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Hormonal induction of gonad maturation in female tinfoil barb fish *Barbonymus schwanenfeldii* using spawnprim hormone

Induksi hormonal terhadap pematangan gonad ikan lemeduk betina *Barbonymus schwanenfeldii* dengan menggunakan hormone spawnprim

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ABSTRACT

The aim of this study was to analyze the effect of spawnprim hormone induction with different doses on gonadal maturity of tinfoil barb fish broodstock (*Barbonymus schwanenfeldii*). A completely randomized design (CRD) was used in this study with 4 treatments and 3 replications. Fish broodstock in group A (control) was not given any treatment, while fish broodstocks in groups B, C, and D were injected with spawnprim hormone at doses of 0.3 ml/kg body weight, 0.6 ml/kg body weight, and 0.9 ml/kg body weight, respectively. The measured parameters were gonad maturity level, broodstock weight gain, egg diameter, and fecundity. Data were analyzed using one way analysis of variance (ANOVA). The result showed that the gonad maturity level (GML) in groups B, C, and D (GML III and IV) was better than group A (GML I). Statistical analysis showed that the induction of spawnprim hormone on tinfoil barb broodstocks significantly affect ($P<0.05$) broodstock weight gain, egg diameter, and fecundity. The optimal dose of spawnprim hormone for tinfoil barb was 0.6 ml/kg body weight, with the average broodstock weight gain, egg diameter, and fecundity were 0.011 kg, 1.55 mm, and 102.15 eggs, respectively. The induction of spawnprim hormone has a positive effect on gonad maturation of tinfoil barb fish (*B. schwanenfeldii*), with the optimum dose is 0.6 ml/kg fish.

Keywords: gonad maturity, tinfoil barb fish, spawnprim

ABSTRAK

Penelitian ini bertujuan untuk menganalisis pengaruh penggunaan hormon spawnprim dengan dosis yang berbeda terhadap kematangan gonad induk ikan lemeduk *Barbonymus schwanenfeldii*. Metode yang digunakan pada penelitian ini adalah metode rancangan acak lengkap (RAL) dengan 4 kelompok perlakuan dan 3 kali pengulangan. Ikan pada kelompok A (kontrol) tidak diberikan perlakuan, sedangkan ikan pada kelompok B, C, dan D masing-masing diinjeksi hormon spawnprim dengan dosis 0.3 ml/kg bobot badan; 0.6 ml/kg bobot badan; dan 0,9 ml/kg bobot badan. Parameter yang diamati adalah persentase induk matang gonad akhir, pertambahan bobot induk, pertambahan diameter telur, dan fekunditas. Data dianalisis dengan menggunakan analisis varians (ANAVA) pola satu arah. Hasil penelitian menunjukkan tingkat kematangan gonad (TKG) pada kelompok perlakuan B, C, dan D (TKG III dan IV) lebih baik dari kelompok A (TKG I). Hasil uji ANAVA menunjukkan bahwa pemberian hormon spawnprim pada induk ikan lemeduk berpengaruh nyata ($P<0.05$) terhadap pertambahan bobot induk, pertambahan diameter telur, dan fekunditas. Tingkat kematangan gonad terbaik pada penelitian ini diperoleh pada kelompok perlakuan C yang diinduksi hormon spawnprim dengan dosis 0.6 ml/kg bobot badan induk ikan dengan rata - rata pertambahan bobot induk, pertambahan diameter telur, dan fekunditas masing-masing adalah 0.011 kg, 1.55 mm, dan 102.15 butir telur. Induksi hormon spawnprim menimbulkan pengaruh yang positif terhadap pertambahan bobot induk, diameter telur dan fekunditas ikan lemeduk (*B. schwanenfeldii*), dengan dosis optimum 0.6 ml/kg bobot badan.

Kata kunci: kematangan gonad, ikan lemeduk, spawnprim

INTRODUCTION

Tinfoil barb fish (*Barbonymus schwanenfeldii*) is one of the freshwater fishery resources found in river waters in Aceh. This fish is also often called lemeduk fish, lampam fish, tengadak fish, kapiek, kapiat, lempem, or lempam. One of the efforts to increase tinfoil barb fish production to meet market demand is through cultivation. Currently, tinfoil barb fish cultivation is still on going at the Lukup Badak Fish Seed Center (UPTD-BBI) in Aceh Tengah. However, it still has several problems, especially those related to hatchery activities. The availability of quality seeds in adequate and continuous quantity is part of the production components that must be fulfilled. The success of the hatchery itself is very dependent on the success of spawning. In new domesticated fish that cannot be spawned naturally, artificial spawning is an alternative that can be done. This is due to the fact that optimal environmental conditions are not yet fully known, or not possible to simulate the required environmental condition for fish to secrete adequate reproductive hormones for natural reproductive performance since environmental condition varies from time to time and greatly affect the fish reproduction (Mylonas *et al*., 2010; Gustiano *et al*., 2020; Servili *et al*., 2020; Zohar, 2021)).

Tinfoil barb domestication efforts for cultivation purposes are deemed necessary, considering that these fish have high economic and ecological value. Currently, the production of tinfoil barb fish (*Barbonymus schwanenfeldii*) seeds carried out at Lukup Badak BBI is constrained by the maturity season of the fish gonads, the long re-maturation process, and the poor quality of the eggs produced. To speed up the gonadal maturation and re-maturation in tinfoil barb fish broodstock, synthetic hormones can be administered. According to Emilda (2015), the choice of hormonal application method is based on the effectiveness, efficiency, palatability, and the cost required. Previous study has been evaluated the effectiveness of ovaprim hormone on *Barbonymus schwanenfeldii* fish which showed the positive effect on spawning variables (Dewantoro *et al*., 2017). However, the use of artificial hormones such as ovaprim has a disadvantage such as the fluctuate price and its availability is sometimes problematic due to ovaprim is an imported product. Alternatively, another hormone that can be used to accelerate the maturation of the gonads of fish is spawnprim.

Spawnprim hormone has been effectively used in the gonad maturation and spawning processes in comet fish (Hidayat, 2010) and red fin shark fish (Islami *et al*., 2017). Spawnprim was also more effective to induce the final gonad maturation in Tor soro fish, which includes ovulation, spawning and the reproductive performances were observed better compared to the use of ovaprim. In contrast, Leonita *et al*. (2021) observed that the spawnprim hormone at dose of 0.5 ml/kg body weight was not effective to enhance the relative fecundity, fertilization rate and hatching rate of *Pangasianodon hypophthalmus* as compared to ovaprim at the same dose.

Although the spawnprim already used for fish spawning, but the dosage used is different for each type of fish. The spawnprim dose needed to stimulate spawning of comet fish was 0.5 mL/kg (Hidayat, 2010), while for *Clarias* sp the injection of spawnprim hormone at dose of 0.5 ml/kg could not enhance the spawning performance as compared to ovaprim (Yulianti *et al*., 2020). Injection of spawnprim hormone at dose of 0.5 mL/kg and 1 ml/kg body weight fish has similar effect in red fin shark with 100 % spawning rate (Islami *et al*., 2017). So far, there are no reports about available regarding the use of spawnprim with the optimal dose on tinfoil barb fish. Therefore, the aim of this study was to analyze the effect of spawnprim hormone at different doses on the gonadal maturity of tinfoil barb fish broodstock, which includes the level of the broodstock gonad maturity, weight gain, increase in egg diameter, and fecundity.

MATERIALS AND METHODS

Experimental design

The design used in this study was a completely randomized design (CRD) consisting of 4 treatments and 3 replications. Determination of the dose of spawnprim in this study was based on the dose of ovaprim given to tinfoil barb fish (Dewantoro *et al*., 2017), namely 0.0; 0.3; 0.6; and 0.9 ml/kg of female broodstock. A total of 12 female tinfoil barb fish were divided into four treatment groups (three fishes per treatment group) and injected with the spawnprim hormone at doses of 0 ml/kg bw (group A, control), 0.3 ml/kg bw (group B), 0.6 ml/kg bw (group C), and 0.9 ml/kg bw (group D).

Experimental procedure

The research was conducted in a concrete fish pond with a size of 1.2x0.40x1 m located at Lukup Badak BBI. Before being used as a research container, the pond was cleaned and dried for one day. Twelve broodstock weighing ± 450g from UPTD-BBI Lukup Badak in Central Aceh were used. The broodstock were given a microchip as a marker of the treatment given. After that, the fish were weighed to determine the initial weight. Egg sampling was done through catheterization which was aimed to ensure the maturity level of the early gonads. Injecting the spawnprim hormone in the test fish was done intramuscularly according to the treatment dose and body weight. The fish were sedated first with a stabilizer (Arowana™) at a dose of 10 ml/30 liters. The spawnprim was then injected to the fish at dose of 0.3 ml/kg, 0.6 ml/kg, and 0.9 ml/kg broodstock according to the treatment dose. The fish were not left for too long in a container containing a stabilizer to avoid stress. Measurement of the parameters of gonadal maturity and fish growth was carried out on days 0 and 20. Measurement on day 0 aims to determine the initial weight of the fish and the initial egg samples.

Parameters

The level of gonad maturity was determined visually based on five stages of gonad maturity according to Haryono *et al*. (2015) which was developed for *B. balleroides*, a relative of *Barbonymus schwanenfeldii.* The classification including gonad maturity level I (immature), gonad maturity level II (developing), gonad maturity level III (develop), gonad maturity level IV (mature), and gonad maturity level V (spent).

The fish weight was measured using the equation of Effendie (1997) as follows:

$$W = Wt - W0$$

W = weight gain (gr)
Wt = average weight of fish at the end of the study (gr)
W0 = average weight of fish at the beginning of the study (gr)

The diameter of fish eggs was measured using ocular millimeters (Farastuti *et al*., 2014). Measuring the increase in egg diameter used the following equation:

$$Ds = Dt - D0$$

Ds = actual egg diameter
Dt = diameter of the final egg
D0 = diameter of the initial egg

The fecundity value was calculated based on the formula used by Effendie (1997).

$$F = \frac{ba - Ba}{Q}$$

F = Fecundity (egg)
Ba = Initial fish weight (g)
ba = Fish weight + egg (g)
Q = Average weight of eggs (g)

Data Analysis

The gonad maturity level was analyzed descriptively, while the broodstock weight gain, fish egg diameter and fecundity values were analyzed using one way analysis of variance followed by Duncan multiple range test at a 95% confidence level.

RESULTS AND DISCUSSION

Results

The results of observations on the gonad maturity level (GML) of tinfoil barb fish after spawnprim hormone induction in 4 treatment groups can be seen in Table 1. Of the 4 treatment groups, the fish in the control group did not lay eggs, while the fish in the groups that were injected with the spawnprim hormone with different doses all laid eggs.

Broodstock weight gain, egg diameter and fecundity value of tinfoil barb fish after spawnprim hormone induction can be seen in Table 2. The use of spawnprim hormone at a dose of 0 ml/kg body weight, 0.3 ml/kg body weight, 0.6 ml/kg body weight and 0.9 ml/kg body weight showed different results on broodstock weight gain, egg diameter and fish fecundity. The administration of spawnprim hormone at doses of 0.3 and 0.6 mg/kg bw showed a significant increase in body weight of the fish ($P < 0.05$) compared to the fish in control group and group D (injected with 0.9 ml/kg bw).

The results obtained for the egg diameter of the tinfoil barb fish which were successfully ovulated after being treated showed that fish in groups B, C, and D had significantly different egg diameters as compared to those in group A

(without spawnprim induction). Based on the values, the largest egg diameter was obtained in group C (1.55 ± 0.0074 mm) and the smallest was in group A (0 ± 0), while in the B and D groups, the diameters were 1.32 ± 0.1259 mm and 1.17 ± 0.2124 mm, respectively (Table 2). The lowest fecundity value was found in group A (0 ± 0), which was significantly lower (P <0.05) compared to groups B, C, and D.

Discussion

Spawnprim hormone injection was able to stimulate the gonadal maturation of tinfoil barb fish. This is based on the value of GML levels, broodstock weight gain, fecundity and egg diameter in group B, C, D as compared to group A (without spawnprjm hormone injection). The increase in the observed parameter values due to the vitellogenesis process. The spawnprim hormone has biological properties such as luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH), with more FSH workforce elements than LH. The function of FSH is to help in the process of egg formation in fish, while LH plays a role in stimulating the maturation process of the gonads which are then ready for vittelogenesis (Farastuti *et al.,* 2014).

The level of gonad maturity varied based on the treatment group. Gonad maturity level I was found in group A (ovaries as thread-like, length up to the front of the abdominal cavity, clear, slippery surface) and GML III was observed in group B (the yellowish ovaries fill half of the abdominal cavity and the eggs begin clearly visible). Fish in groups C and D have similar GML (GML IV) which showed that ovaries dominating the abdominal cavity, the color becomes yellow and darker, and the eggs are visible. The better GML observed in fish injected with spawnprim hormone revealed the effectiveness of this hormone in inducing the gonad maturity in tinfoil barb fish in this study. The administration of spawnprim hormone to tinfoil barb broodstock also provided a positive response to the relative weight gain of the broodstock. The fish weight in group injected with 0.3 and 0.6 ml/kg bw of spawnprim hormone were 0.011 ± 0.0011 and 0.011 ± 0.0003, respectively, and it was significantly increased (P<0.05) as compared to un-injected group (0.0032 ± 0.0004). This was presumably due to the development of oocytes which were filled with vitellogenin, as a response to spawnprim hormone injection. Vitellogenin is the egg yolk, which is the main component of the developing oocyte. The development of the oocyte is marked by the increasing weight of the female broodstock gonads. This happens

Table 1. The gonad maturity level of tinfoil barb fish in 4 treatment groups on day20.

Spawnprim dosage	Replication	Day 20
Group A (0 ml/ kg)	1	GML I
	2	GML I
	3	GML I
Group B (0.3ml/kg)	1	GML III
	2	GML III
	3	GML III
Group C (0.6ml/kg)	1	GML IV
	2	GML IV
	3	GML IV
Group D (0.9ml/kg)	1	GML IV
	2	GML IV
	3	GML IV

Table 2. Broodstock weight gain, egg diameter and fecundity of tinfoil barb fish (*Barbonymus schwanenfeldii*) after being induced with different doses of spawnprim hormone (mean±SE).

	Parameters		
Spawnprim dose	Broodstock weight gain (W) (kg)	Egg diameter (mm)	Fecundity (egg)
A (0 ml/kg)	0.0032 ± 0.0004[a]	0 ± 0[a]	0 ± 0[a]
B (0.3 ml/kg)	0.011 ± 0.0011[b]	1.32 ± 0.1259[c]	102.06 ± 0.00021[b]
C (0.6 ml/kg)	0.011 ± 0.0003[b]	1.55 ± 0.0074[d]	102.15 ± 0.0001[b]
D (0.9 ml/kg)	0.0056 ± 0.0026[a]	1.17 ± 0.2124[b]	80.81 ± 0.00022[b]

[a,b,c,d] Different superscripts in the same column showed significant differences (P <0.05).

because when the vitellogenesis process takes place, the yolk granules increase in number and size, so that the volume of the oocyte becomes enlarged (Pamungkas *et al*., 2019). The induction of spawnprim hormone in Siamese catfish also showed a positive response regarding weight gain of the fish broodstock because the spawnprim hormone increases the appetite of the fish (Islami *et al*., 2017), which also shown in this study.

The diameter of tinfoil barb fish eggs in the spawnprim hormone injection group increased compared to the untreated group. This observation indicated that the induction of this hormone has an influence on the vitellogenesis process. According to Hara *et al*. (2016), vitellogenesis is the combination of vitellogenin proteins carried out by oocytes, which process them into egg yolk protein, thereby increasing the gonad size in female fish until final maturation. Eggs are the final result of the gametogenesis process after the oocyte undergoes a growth phase that is highly dependent on the presence of gonadotropin hormones. Spawnprim hormone contains LHRH-a which can stimulate the hypophysis to produce gonadotropin hormone (Darmawi, 2017), assist the gonad maturity and affect the increasing of egg diameter (Leonita *et al*., 2021) as also observed in this study. Effendie (1997) stated that the higher the level of maturity of the gonads, the larger the egg diameter in the ovary. Furthermore, Farastuti *et al*. (2014) stated that the development of egg diameter in teleosotei oocytes is generally due to the accumulation of egg yolk.

The mechanism of spawnprim hormone in induce gonadal maturation in tinfoil barb fish is similar to that of ovaprim mechanism in general. Hafeez-ur-Rehman *et al*. (2015) mentioned that the component of salmon gonadotropin releasing hormone analog (sGnRH-a) and domperidone in ovaprim which is induced through the bloodstream can replace lost environmental signals to trigger the secretion of gonadotropins hormone (GtH) in the brain. SGnRH-a plays a role in triggering the pituitary gland to secrete GtH which in turn induces the final maturation process, while domperidone is a dopamine antagonist whose role is to help sGnRH-a work by eliminating the inhibitory effect of dopamine on GtH secretion in the brain (Bryant *et al*., 2016; Falahatkar *et al*., 2013, Abdel Latif *et al*., 2021). The dominant GtH is secreted in mature broodstock gonads, namely luteinizing hormone (LH). Fontaine *et al.* (2020) stated that LH secretion occurs caused by negative feedback on FSH due to the accumulation of testosterone in theca cells after developing oocytes reach their maximum size or becoming mature gonads. The accumulation of testosterone in theca cells is caused by the decrease of aromatase activity to produce estradiol-17β in granulose cells from testosterone (Basuki, 2007). LH will induce the final oocyte maturation process by stimulating the follicles that produce 17α, 20β-Dihydroxy-4pregnen-3-one (17α, 20β-DHP) steroid hormone (Hasegawa *et al*., 2022). This hormone has a role as a mediator for further oocyte maturity until it experiences germinal vesicle break down and ends in ovulation (Azarin *et al*., 2016).

From several previous studies it was known that the spawnprim hormone has the same effectiveness as the ovaprim hormone. Hidayat (2010) observed that the GML level (including the ovulation, egg diameter, degree of fertilization, hatching rate, survival rate, feed efficiency) in comet fish given the spawnprim hormone at a dose of 10 ml/kg body weight was similar ($P > 0.05$) compared to the GML in the comet fish group that was given the ovaprim hormone at a dose of 0.5 ml/kg body weight. Furthermore, Basuki (2007) mentioned that treatment using OODEV (another gonadal maturation hormone) also shows a role in gonad maturation which occurs twice in three months. This shows that OODEV, which also contains more FSH hormone like spawnprim hormone used in this study, plays a very important role in the process of vitellogenesis and maturation of the gonads. However, OODEV can only stimulate the maturation process of gonads, unlike spawnprim which can also play a role in stimulating fish spawning.

The results of this study also revealed that of the 3 doses of spawnprim used, the dose of 0.3 ml/kg body weight and 0.6 ml/kg body weight could increase the growth of broodstock, egg diameter and fecundity value of tinfoil barb fish. However based on fecundity value, the dose of 0.6 ml/kg body weight was the optimal dose to induce the gonad maturity of tinfoil barb fish. This dose is similar to the optimal dose of ovaprim used in inducing the gonad maturity of broodstock observed by Dewantoro *et al*. (2017), which was of 0.6 ml/kg body weight.

CONCLUSION

The injection of spawnprim hormone has a positive effect on broodstock weight gain, egg

diameter and fecundity of tinfoil barb fish (*B. schwanenfeldii*), with the optimum dose is 0.6 ml/kg fish.

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