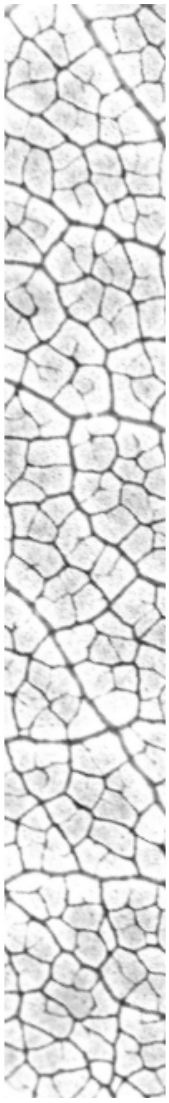

EurAsian Journal of BioSciences
Eurasia J Biosci 13, 177-183 (2019)

Biological study of increasing vitellogenin level and gonado somatic index by laserpuncture exposure at any protein level of dietary on catfish broodstock *(Clarias* sp.*)*

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Abstract
This biological study aims to determine the effect of laserpuncture exposure and protein level of dietary on vitellogenin level and GSI. Three protein levels (30%, 35% and 40%) of dietary were given to the female catfish broodstock along with and without laserpuncture treatment. Laserpuncture exposure was on 2/3 ventral part of the body in 15 sec/week during 8 weeks. We used 172 of mature female catfish broodstock around 1-1.5 years old with 900-1500 grams of body weight. Blood and gonad were taken from 4 samples per treatment group in each week. Vitellogenin level was analyzed by ELISA. This study showed that laserpuncture exposure along with any protein levels of dietary, significantly increases vitellogenin level ($P<0.001$) and GSI number ($P<0.05$). Laserpuncture exposure and 30% protein dietary treatment at third week are most effective to increase vitellogenin level and GSI. It suggested that laserpuncture exposure along with 30% protein level of dietary accelerates gonad maturation 3 weeks faster.

Keywords: Catfish, helium-neon laserpuncture, protein level, vitellogenin

Wisnu Kusuma PS, Hariani D (2019) Biological study of increasing vitellogenin level and gonado somatic index by laserpuncture exposure at any protein level of dietary on catfish broodstock *(Clarias* sp.*)*. Eurasia J Biosci 13: 177-183.

INTRODUCTION

Catfish have the potentiality to be cultivated. Sustainable cultivation of catfish is determined by good seed in quality and quantity. In fact, the quality of seed always decreases and the quantity of seed is not sufficient. The protein dietary is often linked to good egg and acceleration of gonad maturation. Recently, protein dietary is often treated together with laserpuncture exposure.

Laserpuncture exposure was proven to increase Gonad Somatic Index (GSI), accelerate the gonad maturation, spawning, procurement of seed, and shorten the reproductive cycle (Hariani and Kusuma 2008, 2009, Hariani et al. 2010, Kusuma et al. 2007). Manipulation of external and internal factors is also applied to accelerate gonad and spawning to get a number of good seed.

The good seed is gotten by good nutrition. The good nutrition must be contained by sufficient protein. The essential amino acid in a protein is important for body to survive and maintenance. In reproduction system, the essential amino acid is involved in gonad growth and development. Along with estrogen, it also important on vitellogenin synthesize in hepatic (Al-Jiffri and Osama 2017).

Protein is involved in reproductive hormone production (GtH, testosterone, and estrogen) to synthesize vitellogenin. It is also important in follicular growth and development, and oocyte maturation. Lysine and methionine are two essential amino acid that involves in gonad maturation especially on catfish (Al-Jiffri and Osama 2017).

The final stage of oocyte development is indicated by top level of Gonadotrophin Hormone I (GtH-I), estrogen, and vitellogenin. Following this stage, oocyte will mature and be indicated by increasing of Gonado Somatic Index (GSI) until ≥ 20%. In this oocyte maturation, accumulation of yolk increases the diameter and number of oocyte. Therefore, GSI is used as indicator of gonadal maturation (El-Sayed et al. 2003, Khaironizam and Zakaria-Ismail 2013, Shinkafi and Ipinjolu 2012).

Reproductive activity induced by laserpuncture increases estrogen level. Increasing of estrogen level is usually followed by increasing of vitellogenin. Increasing

Received: January 2019
Accepted: March 2019
Printed: May 2019

Table 1. Composition of Formula Catfish Dietary on 30%, 35% and 40% Protein Level

Raw Material	Formula for each protein level on dietary		
	30 %	35 %	40 %
Fish meal	38.94	43.76	48.59
Soya flour	15.60	20.15	24.69
Cornflour	10.00	10.00	10.00
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

of estrogen and vitellogenin level supported by protein dietary can accelerate gonadal maturation and increase seed quality. Along with protein dietary, laserpuncture exposure also has proven trigger gonadal maturation, stimulate egg spawning, increase estrogen and gonadotrophin production level. However, until in this time, the effect of any level protein dietary that given along with laserpuncture exposure on vitellogenin and GSI has been fully elucidated.

MATERIALS AND METHODS

Sample and Dietary Preparation

This research was held in Unit Pengelola Budidaya Air Tawar (UPBAT), Kepanjen, Malang. This experimental research was used Randomized Complete Block Design with two kinds of treatment. The first treatment is protein dietary level (30%, 35%, and 40%). The second treatment is laserpuncture exposure. We divided the population into two treatment groups: laserpuncture exposure and no exposure (control). Laserpuncture was exposed in Once a week (15 sec) during 8 weeks. We repeated each treatment in 4 times repetition.

We used 172 female catfish brood stock (900-1500 grams) and 172 male catfish brood stock (1140-1750 grams). The average of catfish age is 1-1.5 years. Male catfish broodstock was used in spawning process only. The female catfish broodstock was separately maintained in tarpaulin fishpond in 2 m x 2 m x 90 cm size. Acclimatization was held during one week. Initially, all experimental catfish was given by 6% of dietary twice a day (morning and evening) that contain 30% protein.

The composition of catfish broodstock dietary is consist of a fish meal, bran, tapioca flour, fish oil, vitamin, premix mineral, and gluten (carboxyl methyl cellulose). The **Table 1** provides information about catfish dietary composition on 30%-40% protein level.

Protein Dietary and Laserpuncture Treatment

Following the acclimatization, catfish broodstock was spawned in separated tarpaulin fishpond in pairs. Ovum condition after spawning is same. It was assumed that it was not contained mature egg after spawned (0 weeks) (Kusuma et al. 2007). The 30%, 35%, and 40% level of protein on dietary were given to experimental catfish broodstock along with laserpuncture exposure on reproductive acupoint (2/3 ventral part of the body) in 15 sec/week during 8 weeks (Sotolu 2010). This treatment group will be compared to control group (without laserpuncture exposure) (Kusuma et al. 2007).

Vitellogenin Level and GSI Analysis

A blood sample was taken in a tail blood vessel by dispossible squite from 4 samples after spawned (Taghizadeh et al. 2013). Estrogen level was analyzed by Enzyme Linked Immunosorbent Assay (ELISA) with user manual ELISA Kit Grouper Vitellogenin (VTG) (Catalog number: CSB-E14116Fh) (Taghizadeh et al. 2013). Meanwhile, the female catfish broodstock was scaled and operated to get the gonad. Gonad was also scaled. The data of body weight and gonad weight were used to determine GSI. The formula of GSI:

$$\mathrm{GSI} = \frac{\mathrm{Wg\ x\ 100\%}}{\mathrm{Wt - Wg}}$$

Note : GSI (Gonado Somatic Index), Wg (Weight of Gonad in grams), Wt (Weight of Bogy in grams) (Lefler et al. 2008).

The procedure described before was done on each week (from 0 – 8th week). We used 4 samples per treatment for each week.

Statistical Analysis

The present data were expressed as mean ± SD. The vitellogenin level and GSI data were analyzed using one-way analysis of variance (ANOVA) with Gen Stat version 15. Statistic significance was set at P value < 0.001 (vitellogenin level analysis) and P value < 0.05 (GSI).

RESULTS AND DISCUSSION

Plasma Vitellogenin Level of Catfish Broodstock at Post any Protein Level of Dietary along with Laserpuncture Exposure

Profile of plasma vitellogenin level after giving of 30%-40% protein level both in laserpuncture exposure and no laserpuncture exposure on female catfish broodstock during 8 weeks showed increased trend up to certain value and decreased. Plasma vitellogenin level of female catfish broodstock after giving of 40% protein both in laserpuncture exposure and no laserpuncture exposure is higher than 35% and 30% protein level (**Fig. 1**). According to Thome et al. (2005), Ibim and Sikoki (2015), 40% protein dietary level is ideal

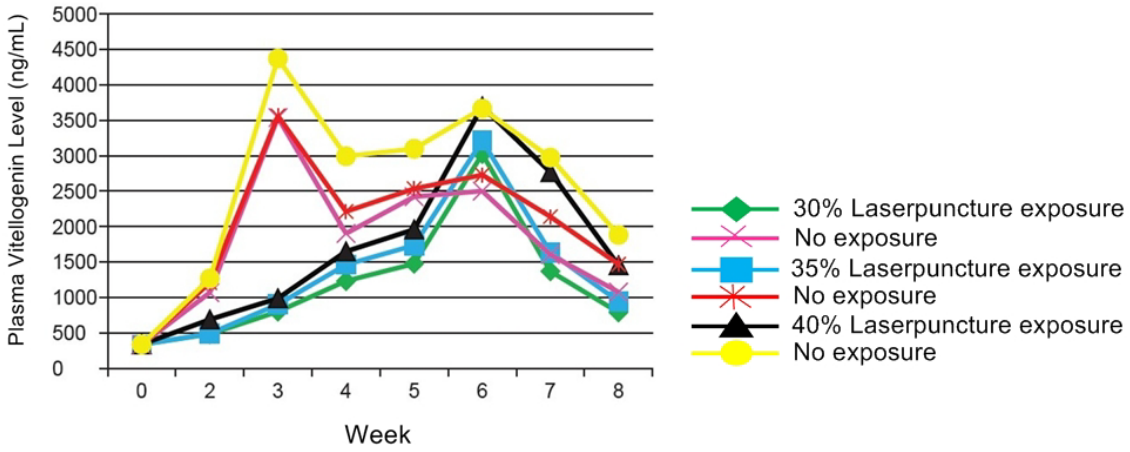

Fig. 1. Profile of Plasma Vitellogenin Level in Female Catfish Broodstock

Table 2. The peak value of vitellogenin level on catfish broodstock *(Claris sp)* at post any protein level of dietary (30%, 35%, and 40%)

Protein Level	Laserpuncture Exposure	Average ± SD of vitellogenin level (ng/mL)
30%	No exposure	3023.88 ± 20.160[a]
	Laserpuncture	3537.628 ± 29.709[a]
35%	No exposure	3211.460 ±7.063[a]
	Laserpuncture	3695.315 ± 37.838[a]
40%	No exposure	3562.815 ± 23.388[a]
	Laserpuncture	4371.735± 25.781[b]

Note: No exposure (3rd week); Laserpuncture exposure (6th week)

level to increase gonad development of African catfish by increasing of vitellogenin level.

This study showed that higher protein dietary level significantly (P<0.001) increase vitellogenin level. The 40% protein dietary level without laserpuncture exposure given between 2nd up to 6th week increase the vitellogenin level of female catfish broodstock. While, it moves to decrease between 7th and 8th week (**Fig. 1**).

Increasing and decreasing of plasma estrogen level is determined by oocyte development stages. The protein dietary level in catfish broodstock is involved in estrogen and vitellogenin synthesize. Availability of good nutrition increases estrogen activity and vitellogenin synthesize. According to Carrillo et al. (2000) and Abidin et al. (2006), the high protein dietary level increases protein level in the egg.

Increasing of vitellogenin level by high protein dietary level is caused by the role of protein as raw material to produce the reproductive hormone, vitellogenin, and yolk. The protein in the yolk is involved in oocyte and larvae development and maturation. Therefore, giving bhigh protein dietary level accelerates vitellogenin synthesis and increases plasma vitellogenin level. According to Ohkubo and Matsubara (2017) and Adewumi et al. (2005), the protein is also the main component in the yolk that contained in the oocyte. Availability of good nutrition supports reproductive process indicated by normal estrogen activity and vitellogenin synthesize.

The plasma vitellogenin level of 30% protein dietary along with laserpuncture exposure at 3rd week is relatively same as 40% protein dietary along with no laserpuncture exposure at 6th week. It means that laserpuncture exposure significantly (P<0.05) increase vitellogenin level in lower protein level equal to higher protein level without exposure. The peak value of vitellogenin level is showed on **Table 2**.

The result of this study is suitable with Hariani (2013) theory implied that 30% protein dietary along with laserpuncture exposure on the catfish broodstock increase plasma estrogen level. Estrogen is involved in regulating vitellogenin synthesize in hepatic. Generally, at 2nd and 3th week, the oocyte is in the previtellogenic stage. The vitellogenin level will gradually increase at 4th and 6th week. In this period, vitellogenin level reaches the peak value. Increasing of vitellogenin level is equal with increasing of estrogen level. Following this stage, vitellogenin level gradually decreases at 7th and 8th week. This period is named postvitellogenic stage because vitellogenin synthesizes in hepatic is stop. In this process, hypothalamus does not produce GnRH. It inhibits pituitary gland to produce GtH and decreases plasma estrogen and vitellogenin level.

In the postvitellogenic stage, estrogen and vitellogenin reach maximal value because of vitellogenin

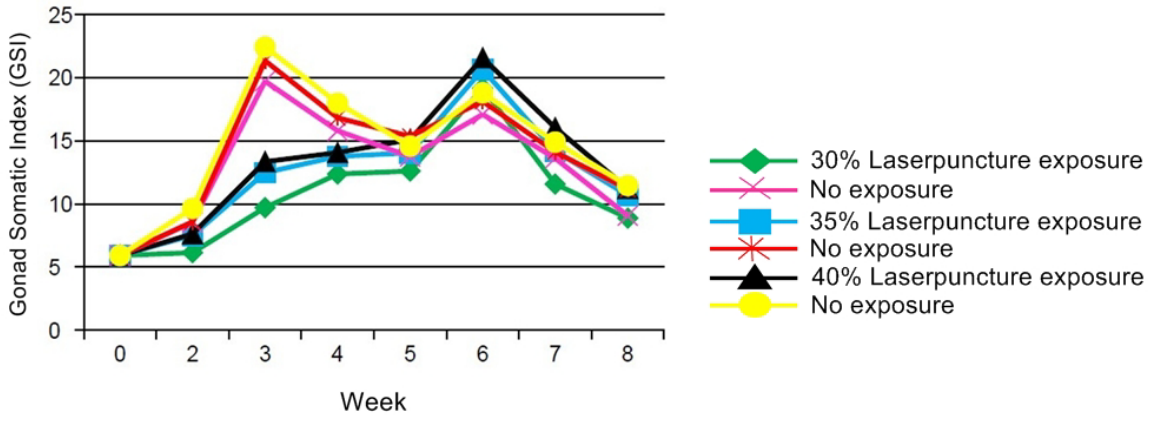

Fig. 2. Gonado Somatic Index (GSI) Value of Female Catfish Broodstock

accumulation in the oocyte. Vitellogenin will change to vitelin. In this stage, oocyte needs good quality and quantity protein supply to produce a good egg. The kind of vitellogenin protein involved in oocyte maturation and embryogenesis is lipovitellin and phosvitin. These proteins will be degraded to free amino acid. This amino acid is involved in oocyte maturation and embryogenesis.

Giving of protein dietary to catfish broodstock along with laserpuncture exposure is involved in oocyte development over time. At 2nd week (pre-vitelogenic stage), estrogen and vitellogenin initially produced. In the middle of the 2nd week up to 3rd week (vitellogenic stage), estrogen and vitellogenin level is high. Following this stage, in the middle of 3rd week up to post-vitellogenin, the estrogen and vitellogenin level reach the peak value. This data prove that laserpuncture exposure accelerates oocyte maturation period in 3 weeks faster. It accelerates previtellogenic and vitellogenic stage in oocyte maturation.

Estrogen level to produce vitellogenin synthesize from pre-vitellogenin to vitellogenin state is also determined by protein dietary level and laserpuncture exposure. Suitable with Kerdivel et al. (2013), this study showed that estrogen in hepatic stimulates vitellogenin synthesize. According to Aizen (2007), Pankhurst (2008) and Kapateh (2009), vitellogenesis is begun by GtH-I release from pituitary gland. GtH-I regulates to produce steroid hormone like estrogen in the gonad. Estrogen is involved in early development of gonad and vitellogenesis. In the postvitellogenic stage, oocyte maturates and release from the ovary (ovulation). Because of this process, the estrogen and vitellogenin level gradually decrease and inhibit GtH-I. In this stage, GtH-II stimulates oocyte maturation. According to Segawa et al. (2015), the estrogen level changing is linked to oocyte development. When oocyte maturates, estrogen and vitellogenin level gradually decrease. The gonad maturation is indicated by plasma vitellogenin level. In the postvetellogenic, the vitellogenin level reaches the maximal value (2011).

Gonad Somatic Index (GSI) of Catfish Broodstock at Post any Protein Dietary Level along with Laserpuncture Exposure

This study showed that highest increasing of GSI value is reached at 3rd week after spawning and gradually decreases. It increases again at 6th week after spawning in the laserpuncture exposure treatment group with 30%, 35% and 40% protein dietary level. At the 7th up tp 8th week, GSI value decreases again. Meanwhile, GSI value gradually increases in control group (no laserpuncture exposure) at all any protein level (30%, 35%, and 40%) between 0–6th week after spawning. After the 6th week, GSI value gradually decreases (**Fig. 2**).

Increasing of GSI value is concomitant with increasing of vitellogenin level. The protein dietary along with no laserpuncture exposure at 2nd and 3th week increase GSI value. In this period, the oocyte is in previtellogenic stage. The GSI value gradually increases between 4th– 6th week (vitellogenic stage). In vitellogenic stage, estrogen level is very high. This estrogen contain stimulates vitellogenin to synthesize in hepatic. This vitellogenin will be saved in oocyte and contained in the yolk. This condition increases GSI value. In the middle of 6th week up to 8th week (postvitellogenic stage), the GSI value gradually decreases (**Fig. 2**).

Gonad maturation is indicated by highest GSI value at 6th week. The vitellogenin accumulation in oocyte stimulate gonad maturation and increase GSI value. Hydrated oocyte increases gonad weight and GSI up to maximal value. This study has supported by previous research that implies GSI value reaches maximal value when oocyte has matured (Araoye 2001, Lalèyè et al. 2006, Shinkafi and Ipinjolu 2012).

In the previtellogenic stage, estrogen level is low but gradually increases at the vitellogenic stage and reaches a peak value at postvitellogenic stage. Based

Table 3. The peak value of GSI on catfish broodstock *(Claris sp)* at post any protein level of dietary (30%, 35%, and 40%) along with laserpuncture exposure (3rd week) and no laserpuncture exposure (6th week)

Protein Level	Laserpuncture Exposure	Average ± SD of GSI
30%	No exposure	19.17 ± 0.87[a]
	Laserpuncture	20.66 ± 0.99[b]
35%	No exposure	19.73 ± 0.82[ab]
	Laserpuncture	21.57 ± 0.99[cd]
40%	No exposure	21.35 ± 0.68[bc]
	Laserpuncture	22.45 ± 0.83[d]

on this trend, vitellogenin increased suitable with estrogen level. Hossenzade et al. (2013) showed that GSI value of female Sturgeon Persia fish *(Acipenser persicus)* gradually increases ovary development. Lefler et al. (2008) showed that GSI value reaches maximal value before spawning.

At 7th and 8th week, GSI value decreases because oocyte is in postvitellogenic stage (final stage of oocyte development). After this stage, estrogen and vitellogenin level will decrease and inhibit GtH-I release from pituitary gland and estrogen production. Decreasing of estrogen stimulates vitellogenin level and GSI value (**Fig. 2**). This study is suitable with previous research. It showed that decreasing of GSI value indicates that vitellogenesis is finished (Utomo et al. 2006).

The GSI values of 40% protein dietary without laserpuncture exposure at 6th week is relatively same as 30% protein dietary along with laserpuncture exposure at 3th week ($P<0.05$). It means that giving of 30% protein dietary level along with laserpuncture exposure has proven increase GSI value 3 weeks faster.

Table 3 show indicates that giving of protein dietary along with laserpuncture exposure significantly accelerates gonad maturation 3 weeks faster. It is indicated by the maximal value of GSI. Laserpuncture exposure on reproductive acupoint during 15 sec accelerates gonad maturation and increases GSI value (Hariani and Kusuma 2008, 2009, Kusuma et al. 2007). According to Cerda et al. (2007), GSI is used as an indicator of gonad catfish maturation.

The acceleration of reproductive physiological activity by laserpuncture exposure must be offset by good quality and quantity of dietary. Akankali et al. (2011) showed that dietary must be composed by sufficient protein, lipid, calcium, and phosphate to produce good yolk in the egg. It means that protein contain determines protein yolk in an egg. Yolk protein containing in egg is involved in oocyte growth and development and increase GSI value.

Increasing of GSI value is determined by protein dietary level and laserpuncture exposure. The catfish broodstock needs sufficient protein to maintenance oocyte growth and development, follicular formation, and yolk protein synthesize. The sufficient protein dietary in catfish is related to amino acid availability for gonad maturation. The previous study showed that the catfish broodstock needs at least 14.76% essential amino acid in reproductive physiological activity. Based on an essential amino acid test of dietary formula showed that 30% protein contains 13.29% essential amino acid, 35% protein contains 15.26% essential amino acid, and 40% protein contains 17.33% essential amino acid.

This study showed that 30% protein dietary level need 0.76% essential amino acid to synthesize normal vitellogenin level. The 35% protein dietary level is good (0.5% excess) to synthesize vitellogenin in the egg of catfish broodstock. While the 40% protein dietary level has 2.57% excess to synthesize vitellogenin. Although 40% protein dietary level is the best level to support good quality and quantity of egg, we must calculate it wisely because of some efficiency reasons.

This study showed that giving of 30%-40% protein dietary level to catfish broodstock is used as energy resources for body maintenance. Furthermore, it also used in somatic and gonad development. Ibim and Sikoki (2015) showed that giving of 40% protein dietary level in African catfish broodstock increase gonad weight and GSI value compared to lower than 30% protein dietary level. Kusuma (2013) showed that laserpuncture exposure on catfish broodstock accelerates gonad maturation and increases GSI value. The acceleration of gonad maturation indicated by increasing of GSI value is supported by good quality and quantity of dietary. It will determine yolk protein containing in the egg.

In conclusion, laserpuncture exposure along with 30% protein dietary at 3rd week is most effective to increase vitellogenin level and GSI value. This condition accelerates gonad maturation 3 weeks faster than no laserpuncture exposure treatment at any protein dietary level.

ACKNOWLEDGEMENTS

The authors would like to thank the Directorate General of Higher Education, the Minister of Research and Higher Education, Indonesia for funding this research through research grant. All authors also thank the Faculty of Medical Sciences, Hang Tuah University Surabaya for the support of research facilities.

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