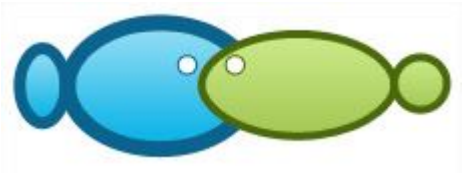

Latency time and egg hatching rate of sangkuriang catfish (*Clarias gariepinus*) using ovaprim hormone and broiler's hypophyseal extract combination

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Abstract. One of the efforts to promote the success of catfish production is hormonal engineering. The use of ovaprim hormone does not only push the broods to ovulate, but it is also related with fertilization, hatching, and larval production. Nevertheless, ovaprim hormone is expensive, IDR. 28,000-30,000 mL^{-1}, so that alternative material needs to be found using broiler's hypophysis to increase egg hatchability. This study aims to know the latency time and the hatching rate of sangkuriang catfish (*Clarias gariepinus*) using ovaprim hormone and broiler's hypohyiseal hormones. The study employed complete randomized design consisting of 5 treatments with 3 replications each. The treatments were ovaprim hormone of 0.3 mL kg^{-1} (100%) (P1), ovaprim hormone of 0.225 mL kg^{-1} (75%) + broiler's hypophysis extract of 125 mg kg^{-1} (25%) (P2), ovaprim hormone of 0.15 mL kg^{-1} (50%) + broiler's hypophysis extract of 250 mg kg^{-1} (50%) (P3), ovaprim hormone of 0.075 mL kg^{-1} (25%) + broiler's hypophysis extract of 375 mg kg^{-1} (75%) (P4), and broiler's hypophysis extract of 500 mg kg^{-1} (100%) (P5). Results showed that the application of ovaprim hormone and broiler's hypophysis extract had better latency time and hatching rate than that of 100% ovaprim hormone. The fastest ovulation latency time and the highest egg hatching rate were recorded in treatment P3, 7 hours 36 minutes and 77.9%, respectively.
Key Words: hormonal engineering, hypophysis, fertilization, larval production.

Introduction. Sangkuriang catfish (*Clarias gariepinus*) is one of the freshwater fish mostly consumed and cultured in Indonesia (Pratiwi 2014). The fish have advantages because they can live in high density condition, are resistant to disease, have good growth, and fast harvest time (Suraya et al 2016). Sangkuriang catfish need to be developed to increase culture production in the next several years. So far, catfish have contributed 10% to the national aquaculture production with a growth rate up to 17-18% per year (Shafrudin et al 2019). One of the important components in fish culture is seed availability. Fish fries are required in sufficient number and quality and always available when needed. Catfish are commonly bred in culture environment, but the management has still been natural and slow, so that yearly seeding productivity is not optimal yet.

To increase catfish production under intensive system, artificial spawning technique can be done in controlled and planned ways. Artificial spawning can yield more fertilized eggs than the natural one, so that more larvae and fries can be obtained (Mayyanti 2013). Artificial spawning can be done through hormonal stimulation, such as ovaprim. Ovaprim is a commercial product as combination of sGnRH-a (salmon gonadotropin hormon-analog) and antidopamine often used in fish spawning induction (Adawiyah et al 2019). The sGnRH-a functions as hypophyseal stimulator to release gonadotropin hormone (GtH), follicle stimulating hormone (FSH, GtH I) and luteinizing hormone (LH, GtH II) (Bosma et al 1997). FSH functions to regulate the yolk synthesis and gametogenesis in male fish, while LH acts to regulate the final stage of egg maturation and spermiation (Slater et al 1994; Moberg et al 1995; Mylonas & Zohar 2001). However, ovaprim hormone is expensive, ranging from IDR. 28,000 to 30,000 mL^{-1}, so that alternative material needs to be studied for sexual activity stimulation in order to reduce the use of ovaprim hormone in artificial spawning technique.

The use of hypophyseal extract has been successful in several fishes including *Clarias* spp. (Wadi et al 2018), but the difficulty to obtain the hypophyseal extract is still a constraint (El-Hawarry et al 2016). Therefore, alternative hypohyseal extract needs to be studied. One of these is broiler's hypophysis. Indonesia is one of the largest broiler-producing countries. Broiler's hypophysis also has ability to secrete GtH, FSH and LH (Andalusia et al 2008). Broiler's hypophysis has been used by Azhar & Masrizal (2007) to accelerate the latency time of catfish spawning. The doses used were 300, 400, 500, 700, and 800 mg kg^{-1}. Their finding indicates that broiler's hypophysis can accelerate the ovulation latency time of catfish *Clarias gariepinus* brood, with the best dose of 743.75 mg kg^{-1} (Azhar & Mazrizal 2007). Andalusia (2008) also added that the administration of broiler's hypophyseal extract at 500 mg kg^{-1} can increase fertilization and hatching rate of carp (*Carassius auratus*). Wadi et al (2018) found that the use of broiler's hypophyseal extract at the dose of 500, 800, and 1000 mg kg^{-1} did not affect the ovulation time, fecundity, egg diameter and egg performance, but 500 mg kg^{-1} of broiler's hypophyseal extract can be used in catfish spawning.

This study aims to know the latency time and the hatching rate of sangkuriang catfish (*C. gariepinus*) using the combination of ovaprim hormone and broiler's hypophyseal extract.

Material and Method. This study was carried out from January 15th to 17th, 2020 in Balai Perikanan Budidaya Air Tawar (Fish Hatchery) of Sungai Gelam, Jambi Province.

The experiment utilized 15 concrete containers of 200 cm x 40 cm x 100 cm at the water level of 50 cm, hypophysis scratcher, centrifuge (DSC 101SD), small plastic cup, wind-blower (ProACO 001), analytical balance (EMD SF-400 of 10.000Gx1 g/ 353ozx0.1 oz) max. and 320 max. gr/d = 0.1 mg, 1 mL-syringe, catheter, and Petri disk. Materials used were 15 mature female sangkuriang catfish, synthetic ovaprim hormone, and broiler's hypophysis extract taken from 40 days-aged chicken's head. Other materials were 96% alcohol and physiological solution, 0.9% NaCl.

This experiment utilized complete randomized design with 5 treatments of 3 replications. The treatments were 100% ovaprim hormone of 0.3 mL kg^{-1} (P1), 75% ovaprim hormone of 0.225 mL kg^{-1} + 25% broiler's hypophysis extract of 125 mg kg^{-1} (P2), 50% ovaprim hormone of 0.15 mL kg^{-1} + 50% broiler's hypophysis extract of 250 mg kg^{-1} (P3), 25% ovaprim hormone of 0.075 mL kg^{-1} + 75% broiler's hypophysis extract of 375 mg kg^{-1} (P4), and 100% broiler's hypophysis extract of 500 mg kg^{-1} (P5).

Broiler's hypophyseal gland was collected by opening the chicken's skull. It was washed in alcohol and stored in 96% alcohol until use. The hypophyseal gland was then weighed following the treatment doses, 125 mg kg^{-1}, 250 mg kg^{-1}, 375 mg kg^{-1}, 500 mg kg^{-1}, using an analytical balance, ground with glassware on the Petri disk, and then added with 0.9% physiological solution, 1.5 mL each. The hypophyseal extract was inserted into the flask and centrifuged at 3,000 rpm for 2-5 min, and two layers were formed, clear liquid and precipitate. The clear liquid was then taken for use (Efrizal et al 1998). On the other hand, the ovaprim hormone was prepared at the doses of 0.075 mL kg^{-1}, 0.15 mL kg^{-1}, 0.225 mL kg^{-1}, and 0.3 mL kg^{-1}.

Furthermore, injection to the broods was conducted on the dorsal fin in the middlle of the body at the syringe angle of 40-45^{o}C and the needle depth of ±1 cm or situated with the fish body size. After ovaprim hormone had been injected, the syringe was taken out and the injection mark was pressed with finger for few seconds to sustain the ovaprim inside the body. The injection to the test animals was done once at the set dose, then the fish were returned to the rearing tank and left for 6 hours to wait for egg collection process (Sinjal 2014). Ovulation observation was carried out after 6 hours of injection. The test fish are said to have ovulation, when the abdomen is pressed toward the cloaca, eggs will go out through the genital hole. If there was no indication of ovulation, the next check was done each 30 min until all eggs were released. Based on preliminary observation, the ovulation time is recorded as the interval between injection time and ovulation time (Harianti 2013). Latency time and hatching rate were observed after the fish had ovulated using 200 egg samples. Parameters observed in this study were latency time of ovulation and the hatching rate.

Ovulation latency time was calculated as the interval between the end of injection time and the ovulation time following Effrizal et al (1998):

Latency time (hour) = ovulation time – end of injection time

Egg hatching rate or the percent of hatching eggs was calculated following (Efrizal et al 1998):

$$\text{Hatching rate} = \frac{\text{Total number of eggs}}{\text{Number of egg samples}} \times 100$$

Water quality parameters observed were water temperature, dissolved oxygen (DO) and pH. Water quality conditions followed Zonneveld (1991), 25-30^{o}C for temperature, DO > 3 mg L^{-1}, pH of 6.5-8. Water quality parameter measurements used thermometer and water test kit (Table 1).

Table 1

Water quality measurement method

Parameter	*Unit*	*Method*	*Remark*
Temperature	^{o}C	ZT 102 Digital thermometer	In situ
Dissolved oxygen	Mg L^{-1}	AZ8403 Digital DO meter	In situ
pH	-	EZ9901Digital pH meter	Ex situ

To know the effect of ovaprim hormone and broiler's hypophyseal extract on the egg hatching rate of the sangkuriang catfish, ANOVA was applied at the significance level of 5%, then continued with Duncan New Multiple Range Test (DNMRT). Other data supporting the study were descriptively analyzed.

Results and Discussion

Ovulation latency time. Injection of 50% ovaprim hormone and 50% broiler's hypophysis mixture to sangkuriang catfish needed 7 hours and 36 min to induce ovulation. This finding had 2 hours and 24 min faster than that of 100% ovaprim hormone injection (Figure 1).

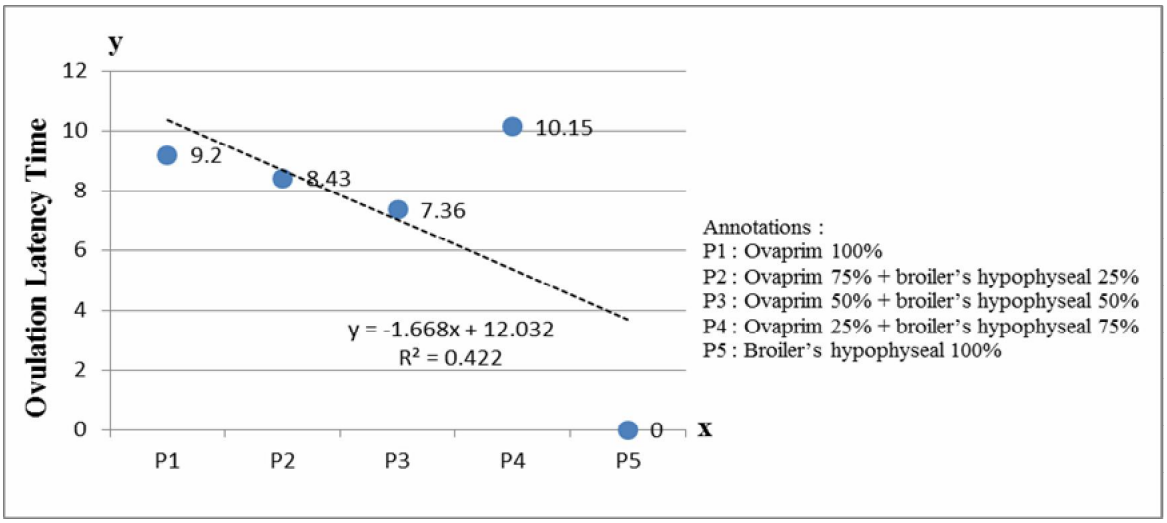

Figure 1. Latency time of sangkuriang catfish ovulation.

The use of 100% broiler's hypophysis extract (P5) did not yield ovulation at all, and the use of 25% ovaprim hormone + 75% broiler's hypophysis extract (P4) did not make all fish ovulate, only one of the three replications. It could result from that the dose used is not appropriate or LH content in the broiler's hypophysis extract is not enough to make

the catfish ovulate, since the broiler used as donor is 40 days old with low reproductive activity and in the period of reproductive organ completion.

Muhammad et al (2001) explained that the ability of fish ovulation is highly related with effective hormone dose usage. According to Azhar & Masrizal (2007), LH functions to stimulate ovulation and spawning in adult female fish. Experiments using the catfish *C. gariepinus* found that LH stimulation induced with gonadotropin (GnRH, LHRH) is inhibited by dopamine (DA) through DA2 receptor at the hypophyseal level and antagonic DA2 receptor, such as domperidone, pimozide, and etc., increases GnRH activity so that ovulation could occur (Chang & Peter 1983; Lin et al 1986; De Leeuw et al 1987; Goos et al 1987; Peter et al 1988; Peter & Yu 1997; Yaron et al 2003; Dufour et al 2005; Zohar et al 2010). Therefore, the combination of GnRH analog and DA2 receptor inhibitor (Linpe method) has been commonly used to induce pre-ovulation LH increment to start gonad maturation and ovulation. Linpe method is a method utilizing antidopamine action as dopamine inhibitor to inhibit LH secretion (Peter et al 1988; Peter & Yu 1997) that is firstly successful in carp culture cooperative in China.

The present study found that mature catfish injected with a combination of 50% ovaprim and 50% broiler's hypophysis extract had the best outcome. Ovulation in treatment 3 (P3) of this ovaprim and broiler's hypophysis extract combination could result from their synergic effect, in which ovaprim works in stimulating the fish ovulation, since ovaprim containing sGnRHa hormone can secrete gonadotropin (GnRH) that directly stimulates the pituitary gland to secrete the LH, while antidopamine in ovaprim functions to block dopamine inhibiting LH secretion (Adawiyah et al 2019). The produced gonadotropin will head to the gonad. Gonadotropin contains FSH that plays important role in vitelogenesis and oocyte development (Adawiyah et al 2019). The broiler's hypophysis extract also has activity to secrete gonadotropin hormone (FSH and LH) (Andalusia et al 2008). According to Wadi et al (2018), broiler's hypothysis extract can enlarge the egg diameter of sangkuriang catfish. Nur et al (2017) stated that bigger egg diameter occurs at the treatment combination of Hcg 500 IU and 0.7 mL ovaprim kg^{-1} body weight. It could result from the role of Hcg to accelerate the egg maturity that could more quickly be ovulated. Ovulation will occur if vitellogenesis is perfect. The accepted model for GTH role in fish indicates that FSH is involved in early gametogenesis regulation and vitellogenesis, while LH stimulates final stage of oocyte maturation and ovulation in female and spermiation in male (Levavi-Sivan et al 2010).

ANOVA revealed that the use of ovaprim hormone and broiler's hypophyseal extract combination influenced the ovulation latency time of sangkuriang catfish at the significance level of 0.05%. Duncan (DNMRT) test indicates that treatments P5 and P4 have no significantly difference effect, but P5 has significantly different effect from treatments P1, P3, and P2 (Table 2). Based on these findings, the brood of sangkuriang catfish injected with 50% ovaprim and 50% broiler's hypophyseal extract mixtures gives the best result. Hollander-Cohen et al (2017) described that two gonadotropins, FSH and LH, play major role at the axis of hypothalamus-hypophysis-gonad (HPG).

Table 2

DNMRT Duncan test on ovulation latency time

Treatment	*Mean*	*Significance level (5%)*
100% broiler's hypophysis extract (P5)	0	a
25% ovaprim + 75% broiler's hypophysis extract (P4)	3.38	ab
50% ovaprim + 50% broiler's hypophysis extract (P3)	7.36	bc
75% ovaprim + 25% broiler hypophysis extract (P2)	8.43	c
100% ovaprim (P1)	9.2	c

Note: similar alphabet in the same column indicates not significant difference.

Egg hatching rate (%). This study found that the highest hatching rate was recorded in treatment P3 (50% ovaprim + 50% broiler's hypophysis extract), 77.9%. It is evident that the use of ovaprim hormone and broiler's hypophyseal extract combination was better than that of 100% ovaprim hormone (Figure 2).

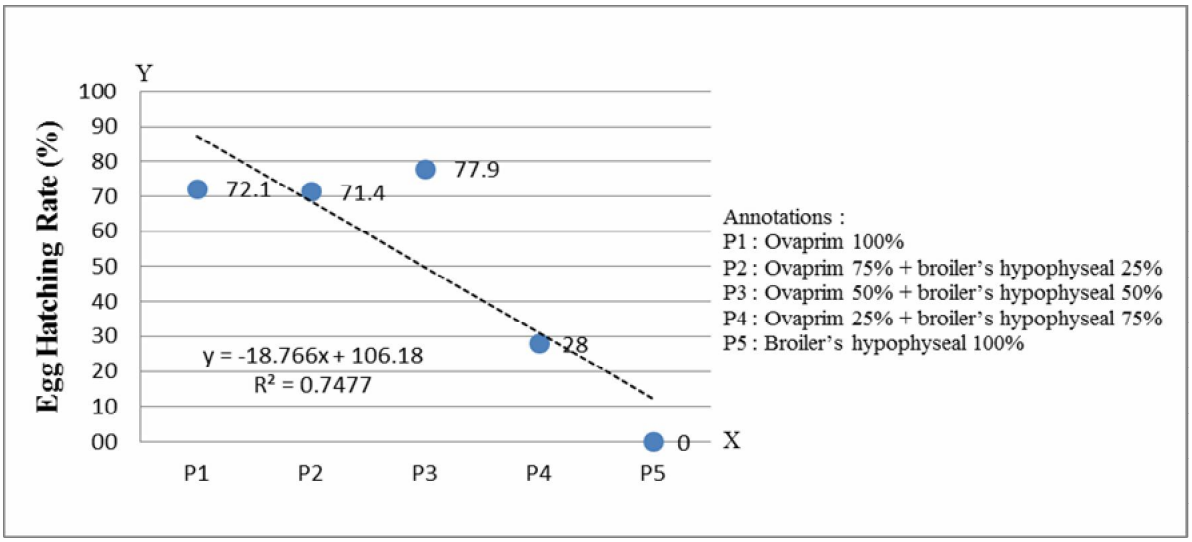

Figure 2. Egg hatching rate of sangkuriang catfish (%).

Figure 1 demonstrates that the egg hatchability in treatment P3 is better than that in treatment P5. It could be caused by the effect of ovaprim and broiler's hypophysis that hold FSH to support the final stage maturation of the fish oocyte. ANOVA revealed that the use of ovaprim hormone and broiler's hypophyseal extract combination significantly influenced the egg hatching rate of sangkuriang catfish at the significance level of 0.05. DNMRT test indicates that treatment P5 is significantly different from P3, P1, and P2, but non-significantly different from P4 (Table 3).

Table 3

DNMRT Duncan test on egg hatching rate

Treatment	*Mean*	*Significance level (5%)*
100% broiler's hypophysis extract (P5)	0	a
(25% ovaprim + 75% broiler's hypophysis extract (P4)	28	ab
50% ovaprim + 50% broiler's hypophysis extract (P3)	77.9	b
75% ovaprim + 25% broiler hypophysis extract (P2)	71.4	b
100% ovaprim (P1)	72.13	b

Note: similar alphabet in the same column indicates not significant difference.

The highest hatching rate was recorded in treatment P3. It could result from the use of ovaprim hormone and broiler's hypophyseal extract combination containing FSH hormone that plays important role in the final stage of oocyte maturation in fish. Sahoo et al (2007) and Nandeesha et al (1990) stated that ovaprim is a combination of salmon Gonadotropin Releasing Hormone analogue (sGnRHa) with antidopamine in which each 1 mL of ovaprim contains 20 µg of sGnRHa (DArg6, Trp7, Leu8, Pro9-NET)-LHRH and 10 µg of antidopamine that functions in the final stage maturation of the fish oocyte. It is in line with Sugistia et al (2017) that the use of ovaprim hormone does not only support the broods to ovulate, but it is associated with fertilization, hatching and larvae produced as well. Andalusia et al (2008) also stated that the addition of broiler's hypophyseal extract could not speed up the latency time, but it could increase the fertilization and hatching success. Besides the hormonal effect on the fish brood, there are several factors affecting the hatching success. Oyen et al (1991) described that internal factor influencing the egg hatching rate is delayed embryonic development due to poor quality of spermatozoa and ovum, while the external factor is environmental factors, such as water temperature, DO, pH, and ammonia concentration. According to Kucharczyk et al (2019), fish sperms usually have sufficiently short motility period after the activity before loss of their viability. After the contact with water or liquid activator, the sperms generally lose the

activity for motility and egg fertilization in few seconds (Cejko et al 2013, 2016). Biegniewska et al (2010) reported that sperms of African catfish lose their motility capacity in very short time after activation. Several species need the longest time of 180 seconds of the egg activation to fertilization, in which there are no reduced fertilized eggs during this period (Kucharczyk et al 2016). Baidya & Senoo (2004) also added that egg hatching rate is also influenced by egg quality or overmaturation. Delayed egg stripping after ovulation causes an overmaturation phenomenon that can make low fertilization rate and hatching with increased number of flawed larvae (Sakai et al 1975; Springate et al 1984; Legendre & Oteme 1995). During the overmaturation, the egg experiences morphological and composition alteration and loss of progressive viability (Sakai et al 1975; Springate et al 1984).

Water quality. Water quality plays an important role in reproductive process and egg hatching. It, in general, occurs faster in higher temperature, since metabolism occurs faster at higher temperature, so that the embryonic development is faster. Water quality data measured in this study are presented in Table 4.

Water quality conditions are in the safe range for spawning and hatching of sangkuriang catfish (Table 4). Water temperature is an important factor in influencing the embryonic development, egg hatching rate, and yolk absorption rate. Low water temperature causes the enzyme (chorion) unable to work well on the egg skin and make the embryos need longer time to dissolve the egg skin, so that the embryo will hatch longer. In contrast, high temperature could cause premature hatching and it makes the larvae have low survival.

Table 4

Water quality measurements

Parameter	*Treatment*					*Range*
	P1	*P2*	*P3*	*P4*	*P5*	
Temperature (^oC)	27.28	27.28	27.7	27.7	27.7	25-30^oC (Zonneveld 1991)
DO (mg L^{-1})	4.6	7.8	7.1	6.8	6.6	> 3 mg L^{-1} (Zonneveld 1991)
pH	6.7	6.8	6.6	6.7	6.6	6.5-8 (Zonneveld 1991)

Eggs need enough oxygen for their survival. Oxygen diffusively goes into the eggs through the surface layer of the egg shell. The measurements showed that dissolved oxygen in the water was safe during the experiment and supported the egg hatching.

Water pH in culture media also affects the egg hatching, but pH level could also influence the toxicity of chemical compounds. Ionized ammonium is usually found in low pH waters. Ammonium is not toxic, but at high pH, more unionized ammonia (NH_3) are found and toxic. Optimum fish egg hatching occurs in alkaline waters. The pH value for egg hatching ranges from 6.5 to 8 (Zonneveld et al 1991).

Conclusions. Combination of ovaprim hormone and broiler's hypophyseal extract influenced the latency time with the fastest time in treatment P3 (ovaprim 50% + broiler's hypophysis extract 50%), 7 hours and 36 minutes. This finding yielded ovulation time of 2 hours and 24 minutes faster than the use of only 100% ovaprim hormone, while the use of 100% broiler's hypophysis extract gave no ovulation in sangkuriang catfish. Similarly, the highest effect on egg hatching rate was recorded in the same treatment (P3), averagely 77.9 %. Water quality conditions during the study gave good support on the egg survival up to hatching.

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