

Original article

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Hormonal induction maturation of silver pompano *Trachinotus blochii*

Induksi maturasi secara hormonal pada ikan bawal bintang *Trachinotus blochii*

Wiwin Kusuma Atmaja Putra^{1*}, Tengku Said Raza¹

¹Aquaculture Departement, Marine Science and Fisheries Faculty, Raja Ali Haji Maritime University
Politeknik Streat, Senggarang, Tanjungpinang, Riau Island

*Corresponding author: wiwin.bdp@umrah.ac.id

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ABSTRACT

This study was aimed to acquire the most suitable hormone treatment to induce gonad maturation of silver pompano *Trachinotus blochii*. This study used a completely randomized design with four treatments and three replications, i.e NaCl 90% (control) (0.5 mL/kg), hCG (20 IU/kg fish body weight), PMSG (20 IU/kg fish body weight), and PG600 (20 IU/kg fish body weight). Parameters measured were gonadosomatic index (GSI), hepatosomatic index (HSI), gonad maturity level (TKG), gonad histology, and absolute growth. The best result was PG600 treatment with 0.12% gonadosomatic index, 2.29% hepatosomatic index, TKG IV, and 85 g absolute growth for four weeks. This study concludes that the most suitable hormone to induce gonad maturation of silver pompano *Trachinotus blochii* is PG600 with 20 IU/kg body weight, which effectively affects GSI, HSI, and absolute growth of silver pompano.

Keywords: hCG, maturation, PG600, PMSG, silver pompano.

ABSTRAK

Penelitian ini bertujuan mendapatkan hormon yang terbaik untuk menginduksi maturasi gonad ikan bawal bintang. Hormon yang digunakan pada penelitian ini adalah kontrol NaCl 90% (dosis 0,5 mL/kg), hCG (dosis 20 IU/kg bobot tubuh ikan), PMSG (dosis 20 IU/kg bobot tubuh ikan), dan PG600 (dosis 20 IU/kg bobot tubuh ikan). Parameter yang diamati adalah indeks gonadosomatik (GSI), indeks hepatosomatik (HSI), tingkat kematangan gonad (TKG), histologi gonad, dan pertumbuhan bobot mutlak. Hasil terbaik adalah perlakuan PG600 dengan gonadosomatik indeks (0,12%), hepatosomatik indeks (2,29%), TKG IV, dan pertumbuhan mutlak (85 g) selama empat minggu. Kesimpulan penelitian ini adalah perlakuan hormon yang berpotensi menginduksi maturasi gonad ikan bawal bintang adalah hormon PG600 dengan dosis 20 IU/kg bobot tubuh dikarenakan adanya pengaruh nyata terhadap parameter GSI, HSI dan pertumbuhan mutlak ikan bawal bintang.

Kata kunci: ikan bawal bintang, hCG, PMSG, PG600, maturasi.

INTRODUCTION

Silver pompano is one of superior commodities in Riau Islands Province. Problems on silver pompano culture in Riau Islands Province are low fingerling supply, insufficient fingerling production in culture center or fish farmers, limited spawning period (silver pompano only spawns on moon period), as well as limited matured broodstocks. These problems can be overcome by maximizing the broodstock production and improving the gonad maturation through a hormonal induction. Hormonal induction is performed as this technology minimizes the natural factors influencing the reproductive hormone performance of silver pompano to induce the gonad maturation process compared to the natural signals and nutrients. Gonad maturation is a gonad development before ovulation and spawning controlled by gonadotropin I hormone (follicle stimulating hormone (FSH)).

Gonadotropin hormones are hormones that act in the gonad maturation process of fish. Gonadotropin hormones are divided into two types, namely follicle stimulating hormone (FSH) and luteinizing hormone (LH). The gonad maturation rate can be triggered by inducing the fish with FSH and LH hormone. FSH and LH hormone can be obtained from commercial products, such as human chorionic gonadotropin (hCG), pregnant mare serum gonadotropin (PMSG), and PG600. PMSG hormone contains FSH+LH with higher FSH concentration, while hCG also contains FSH+LH but higher LH concentration than FSH (Guzmán *et al.*, 2011). Gonadotropin hormones have functions to induce egg developmental process during the maturation process, ovulation, and spawning. Gonadotropin hormones enter theca cells and induce testosterone hormone production, then alter testosterone into estradiol hormones with the help of aromatase enzyme. Estradiols will induce liver to produce vitelogenins (egg yolk candidate), which are

brought in the bloodstream to granulose cells and absorbed into the egg developmental process until reaching a maximum diameter (maturation), then continued with ovulation and spawning process (Nagahama, 2011).

Studies about the gonad maturation of silver pompano using PMSG, hCG, and PG600 hormone are still rarely performed, especially in Indonesia. The latest study has performed hCG and PMSG hormone induction in silver pompano (Putra & Razai, 2017) to reach a gonad maturation rate of TKG III for four weeks (four times injection) and spawning process (Putra *et al.*, 2018a; Putra *et al.*, 2018b). Studies about fish maturation induction using PMSG and hCG have many been performed in swamp eels (Putra, 2013; Putra, 2017), tor soro (Farastuti, 2014; Rahimi *et al.*, 2019), eel (Handoyo *et al.*, 2012), silver pompano (Gopakumar *et al.*, 2012; Putra *et al.*, 2018b; Saputra *et al.*, 2019b), catfish (Ahmed, 2017), striped catfish (Samara *et al.*, 2019), Nile tilapia (Darliansyah *et al.*, 2017), eel tail catfish (Anggraini *et al.*, 2019; Yulianto *et al.*, 2019), and sea bass (Cerda *et al.*, 1997; Saputra *et al.*, 2019a). This study was performed to analyze the gonad maturation rate of silver pompano induced by different gonadotropin hormones and obtain a potential hormone type for gonad maturation inducement in silver pompano.

MATERIALS AND METHODS

This study contained four treatments, namely control with NaCl 90% (0.5 ml/kg body weight), PMSG hormone (20 IU/kg body weight), hCG hormone (20 IU/kg body weight), and PG600 hormone (20 IU/kg body weight). The use of 20 IU dose was an applied hormone dose from the previous study of Putra (2013) about a hormonal maturation induction of swamp eel and silver pompano. All treatments induced three broodstock candidates on each treatment. This study comprised some steps.



Figure 1. Container position

Broodstock selection

Selection process was performed on the broodstock used. The broodstock candidate weight used reached 500 g/broodstock with 7 months old range. The broodstock candidates used were 12 fish and obtained from Fish Fingerling Center, Pengujan, Bintan, Riau Islands.

Container preparation

The container used in the study was a floating net cage. The floating net cages used were four units with the size of 3×3 m² as presented on Figure 1.

Silver pompano rearing

The rearing step of silver pompano contained some activities, namely feeding and fish condition control. Feeding was performed by giving a commercial pelleted feed (37% protein) with the feeding rate of 3–5% from fish weight/day. The feeding period was twice, i.e morning (07.00) and afternoon (17.00).

Hormonal injection

The injection was performed intramuscularly in dorsal part of the fish. Before injected, fish were anesthetized using a clove oil (1 ml/250L). The hormonal injection doze was based on fish body weight. The injection was performed in four weeks, namely 0th week (W0), 1st week (W1), 2nd week (W2), and 3rd week (W3).

Parameter observation

Parameters observed were body weight, gonadosomatic index (GSI), hepatosomatic index (HSI), gonad maturity level (TKG I–IV), gonad histology, and absolute growth. Data sampling was performed at the initial study period (M0) and 4th week of study period (M4). Fish samples taken on the initial and final study period were three fish in each treatment.

Body weight

Absolute body weight parameter measurement of silver pompano was performed using a digital scale with 1 g accuracy. Fish were anesthetized first, then measured and noted the measurement result in a gram unit (g). Measurement was performed at the 0th week (W0), 1st week (W1), 2nd week (W2), 3rd week (W3), and 4th week (M4).

Gonadosomatic index (GSI)

GSI is the measurement result of gonad weight divided with fish body weight multiplied by 100 in a percentage unit (%). The GSI measurement

was performed on the initial (M0) and final (M4) study period. Silver pompano fish were measured their body weight before dissected, then dissection was performed to take the gonad, which was measured using a digital scale (0.01 g accuracy). GSI calculation formula based on (Bromage *et al.*, 2001):

$$GSI = \frac{W_g}{W} \times 100$$

Note:

GSI : Gonadosomatic index (%)
 W_g : Silver pompano gonad weight (g)
 W : Silver pompano body weight (g)

Hepatosomatic index (HSI)

Hepatosomatic index (HSI) is the calculation result of liver weight divided with body weight multiplied by 100 in a percentage unit (%). The procedure was performed by measuring the body weight (W), dissection, and measuring the liver weight (W_h). HSI calculation was performed on the initial (M0) and final (M4) study period. HSI calculation used the following formula (Cek *et al.*, 2001):

$$HSI = \frac{W_h}{W} \times 100$$

Note:

HSI : Hepatosomatic index (%)
 W_h : Fish liver weight (g)
 W : Fish body weight (g)

Gonad maturity level (TKG)

The gonad maturity level can be measured through either a visual or microscopical observation (histology). This study used a visual observation based on the egg nucleus movement and gonad maturity level standard on the following Table 1.

Gonad histology

Gonad histological observation was performed to identify the microscopical gonad maturation level. The gonad development observation through a histological figure was performed on the initial and final study period. Gonad samples were measured using an analytical scale with 0.01 g accuracy and preserved using formalin 10%. The histological preparation was performed by sending the gonad samples to the laboratory of pathology, Faculty of Animal Medicines, IPB University. A prepared histological sample

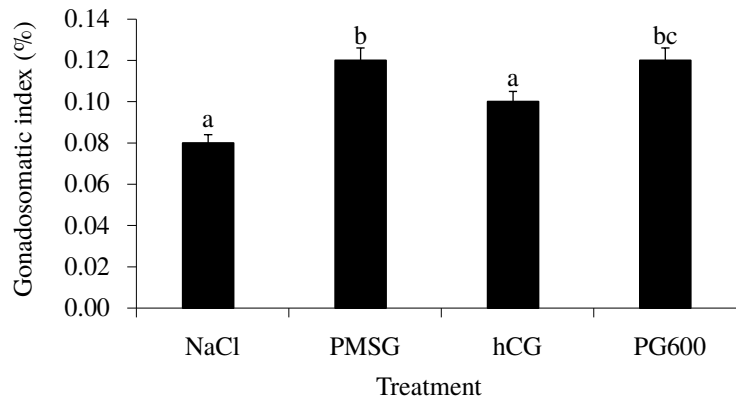


Figure 2. Gonadosomatic index of silver pompano on the final study period. Different letters in graphic indicate a significant difference ($P < 0.01$).

Table 1. General gonad maturity level (Yaron & Levavi, 2011); Han *et al.*, 2003)

TKG	Visual	Microscopical
I Immature	Small ovary and testis is 1/3 of body cavity, oval egg shape. Pink colored ovary and transparent white colored testis.	Small egg with low invisibility, 1–16 μm diameter, transparent.
II Mature	Small ovary and testis covers 1/2 of body cavity, expanded. Transparent pink colored ovary and quite symmetrical white colored testis.	Egg is invisible with naked-eyes, clear egg with 10–21 μm diameter.
III Mature-to-Ripe	Small ovary and testis is 1/2–2/3 of body cavity, asymmetrical gonad. Yellow colored ovary, granule and blood stream existence in the surface, white colored testis.	Egg occurs to be opaque and intransparent with 29–52 μm diameter.
IV Ripe	Ovary and testis fill 2/3 until fully in the body cavity, orange-pink color, bloodstream in the surface, soft and grey colored testis	Semi-transparent riped egg with 45–70 μm diameter.
V Spent	Ovary and testis fill 2/3 until fully in the body cavity, orange-pink color, bloodstream in the surface, soft and grey colored testis	Semi-transparent riped egg with 51–93 μm diameter.

was then observed under a microscope with 100 times magnification. The observation result was analyzed descriptively.

The histological preparation procedure was begun as the preserved gonad samples were dried and separated. Gonad samples was cut transversely and soaked into an alcohol 70%. The first procedure of histological sample preparation (Saeed *et al.*, 2010) was a prepared organ cutting, which was then moved into a cassette and coded based on the treatments. Cassette was moved into a tissue-processor machine in one night for a dehydration process. The embedding process was performed with a paraffin block on a tissue-tek machine for four hours. After four hours, sample was cut with a microtom at 5 μm thick, and the cutting result was enclosed into an object glass, then moved into a waterbath for 15 minutes. The glass sample was pulled and air dried, then moved into an incubator for two hours. The final procedure

was a haematoxylin-eosin (HE) staining, which was then closed with a cover glass.

Data analysis

The study data were analyzed statistically using an anova with the parameters contained gonadosomatic index, hepatosomatic index, and absolute growth, while a descriptive analysis was performed on the gonad maturity level and gonad histology. When the results showed a significant difference ($P < 0.01$), then performed a continuous Tukey test.

RESULT AND DISCUSSION

Gonadosomatic index

The measurement result of gonadosomatic index indicated that the hormone treatments to fish had a significant effect against the gonad

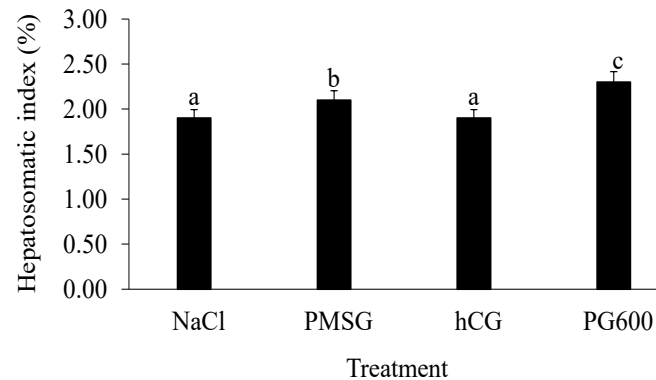


Figure 3. The hepatosomatic index of silver pompano on the final study period in each treatment. Different letters in the graphic indicate a significant difference ($P < 0.01$).

development. The GSI value is a quantitative value, which draws an alteration during the gonad development and will reach a maximum value when the spawning begins (Putra, 2017; Cek *et al.*, 2001). Increased GSI indicated vitellogenesis and gonad development occurrence during the study period (Magalhães *et al.*, 2012). The vitellogenesis activity caused increased HSI and GSI of fish (Cerdeira *et al.*, 1997). The GSI values of silver pompano on control, PMSG, hCG, and PG600 treatment were $0.08 \pm 0.03\%$; $0.12 \pm 0.04\%$; $0.09 \pm 0.09\%$; and $0.12 \pm 0.06\%$ of body weight (Figure 2). The best GSI value in this study was found on PG600 treatment. The result of $0.12 \pm 0.06\%$ meant that the gonad development of 500 g silver pompano was 0.12% of its body weight. The gonadosomatic index result on PG600 hormone treatment was insignificantly different from PMSG hormone treatment ($P < 0.01$). Based on the ANOVA analysis, the hormone induction treatments which had a significant effect against the gonadosomatic index of silver pompano were found on PMSG and PG600 treatment.

The improved gonad development along with vitellogenesis were respectively influenced by FSH and LH level (Rafiuddin, 2014), which contained in PMSG and hCG hormone. The combination of PMSG and hCG as the main component of PG600 hormone caused excessive FSH and LH hormone to induce a vitellogenin production. In sockeye salmon, the salmon GnRH (sGnRH) is distributed from olfactory nerves through hypothalamus along with the gonadotropins (GTH) and growth hormones (GH) release (Kim *et al.*, 2011; Heyrati *et al.*, 2010). PMSG hormone contains more follicle stimulating hormone (FSH) and less luteinizing hormone (LH) (Rafiuddin, 2014), thus can induce a gonad maturity process well.

A hormone that influences the vitellogenesis process is FSH or gonadotropin I (Yaron & Levavi, 2011; Voronina & Wessel, 2003; Wu *et al.*, 2012). The GSI value in this study was lower than Putra & Razai (2017) with 0.6% after induced with PMSG hormone on silver pompano. This was suspected due to a different fish body weight used which reached 1 kg size, therefore the gonad weight influenced the GSI calculation. A study about the induction of PMSG hormone on swamp eel could improve the GSI value until 0.3% of body weight after four week rearing and four time injections (Putra, 2017). Furthermore, the utilization of PG600 hormone on swamp eel could improve the GSI value until 0.74% of body weight (Putra, 2013). The improved GSI value was respectively influenced by the fish weight. Improved GSI value is also followed with an improved HSI value. The utilization of hCG and PMSG hormone could produce lower GSI values than PG600 hormone utilization. This was suspected due to low FSH level, which induced the gonad development, causing a low GSI value. The utilization of hCG and PMSG hormone that still had lower GSI values than PG600 hormone was considered as a less FSH level in the hormones, which induced the gonad development, causing low GSI value obtained (Putra, 2017; Zadmajid *et al.*, 2018).

Hepatosomatic index

The hepatosomatic index of silver pompano given the hormone treatments can be seen on Figure 3. The hepatosomatic index (HSI) is a quantitative value which draws the increased liver weight along with gonad development and increased GSI (Cek *et al.*, 2001; Kim *et al.*, 2011). The study result showed that HSI value on each treatment, namely control, PMSG, hCG, and

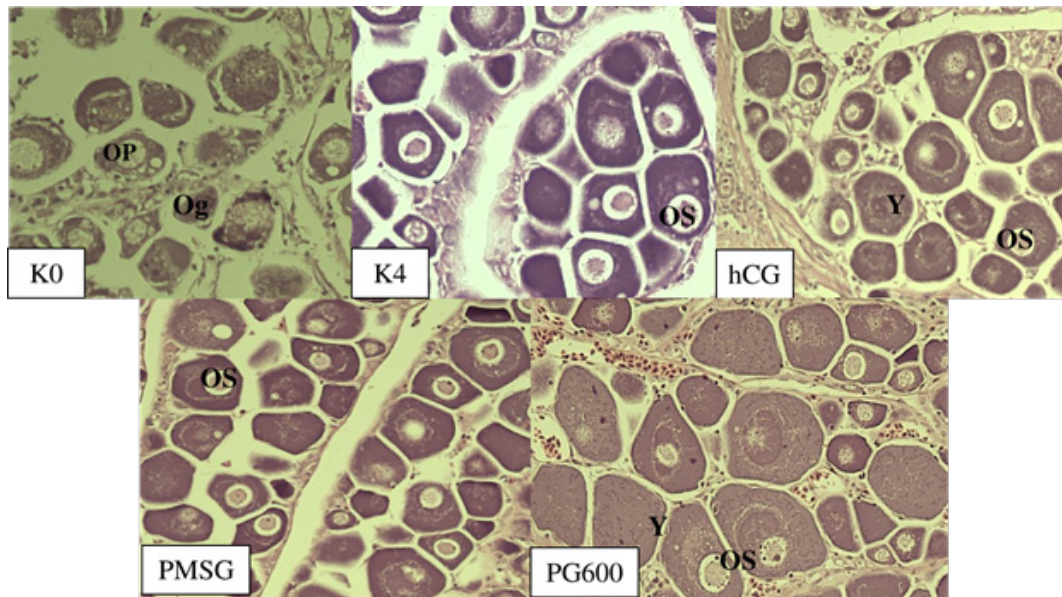


Figure 4. Gonad histology of silver pompano in each treatment on the final study period. Note: KO (control on the initial period); K4 (control on the 4th week period); Y (egg yolk); OS (secondary oocyte); OP (primary oocyte); Oog (oogonium) (Esmacili *et al.*, 2017; Behera, 2012; Duarte *et al.*, 2017). 100 times magnification.

PG600, was $1.90 \pm 0.32\%$, $2.08 \pm 0.40\%$, $1.91 \pm 0.32\%$, and $2.30 \pm 0.15\%$ respectively. Based on the ANOVA analysis, the hormonal induction treatment gave a significant influence against the hepatosomatic index of silver pompano ($P < 0.01$). Liver did not only develop the weight during the gonad maturation process, but also occurring a darker red whenever approaching the final maturation level (TKG IV). The best result was obtained from PG600 treatment with 2.30% , which indicated that there was an increased liver weight as the gonad maturation process occurred. PMSG hormone contained more follicle stimulating hormone (FSH) and less luteinizing hormone (LH) (Putra, 2017). The activity of hCG hormone is more similar to LH and less similar to FSH. hCG hormone induced the gonadotropin concentration during the vitellogenesis process and final maturation (Mehdi & Ehsan, 2011). PG600 hormone was a combination of PMSG and hCG, therefore having more composition and concentration of FSH and LH, which worked more than PMSG and hCG, affecting a faster gonad development, as well as higher GSI and HSI value.

The increased concentration of FSH (GTH-I) induced the aromatase enzyme which converted testosterone into 17β -estradiol (Isriansyah, 2011), therefore the vitellogenin synthesis in liver could be conducted (Mylonas *et al.*, 2010; Francis *et al.*, 2011). The gonad maturation is closely related

to vitellogenesis process. This was based on the statement of Cek *et al.* (2001) that liver has a role in synthesizing some materials supplied into the ovary during the reproductive period. The ratio of liver and body weight on gonad matured fish increases during the vitellogenesis process and decreases during the ovulation process.

Gonad maturity level

The gonad maturity level of silver pompano on the final study period is presented in the following Table 2.

Table 2. Gonad maturity level of silver pompano on the final study period

Treatment	Fish		
	1	2	3
Control	I	I	II
PMSG	II	III	III
hCG	II	III	III–IV
PG600	III	IV	IV

Tang and Affandi (2004) stated that the gonad maturity level I was marked from the egg cells majority on the oogonium phase (vitellogenin droplet had no egg nucleus), gonad maturity level II was marked from a perinucleolar stage (PNS) as yolk started to form and had a dotted egg nucleus. Gonad maturity level III was a cortical alveolar stage (CAS) with developed and unmerged egg nucleus, brighter colored yolk, and lipid droplets.

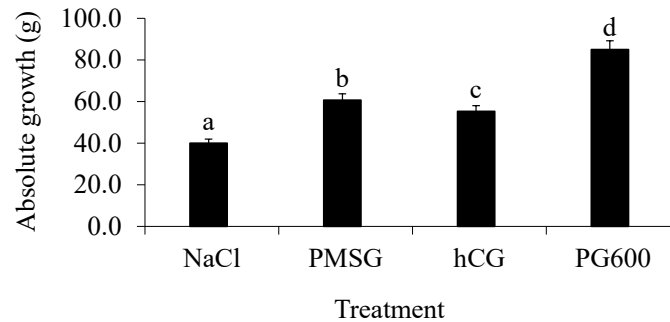


Figure 5. The absolute weight growth of silver pompano in each treatment on the final study period. Different letters in the graphic indicate a significant difference ($P < 0.01$).



Figure 6. Abdominal contents and gonad of silver pompano (Study documentation, 2017)

Gonad maturity level IV is an oocyte included in the vitelogenic stage (VS), meanwhile oocyte moved into a maturation stage (MS) with the egg nucleus was merged and maximum egg diameter. TKG V was marked from less number of oocytes after spawning process, while resting range happened as the resting period for preparing the initial phase (Lemos *et al.*, 2011). The anatomical observation result in gonad based on Yaron&Levavi (2011) was that the application of PG600 hormone cause two of three broodstocks reached TKG IV. These TKG results followed Handrianto *et al.* (2017), who stated that the induction of PMSG hormone in silver pompano reached a gonad maturity level (TKG) III for four weeks after injected with the doze of 20 IU/kg body weight. Increased gonad maturation will impact on the improved FSH and LH hormone concentration (Zahri *et al.*, 2018). Increased gonad maturity level with hCG induction also occurred on grey mullet (Cahyono *et al.*, 2019).

Gonad histology

The gonad histologies of silver pompano on the initial and final study period are presented on Figure 4. Based on Figure 4, the best result was obtained from PG600 treatment as egg nucleus was merged, although there were some

egg cells were in the secondary oocyte phase. This proved that PG600 treatment could reach a gonad maturity level III. This result was influenced by some factors, i.e hormone contents, doze, and nutrient levels to support the gonad maturation process. PG600 is composed of PMSG (containing FSH+LH) (Hutagalung *et al.*, 2015) and hCG (FSH+LH) (Nuraini *et al.*, 2008), therefore PG600 was possibly better to induce the fish gonad maturation. Another treatment, such as control, still included in TKG I due to the existence of oogonium that was dominantly found on the egg cells histological figure, while hCG and PMSG treatment showed a gonad development as egg nucleus occurred and moved at the egg corner, but not yet reaching a phase when the egg cell merged as seen on PG600 treatment. The hCG and PMSG treatment were only capable of reaching gonad maturity level (TKG) II. This was possibly influenced by the dozes given, hormone contents, and a possibility that only happened on the seawater fish, i.e moon period (date of 15th until 2nd) and dark moon period (date of 4th until 14th). For moon and dark moon period, studies following this concern has never been proven scientifically, however a broodstock selection is performed on the dark moon period (around date of 15th until 2nd or 3rd on each month) as learned from the experience of fish fingerling center.

Absolute weight growth

The weight growth of silver pompano on hormone treatments is presented on Figure 5. The PG600 hormone treatment caused an absolute weight growth of 85 g. This increased weight was suspected due to the influence of feed, gonad development, and lipid deposits (Putra, 2017) which contained in the digestive tract of silver pompano (Hamed *et al.*, 2016; Kumar *et al.*, 2015; Ma *et al.*, 2016; Handrianto *et al.*, 2016; Lemos *et al.*, 2011; Jayakumar *et al.*, 2014; Cavalin & Weirich, 2009; Belfranin, 2016). Growth is the increased body weight and length as body weight has the supportive components, namely meat, body skeleton (body), skin, body organs, digestive tract, and abdominal lipids. Growth in silver pompano is specifically influenced by the increased lipid weight or also gonad development, although this is less possible as the gonad weight of silver pompano is not much great. The body and abdominal contents of silver pompano are visually seen on the following figure:

Vitellogenesis is the vitellogenin induction and synthesis process in liver by 17β -estradiol hormone (Rehman *et al.*, 2015), as well as vitellogenin absorption carried by bloodstream into oocytes (Tyler *et al.*, 1991). In vitellogenesis, the environmental factors, such as rain and media temperature, are accepted by the central nervous system and transmitted into the hypothalamus. The GnRH hormone release is a response of hypothalamus against the stimulation of both natural and exogenous hormones injected into the fish body. Furthermore, Hypophysis synthesizes FSH hormone that works on theca cells on the oocytes, resulting a testosterone production the theca cell layer (Nagahama, 2011). The testosterone hormone is converted into 17β -estradiol by the aromatase enzyme on the granulosa layer. 17β -estradiol stimulates the liver to synthesize vitellogenin as a yolk candidate (Schaefer *et al.*, 2016).

Vitellogenin is brought by the bloodstream to gonad and absorbed by oocyte follicle layer (Yaron & Levavi, 2011). The vitellogenin absorption results an enlarged oocyte growth and stops whenever reaching the maximum diameter (Chattopadhyay & Chattoraj, 2017). In this circumstance, eggs wait for the environmental signal to perform spawning. The gonadosomatic index (GSI) (Fontoura *et al.*, 2018) and hepatosomatic index (HSI) will increase simultaneously along with the vitellogenesis process (Cerda *et al.*, 1997;

Heyrati *et al.*, 2010). The vitellogenin synthesis is influenced by 17β -estradiol as vitellogenin and androgens biosynthesis stimulator presented in fish body. This happens as androgens will converted into estrogens by liver aromatase (Elakkanai *et al.*, 2017) and suspected phytoestrogens supplemented in fish feed such as soy beans and alfalfa plant have a positive influence on the fish vitellogenesis (Mehdi *et al.*, 2011; Yaron & Levavi, 2011). According to Yaron and Levavi (2011), and Ekokotu and Olele (2014), when the vitellogenesis process occurs, the egg yolk granules or globules increase in number and size, resulting an enlarged oocyte volume.

CONCLUSION

The use of gonadotropin hormones could induce the gonad maturation of silver pompano until gonad maturation level III and IV for four weeks and the best result was obtained from PG600 hormone compared to control and other treatments ($P < 0.01$).

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