study revealed that crossbreed male catfish var. Paiton and female var. Sangkuriang broodstock supplemented with 35% protein of feed before spawning process then induced with laserpuncture has proven that had increased FR rather than the without laser induced broodstock.

The egg quality of crossbreed male catfish Paiton and female var. Sangkuriang with and without laserpuncture induced treatment according to egg hatching rate (HR)

The results of this study show that the crossbreed male catfish var. Paiton and female var. Sangkuriang both with and without laserpuncture induction have a significant impact in the egg hatching rate (HR) larvae ($P < 0{,}00$) (Table 2).

The quality of semen and ovum from crossbreed broodstock will determine the egg production and influenced by liver vitellogenin. Vitellogenin is a glicophospo lipoprotein which contains 90% protein and 20% lipid, especially phospolipid, trigliserida, cholesterol and lipoprotein. Vitellogenin formation is influenced by food quality and the laserpuncture induction for increasing the broodstock metabolism. Therefore, the vitellogenin synthesis process is determined by estradiol activity as the result of laserpuncture induction. Vitellogenin is the energy source for oocyte formation which is very important for the embryo development and elongating egg lifespan. It is quite sure that the availability of protein in the broodstock feed will accelerate gonad development and maturation wherewith improving the egg quality (Pandey, 2013).

The protein presence in the feed has an important role to affect the weight of gonad and oocyte in the ovarium and sperm quantity and quality in testis. The protein feed combined with laserpuncture induction would make the testis and ovarium develop which further affected the quality of sperm, ovum, and semen according to HR much faster.

The results show that the treatments could accelerate vitellogenesis synthesis in the catfish liver. Furthermore, vitellogenin will be used as a base material of egg yolk formation and stimulate the Sertoli cells to synthesize ABP (*Androgen Binding Protein*) which plays a role in the spermatogenesis.

It has been reported that protein presence in feed has an important role in the yolk protein formation (Kapateh, 2009) because protein presence in the feed of crossbreed broodstock has a important role as a material in the vitellogenin synthesize which henceforth vitellogenin will be changed to yolk protein. A high value of protein yolk in the oocyte is very important to bring egg to a higher quality. The production of high quality yolk will lead to the possibility of fertilized egg. Thus, the value of FR would be high, and so would the HR if the environment condition supported the hatching mechanism.

It proves that the protein consumption will be useful for fertilization process by increasing the protein amount in the yolk and sperm of broodstock. The fertilized egg will hatch to become larvae. The larvae HR of the laserpuncture induced crossbreed broodstock is higher than larvae HR without laserpuncture induced, because of the excess dose of nutrition of food that brings ovum and sperm of good quality.

This study shows that 35% protein feed consumption before spawning the crossbreed using laserpuncture induction treatment could increase the seed HR higher than the crossbreed without induction treatment.

Larvae quality from crossbreed male catfish var. Paiton and female var. Sangkuriang using and without laserpuncture induction according larvae survival rate (SR)

The crossbreed broodstock male catfish var. Paiton and female var. Sangkuriang both with and without laserpuncture induction has a significant impact on the larvae survival rate (SR) ($P < 0{,}00$) (Table 3).

The crossbreed broodstock which consumed feed contains 35% protein then induced by laserpuncture has bigger larvae SR for 9 days after hatching than the other without laserpuncture induction.

This is probably due to the fact that previous feed consumption and induction treatments are proven to highly increase the egg mean value FR and HR using FR and HR

Table 2 Persentage mean of egg HR (%) from crossbreed male catfish var. Paiton and female var. Sangkuriang with and without laserpuncture induction.

Treatment	Crossbreed	*N*	Mean HR (%) ± SD
Without laser	PT male vs SK female	8	94.04 ± 0.96
Laser	PT male vs SK female	8	96.76 ± 1.04

Description: PT = Paiton; SK = Sangkuriang.

Table 3 Percentage of 9 day old larvae SR (%) from crossbreed catfish with and without laserpuncture induction.

Treatment	Cross-breed	*N*	Mean of SR (%) ± SD
Without laser	PT male vs SK female	8	91.52 ± 0.86
Laser	PT male vs SK female	8	93.18 ± 1.18

Description: PT = Paiton; SK = Sangkuriang.

as the indicators which affect larvae SR. The consumption of 35% protein feed and laserpuncture induction to the crossbreed broodstock could provide the energy for the semen formation and produce bigger size which has homogen diameter relatively, therefore, the bigger yolk amount is useful to help larvae to survive in the new environment. These good quality homogen diameter semen 90% affected the crossbreed fertilization, hatching process, and survival of the five day old larvae better in which had been induced by laserpuncture than those without one.

It has proven that consumption of 35% protein before laserpuncture induction toward crossbreed catfish could increase the fry quality which is shown by a high number of larvae SR. The SR number depends on the yolk protein due to its supporting roles in embryogenesis. It shows that the yolk protein is very important to increase the sperm quality in order to succeed fertilization process. Yolk protein also supported hatching process and larvae development until five days. Therefore, there is a close correlation between yolk protein and consumption of 35% protein before crossbreeding.

The quality of larvae SR from crossbreed catfish with and without laserpuncture induction is greatly affected by ovum diameter from female broodstock. The larvae which are derived from long diameter ovum are more resistant to the environment changes than from small diameter ovum. The seed with bigger diameter leads to high survival rate and healthy larvae, shown by the fast growth and resistance to high environment changes (Kjørsvik et al., 2003; Giménez et al., 2006).

Larvae SR from crossbreed catfish which was induced by laserpuncture and consume 35% protein has higher value than non-induced larvae SR, on the 9th day after, non-induced crossbreed catfish shows lower value than the induced one, respectively 91,5% versus 93,2% larvae.

It might be caused by protein consumption before laserpuncture induction to the 2/3 parts of the body can accelerate vitellogenin synthesize then it will be absorbed and accumulated in the oocyte, proven by the oocyte will enter vitellogenic final stage. The vitellogenic final stage happens as the initiation of oocyte maturation which is affected by protein in the broodstock feed and laserpuncture induction. Laserpuncture induction supports the release of GtH-II hormone, then it will stimulate MIH formation in the gonad to stimulate MPF and it will result in GVBD production, which is shown by the mature oocyte which will be ovulated soon. This condition will lead to spawning, fertilizing, hatching and larvae development.

This study was supported by Nagahama (1994) and Clelland and Peng (2009) who explained that GtH-II stimulates oocyte maturation and *Maturation Inducing Hormone* (MIH), MIH will stimulate *Maturation Promoting Factor* (MPF), then MPF will stimulate oocyte which is marked by the increase in vitellogenin and oocyte diameter because of being filled by homogen yolk mass. MPF also plays a role in the final stage of oocyte maturation which is marked by the oocyte core through the animal pole and stimulates *Germinal Vesicle Break Down* (GVBD) where the mature oocyte will soon be ovulated and spawned.

Physiological activity of laserpuncture induced crossbreed broodstock is more accelerated than that the without one. It proves that protein consumed before crossbreeding and the induction will enhance the cellular activity because it needs the nutrition from the broodstock feed. The environmental factors such as, temperature, pH and dissolved oxygen might contribute in the spawning and seed hatching process. The incubated water temperature in this study is around 27,30 °C–27,60 °C, pH 7, and dissolved oxygen amount is 8, this condition sustains the larvae growth and development stability, thus, the larvae will increase its metabolism rate to perform its faster movements (Kelly, 2004; Oyelese, 2006). Puvaneswari et al. (2009) said that the environment condition, such as temperature affected the egg hatching. The optimum temperature for incubation is around 26 °C–30 °C. At this temperature the absorption of protein by the egg ends three days earlier after the egg is hatched which is indicated by the transformation to larvae.

Beside the support of environment condition, the biochemical composition of egg also supports the larvae SR because the biochemical composition of egg defines the embryo needs in order to develop into larvae. Therefore, the good quality feed for the crossbreed broodstock is very much needed due to its effect on the larvae growth and development. The food nutrition should be in accordance with the semen biochemical composition because it is an essential component and also as one of the egg quality parameters which can be used to evaluate its quality before fertilization. In addition the egg biochemical component acts as a metabolism energy resource for embryonic tissue synthesis in the larvae development.

This study proves that consumption of 35% protein feed before crossbreeding process and laserpuncture induction could support the egg biochemical component which will be used as a metabolism energy resource for embryonic tissue synthesis, thus it will increase the larvae survival rate than the larvae survival rate from crossbreed broodstock without laserpuncture induction.

Conclusion

Laserpuncture induction has been proved to improve egg fertilization rate (FR), hatching rate (HR) and increased the larvae survival rate (SR) of crossbreed male catfish var. Paiton and female var. Sangkuriang broodstock compared with the results of non-induced laserpuncture broodstock.

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