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Effects of dietary supplementation with 17β-estradiol on the Steroid Hormone Levels, Gonadosomatic Index, and Gonadal Histology of Female Silver Pompano (*Trachinotus blochii*) Broodstock

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ABSTRACT

Silver pompano (*Trachinotus blochii*) is a mariculture commodity with fast growth characteristics and easy adaptation to the environment. However, the production of its seeds is hindered by the maturation of the parent's gonads out-side the spawning season. Hormonal approaches are needed for the maturation of the parent's gonads. The aim of this study is to investigate the level of steroid hormones, gonadosomatic index, and gonad histology in female silver pompano (*Trachinotus blochii*) broodstock after dietary supplementation with 17β-estradiol. This research is crucial for the advancement of effective and efficient techniques for rearing this fish. The study used a completely randomized design (CRD) consisting of three different 17β–estradiol concentrations (0 mg/kg (E1), 30 mg/kg (E2), and 60 mg/kg (E3) fed to female silver pompano broodstock for 30 days, beginning at initial feeding, each with eight individual replicates. Absolute body weight growth, steroid hormone levels, gonadal maturation index, gonadal histology, fecundity, and egg diameter of female silver pompano broodstock were all examined in this study. The incorporation of 17β-estradiol hormone into the sustenance amplifies the reproductive capabilities of the female silver pompano broodstock. The inclusion of 17β-estradiol hormone in the diet renders the most elevated values regarding fecundity and egg diameter in the female silver pompano broodstock. Histological examinations expose that the development of the gonads in the female broodstock treated with 17β-estradiol hormone progresses towards maturation. The inclusion of 17β- estradiol hormone in the diet at doses of 20 and 60 mg/kg feed yields the highest values in terms of fecundity and egg diameter in female silver pompano broodstock.

Introduction

The silver pompano, *Trachinotus blochii*, is considered a promising species for aquaculture due to its fast growth and ease of maintenance (Prabu *et al.*, 2021; Weirich *et al.*, 2021). With its high nutritional content and delicious taste, this fish is highly sought after in both local and international markets, such as Singapore, Taiwan, China, and Hong Kong (Ebeneezar *et al.*, 2020). Mass production of silver pompano can be achieved through intensive aquaculture, which requires a large and sustainable supply of fingerlings. In this regard, there is a need to increase the population of broodstock that is ready for adequate breeding purposes.

The ability to obtain high-quality seeds and the ability to control fish reproduction are limiting factors for fish farming. Therefore, sufficient information, both quantitative and qualitative, regarding the reproduction of silver pompano is necessary for the development of silver pompano aquaculture. Quantitative observations Include Gonadosomatic Index (GSI), steroid hormone level, and reproductive performance, and qualitative observations include gonad development and gonad histology that can describe the reproductive system

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(gonad development) of potential silver pompano broodstock.

The hormone commonly used for enhancing reproductive performance in female aquaculture species is 17β-estradiol. 17β-estradiol hormone can be utilized for sex differentiation, growth enhancement, vitellogenin protein production, gonad development, and fish steroid plasma levels (Hu *et al.*, 2023; Rocha *et al.*, 2023; Zarragoitia *et al.*, 2014). During vitellogenesis process, an increase in estradiol hormone leads to higher oocyte size. The elevation of estradiol concentration in the blood stimulates the liver to initiate the vitellogenesis process, subsequently accelerating gonadal maturation in orange-spotted grouper (*Epinephelus coioides*) (Ye *et al.*, 2022). Proper administration of estradiol in Striped catfish (*Pangasianodon hypophthalmus*) with appropriate dosage influences the conversion of estradiol into vitellogenin in the liver, allowing a continuous and uninterrupted vitellogenesis process with final oocyte maturation (Pamungkas *et al.*, 2019).

The hormone 17β-estradiol has been found to be effective in accelerating the maturation of the gonads of prospective breeding individuals, as evidenced by an increase in the level of 17β-estradiol in the blood plasma. Several reproductive studies have applied 17β-estradiol, including Cahyono *et al.* (2019), who investigated the stimulation of gonad development in mullet fish (*Mugil dussumieri*) and found that administering 0.07 mg/kg of estradiol hormone increased the gonad maturation index. In another study, Muzaki *et al.* (2017) administered oral estradiol hormone at a dose of 50 µg/kg body weight to promote gonad development in prospective breeding individuals of humpback grouper, which resulted in increased growth (weight), estradiol hormone levels in the fish's blood, and gonad development rates.

Currently, there is limited research on the addition of 17β-estradiol in marine fish species, including silver pompano. Therefore, it is necessary to conduct a study on the addition of 17β-estradiol in silver pompano by analyzing steroid hormone levels, reproductive indices, and gonadal histology, in order to provide a reference for studying the reproductive system (gonadal development) of silver pompano. The results of this research are expected to help improve the productivity of silver pompano aquaculture in the future, in an effort to produce high-quality and sustainable silver pompano seed production technology innovations.

Materials and Methods

Experimental Fishes

The broodstock of female silver pompano were obtained from the Marine Aquaculture Center, Lampung. A total of 24 female broodstock of silver pompano were collected in this study. The broodstock criteria used are health, absence of any defects and lack of injuries on their entire body. The average weight is 2404.54 ± 210.26 g and the average length is 51.96 ± 1.99 cm. Before being treated, the fishes were adapted to a preservation container for 7 days.

Experimental Design

During the maintenance period, three units of floating net cages measuring 3 x 3 x 3 m^3.The research design employed in this study utilized a Completely Randomized Design (CRD) with 3 treatments of different concentrations of estradiol/kg of feed. A total of 24 female broodstock of silver pompano were divided into three treatment group (eight fishes per treatment group and gived with 17β-estradiol hormone at doses of E1 (0 mg/kg), E2 (20 mg/kg), dan E3 (60 mg/kg). The maintenance of hormone-fed broodstock was conducted for a duration of 30 days. The feeding regimen consisted of two daily feedings, one in the morning at 08:00 a.m. and another in the afternoon at 02:00 p.m. The amount of feed provided was equivalent to 2% of the total biomass weight of the fish.

Diet Preparation

The feed utilized in this study was a commercial pellet feed for marine fish with a protein content of 50%. The administration of hormone treatment was based on the methodology of Vidal-López *et al.* (2019). The hormone utilized in this study was 17β-estradiol, which was in the form of a fine white powder (Argent Chemical Laboratories, USA). The hormone was weighed according to the treatment dosage (0, 20, and 60 mg/kg of feed) and then placed in a tube. Subsequently, it was dissolved in 96% alcohol at a dosage of 100 ml per 1 kg of feed. The solution was then sprayed onto the feed. The drying process was carried out by spreading the feed thinly on the surface of a plastic tray and allowing it to air-dry at room temperature for 12 hours to evaporate the solvent or until the feed was completely dry and free of alcohol aroma. The feed was then stored in a sealed container and kept in a freezer at -20°C.

Blood Collection Sampling

Two randomly selected fish from each treatment were used for blood samples. Blood was extracted from the caudal vein using a 1 ml syringe at day 0, 7, 21, and 28 days. The blood samples were transferred

to a 1.5 ml microtube and kept in a cool box. After the last sample was taken, the blood samples were centrifuged at 10000 rpm for 5 min. The plasma obtained is subsequently collected in microtubes and stored at a temperature of -20°C for further use in the determination of plasma E2 levels. The measurement of 17β-estradiol levels in the blood plasma was conducted using the Enzyme-Linked Immunosorbent Assay (ELISA) method with a commercial kit. The measurement of estradiol hormone levels in this study was performed at the Agroindustry and Biomedical Laboratory-Agricultural Production, Puspitek-Serpong.

Histological Examination of Gonads

Histological analysis of gonads was performed by randomly sampling fish at the beginning of the experiment (n = 4) and at the end of the experiment (n = 2 of each treatment). Anesthesia was performed using dose of 1 ml L^{-1} of clove oil. The anesthetization process of fish using clove oil is conducted for a duration of 20-30 minutes. The weight of the dead fish was measured. The gonads were removed, weighed, and fixed in 10% formalin buffered. After 24 h of post-fixation, the gonads were transferred and preserved in 70% ethanol. The preparation of histological slides in this study was conducted at the Fish Health Laboratory, Bogor Agricultural University, Bogor. Suitable-sized pieces from each fixed gonad were processed using stained with hematoxylin and eosin. Classified the stages of gonadal development, microscopically following Sun *et al*. (2022).

Zootechnical variables

The gonadosomatic index (GSI) was calculated by dividing the gonad weight by the total fish weight and multiplying the result by 100. This was done for the fish sampled at the beginning of the experiment (n = 4 fish), at the end of the experiment (n = 2 of each treatment). The following zootechnical variables were measured or calculated at the beginning and end of the experiment: weight gain (Δw) which was determined by subtracting the initial weight from the final weight. Fecundity measurement was done by counting the number of eggs released by the female broodstock after the gonads were removed. Fecundity was calculated using the gravimetric method with the following formula: fecundity (grains) = [number of eggs taken (grains) x gonad weight (g) / weight of sample eggs (g)]. The calculation of egg diameter used methods by egg samples were taken as many as 50 eggs/treatment and carried out 2 times, then observed and measured under a microscope equipped with 40x magnification used a micrometer.

Statistical Analysis

All values were reported as mean ± standard error mean (SEM). The one-way analysis of variance (ANOVA) followed by Duncan test was used for multiple comparisons among treatments if the assumptions were met (Kolmogorov-Smirnov one sample test). Statistical significance was tested at $P < 0.10$. All analysis and data visualization were performed using SPSS ver. 26.

Results

The absolute weight growth of female silver pompano broodstock given estradiol hormone treatment through feed, as presented in Figure 1, showed that the absolute weight growth of each 17β-estradiol treatment was higher compared to the 0 mg/kg of estradiol hormone (control). The highest absolute weight growth of female silver pompano broodstock was observed in the 17β- estradiol hormone at doses of 20 mg/kg, with a value of 279.00 g, followed by the 17β- estradiol hormone at doses of 60 mg/kg (265.13 g) and the 0 mg/kg of estradiol hormone (251.25 g). Based on the analysis of variance, it was found that the induction of estradiol hormone with different doses not significantly influenced ($P>0.10$) the absolute weight growth.

The gonad maturity index of female silver pompano broodstock given estradiol hormone treatment through feed, as presented in Table 1, increased at the end of the 30-day rearing period. The gonad maturity index values for each treatment ranged from 0.70 to 0.89%. The treatments applied to female silver pompano broodstock had a similar effect on the gonad maturity index at the beginning and end of the rearing period ($p>0.10$).

The plasma estradiol hormone levels in the blood of female silver pompano broodstock given different doses of 17β-estradiol treatment during the study are presented in Figure 2. The results showed that the estradiol levels increased on day 21 and decreased on day 28 for all treatments except for the 17β- estradiol hormone at doses of 60 mg/kg. The highest increase in estradiol levels was observed in the 17β- estradiol hormone at doses of 20 mg/kg (1.59 ng/mL) compared to the 0 mg/kg of estradiol hormone (0.81 ng/mL) and 17β- estradiol hormone at doses of 60 mg/kg (0.68 ng/mL) on day 21. At the end of the study, only the 17β- estradiol hormone at doses of 60 mg/kg showed an increase in estradiol levels, with a value of 0.76 ng/mL. The 17B- estradiol hormone at doses of 20 mg/kg experienced a decrease in estradiol levels, with a value of 0.86 ng/mL.

Meanwhile, estradiol levels were not detected in the 0 mg/kg of estradiol hormone.

Histological results of the gonads indicated that the developmental stage of female silver pompano broodstock varied from level 1 to 3. The histological results of the gonads can be seen in Figure 3. The histological appearance of the gonads at each data collection showed that the ovaries of female silver pompano broodstock treated with different doses of 17β-estradiol hormone underwent development towards maturity, while in the 0 mg/kg of estradiol hormone treatment, the development occurred very slowly and was still in the immature stage.

The fecundity of female silver pompano broodstock treated with different doses of 17β-estradiol hormone during the study is presented in Table 1. The fecundity of the treatments increased at the end of the study during the 30-day maintenance period. The highest fecundity of female silver pompano broodstock treated with 17β- estradiol hormone at doses of 20 mg/kg was 188,673 eggs at the end of the study. Based on the analysis of variance, it was found that the induction of estradiol hormone with different doses had a significant effect ($P<0.10$) on fecundity. Furthermore, based on the Duncan's post hoc test, it was found that the 0 mg/kg of estradiol hormone treatment did not significantly differ from the 17β- estradiol hormone at doses of 20 mg/kg and 17β- estradiol hormone at doses of 60 mg/kgs, while the 17β- estradiol hormone at doses of 20 mg/kg and 17β- estradiol hormone at doses of 60 mg/kg significantly differed from each other.

The egg diameter of female silver pompano broodstock treated with different doses of 17β-estradiol hormone during the study is presented in Table 1. The results showed an increase in egg diameter at the end of the study during the 30-day maintenance period. The egg diameter in each treatment ranged from 0.94 to 1.06 mm, with the highest egg diameter observed in the 17B- estradiol hormone at doses of 20 mg/kg at 1.06 mm. Based on the analysis of variance, it was found that the induction of estradiol hormone with different doses had a significant effect ($P<0.10$) on egg diameter. Furthermore, based on the Duncan's post hoc test, it was found that the 17β- estradiol hormone at doses of 60 mg/kg did not significantly differ from the 0 mg/kg of estradiol hormone and 17B- estradiol hormone at doses of 20 mg/kg, while the 0 mg/kg of estradiol hormone and 17β- estradiol hormone at doses of 20 mg/kg significantly differed from each other.

Discussion

Hormonal activities regulate the reproductive activities of fish. The induction of gonad development in immature female silver pompano (*Trachinotus blochii*) potential broodstock is expected to be achieved through the administration of 17β-estradiol hormone. In this study, the effects of treatment on the reproductive response of female silver pompano potential broodstock were comprehensively observed, including absolute body weight growth, 17β-estradiol hormone levels in blood plasma, gonadosomatic index, gonad development, fecundity, and egg diameter.

The results of the study showed that the administration of 17β-estradiol hormone at different doses did not have a significant effect on the absolute weight growth of female silver pompano potential broodstock ($P>0.10$). This indicates that the given estradiol hormone treatment did not affect the metabolism of the test fish. According to Ramos-Júdez *et al.* (2023), during the gonad maturation phase, a significant amount of energy stored in the liver is allocated to the ovaries for vitellogenesis processes. Furthermore, Mobley *et al.* (2021) stated that gonad development processes have an impact on energy consumption, requiring more energy for gamete formation in potential broodstock.

The gonadosomatic index (GSI) of the treatment showed an increase at the end of the 30-day maintenance period. The increase in GSI values indicates an increase in gonad weight due to the positive response of the treatment in the gonad development process. The increase in the gonad maturation index indicates the occurrence of vitellogenesis and gonad development during the study. The GSI values in this study ranged from 0.70% to 0.89%. These values are higher compared to the study conducted by Handrianto *et al.* (2017), which reported a GSI value of 0.6% with PMSG hormone induction treatment in Asian seabass (*Lates calcarifer*). The GSI value will continue to increase as the fish's gonads mature and will reach their maximum value during the peak period of gonad maturation (Chen *et al.*, 2021).

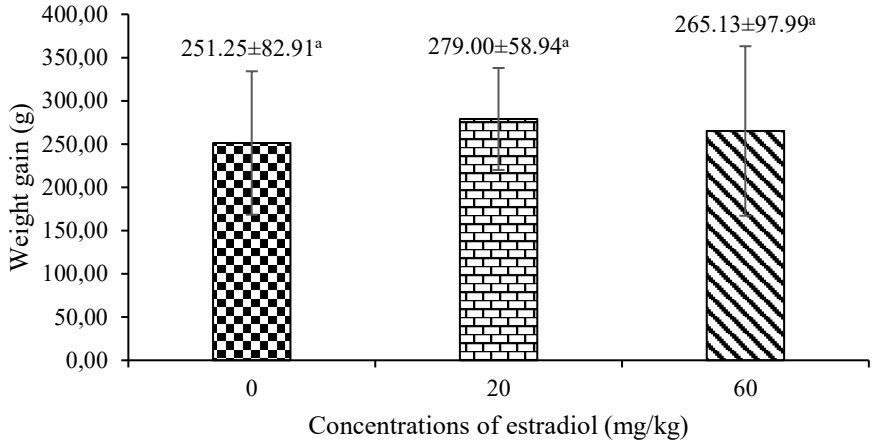

Figure 1. Mean (±SE) weight gain for female broodstock of silver pompano *Trachinotus blochii* at three different concentrations of estradiol/kg of feed for 30 days starting at initial feeding. Means with different letters in the same row differ significantly ($p < 0.10$).

Table 1. Mean (±SE) gonadosomatic index, fecundity, and egg diameter for female broodstock of silver pompano *Trachinotus blochii* at three different concentrations of 17β-estradiol /kg of feed for 30 days starting at initial feeding. Means with different letters in the same row differ significantly ($p < 0.10$).

Parameter	Data observation	Estradiol (mg/kg)		
		0	20	60
Gonadosomatic index (%)	Beginning	0.70±0.14[a]	0.70±0.14[a]	0.70±0.14[a]
	End	0.84±0.06[a]	0.89±0.01[a]	0.87±0.03[a]
Fecundity (egg)	Beginning	133449±16724[a]	133449±16724[a]	133449±16724[a]
	End	171546±33374[ab]	188673±26042[a]	178153±25036[b]
Egg diameter (mm)	Beginning	0.94±0.21[a]	0.94±0.21[a]	0.94±0.21[a]
	End	1.05±0.20[a]	1.06±0.25[b]	1.03±0.22[ab]

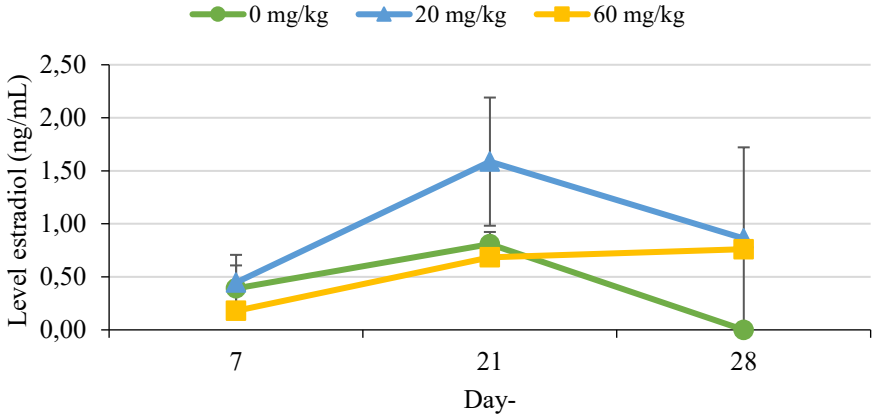

Figure 2. Mean (±SE) the plasma E2 levels for female broodstock of silver pompano *Trachinotus blochii* at three different concentrations of estradiol/kg of feed for 30 days starting at initial feeding. Means with different letters in the same row differ significantly ($p < 0.10$).

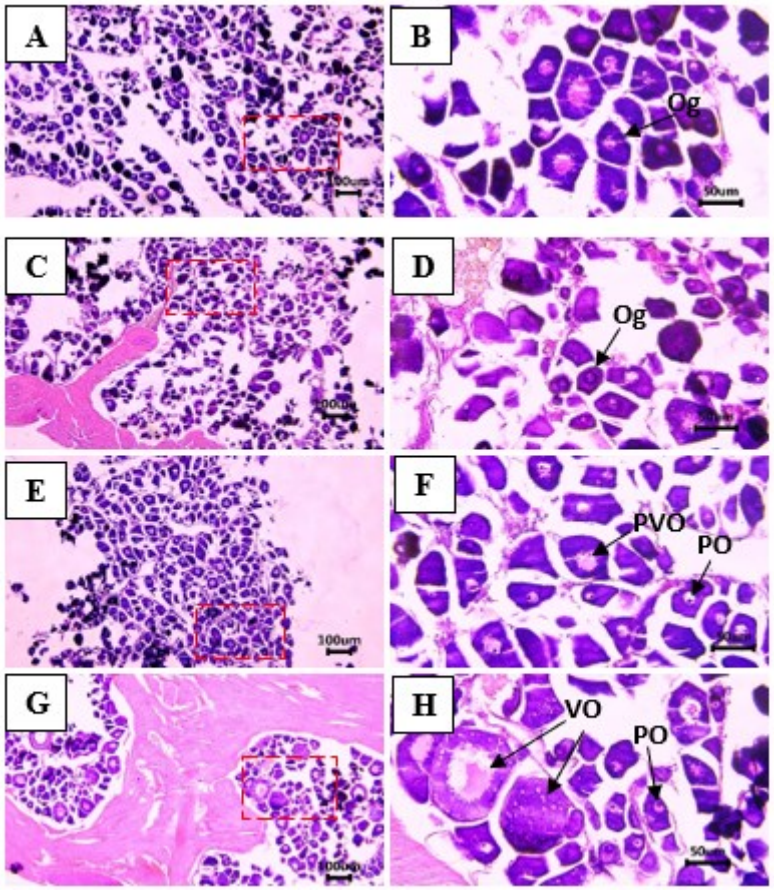

Figure 3. The representative images of the histological structures of the ovary for female broodstock of silver pompano *Trachinotus blochii* at three different concentrations of estradiol/kg of feed for 30 days starting at initial feeding. **A-B**: ovary day-0, **C-D**: ovary day-28, 0 mg/kg of estradiol hormone, **E-F**: ovary day-28, 17β- estradiol hormone at doses of 20 mg/kg,**G-H**: ovary day-28, 17β- estradiol hormone at doses of 60 mg/kg.Og: Oogonia, PO: Primary Oocyte, PVO: Previtellogenic Oocyte, VO: Vitelogenic Oocyte. Hematoxylin-Eosin staining. Scale bar = 100 and 50 μm (100x and 400x).

During the study, the estradiol levels showed an increase on day 21 for all treatments. This increase in 17β-estradiol levels is believed to be due to the presence or action of 17β-estradiol hormone, which acts as an estrogen and can elevate the levels of estradiol in the blood. The high concentration of 17β-estradiol in the blood indicates that the fish are ready to initiate gonadal development. The changes in 17β-estradiol are associated with oocyte development and an increase in the gonadosomatic index (Banh *et al*., 2021). The plasma estradiol levels gradually increase during the vitellogenesis phase, in parallel with the increase in oocyte diameter (Kong *et al*., 2020; Simchick *et al*., 2024). The elevated concentration of estradiol in the blood stimulates the liver to undergo vitellogenesis and subsequently accelerates the gonad maturation process (Chen *et al*., 2021; dos Santos Ribeiro *et al* 2023).

At the end of the study (day 28), the estradiol levels in the plasma decrease in treatments 0 mg/kg of estradiol hormone and 17β- estradiol hormone at doses of 20 mg/kg, except for treatment E3, which shows an increase. This is because the administration of estradiol hormone through low-dose feed is believed to be sufficient to stimulate a rapid hormonal response. On the other hand, high doses of estradiol hormone administered through feed may require more time to reach significant estradiol levels in the plasma. This is because the body tends to carefully regulate hormone balance when exposed to high doses, and the metabolism of these hormones may require more time. According to Takahashi & Ogiwara (2021), the decrease in plasma estradiol levels is also necessary for the resumption of meiosis during the pre-ovulatory phase (FOM or pre-spawning phase), and for the release of a large number of mature eggs that can be fertilized for successful spawning. Furthermore, Sattang *et al*. (2021) state that naturally high concentrations of estradiol hormone occur during the vitellogenesis phase, reaching its peak during the mGV (germinal vesicle migration) phase and then decreasing during the pGV (germinal vesicle peripheral) phase.

The stage of gonad development can be determined through the observation of gonadal histology. The histological results of the gonads show that as the maintenance time of female silver pompano broodstock increases, the diameter of the oocytes becomes larger. At the beginning of the experiment, the ovaries were observed to be in stage I oocytes. According to Sun *et al.* (2022), stage I oocytes only contain oogonia. At the end of the study, the ovaries of female silver pompano broodstock treated with different doses of 17β-estradiol showed development towards maturity in the 17B- estradiol hormone at doses of 20 mg/kg and 17β- estradiol hormone at doses of 60 mg/kg, while in the 0 mg/kg of estradiol hormone, development occurred very slowly and was still in an immature stage. The gonadal histology in the 17B- estradiol hormone at doses of 20 mg/kgentered stage II. Stage II oocytes, also known as early maturation, are characterized by the development of oocytes and the presence of numerous primary oocytes, previtellogenic oocytes, an increase in cell volume and cytoplasm content, as well as an enlargement of the nucleus diameter (Sun *et al.*, 2022). In the 17β-estradiol hormone at doses of 60 mg/kg, the gonadal histology entered stage III or mature gonads, which is characterized by the presence of vitellogenic oocytes, as well as some primary and previtellogenic oocytes.

The 17β- estradiol hormone at doses of 20 mg/kg treatment exhibited a lower stage of gonad development compared to the 17β- estradiol hormone at doses of 60 mg/kg, but the pattern of changes in plasma estradiol levels showed higher values. The lower stage of gonad development in the 17β - estradiol hormone at doses of 20 mg/kg treatment can be attributed to several factors. The first possibility is that the rapid maturation process, indicated by the high increase in plasma estradiol levels on day 21, resulted in the missed observation time, leading to the reabsorption of oocytes that had already passed the maturation period. Based on Tronbøl *et al.* (2022), the reproductive cycle of some females may, however, get arrested at this advanced PVO phase (resting skipper). Alternatively, oocytes might be reabsorbed at the early CAO phase, a process which has been associated with insufficient energy reserves (reabsorbing-CAO skipper). Another possibility is that high plasma estradiol levels can occur if the fish's body responds by increasing estradiol production to compensate for the low dose (Rutherford *et al.*, 2020). However, high estradiol levels do not always necessarily result in the expected effects on gonadal development (Langston *et al.*, 2020; Kadlec *et al.*, 2022). Then, the high level of gonad maturity in the estradiol 60 mg/kg treatment is caused by the difference in the pattern of increasing estradiol plasma levels in the blood. In the estradiol 20 mg/kg treatment, there is an increase in estradiol levels on day 21 and a decrease on day 28. Conversely, in the estradiol 60 mg/kg treatment, the estradiol levels continue to increase from the beginning to the end of observation period. This results in a higher level of gonad maturity in the estradiol 60 mg/kg treatment compared to the estradiol 20 mg/kg treatment, whre the observation of gonad maturity is conducted on day 28.

Based on the observation results, the administration of 17β-estradiol hormone at different doses has an effect on the fecundity of female silver pompano prospective broodstock ($P<0.10$). The highest fecundity of female silver pompano prospective broodstock treated with 17β- estradiol hormone at doses of 20 mg/kg was 188,673 eggs at the end of the study. According to Gopakumar *et al.* (2012), the number of eggs produced in a single spawning ranges from 80,000 to 184,000. The fertilized egg count ranges from 60,000 to 175,000. The high fecundity in the 17β- estradiol hormone at doses of 20 mg/kg is consistent with the high value of IKG. This is in line with the findings of Arafat & Bakhtiyar (2022) that higher IKG values and fecundity of broodstock lead to higher productivity. According to Hua *et al.* (2023), the increase in fecundity is also believed to be due to an increase in vitellogenin synthesis, which subsequently increases the number of oocytes containing vitellogenin.

The administration of 17β-estradiol hormone at different doses also affects the egg diameter of female silver pompano prospective broodstock ($P<0.10$). The egg diameter increased at the end of the study during a 30-day rearing period. The egg diameter in each treatment ranged from 0.94 to 1.06 mm. According to Gopakumar *et al.* (2012), the egg diameter of *Trachinotus blochii* ranges from 900 to 1000 µm. Changes in egg diameter are also related to oocyte maturation processes. The administration of 17β-estradiol hormone in the experiment resulted in an increase in vitellogenin synthesis, followed by an increase in the number of oocytes that absorb the vitellogenin. According to Takahashi & Ogiwara (2022), the active synthesis of vitellogenin in the liver leads to an increase in the number of germ cells in the ovaries filled with vitellogenin. This vitellogenin becomes the building material for the eggs deposited inside the oocytes during gonad development, thereby triggering an increase in egg diameter.

Conclusion

The addition of 17β-estradiol hormone in the feed enhances the reproductive performance of female silver pompano broodstock. The inclusion of 17β-estradiol hormone in the diet at doses of 20 and 60 mg/kg feed yields the highest values in terms of fecundity and egg diameter in female silver pompano broodstock. Histological observations reveal that the gonadal development of female broodstock treated with 17β-estradiol hormone progresses towards maturation.

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