Pregnant mare's serum gonad hormones and anti-dopamine treatments on the maturation of snakehead (*Channa striata*)

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Abstract. The application of hormonal treatments to accelerate fish maturation has been well implemented in aquaculture. However, the optimal results of hormonal treatment much depend on target species relation to dose, frequency and time periods of implementation. This research aimed to determine the optimal dose of a combination of hormone from pregnant mare serum gonad (PMSG) hormone and anti-dopamine (AD) to induce the gonad maturation on snakehead (*Channa striata*). Hormonal treatments of 1.5 mL, 1.75 mL, and 2 mL per kg of body weight were induced intramuscularly, in the dorsal part of broodstocks with a mean weight of 261.74 ±6.38 g, and a mean length of 32.9±1.9 cm, with 3 fish in each treatment. The effects of the treatments on gonadal maturation were observed, egg diameter was measured and estradiol-17β concentrations were determined every 30 days for 90 days. The results showed that the PMSG+AD dose of 2 mL kg^{-1} body weight produced an egg diameter of 1.45±0.023 mm, significantly different from the other treatments, 1.32±0.073 mm for the dose of 1.5 mL kg^{-1} body weight and 1.33±0.035 for the dose of 1.75 mL kg^{-1} body weight. Congruent with egg diameter, the dose of 2 mL kg^{-1} body weigth produced the highest estradiol-17β concentration, 105.07±0.550 ng mL^{-1} ($P<0.05$), while the concentration were 79.07±0.378 ng mL^{-1} for the dose hormone of 1.5 mL kg^{-1} body weight, and 75.03±0.585 ng mL^{-1} for the dose hormone of 1.75 mL kg^{-1} body weight. The study concludes that the highest dose of hormone treatment enables better results, but the optimum dose still needs to be explored.

Key Words: AD, estradiol-17β, hormonal application, induced breeding, PMSG.

Introduction. Snakehead (*Channa striata*) is an economically important native freshwater fish in Indonesia (Gustiano et al 2013). This species is well known for its high nutritional content in meat, as well as albumin monomer protein. Albumin is very important for pharmaceutical uses, in helping healing different types of wounds and even cancer (Yaakov & Ali 1992; Firlianty et al 2013; Chasanah et al 2015). On the market, snakehead price ranged between 4 and 6 USD per kg in 2016 (Kusmini et al 2016) and recently, it increases to 6-10 USD per kg. Snakehead production in Indonesia was 36.155 tons in 2017, with 99.35% of production from capture fisheries (MMAF, 2018). Kalimantan Island is the largest producer of snakehead followed by Sumatera and Java regions; the snakehead production from these regions in 2010 and 2017 increased from 86% to 95% of total production (MMAF 2018).

The high demand of snakehead threatens the natural population in the wild due to overfishing. In the future, breeding and culture will be needed to keep the natural population richness and market supply. Various studies of snakehead in Indonesia have been published since the last decade on population genetics, reproduction (Kartamihardja 1994; Saputra et al 2017; Gustiano et al 2019), or breeding (Ath-thar et al 2017; Gustiano et al 2019). Even though breeding technology has been developed, it is still much influenced by the season. Thus, well domesticated broodstock with the capability to spawn throughout the year should be established.

Hormonal treatment to accelerate broodstock maturation is already widely implemented on various cultured species. This method is an essential technique for maintaining the sustainability of intensive aquaculture production (Mylonas et al 2010). Several common hormones that induce fish maturation and spawning are gonadotropin hormone, human chorionic gonadotropin (HCG), pregnant mare serum gonadotropin (PMSG) and anti-dopamine (AD) (Ferchaud et al 2010). PMSG containing follicle-stimulating hormone (FSH) plays an important role in the initiation of gametogenesis and gonadal maturation (Nagahama & Yamashita 2008). Dopamine inhibits the release of hormones from the pituitary involved in the action of gonadotropin-releasing hormones (GnRH) and the release of gonadotropins (including FSH and LH - luteinizing hormone) (Fontaine et al 2013). To address this mechanism, AD is mainly used to block the dopamine inhibitor system and to elevate GnRH secretion (Zohar et al 2009; Mehdi & Ehsan 2011).

The use of a combination between PMSG and AD to enhance gonadal maturation in freshwater fish has been carried out on the climbing perch (*Anabas testudineus*) (Rozikin et al 2016), mahseer (*Tor soro*) (Farastuti et al 2014), Asian catfish (*Pangasius hypopthalmus*) (Agustinus 2013; Arfah et al 2018), Indonesian shortfin eel (*Anguilla bicolor*) (Ahlina et al 2015), African catfish (*Clarias* sp.) (Nainggolan et al 2015), and redfin (*Epalzeorhynchos frenatus*) (Islami et al 2017).

The results of hormone treatments depend on the target species, applied doses, frequency of induction, time period of implementation. The most important points in hormonal therapy for fish reproduction are the dose of hormone used and the stage of ovarian development (Duncan et al 2013). Hutagalung et al (2015) reported that the best dose of PMSG and AD administered in snakehead was 1.25 mL kg^{-1}, with the average value of the gonadosomatic index (GSI) of 3.35%, and hepatosomatic index (HSI) of 1.37%, but the study did not refer to the level of gonad maturity of target fish. According to Ath-thar et al (2017), a dose of 1.5 mL kg^{-1} of PMSG combined with AD enable a better acceleration in maturation of the gonad. The objective of the present study is to determine the optimal dose hormone combination between PMSG and AD to induce the gonad maturation of the snakehead.

Material and Method

Broodstock. The broodstocks of snakehead were collected from a fish farm in Bogor Regency, West Java Province, Indonesia. They were then transferred to the research station of the Research Institute for Freshwater Aquaculture and Fisheries Extension, in Cijeruk, Bogor. All the broodstock were reared in a concrete pond, with the size of 8x5x1 and fed with commercial pellets (3% body weight), twice per day. After a month, 9 mature females with an average weight of 261.74±6.38 g and a mean length of 32.9±1.9 cm were selected for the experiment. Subsequently, the selected females were marked with electronic tags to regularly evaluate the gonadal development of each fish every month.

Induced hormonal treatment. Hormonal treatments administrated on the target broodfish contained PMSG and AD. Prior to hormonal manipulations, fish were anesthetized with fish stabilizer anesthetic at a dose of 1 mL per 4 L of water. Hormonal treatments in 3 different doses (1.5 mL, 1.75 mL, and 2 mL kg^{-1} body weight) were induced intramuscularly in the dorsal part of the fish (3 fish per treatment), using a hypodermic syringe. Hormonal injections were conducted repeatedly, once every month. Fish observation was conducted every 30 days during the 90 days of experiment, starting from September to November. The experiment used a completely randomized design.

Observation of egg diameter and estradiol-17β concentration. The parameters observed in determining gonadal maturation were the egg diameter and the estradiol-17β concentration in the blood plasma of examined broodfish. The indication of gonadal maturation process in fish reproduction could be observed based on the 17ß-estradiol and vitellogenin content in the blood plasma (Baumann et al 2013; Tirado et al 2017). Egg diameter and estradiol-17β concentrations were sampled every 30 days prior to the hormonal injection. The eggs were observed by the cannulation method for all fish in each treatment. 30 eggs were measured to determine the diameter under a binocular microscope. To determine the estradiol-17β concentration, 1 mL of blood plasma was collected using a 3 mL syringe with anti-coagulant from caudal venipuncture. Blood plasma was then stored in a 1.5 mL microtube and placed in a cool box. In the laboratory, blood samples were centrifuged at 10000 rpm for 10 minutes to collect the supernatant, and stored in a freezer at -4°C for the ELISA analysis of estradiol-17β concentration.

Data analysis. Data was statistically analyzed using Microsoft Excel 2016 and SPSS version 25. The egg diameter and the estradiol-17ß levels were presented descriptively to determine egg diameter development and changes of 17ß-estradiol level based on a different dose of hormonal treatments.

Results and Discussion

Egg diameter development. Different doses of hormonal treatments with a combination of PMSG hormone and AD triggered the early stages of gonad maturity to develop. There was a significant difference in the egg diameter between the 2 mL kg^{-1} body weight hormone combination treatment and the other treatments ($P<0.05$). The broodstock induced with 2 mL hormone combination produced the largest egg diameter (Table 1).

Table 1

Egg diameter (mm) resulted from different doses of pregnant mare serum gonad hormone and anti-dopamine treatments on *Channa striata*

Hormone dose	*N*	*Initial egg diameter (mm)*	*Final egg diameter (mm)*	*Mean egg diameter (mm)*
1.5 mL kg^{-1}	3	0.48 ± 0.087^a	1.32 ± 0.073^b	0.84 ± 0.015^b
1.75 mL kg^{-1}	3	0.51 ± 0.023^a	1.33 ± 0.035^b	0.81 ± 0.018^b
2 mL kg^{-1}	3	0.47 ± 0.021^a	1.45 ± 0.023^a	0.98 ± 0.015^a

Note: different superscripts show significant differences ($P<0.05$).

The development of egg diameter had a similar pattern in all treatments. The egg diameter increased in line with the time of rearing. Generally, up to 30 and 60 days of rearing, the egg diameter tended to grow quickly (Figure 1), then slowed to the end of the experiment. The results illustrated that the majority of egg diameters reach optimum size about 1.45±0.023 before 90 days (Marimuthu et al 2007). The optimal dose hormone treatment produced a consistently bigger egg diameter than other treatments.

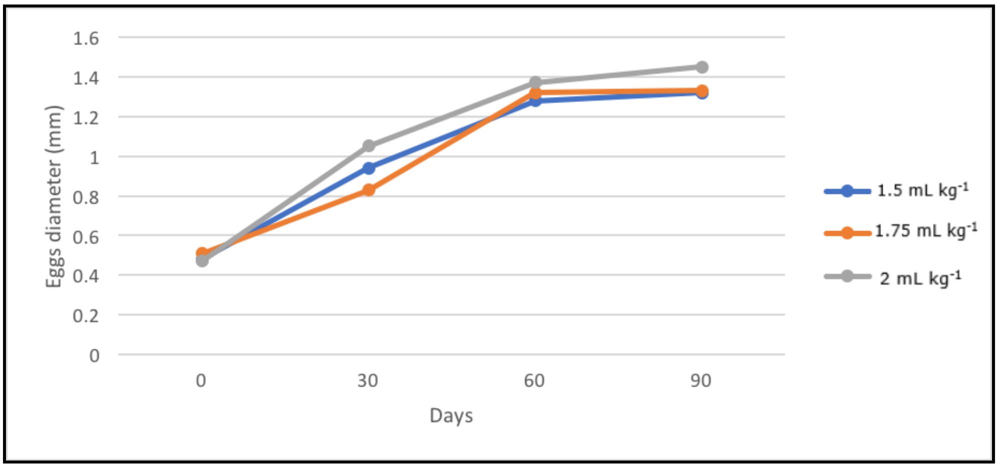

Figure 1. The development of egg diameter (mm) induced with different dose of combination of pregnant mare serum gonad hormone and anti-dopamine.

Estradiol-17β concentration. The effect of different doses of hormone treatments on the estradiol-17β concentration generally showed a similar pattern, within 90 days of observation. Estradiol concentration gradually increased up to the 60th day, except for the dose of 1.5 mL kg^{-1}, which increased until the 30th day, and declined until the 90th day (Figure 2). The dose of 2 mL kg^{-1} body weight showed the highest estradiol-17β concentration among the treatments at the 60th day ($P<0.05$).

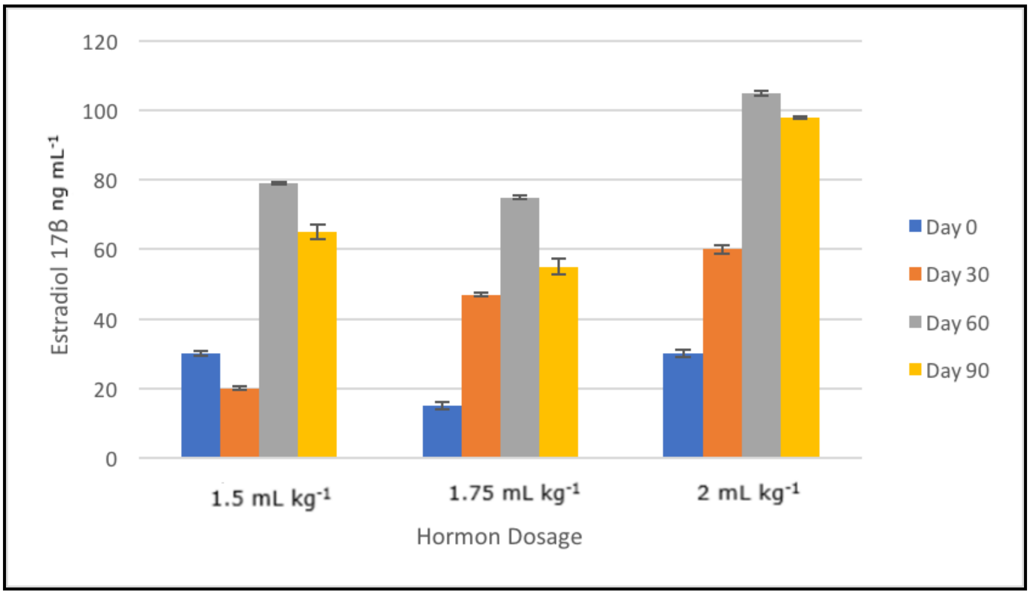

Figure 2. The performance of estradiol-17β concentration resulted from different doses of hormonal treatments on *Channa striata*.

The egg diameter recommended for the success of spawning activity in the snakehead is ranged from 1.2-1.4 mm (Marimuthu et al 2007). The final egg diameter of the snakehead broodstock in the present study (1.45 mm) was bigger than the egg diameter of *C. striata* observed in Pakistan (1.3 mm) and Malaysia (1.33 mm) (Ghaedi et al 2013; Narejo et al 2015), but it was smaller than the egg diameter of snakehead in India (1.53 mm) (Sakhare 2015). The stage of maturity in fish can be observed from the development of egg diameter indicated by the vitellogenesis process. This process can be accelerated by hormonal induction, which plays a vital role in early gonad maturation or vitellogenesis (Rottmann et al 1991; Nagahama et al 1995; García Ayala et al 2010). The effect of hormone treatments on egg maturation in fish is essential to control reproduction out of natural habitats and seasons. Some studies reported that in Indonesia (Java, Sumatra, Kalimantan and Sulawesi Island) various gonad maturation stages of snakehead occur throughout the year (Kartamihardja 1994; Makmur 2006; Harianti 2013). Thus, it is assumed that snakehead is able to spawn throughout the year. Based on the observations of egg diameter, fecundity and GSI as an indicator of reproduction status, the peak season of optimum gonad maturation in nature occurs once within a year (Bijaksana & Balantek 2016). Saputra et al (2017) support the previously mentioned study, stating that the peak of snakehead spawning in nature occurs in the rainy season.

The right time to implement hormone treatment for gonad maturation is several months before spawning season starting from October every year. The proper understanding of the reproductive cycle could be very useful to manage gonad maturation of fish and to control the mass spawning for fry production. Using the effective hormone and the optimum dose, the spawning of snakehead broodstock enables a periodical management. Theoretically, the reproductive hormone mechanism of fish in the spawning season is controlled by connection of the brain - the hypothalamus-pituitary - gonad axis (Rottmann et al 1991; García Ayala et al 2010). Environmental signals such as rain, temperature, and media are received by the brain and forwarded to the hypothalamus (Bromage et al 2001; Cardinaletti et al 2010). The hypothalamus responds by releasing gonadotropin-releasing hormone (GnRH) and dopamine that affect the pituitary gland. Furthermore, gonadotropin hormone contains follicle stimulating hormone (FSH) and luteinizing hormone (LH) that act on gonads as target organs.

The elevated estradiol-17β concentration indicates the start of gonad developmental stage and gonadosomatic index improvement (Lee & Yang 2002). It can be suggested that the increase in estradiol concentration will be followed by increasing the size of the egg diameter. The present study showed that the largest egg diameter was in the 2 mL kg^{-1} body weight hormone treatment, whereas the highest concentration of the estradiol-17ß was 105 ng mL^{-1}. The estradiol-17ß is generally used as an indicator of vitellogenin synthesis in the detection of the level of maturity in fish based on oocyte formation, ovarian growth, and steroidogenesis (Shappell et al 2010; Dammann et al 2011; Chatakondi & Kelly 2013). The gradual increase of estradiol-17ß in blood plasma during vitellogenesis is in line with the increasing ovarian weight and it mainly reaches a peak in the pre-spawning stage (Ghosh et al 2016). Based on Figure 2, the increasing level of estradiol-17ß in the blood plasma of mature snakehead broodstock was predicted to reach the peak of vitellogenesis process on 60^{th} day maturation stage and subsequently, in 90^{th} day, the concentration of estradiol-17ß decreased due to the absorption of vitellogenin by oocytes in the final maturation stage. The estradiol-17ß level in fish commonly decreases after gonad maturation, being indicated by the completion of the vitellogenesis process (Reading et al 2017; Zupa et al 2017).

Conclusions. The combination of PMSG and AD hormone of 2 mL kg^{-1} body weight for *Channa striata* enables the best results among the doses implemented, with the egg diameter of 1.45±0.023 mm and estradiol concentration of 105.07±0.550 ng mL^{-1}. The doses still needed to be explored to determine whether a better dose exists (possibly over 2 mL kg^{-1} body weight).

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