The Effect of Combine Between Dietary Protein Level Variation and Laserpuncture Induction on Catfish (Clarias sp) Oocyte Development

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RESEARCH ARTICLE

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The Effect of Combine Between Dietary Protein Level Variation and Laserpuncture Induction on Catfish (*Clarias* sp) Oocyte Development

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Dietary protein was involved in accelerating the oocyte development. Nowadays, the effect of dietary protein level and laserpuncture induction on oocyte development is widely studied. However, the effect of combine between dietary protein level variation and laserpuncture induction on oocyte development to accelerate spawning process is not fully elucidated. This study aims to demonstrate that the treatment combination between dietary protein level and laserpuncture inductions are involved in oocyte development. This study used Randomized Complete Block Design with three kinds of treatment with four times repetition. The treatment is consisting of three factors: (1) Factor I—treatment protein level (30%, 35%, and 40%), (2) Factor II—treatment laserpuncture induction (without or control and with laserpuncture induction), (3) Factor III—treatment duration (8 weeks). Gonado somatic index (GSI) and oocyte diameter data are analyzed by analysis of variance. The gonad maturity stage is analyzed descriptively. The result showed that 40% dietary protein and laserpuncture induction significantly ($P < 0.001$) increased oocyte development (GSI and oocyte diameter). This combination treatment accelerated gonad maturation 3 weeks faster than without laserpuncture induction. In conclusion suggested that 30% dietary protein and laserpuncture induction is treatment to increase oocyte development and accelerate reproductive cycles from 6 weeks to 3 weeks.

Keywords: Laserpuncture Induction, Dietary Protein, GSI, Oocyte Diameter, Gonad Maturity Stage, Catfish.

1. INTRODUCTION

The fish oocyte develops within the ovarian occurs in the early stages of oogenesis before yolk formation is extensively reviewed.[1–3] The sufficient dietary protein level accelerates oocyte development.[4–8] The of final oocyte development and oocyte size at the end of vitellogenesis is shown to exhibit rather constant values in fish at final oocyte maturation.[3,9] Oocyte development is regulated by the brain-pituitary-gonad-liver axis of female fish to oogenesis, such as vitellogenesis and oocyte maturation. The pituitary gland is the regulator that reproductive hormone production. Gonadotropin Releasing Hormone (GnRH) is released by the hypothalamus. GnRH stimulates Gonadotropin Hormone (GtH-I and GtH-II).[10,11] To those activities necessary nutrition. Nutrition determines reproductive activity especially dietary protein is component in vitellogenin and gonad maturation process especially to determine oocyte size and maturation.[12–14] The reproductive action is indicated by oocyte quality such as oocyte diameter, gonado somatic index (GSI), and gonad maturity stage.[9,15,16]

The high stage of oocyte development is comparable with gonad weight and gonad maturity stage.[17] Gonad maturity stage is used as an indicator to determine spawning time. Gonad development can be determined by GSI value.[18–20] The female catfish broodstock weight in mature gonad increase 1–20%.[21] GSI value correlates with gonad development. The highest GSI value indicates oocyte maturation.[22,23] Gonad maturation process until ovulation can be accelerated by proper dietary protein level.[14,24] This nutrition determines enzyme activity and GtH-I and II production. On the other hand, laserpuncture induction has been proven to increase this action too. Studies are done by using lasers very low output power, laser systems to influence cellular repair and reproduction.[25–29]

Helium-Neon (He–Ne) laserpuncture is a short light wave with low power (4–5 mW), 632.8 nm wavelengths, and 0.2 cm^2 light square output. This laserpuncture uses save wavelength range (600–950 nm). These devices have been called low-level lasers or cold lasers. The important thing to acknowledge is that the increase in temperature is not the cause of the cellular changes and the trigger of this activity is caused by a photochemical responseser.[30,31]

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Laserpuncture induction is a biostimulator in axis part of the brain, pituitary gland, gonad, and liver in reproduction activity such as reproductive enzyme and hormone production also stimulates membrane receptor activity, stimulates gonad maturation and spawning in catfish.[32,33] The pituitary is the production of GtH-I and II. GtH-I and II systemically will deliver to granulosa cell in the gonad and increase reproductive hormone level in blood plasma[31] such as estrogen (estradiol-17β).[34] Estrogen will regulate vitellogenesis and deposition vitellogenin in oocyte cause oocyte diameter increased.[35] In the serum, the estradiol-17β level is dependent on the developmental stage of the ovary.[36] Increasing of estrogen production can be stimulated by laserpuncture induction and proper dietary level.

Dietary is protein and lipid as energy resources to keep metabolic and physiological balances and produce good yolk.[14] Based on this background, we need to know the effect of treatment combine between dietary protein level variation and laserpuncture induction to accelerate of oocyte development (GSI, oocyte diameter, and gonad maturity stage) in catfish (*Clarias* sp).

2. METHOD

This research is held in Unit Pengelola Budidaya Air Tawar (UPBAT), Kepanjen, Malang. This experimental research was used Randomized Complete Block Design with three kinds of treatment. The first treatment is dietary protein level (30%, 35%, and 40%). The second treatment is laserpuncture induction. The third treatment we divided the population into two treatment groups: laserpuncture induction and without induction (control). Laserpuncture is inducted in once a week (15 sec) during eight weeks. We repeated each treatment in four times repetition.

We used 172 female catfish broodstock (900–1500 grams) and 172 male catfish broodstock (1140–1750 grams). The average of catfish age is 1–1.5 years. Male catfish broodstock is used in spawning process only. The female catfish broodstock is separately maintained in tarpaulin fishpond in 2 m × 2 m × 90 cm size. Acclimatization is held during one week. Initially, all experimental catfish is given by 6% of dietary twice a day (morning and evening) that contain 30% protein. The composition of catfish broodstock dietary consists of a fish meal, rice bran, tapioca flour, fish oil, vitamin, premix mineral, and gluten (carboxyl methyl cellulose). Table I provides information about catfish dietary composition on 30%–40% protein level and Table II about proximate analysis.

Table I. Composition dietary on 40% protein level showed the highest feed quality.

	Protein level (%)		
Raw material	30	35	40
Fish meal	8.94	43.76	48.59
Soya flour	15.60	20.15	24.69
Corn flour	10.00	10.00	10.00
Rice bran	10.00	10.00	10.00
Tapioca flour	13.46	6.92	0.37
Fish oil	3.85	2.42	1.00
Vitamin-mineral mix	2.00	2.00	2.00
CMC	6.15	4.75	3.36
Total	100.00	100.00	100.01

Table II. Proximate analysis result of catfish broodstock dietary formula.

	Protein level (%)		
Proximate items (%)	30	35	40
Dry matter	87.35	86.37	86.45
Ash	15.88	17.21	19.31
Crude protein	30.15	34.72	40.05
Crude fiber	3.6	3.78	3.89
Crude lipid	8.07	8.56	8.62
Energy gross (kcal/kg)	3959.18	4018.14	4057.35

Dietary Protein and Laserpuncture Treatment. Following the acclimatization, catfish broodstock is spawned in separated tarpaulin fishpond in pairs (1 male: 1 female). Ovum condition after spawning is same. It is assumed that it is without contained mature egg after spawned (0 weeks).[34] The 30%, 35%, and 40% level of protein on dietary are given to experimental catfish broodstock[37] along with laserpuncture induction on reproductive acupoint (2/3 ventral part of the body) in 15 sec/week during eight weeks (Fig. 1). This treatment group will be compared to control group (without laserpuncture induction).[31] Table I provides information about catfish dietary composition on 30%–40% protein level. Composition dietary on 40% protein level showed the highest feed quality. Table II indicated that proximate analysis result of catfish broodstock dietary formula isoenergy and 30%, 35%, and 40% level of protein on dietary were given to experimental catfish broodstock.

Figure 1 indicated that laserpuncture induction on reproductive acupoint (2/3 ventral part of the body) in 15 sec/week during 8 weeks to observe development oocytes.

2.1. GSI Analysis

The female catfish broodstock is scaled and operated to get the gonad. Gonad is also scaled. The data of bodyweight and gonad weight are used to determine GSI. The formula of GSI (Gonado Somatic Index).[38] The procedure described before was done on each week (from 0–8th week). We used four samples per treatment for each week.

2.2. Oocyte Diameter Analysis

Oocyte diameter is determined by taking of oocyte catfish using section needle. A hundred oocytes are arranged on object glass.

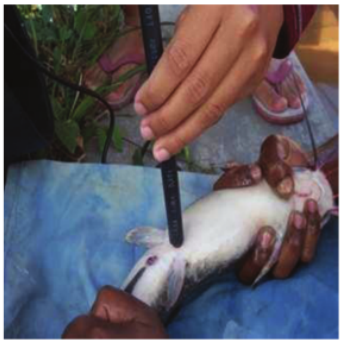

Fig. 1. Laserpuncture induction on reproductive acupoint (2/3 ventral part of the body) in 15 sec/week.

The oocyte diameter was measured using an ocular micrometer with 4×10 times magnification.[39]

2.3. Gonad Maturity Stage Analysis

Gonad maturity stage is determined by tissue slide and Hematoxylin-Eosin staining techniques of gonad for all samples.

2.4. Statistical Analysis

The present data are expressed as mean $\pm$ SD. GSI value and oocyte diameter data are analyzed using two-way analysis of variance (ANOVA) with Gen Stat version 15. Statistic significance is set at P value <0.001. Meanwhile, gonad maturity stage data is analyzed by description. Giving of treatment combination between dietary protein level variation (30%, 35%, and 40%) along with laserpuncture induction during eight weeks significantly ($p < 0.001$) accelerated gonad maturation based on all parameter.

3. RESULTS AND DISCUSSION

Giving of treatment combination between dietary protein level variation (30%, 35%, and 40%) along with laserpuncture induction during eight weeks significantly ($p < 0.001$) accelerated gonad maturation based on all parameter.

Gonado Somatic Index (*GSI*) value resulted by giving of treatment combination between dietary protein level variation and laserpuncture induction on female catfish broodstock is showed in Figure 2. Mean of GSI value in female catfish broodstock after giving of 40% dietary protein level was higher than 35% and 30% in both with and without laserpuncture induction ($P < 0.005$). In 30% dietary protein level, laserpuncture induction increased 9.33% GSI value than without laserpuncture induction. In 35% dietary protein level, laserpuncture induction increased 7.77% GSI value than without laserpuncture induction. In 40% dietary protein level, laserpuncture induction increased 5.15% GSI value than without laserpuncture induction.

This study showed that treatment combination between dietary protein level variation and laserpuncture induction generated two peaks of GSI values: at 3rd week (GSI value = 19.23–22.45) and at 6th week (GSI value = 19.22–21.71). However, giving of dietary protein level variation without laserpuncture induction generated only one peak of GSI value: 6th week (GSI = 19.42–21.85). GSI value in 3rd week (in laserpuncture induction treatment) showed that gonad in pair condition and ready to be spawned.

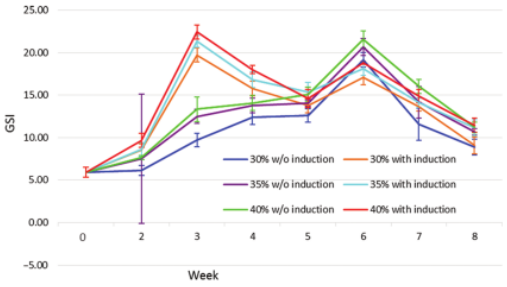

Fig. 2. Gonado somatic index (GSI) maximal to catfish (*Clarias* sp) after giving combination protein level 30%, 35%, and 40% and induction laserpuncture have two peaks at three and six weeks and without induction laserpuncture, GSI has one peak at six weeks.

This study showed that giving of 30–40% dietary protein level with laserpuncture induction generated two peaks of GSI value (at 3rd and 6th week) (Fig. 2). It might cause by laserpuncture activity in physiological reproductive stimulation to synthesize vitellogenin. However, to support the physiological activity, the good dietary is an important factor. Protein is involved in yolk formation and accumulation in the oocyte. This study showed that yolk accumulation and oocyte maturation is three weeks faster than without laserpuncture induction. However, the oocyte quality is good. Yolk accumulation caused oocyte become to bigger, gonad becomes to heavier, and GSI value becomes to higher and GSI was independent of the size of fish and has significant correlations with total length, total weight and gonad maturation stage in females.[40]

GSI value has been proven increased caused by treatment combination between dietary protein level variation and laserpuncture induction. Catfish broodstock needs sufficient protein to support growth and development of oocyte, follicular and yolk formation. This study showed that 30%, 35%, and 40% dietary protein level need to synthesize normal vitellogenin level (Fig. 2). Although 40% dietary protein level is the best level to support good quality and quantity of egg, we must calculate it wisely because of some efficiency reasons. Therefore, giving of 30% dietary protein level along with laserpuncture induction was a most efficient treatment to accelerate oocyte maturation and reproductive activity.

Giving of commercial dietary along with laserpuncture induction in reproductive acupoint increased GSI value and accelerate gonad maturation until broodstock is ready to spawn in three weeks faster.[33] Another result showed that catfish broodstock is given sufficient protein, lipid, calcium, and phosphate in dietary to produce good yolk.[41] It proved that dietary protein level determined yolk protein level in the egg. The protein in the yolk is involved in growth and development of oocyte and can increase GSI value.

Oocyte diameter resulted by giving of treatment combination between 40% dietary protein was higher than 35% and 30% dietary protein in both with and without laserpuncture induction ($P < 0.05$). In 30% dietary protein, laserpuncture induction increased 9.90% oocyte diameter than without laserpuncture induction. In 35% dietary protein, laserpuncture induction increased 8.79% oocyte diameter than without laserpuncture induction. In 40% dietary protein, laserpuncture induction increased 14.12% oocyte diameter than without laserpuncture induction.

This study showed that giving of dietary protein level variation along with laserpuncture induction generated two peaks of oocyte diameter value: at 3rd week (0.955–1.105 mm) and 6th week (0.923–1.003 mm). However, giving of dietary protein level variation without laserpuncture induction generated only one peak of oocyte diameter number: at 6th week (0.879–0.970 mm) (Fig. 3).

This study showed that giving of 30%–40% dietary protein along with laserpuncture induction generated two peak value of oocyte diameter (3rd and 6th week) (Fig. 3). In the previous research showed that laserpuncture induction has been proven stimulated pituitary neuron to produce GtH-I. GtH-I in theca and granulosa cell stimulated high testosterone and estrogen level in blood. These hormones will be delivered to a liver cell to regulate vitellogenin synthesize. Steroid hormone (testosterone and estrogen) formation and vitellogenin synthesize would be faster if it is

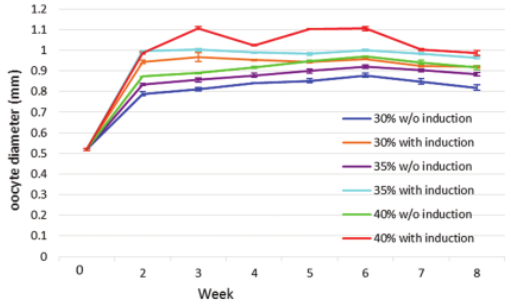

Fig. 3. Oocyte diameter (mm) maximal to catfish (*Clarias* sp) after giving combination protein level 30%, 35%, and 40% and induction laserpuncture have two peaks at three and six weeks and without induction laserpuncture, GSI has one peak at six weeks.

supported by good dietary. This dietary is used as raw material of suitable with catfish broodstock necessary. It proved that treatment combination between giving of dietary protein level variation and laserpuncture induction produced bigger oocyte diameter caused higher accumulation of yolk in the oocyte.

Oocyte diameter indicated stored of energy amount in oocyte that is used in oocyte maturation and embryo development. Oocyte that is ready to be ovulated and oviposition had the same size, but the diameter was bigger than ones. The mature oocyte is ovulated in next reproductive cycle. The dietary protein could accelerates gonad maturation and improve egg quality[12] and the nutrient is involved as catabolic substrate (energy resources),[42] the main substrate in bioactive and protein synthesize. The energy contained in the yolk is needed in oocyte development.

Giving of treatment combination between 30%–40% dietary protein level and laserpuncture induction are proven increase oocyte diameter and accelerate mature condition. The dietary protein level increase oocyte diameter, size, and heavy of catfish gonad.[43] The mature oocyte and ready to have homogeny maximal diameter[44] and the oocyte in the final stage has homogeny diameter.[38] Laserpuncture induction in the black parrot fish with commercial dietary accelerates gonad maturation.

The oocyte diameter in this treatment combination is relatively homogeny. The laserpuncture induction must be supported by dietary[33] because it is very important to form high oocyte diameter. Laserpuncture induction accelerates oocyte maturation in the final stage. The mature oocyte will be released simultaneously.

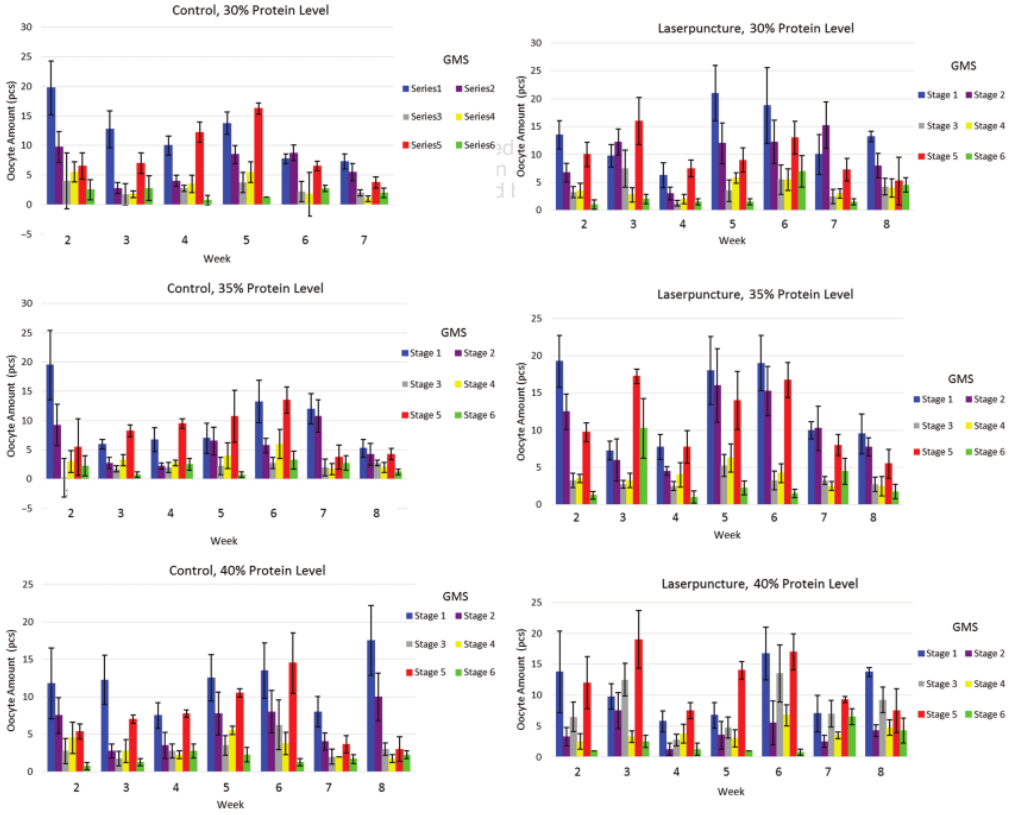

Fig. 4. Gonado maturation stage (GMS) to catfish (*Clarias* sp) after giving combination protein level 30%, 35%, and 40% and induction laserpuncture and without induction laserpuncture every week. Blue: GMS stage 1; purple: Stage 2; grey: Stage 3; yellow: Stage 4; red: Stage 5; green: Stage 6.

However, mature oocyte will be released gradually in without laserpuncture induction.

3.1. Gonad Maturity Stage (GMS)

Oocyte development from all treatment (treatment combination between dietary protein level and without laserpuncture induction) was same from the first stage until 6th stage. However, oocyte number for each week is dominated in this stain stage (Fig. 4).

Giving of 30%–40% dietary protein and laserpuncture induction accelerates gonad maturity stage. Gonad maturation is accelerated three weeks faster. It also generated two peak value of gonad maturity stage (at 3rd and 6th week). Giving of dietary protein level and laserpuncture induction accelerates oocyte maturation (at III, IV, and V stage). This acceleration is determined by GtH-I and GtH-II. At I–III stage, GtH-I, and estrogen have a role. The IV stage is determined by GtH-II. Meanwhile, the V and VI stage are determined by GtH-II, MIH, MPF, and prostaglandin. There are main unsure in dietary that determine in vitellogenesis[45, 46] and dietary is very important in those process. The high vitellogenin content in the oocyte is in the almost mature oocyte. It is determined by GtH-I and estrogen activity. Aizen et al.,[47] Pakhurst,[49] and Hosseinzadeh et al.[48] showed that GtH-I regulates initial stage of oocyte maturation and vitellogenesis.[47–49] The hormone that regulates vitellogenesis is estradiol-17β (estrogen/E2). Meanwhile, GtH-II regulates the next stage in oocyte maturation.

Laserpuncture induction that is induced in precisely 2/3 ventral part of the body (reproductive acupoint) during 15 sec stimulates axis hypothalamus—pituitary gland—gonad to release GtH. GtH-I and II are brought by the bloodstream to the gonad. These hormones were gradual increases. Increasing of GtH-I determines steroid hormone production such as estrogen. This high estrogen level increases vitellogenesis. Vitellogenin brought by bloodstream into oocyte is absorbed by endocytosis. Vitellogenin will be changed into cathepsin and vitelline (yolk). The yolk is accumulated in the oocyte, increase oocyte diameter, and increase GSI value, finally, increase gonad maturity stage. GtH-II regulates mature oocyte activity. In immature gonad, MPF has not active. That hormone activity stimulates Germinal Vesicle Break Down (GVBD), chromosome condensation, spindle formation before ovulation. The mature oocyte and ovulation process can be stimulated by prostaglandin hormone. The external factor like male broodstock can stimulate ovulation, oviposition, and spawning. Oocyte maturation is regulated by GtH-II. GtH-II stimulates theca cell to release MIH and it will bind to membrane receptor in granulosa cell to induce protein factor formation such as (MPF, cdc two kinases, and protein cyclin B). MIH activates MPF and stimulates GVBD. In this condition MPF is active and gonad will become too mature. This study showed that giving of 30%–40% dietary protein and laserpuncture induction in catfish broodstock accelerates gonad maturation three weeks faster than without laserpuncture induction.

4. CONCLUSIONS

The implication of 30% dietary protein with laserpuncture induction was a most effective treatment increased oocyte development (GSI), oocyte diameter, and gonad maturity stage. This treatment combination accelerated reproductive cycle from 6 weeks become to 3 weeks faster (2 cycles in 6 weeks) than without laserpuncture induction (1 cycle in 6 weeks).

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