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Effect of Oodev (Oocyte Developer) Hormonal Induction on Estradiol, Vitellogenin and Cortisol Profiles of Putak Fish (*Notopterus notopterus*, Pallas 1769)

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ABSTRACT

The putak fish (*Notopterus notopterus*) is a freshwater species native to Indonesia, protected due to its ecological importance. Oodev, a hormonal inducer, accelerates gonad maturation in the early stage. Estradiol and vitellogenin can serve as indicators of gonad maturation, while cortisol indicates stress response. This study aimed to profile estradiol, vitellogenin, and cortisol in putak fish induced with different doses of Oodev. A completely randomized design was used with four treatments and five replicates: control (0mL/kg), P1 (0.5mL/kg), P2 (0.7mL/kg), and P3 (0.9mL/kg). Twenty fish (19–25.6cm length) were acclimatized for 30 days and treated for 90 days via intramuscular injection. Hormone profiles in blood plasma were measured using ELISA and analyzed by one-way ANOVA. Results showed the highest estradiol concentration in P1 (3450.01±47.05pg/mL) and the lowest in P2 (2886.73±214.73pg/mL). P2 was significantly lower than the control and P1 ($P<0.05$). Vitellogenin concentration was highest in P1 (63.05±6.56µg/mL) and lowest in P3 (41.27±7.71µg/mL). P1 and P2 were significantly higher than in the control and P3 ($P<0.05$). The highest cortisol concentration was in P2 (1132.09±17.19 ng/mL), and the lowest in P1 (911.34±69.69ng/mL). P1 was significantly lower than in the control and P2 ($P<0.05$). No significant differences were observed in body length ($P>0.05$). These results suggest that Oodev at 0.5mL/kg effectively stimulates estradiol synthesis and vitellogenesis while minimizing stress responses in putak fish.

Keywords: Cortisol, Estradiol, Oodev, *Notopterus notopterus*, Vitellogenin.

INTRODUCTION

The putak fish (*Notopterus notopterus*), also known as the Javanese belida or bronze featherback, is an Indonesia freshwater species that belongs to the family Notopteridae, order Osteoglossiformes. This species in Indonesia is distributed across Sundaland, Sumatra, Java, and Kalimantan (Khansa et al. 2023; Wibowo et al. 2024). Putak fish has a bronze-colored body, a small knife-like dorsal fin, and a long anal fin that merges with the caudal fin on the incomplete anterior pelvic fin (Yanwirsal et al. 2017). The International Union for Conservation of Nature (IUCN) listed *Notopterus notopterus* as Least Concern (Ng 2020). Putak fish is a protected freshwater species under Government Regulation Number 7 of 1999 on the preservation of plant and animal species. The Ministry of Environment and Forestry implemented Minister of Environment Forestry Regulation Number 106 of 2018 to

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protect *Notopterus notopterus,* which was strengthened by the Decree Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number 83 of 2024 to determine *N. notopterus* with a limited protection status so that fishing and utilization are restricted.

Putak fish are partial, fractional, and substrate spawners. (Gustomi et al. 2016; Muslim et al. 2024) . This species spawns during the rainy season, April – September (Yanwirsal et al. 2017; Ahmadi et al. 2019; Khowhit et al. 2024). Putak fish migrate from rivers to floodplains for spawning and foraging during the rainy season and then return to the river or their habitats during the dry season (Takagi et al. 2010). However, due to habitat alterations and environmental pressures that can disrupt natural spawning migrations, there is an increasing need for controlled aquaculture as an ex-situ conservation strategy to ensure spawning success, which depends on broodstock and gonad maturation (Pamungkas et al. 2019).

To optimize these outcomes in a controlled environment, hormonal treatments are often used to stimulate reproductive development. One example is hormonal induction, using Oodev (Oocyte Developer), which accelerates gonad maturation in the early stage. Oodev contains Pregnant Mare Serum Gonadotropin (PMSG) and anti-dopamine (AD) (Zulfadhli et al. 2024). PMSG, also known as equine chorionic gonadotropin (eCG), is a non-pituitary gonadotropin hormone produced by the endometrium of pregnant mares. PMSG stimulates gonadotropin secretion by mimicking endogenous gonadotropins (Murphy 2012; Somanjaya et al. 2021). Meanwhile, domperidone, is an anti-dopamine drug contained in Oodev, inhibiting dopamine suppression of the hypothalamus (Pham and Le 2016).

Previous studies have demonstrated the effectiveness of Oodev in accelerating gonadal maturation in various fish species. For instance, Nainggolan et al. (2014) reported that Oodev injection increased estradiol levels in the blood plasma of *Clarias* sp. (Asian catfish). Research by Zulfadhli et al. (2024) showed that Oodev can accelerate gonadal maturation of bileh fish (*Rasbora maninjau*) by increasing estradiol levels, which play a role in vitellogenesis so that it can increase GSI, HSI, fecundity, and egg diameter. Oodev injection had a significant effect in speeding up the gonadal rematuration in snakehead (*Channa striata*) (Anwar et al. 2018); *Osteochilus melanopleurus* (Asiah et al. 2021); *Poropuntius tawarensis* (Mellisa et al. 2022).

Based on these considerations, this study aims to determine the profiles of estradiol, vitellogenin, and cortisol in putak fish after being induced with the Oodev hormone with different doses.

MATERIALS AND METHODS

Study site and experimental condition

The study was conducted from July 2024 to April 2025 at the Aquaculture Research Facility, PGRI University, Palembang, South Sumatera. Hormone profile analysis of putak fish was performed at the Bacteriology Laboratory, KST Soekarno, BRIN Cibinong, Bogor Regency, Indonesia. Twenty putak fish with a total length of 19–25.6cm were obtained from Kelekar River, South Sumatra (Fig. 1).

Fig. 1: *Notopterus notopterus* used as the experimental animal in this study.

After collection, putak fish were acclimatized for 30 days in a round tarpaulin pond with a diameter of 3.0m and a height of 0.8m at Palembang. The fish were fed to satiation daily at 4 PM with white shrimp (*Litopenaeus vannamei*) and catfish seeds (*Clarias* sp.). Water quality parameters consisting of temperature, pH, dissolved oxygen (DO), and total dissolved solids (TDS) were measured on days 15, 30, 45, 60, 75, and 90 at 8 AM.

Experimental design and hormone injection

A Completely Randomized Design (CRD) was employed with four treatments and five replicates. The fish were treated for 90 days with the following treatments: Control, P1, P2, and P3 (Table 1). Oodev hormone was injected intramuscularly into the dorsal saddle muscle, located near the dorsal fin above the lateral line, between the operculum and one–third of the distance to the caudal fin. This area was selected due to its thick muscle tissue and lower risk of injuring internal organs (Neiffer and Stamper 2009). Oodev injections were administered on days 15, 30, 45, 60, 75, and 90 at 8 AM.

Table 1: Experimental treatment group

Treatment	Dose of Oodev
Control	0mL/kg
P1	0.5mL/kg
P2	0.7mL/kg
P3	0.9mL/kg

Blood plasma samples collection

Blood plasma samples were collected at the end of the study. A 0.2mL blood was collected from the caudal vein near the lateral line of putak fish using a 1mL syringe, a method considered low risk for internal organ injury (Sadoul and Geffroy 2019). Blood samples were placed in 1.5mL microtubes containing one drop of sodium citrate as an anticoagulant, then centrifuged at 8000rpm for 8 minutes. The plasma (supernatant) transferred to new 1.5mL microtubes using a micropipette. All plasma samples were stored at -20℃ until ELISA analysis.

Hormone profile measurement by ELISA

The hormone profiles of estradiol, vitellogenin, and cortisol were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The instrument used was the iMark^{TM} Microplate Absorbance Reader Bio-RAD with a wavelength of 450nm. The ELISA procedure followed the product catalog protocols of DRG® Estradiol ELISA (EIA-2693), Bioenzy ® Fish Vitellogenin ELISA KIT BZ-08060200-EB, and DRG® Cortisol ELISA (EIA-

1887). The analysis was performed in duplicate.

Data Analysis

The normality and homogeneity of the data were analyzed using Kolmogorov- Smirnov test and Levene's test. Non–normal and non–homogenous data were transformed logarithmically. The data were analyzed by one–way ANOVA. All statistical analyses were conducted at a 0.05 significance level using SPSS software, version 23.

RESULTS

Body length of putak fish

The highest increase in body length was observed in the P1 (Oodev at 0.5mL/kg), with an average growth of 0.9±0.9cm (Table 2). The smallest increase was recorded in Oodev at 0.7mL/kg with an average growth of 0.4±0.47cm. Based on statistical analysis, there was no significant difference among treatments (P>0.05).

Hormone profiles of putak fish

The highest estradiol concentration was observed in putak fish injected with Oodev at a dose of 0.5mL/kg (P1) at 3450.01±47.05pg/mL (Table 3). The lowest estradiol concentration was recorded in P2 (Oodev at 0.7mL/kg) at 2886.73±214.73pg/mL. Statistical analysis revealed that the estradiol level in P2 was significantly lower than in both the control and P1 groups (P<0.05). Meanwhile, P3 (Oodev at 0.9mL/kg) did not differ significantly from the other treatments, as indicated by overlapping superscript letters (ab). For Vitellogenin, the highest concentration was found in P1 at 63.05±6.56µg/mL while the lowest was in P3 at 41.27±7.71µg/mL. Vitellogenin levels in P1 and P2 were significantly higher than those in the control and P3 (P<0.05). Regarding cortisol, the highest concentration was observed in P2 at 1132.09±17.19ng/mL, and the lowest in P1 at 911.34±69.69 ng/mL. Cortisol levels in P1 were significantly lower than those in the control and P2 groups (P<0.05), whereas P3 did not differ significantly from either treatment.

Water quality during the study

Water quality parameters measured during the study were within acceptable ranges for putak fish (Table 4). The observed temperature ranged from 28 to 30.7°C, while dissolved oxygen levels ranged from 9.5 to 15.5mg/L. The water pH was relatively alkaline, ranging from 8.9 to 9. Total dissolved solids (TDS) values varied from 91.8 to 316.7ppm.

DISCUSSION

Based on the study results, estradiol concentrations were highest in fish treated with 0.5 mL/kg of Oodev (P1) and lowest in those treated with 0.7mL/kg (P2). This suggests that Oodev at 0.5mL/kg may be optimal for stimulating estradiol synthesis in *Notopterus notopterus*. Although specific studies on gonadotropin levels following Oodev injection are limited, its potential to stimulate estradiol synthesis is supported by its hormonal profile. Oodev consists of PMSG and anti-dopamine, both of which have been shown to influence gonadotropin secretion.

Tomasoa et al. (2015) Reported that a combination of PMSG and AD increased FSH levels to 2.19–2.51mIU/mL and LH levels to 1.94-2.11mIU/mL in *Anguilla* sp. Furthermore, Zulfadhli et al. (2024) Found that the highest estradiol levels in *Rasbora maninjau* (32.3±6.82pg/mL) occurred with an Oodev dose of 2mL/kg. These findings suggest that the optimal Oodev dose for hormone induction varies across species.

Anti-dopamine in Oodev blocks D2 receptors, thereby inhibiting dopamine's suppressive effect on the hypothalamus and increasing GnRH release to the anterior pituitary.(Mellisa et al. 2022; Pham and Le 2016). PMSG in Oodev mimics the function of endogenous gonadotropins because of its structural similarity.

Table 2: Comparison of putak fish body length before and after Oodev injection

Parameter	Oodev Treatment			
	Control (0mL/kg)	P1 (0.5mL/kg)	P2 (0.7mL/kg)	P3 (0.9mL/kg)
Initial Length (cm)	20.38±0.86^{a}	22.1±2.43^{a}	22.3±1.61^{a}	23.24±1.15^{a}
Final Length (cm)	21±1.22^{a}	23±2.24^{a}	22.7±1.92^{a}	23.96±0.65^{a}
Δ Length (cm)	0.62±0.43^{a}	0.9±0.9^{a}	0.4±0.47^{a}	0.72±1.08^{a}

Note: The same superscript letters in the same column indicate no significant differences among treatments (P>0.05).

Table 3: Hormone profiles of putak fish after Oodev injection

Parameter	Oodev Treatment			
	Control (0mL/kg)	P1 (0.5mL/kg)	P2 (0.7mL/kg)	P3 (0.9mL/kg)
Estradiol Concentration (pg/mL)	3368.79±49.53^{b}	3450.01±47.05^{b}	2886.73±214.73^{a}	3124.54±40.27^{ab}
Vitellogenin Concentration (µg/mL)	44.47±7.52^{a}	63.05±6.56^{b}	56.80±2.25^{b}	41.27±7.71^{a}
Cortisol Concentration (ng/mL)	1050.11±126.58^{b}	911.34±69.69^{a}	1132.09±17.19^{b}	981.18±113.22^{ab}

Note: Different superscript letters in the same row indicate significant differences among treatments (P<0.05), while the same superscript letters indicate no significant differences among treatments (P>0.05).

Table 4: Water quality parameters measured during the study

Parameter	Value	References
Temperature (°C)	28–30.7	29–30 (Kulkarni 2021)
Dissolved Oxygen (mg/L)	9.5–15.5	9.6–10.9 (Kulkarni 2021)
pH	8.9–9	8.4–8.9 (Kulkarni 2021)
Total Dissolved Solids (ppm)	91.8–316.7	64.9–360 (Gupta et al. 2012)

PMSG consists of non-covalent α and β subunits that bind to FSH receptors (FSHRs) in granulosa cells and LH receptors (LHRs) in theca cells (Murphy 2012). This binding activates signaling pathways that enhance the transcription of StAR (steroidogenic acute regulatory protein), which mobilizes cholesterol to the mitochondrial membrane for steroidogenesis. Cholesterol is converted to pregnenolone by the P450scc enzyme, which is then further converted to testosterone in the theca cells. Testosterone then diffuses into the granulosa cells, where it is converted into estradiol by the aromatase enzyme (CYP19A1) (Esteves and Alviggi 2015; Fuentes and Silveyra 2019; Tenugu et al. 2021).

The combined action of PMSG and anti-dopamine enhances estradiol production and stimulates the release of endogenous gonadotropins, particularly LH. Elevated estradiol levels provide positive feedback to the brain, increasing the secretion of endogenous FSH and, especially, LH. This leads to increased aromatase activity and higher estradiol concentrations in the blood plasma during oocyte maturation. (Pham and Le 2016; Kauffman 2022).

Higher doses of Oodev (0.7mL/kg and 0.9mL/kg) may exceed the physiological threshold, triggering negative feedback on estradiol secretion. Excessive estradiol levels can cause the ovaries to send inhibitory signals to the hypothalamus, reducing gonadotropin secretion and estradiol synthesis. According to Senthilkumaran (2013) and (Zheng et al. 2024). The hypothalamic-pituitary-gonadal (HPG) axis regulates homeostasis through sex steroid feedback mechanisms. (Sanz-Pastor et al. 2024). Estradiol directly lowers gonadotropin levels by binding to ERα in the pituitary, suppressing FSHβ and LHβ transcription. Indirectly, estradiol inhibits kiss1/kiss2 expression, reducing kisspeptin levels and decreasing GnRH pulse amplitude to the pituitary. (Christian et al. 2008; Fontaine et al. 2020).

Estradiol plays a critical role in stimulating vitellogenesis. Estradiol binds to the estradiol receptors (ERs) in hepatocyte cells. It forms homodimers or heterodimers and then binds to the estrogen response element (ERE) on the vitellogenin gene promoter. E2 stimulates vitellogenesis through genomic and non-genomic pathways. (Mahalingam and Santhanam 2023). Vitellogenin is released into the blood circulation as a homomeric complex, then absorbed by developing oocytes via endocytosis. (Gupta et al. 2021). Estradiol concentration induced by different doses of Oodev influences vitellogenin levels. The dose of 0.5mL/kg appears to optimize vitellogenesis, as indicated by high levels of vitellogenin in blood plasma that oocytes have not yet absorbed. In contrast, doses of 0.9 mL/kg reduced vitellogenin levels by decreasing estradiol levels through negative feedback inhibition.

The estradiol and vitellogenin concentrations also interact with cortisol regulation through the HPG axis and hypothalamic-pituitary-interrenal axis (HPI axis) (Fuzzen et al. 2024). The dose of 0.5 mL/kg increased estradiol and vitellogenin concentration while significantly suppressing cortisol, indicating that optimal stimulation of vitellogenesis may mitigate stress responses. Higher doses of Oodev increased cortisol levels as a physiological stress response, which can suppress the HPG axis function to conserve energy. ACTH (adrenocorticotropic hormone) can bind to MC2R (melanocortin-2 receptor) at the ER gene promoter in the liver, reducing ER expression and sensitivity to estradiol, thereby inhibiting vitellogenesis (Navarro et al. 2022; Shaughnessy et al. 2023). Cortisol inhibits GnRH expression through glucocorticoid receptors (GRs) on GnRH neurons. Glucocorticoid response elements (GREs) have been identified in the promoters of GnRH genes in various fish species, including zebrafish, suggesting that cortisol can suppress GnRH transcription. (Khor et al. 2016). Reduced GnRH expression lowers FSH and LH transcription and translation, decreasing estradiol synthesis. (Fuzzen et al. 2010).

The change in body length (Δ Length (cm) was measured to evaluate growth performance among treatments. *N. notopterus* with a length of 20-25cm, particularly those exceeding 24cm, are classified as mature adults, as they have attained gonadal maturity stages IV and V, indicating readiness to spawn (Shankar et al. 2015; Rahmadhani et al. 2024). There was no significant difference among treatments ($P>0.05$), indicating that the Oodev doses in this study did not significantly affect the growth of the putak fish during the experimental period.

Water quality can influence the plasticity of gonadotrope cells in the fish pituitary, which regulate the secretion of FSH and LH, thereby altering reproductive hormone profiles according to environmental conditions (Fontaine et al. 2020). The water quality parameters during the study were within the acceptable range for *N. notopterus*, as supported by previous studies. Temperature ranged from 28 to 30.7℃, DO levels ranged from 9.5 to 15.5mg/L, pH ranged from 8.9 to 9 and TDS values varied from 91.8 to 316.7ppm, remaining within or close to the optimal ranges reported by Gupta et al. (2012) and Kulkarni (2021). These conditions are suitable for maintaining physiological functions, including metabolism and reproduction. Therefore, environmental factors were unlikely to influence the hormonal responses observed in this study negatively.

Conclusion

Oodev at a dose of 0.5mL/kg (P1) resulted in the highest estradiol and vitellogenin concentrations and the lowest cortisol levels. In contrast, 0.7mL/kg (P2) produced the lowest estradiol and highest cortisol levels, indicating a less favorable hormonal profile. Therefore, 0.5mL/kg is the optimal dose for enhancing reproduction in *Notopterus notopterus*, supporting its use in aquaculture and conservation.

DECLARATIONS

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Author's Contribution: Yasmin Ipak Sinantein: Experimental research, Data curation and investigation, laboratory analysis, and writing a draft paper. Sri Rahayu: Conceptualization, Data analysis, and finalization of the paper. Arif Wibowo: Conceptualization, Funding acquisition, Review. Boby Muslimin: Conceptualization, Funding acquisition, and writing a draft paper. Nunak Nafiqoh: Experimental research, Laboratory analysis. Bastiar Nur: Experimental research, Laboratory analysis. Andi Chadijah: Experimental research, Laboratory analysis. Rendy Ginanjar: Format analysis, methodology, Review. Tutik Kadarini: Conceptualization, Review. Mirna Dwirastina: Experimental research, Laboratory analysis. Dewi Apriyani: Experimental research, Laboratory analysis. Mochammad Zamroni: Conceptualization, Data analysis, finalization of the paper.

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