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Induced breeding of climbing perch *Anabas testudineus* using OVASPEC hormone

Induzierte Zucht des Kletterbarsches *Anabas testudineus* mittels des OVASPEC-Hormons

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Summary

Climbing perch, *Anabas testudineus*, is one of the important freshwater fish species with a high economic value. Currently, the climbing perch fish has been cultivated in Indonesia and other Asian countries. But, breeding has not been supported by good hatchery technology, so the availability of high-quality larvae in sufficient quantities has not been resolved. In artificial spawning, hormone injections are often carried out to stimulate the ovulation process in fish. In this study, the OVASPEC was tested for climbing perch. Ovaspec contains the sGnRHa hormone and anti-dopamine. Therefore, the objective of the present study is to determine the optimum dosage of OVASPEC for climbing perch female broodfish. Four dosages of OVASPEC were tested, namely, 0.1 ml kg^{-1} body weight (BW), 0.3 ml kg^{-1} BW, 0.5 ml kg^{-1} BW, and 0.7 ml kg^{-1} BW of broodfish. The results show that the administration of OVASPEC with different doses had a significant effect ($p < 0.05$) on the latency period, fertilization, hatching, and survival rates of climbing perch. Fish injected with OVASPEC at a dose range of 0.1–0.7 ml kg^{-1} BW succeeded in inducing ovulation in climbing perch. The best results were recorded at the dosage of 0.5 ml kg^{-1} BW.

Keywords: kruep; fish breeding; reproductive hormone; sGnRH-a

Zusammenfassung

Der Kletterbarsch *Anabas testudineus* ist ein Süßwasserfisch mit hohem ökonomischem Wert. Derzeit wird der Kletterbarsch in Indonesien und anderen asiatischen Ländern gezüchtet. Die Zucht wird jedoch nicht durch eine gute Bruttechnologie unterstützt und deshalb ist keine ausreichende Menge hochwertiger Larven vorhanden. Beim künstlichen Laichen werden häufig Hormoninjektionen verabreicht, um den Eisprung bei den Fischen anzuregen. In dieser Studie wurde OVASPEC für Kletterbarsche getestet. Das Ziel der vorliegenden Studie war es, die optimale OVASPEC-Dosierung für weibliche Kletterbarsch-Brutfische zu bestimmen. Es wurden vier OVASPEC-Dosierungen getestet, nämlich 0.1 ml kg^{-1} Körpergewicht (KG), 0.3 ml kg^{-1} KG, 0.5 ml kg^{-1} KG und 0.7 ml kg^{-1} KG des Brutfisches. Der einfaktorielle ANOVA-Test zeigte, dass die Verabreichung von OVASPEC in unterschiedlichen Dosen eine signifikante Wirkung ($p < 0,05$) auf die Latenzzeit, Befruchtung und die Schlupf- und Überlebensraten der Kletterbarsche hatte. Bei Fischen, denen OVASPEC in einer Dosierung von 0.1–0.7 ml kg^{-1} KG injiziert wurde, gelang es, bei Kletterbarschen den Eisprung auszulösen. Im Allgemeinen wurden jedoch die besten Ergebnisse bei einer Dosierung von 5 ml kg^{-1} KG erzielt.

Schlagwörter: Kruep; Fischzucht, Fortpflanzungshormone, sGnRH-a

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1. Introduction

Climbing perch, *Anabas testudineus*, is one of the important freshwater fish species distributed in Asia, mostly in Bangladesh, India, Malaysia, Thailand, Vietnam, and Indonesia (Muchlisin and Siti-Azizah, 2009; Hossain et al., 2015; Khatun et al., 2019). This fish has a high commercial value and consumer preference and is favored by the local people because it has good taste and nutritive values (Suriansyah et al., 2011; Akbar, 2012; Muchlisin, 2013). They inhabit in swamps, small rivers, creeks, irrigation canals, and rice fields (Dekar et al., 2018; Nur et al., 2019) and can tolerate estuary conditions and salinities up to 10.2 ppt (Chotipuntu and Avakul, 2010). In Indonesia, the climbing perch is widely distributed in Kalimantan, Java, Sulawesi, and Sumatra, including Aceh province (Bungas et al., 2013; Muchlisin et al., 2015). In the local language of Acehnese, this fish is known as *kruep* (Muchlisin and Siti-Azizah, 2009). Climbing perch farming has been initiated by several fish farmers. However, this business is still facing obstacles, including the scarcity of high-quality larvae, due to underdeveloped hatchery technology for this fish. Therefore, intensive efforts are crucially needed to overcome this problem.

Hormone manipulation is frequently applied in hatcheries to stimulate gonadal maturation and ovulation in fish (Santika, 201). There are several synthetic hormones available in the market and are often used for fish breeding and seed production, for instance, Ovaprim, Oodev, HCG, Spawnprim, and Oxytocin (Muchlisin et al., 2014; Saptono et al., 2018; Rahmi et al., 2020; Darliansyah et al., 2017; Siregar et al., 2018; Leonita et al., 2021). The application of the synthetic hormones for induced breeding of climbing perch has been reported by several researchers. Saptono et al. (2018) reported that Ovaprim at a dose of 0.5 ml kg^{-1} body weight (BW) was the best dosage for climbing perch; Siregar et al. (2018) reported that 0.5 ml kg^{-1} BW was the best dosage for HCG, and Rahmi et al. (2020) tested Oodev hormone and reported that 0.7 ml kg^{-1} BW was the best dose for this species. In addition, Aya et al. (2023) demonstrated the effectiveness of the combination of 0.5 ml IU HCG and 0.5 ml kg^{-1} Ovaprim for inducing the breeding of climbing perch. However, the application of the OVASPEC hormone in climbing perch, *A. testudineus*, has not been reported despite its potential importance as cost-effective and widely available alternative for induced breeding of fish.

OVASPEC is a synthetic reproductive hormone that contains a salmon analog gonadotrophin-releasing hormone (sGnRH-a) and anti-dopamine. This hormone plays an important role in triggering the endogenous gonadotropin hormone release that further induces ovulation and the spawning process in fish (Sukendi, 1995). The successful breeding and seed production using OVASPEC hormone have been reported in two species of fish, namely, peres fish *Osteochilus kappeni* (Faisal and Komariyah, 2021) and seluang fish *Rasbora einthovenii* (Syarif et al., 2021). However, the use of OVASPEC hormone in climbing perch has not been reported previously. Therefore, this study aims to determine the optimal dose of OVASPEC hormone for the induced breeding and seed production in climbing perch.

2. Material and Methods

2.1. Site and time

The study was done in the Laboratory of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Padang, and in the Bungus Hatchery Center Padang, Sumatra Barat, from June to July 2022.

2.2. Experimental design

The non-factorial completely randomized design (CRD) was employed in this study. The tested factor was the differences of OVASPEC hormone (Spectrum Asia) at four dosages, namely, 0.1 ml kg^{-1} body weight (BW), 0.3 ml kg^{-1} BW, 0.5 ml kg^{-1} BW, and 0.7 ml kg^{-1} BW of female broodfish. Each treatment was performed at three replications.

2.3. Broodfish

A total of 50 matured male broodfish and 15 female broodfish were collected from a local fish farmer in Padang city. The male broodfish body weight ranges from 8 to 12 g and the total length measures 6–8 cm, while the female ranges from 35 to 45 g body weight and 10–13 cm in total length. The broodfish was acclimatized for 1 week in the broodfish tanks. During this process, the fish fed on a commercial fish diet contains 40% crude protein, two times a day at 08.00 AM and 16.00 PM.

2.4. Spawning process and egg incubation

Fish was injected with OVASPEC with respective dosages that were divided into two injections (half dosage for every

injection). The first injection was done at 12.00 PM, and the second injection was carried out 6 hours after the first injection (at 06.00 AM). The male fish were injected with a dosage of 0.25 ml kg^{-1} with a single dose at 06.00 AM. The injected female and male fish were paired at a 1:4 ratio (female: male) and placed in styrofoam containers (48 cm × 32 cm × 29 cm). Breeding and ovulation success were monitored in two-hour intervals.

After ovulation occurs, the genital area was cleaned with dry tissue paper; then, the abdomen was gently pressured, and the eggs and sperm were collected in a plastic basin and placed in an icebox at 4 °C. The sperm was diluted with a physiological solution (NaCl 0.9%) as an extender with a ratio of 1:60 (sperm: extender, v/v). Then, the diluted sperm was mixed with eggs in a ratio of 1:4 (v/v), and then, 2 drops of tap water were mixed to activate the sperm. Then, the mixture was stirred evenly using chicken feathers and left for about 5 min to allow the sperm to fertilize the egg.

A total of 100 eggs were sampled randomly and incubated in the plastic basin. The fertilization rate was determined after 2 hours of incubation for each hormone dose. The fertilized eggs are characterized by transparent color characteristics, while the unfertilized eggs are opaque white (Muchlisin et al., 2015b; Muthmainnah, 2018). Unfertilized eggs were removed from the incubation basin. The fertilized eggs were monitored every 2 hours until hatching. After hatching, the larvae were reared for 2 weeks, and the larvae were fed with *Artemia nauplii* three times a day at satiation.

2.5. Measured parameter

Latency period and egg size: The latency period was calculated following Manantung et al. (2013) as follows: Latency period = (Ovulation time – hormone injection time). The egg diameter was measured by selecting 30 eggs randomly and then measuring them using a stereo microscope at 200X magnification.

Fertilization, hatching, and survival rates: The percentage fertilization rate was calculated based on Maulida et al. (2021) as follows: Fertilization rate (%) = (The fertilized eggs/the total of incubation eggs) × 100. The percentage hatching rate was calculated based on Muchlisin et al. (2016) as follows: Hatching rate (%) = (Total of hatched eggs/the fertilized eggs) × 100. The survival of the larvae was calculated according to Muchlisin et al. (2017) as follows: Survival rate (%) = $(N_o - N_t)/No \times 100$, where N_t is the total larvae died during the study and N_o is the total larvae at the beginning of the study.

Table 1. Latency time, fertility, hatching, and survival rate of climbing perch *A. testudineus* according to the dosage treatment of the OVASPEC. Mean values ± SD at the same column followed by the different superscripts are significantly different ($p < 0.05$)
Tabelle 1. Latenzzeit, Fruchtbarkeit, Schlupf- und Überlebensrate des Kletterbarsches *A. testudineus* je nach Dosierungsbehandlung mit OVASPEC. Mittelwerte±SD in derselben Spalte, gefolgt von den verschiedenen hochgestellten Buchstaben, unterscheiden sich signifikant ($p < 0,05$).

OVASPEC dosage (ml/kg BW)	The latency period (min)	Fertilization (%)	Hatching (%)	Survival (%)
0.1	441.6±10.4^{c}	65.3±8.38^{d}	44.59±1.08^{c}	85.00±4.86^{b}
0.3	428.3±10.4^{bc}	74.3±4.50^{c}	73.21±4.91^{b}	95.52±0.87^{a}
0.5	415.6±2.51^{ab}	91.3±3.21^{a}	92.34±0.87^{a}	97.38±0.66^{a}
0.7	398.8±7.63^{a}	84.3±4.16^{ab}	90.91±1.72^{a}	96.05±1.40^{a}

Table 2. The egg size of climbing perch *A. testudineus* before and after injection of the OVASPEC. Mean values ± SD at the same column followed by the different superscripts are significantly different ($p < 0.05$)
Tabelle 2. Die Eigröße des Kletterbarsches *A. testudineus* vor und nach der Injektion von OVASPEC. Mittelwerte ± SD in derselben Spalte, gefolgt von den verschiedenen hochgestellten Buchstaben, unterscheiden sich signifikant ($p < 0,05$)

OVASPEC dosage (ml/kg BW)	Egg size pre-hormone injection (µm)	Ovulated egg size (µm)	Increased egg size (µm)
0.1	41.336	43.369	2.07±0.8^{a}
0.3	41.336	43.3338	2.58±0.6^{a}
0.5	41.336	43.5654	2.47±0.5^{a}
0.7	41.336	45.6752	4.37±0.1^{b}

2.6. Data analysis

Data were analyzed using one-way of analysis of variance (ANOVA) at 95% confidence limits and then followed by Duncan's multiple range test to determine the significant difference among the hormone dosages.

3. Results

The ANOVA test results show that differences in OVASPEC dose had a significant effect on latency period, fertilization, hatching, and survival rates of climbing perch ($p < 0.05$). The results indicated that a significantly low latency period was observed in the dosage of 0.7 ml kg^{-1} BW (398.3 min) compared to other treatments ($p < 0.05$). The highest fertilization rate and hatching rates were obtained in the dosage of 0.5 ml kg^{-1} BW with the value of 91.33% and 92.34%, respectively.

These values were significantly different from the 0.1 ml kg^{-1} BW and 0.3 ml kg^{-1} BW dosages but not significantly different from the dosage of 0.7 ml kg^{-1} BW ($p > 0.05$). The highest percentage of larval survival was also obtained by 0.5 ml kg^{-1} BW treatment (97.38%), and this value was significantly different from the 0.1 ml kg^{-1} BW treatment but was not significantly different from other treatments ($p > 0.05$) (Table 1).

The ANOVA test results also reveal that the differences in the dose of OVASPEC hormone had a significant effect on egg diameter ($p < 0.05$). The results showed that the highest egg diameter was found in the dosage of 0.7 ml kg^{-1} BW (4,372 μm), and this value was significantly different from other treatments (Table 2).

4. Discussion

The study showed that increasing the dose of OVASPEC hormone resulted in reduction in the latency period and effectively accelerated the ovulation process. This effect is probably due to the dose-dependent increase in GnRH hormone, which in turn accelerates the stimulation of the pituitary gland to produce enhanced gonadotropin hormones such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH plays a role in oocyte development (vitellogenesis), while LH functions in oocyte maturation to accelerate the process of ovulation (Sukendi, 2001). The OVASPEC also contains an anti-dopamine (dopamine blocker), which functions to inhibit the action of dopamine. This dopamine hormone can block the production of gonadotropin hormones (LH and FSH) (Rottmann et al., 1991; Bryant et al., 2016). Hence, in the presence of a dopamine blocker, the dopamine hormone is not secreted, allowing the LH and FSH hormones to act optimally and to accelerate gonadal maturation (Hoar et al., 1983; Hill et al., 2009).

This study shows that the application of OVAPSEC at the dose of 0.5 ml kg^{-1} BW gave better results compared to other tested hormone doses, with fertilization, hatching, and survival rates of 91.3%, 92.34%, and 97.38%, respectively. However, the recorded fertilization and hatching rates in this study were lower than those reported by Choresca et al. (2024) with the values of 99.13% and 97.50%, but the larval survival rates were higher than those reported in previous reports of 84.69%.

In addition, the results of the present study were higher than the performance of Ovaprim at the same dose on climbing perch, which produced fertilization and hatching rates of 75.11% and 78.09%, respectively (Saptono et al., 2018). This suggests that OVASPEC is found to be more effective than Ovaprim in climbing perch. Further, the study also shows that the hormone dose of 0.5 ml kg^{-1} BW OVASPEC increased hatching and a larval survival rates above 90%. However, the increased hormone dose of OVASPEC more than 0.5 ml kg^{-1} BW decreased egg quality, fertilization, and hatching rates at the dosage of 0.7 ml kg^{-1} BW. Increasing the OVASPEC dosage, in addition to resulting in a short latency period, also causes the eggs to develop bigger, but the fertility and hatching rates decreased. This indicates that although the egg size was increasing, their quality was decreasing as described above. This is likely due to the hormone dosage exceeding the optimum limit (overdose), resulting in an excessively high level of gonadotropin in the blood, this causes the vitellogenesis process to accelerate, leading to overripe eggs. This is in agreement with I'tishom (2008), who stated that hormones work optimally at the suitable dose. The use of an inappropriate dosage reduces the biological potential of fish, as seen in the fertilization and hatching rates recorded in this study.

The application of high doses of productive hormones caused early ovulation and ovulatory eggs to remain in the ovarian lumen in hypoxic conditions, which led to a decrease in egg quality (Dewantoro, 2017). According to Fujaya (2004), the administration of overdose hormones can reduce the potential of fertilization and hatching rate, result in low-quality larvae, and also decrease the survival rate. However, egg hatching and survival are not only influenced by egg quality but also affected by environmental factors such as temperature, dissolved oxygen, and pH. The water quality parameters for the experimental water were a temperature of 26–27 °C, dissolved oxygen levels of 5.40–5.43 mg L^{-1}, and a pH of 7.0. However, the optimum temperature for the hatching of climbing perch fish eggs was 26–29 °C, dissolved oxygen 2–5 mg L^{-1}, and pH 6.5–7.5 (Marlida, 2001). Therefore, the water quality ranges are still suitable for the hatching and survival of climbing perch *A. testudineus* larvae.

This study, along with previous reports by Choresca et al. (2024), Aya et al. (2023), and Maulida et al. (2022; 2023; 2024), has addressed the problems of climbing perch breeding. However, still other challenges remain; for instance, the nutritional requirements of larval stages are crucial and need to be explored intensively (Putra et al., 2016) for hatchery seed production and commercial culture of this valuable fish species.

5. Conclusion

The dose of OVASPEC hormone had a significant effect on the latency period, fertilization rate, hatching of eggs, and survival rates of climbing perch *Anabas testudineus* larvae.

The study recommends 0.5 ml kg^{-1} BW as the optimal dose of OVASPEC for successful breeding and seed production of climbing perch.

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