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|  | EurAsian Journal of BioSciences  Eurasia J Biosci 14, 1-7 (2020)  The role of HCG in jelawat fish (*Leptobarbus hoevenii*) breeding in Indonesia  **Siti Hudaidah 1\*, Maulana Iqbal Abdul Aziz 1, Muhammad Browijoyo Santanumurti 2, Tarsim 1**  1 Department of Fisheries and Marine Science, Faculty of Agriculture, University of Lampung, Jl. Prof. Sumantri Brojonegoro, Bandar Lampung, Lampung 35141, INDONESIA  2 Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Mulyorejo, Mulyorejo, Surabaya 60115, INDONESIA  \*Corresponding author: [idahasan64@gmail.com](mailto:idahasan64@gmail.com)   |  | | --- | | **Abstract**  Jelawat fish (*L. hoevenii*) is a fish that is easily found in Southeast Asia, including Indonesia. It has high economic value and good nutrition content so many farmers are encouraged to do Jelawat fish aquaculture activity. One of the problem is the availability of quality seeds that cannot be fulfilled and depend on natural stock. The solution is using HCG (Human Chrorionic Gonadotropin). This study used three treatments, P1 (HCG 0 IU/kg), P2 (HCG 300 IU/kg) and P3 (HCG 600 IU/kg). The results showed that the injection of HCG was effective to increase the spawning and reproductive performance of jelawat fish with optimal results in the 600 IU/kg treatment. These treatments showed a 100% ovulation rate, 8 hours latency time, 121.333 eggs fecundity, 1.183 mm egg diameter, 86.16% fertilization rate, 87.48% hatching rate, 94.88% survival rate and larvae abnormality of 0.  **Keywords:** food security, HCG, hatchery, jelawat fish  Hudaidah S, Aziz MIA, Santanumurti MB, Tarsim (2020) The role of HCG in jelawat fish (*Leptobarbus hoevenii*) breeding in Indonesia. Eurasia J Biosci 14: 1-7.  © 2020 Hudaidah et al.  This is an open-access article distributed under the terms of the Creative Commons Attribution License. | |

# INTRODUCTION

Jelawat fish (*L. hoevenii*) is a fish that is easily found in Southeast Asia, such as Laos, Thailand, Malaysia and Indonesia (Srithongthum et al. 2020). In Indonesia itself, jelawat fish is a freshwater fish found in Indonesian public waters, such as in Sumatra and Kalimantan (Sintia et al. 2020). These fish have high economic value. The selling price can reach 4 USD/kg in and is imported to Malaysia and Brunei (Selawati et al. 2019). Not only economical, jelawat fish also has good nutritional content, such as 61.33% protein, 11.9% fat, vitamins B and minerals (calcium, phosphorus and iron) (Au et al. 2020, Aryani et al. 2012). Therefore, the activity of jelawat fish farming continues to be increased in Indonesia.

In the aquaculture of jelawat fish, the availability of quality seeds cannot be fulfilled, so it still depends a lot on nature (Rimalia 2014). In the seedling process, it is still determined by the season so that seeds cannot be available all the time (Cho et al. 1985). This is not a good thing to do because it will disturb the sustainability of what is in nature. To overcome this, it is necessary to provide hormones to accelerate egg development so that it will increase the effectiveness of hatchery activities.

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One of the hormones that plays a role in spurring reproductive development is HCG (Human Chrorionic Gonadotropin) (Mahadevi et al. 2020). HCG has been used in cultured fish since the 1930s to promote ovulation and spawning (Badran et al. 2019). Giving HCG to *Clarias lazera* is able to speed up latent time and increase gonad weight and the number of eggs released (Ahmed 2018). Giving HCG for 4 weeks has an effect on gonad maturation of pomfret fish (Handrianto et al. 2017). In *Sparus aurata*, giving a combination of HCG and LHRHa resulted in better reproductive performance than giving one type of hormone (Wahbi et al. 2017). Giving a combination of HCG and ovaprim to kelabau fish (*Osteochilus melanopleura*) can shorten the latency time, increase the degree of fertilization and the degree of hatching (Rachimi et al. 2015). The effectiveness of HCG application to jelawat fish in Indonesia has not been widely reported. This study aims to determine the effect of HCG on the reproductive performance of jelawat fish and to determine the optimal dosage for its use. The results of this study are expected to increase the development of jelawat fish as food security in Indonesia.

# MATERIAL AND METHODS

**Materials**

The material used in this study was jelawat fish (*L. hoevenii*) obtained from the Freshwater Aquaculture Fishery Sungai Glam, Muaro Jambi, Jambi, Indonesia. HCG used in this study was a product of Intervet Intl. B.V. Netherlands while ovaprim was from Syndel Laboratories LTD.

## Research procedure

The research procedure was in accordance with previous research (Rimalia 2014). HCG and ovaprim were injected to stimulate the maturation of the gonads. The injection was carried out intramuscularly to the female broodstock at a predetermined dose with a tuberculine syringe measuring 1 ml. This study used three treatments, P1 (HCG 0 IU/kg), P2 (HCG 300 IU/kg) and P3 (HCG 600 IU/kg). HCG injection was carried out 7 days before the injection of the Ovaprim hormone 0.7 ml/kg. The injection of the ovaprim hormone was done in three stages of injection, the first injection of dose, 5 hours intervals of a second injection of dose, and an interval of 7 hours, a third injection of ½ dose.

Broodstock that had been injected were inserted into a 3 x 4 meter spawning pond with a water depth of 1.2 meters with a male to female parent ratio of 2: 1. Then the female was transferred to a hatchery in the form of a fiber tub with a capacity of 250 liters.

### Parameter analyzing

The analysis parameters consisted of latent time and ovulation rate, fecundity, egg diameter, fertilization rate, harching rate, survival rate and larval abnormality. The latent time for jelawat fish spawning was calculated based on data taken during the spawning process by calculating the time difference from the last injection to the release of the egg or ovulation. The formula used was followed (Adriana et al. 2013):

Latent time (hour) = Ovulation time (hour) - Time of last hormone injection (hour)

Meanwhile, the ovulation rate was calculated according to previous research (Mahla et al. 2017).

Fecundity measurement was done by counting the number of eggs released by the female broodstock after stripping. Fecundity was calculated using the gravimetric method with the following formula (Saha et al. 2017):

Number of eggs sought (grains): Number of sample eggs (grains) = Weight of all eggs (grams): Weight of sample eggs (grams)

The calculation of egg diameter used methods from previous studies (Boonkusol et al. 2020). Egg samples were taken as many as 100 eggs/treatment and carried out 3 times, then observed and measured under a microscope equipped with a micrometer.

Fertilization rate in jelawat fish eggs was calculated by looking at the fertilized eggs. The fertilized eggs had clear color, while unfertilized eggs were white. The percentage of fertilized eggs was calculated based on the following formula (Saha et al. 2017):

Fertilization Rate = Number of fertilized eggs : Total eggs x 100%

The percentage of hatching rate was observed during the research. This was done to determine the percentage of the number of eggs that hatched. The percentage of hatching eggs was calculated using the following formula (Fanni et al. 2019):

Hatching Rate = Number of eggs hatched : Total number of fertilized eggs x 100%

Survival rate was observed at the end of the study to determine the percentage of surviving larvae. Survival rate was calculated using the following formula (Akbarurrasyid et al. 2020).

Survival Rate= Total live larvae at the end of the study : Hacthed eggs x 100 %

Observation of larva abnormality in this study included head shape, body shape and tail shape. Calculations carried out to determine the magnitude of larval abnormality use the following formula (Aidil et al. 2016):

Larva abnormality = Number of abnormal larvae : Total larvae x 100 %

**Water quality parameters**

Temperature, pH and dissolved oxygen (DO) were still controlled during egg incubation until larvae according to previous research methods (Santanumurti et al. 2019). Water quality conditions were maintained by measuring the temperature three times a day in the morning, afternoon and evening (06.00 AM, 12.00 PM, and 06.00 PM). pH and dissolved oxygen were calculated twice a day in the morning (06.00 AM) and afternoon (12.00 PM) while ammonia once a day in the morning (06.00 AM).

**Statistical calculations**

Data obtained during the study were analyzed using Microsoft Excel and Minitab 16. Parameters of egg diameter, fecundity, fertilization rate, hatching rate, survival rate, and larval abnormality were analyzed for variance (ANOVA). If there was a significant effect or difference, the BNT further test was carried out with a confidence level of 95%. The latent time and water quality parameters were analyzed descriptively.

# RESULTS

The latent time and ovulation rate in this study were presented in Table 1.

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| **Table 1.** Latent time and ovulation rate in Jelawat fish. |
| |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | | **Treatments** | **Broodstock (fish)** | **W0 (kg)** | **Wt (kg)** | **Ovulation rate (%)** | **Latent time (h)** | **Average latent time**  **(h)** | | P1 | 3 | 2,88 | 2,73 | 66,66a | 4 ; 11 ; NS | 5a | | P2 | 3 | 2,86 | 2,58 | 66,66a | 9; 10 ; NS | 6.33 a | | P3 | 3 | 2,85 | 2,53 | 100b | 8; 8; 8 | 8 a |   Note : Wo : Weight before spawning  Wt : Weight after spawning  NS : Not Spawning |

The results showed that all treatments were able to stimulate gonad maturation with success rates ranging from 66.66–100%. In P3 treatment, the success rate was 100%, while P1 and P2 had success rate of 66.66%.

The results of the reproductive performance of jelawat fish were presented in Table 2.

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| **Table 2.** Reproductive performance of jelawat fish. |
| |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | | Treatment | Fecundity  (eggs) | Egg diameter (mm) | Fertilization rate (%) | Hatching rate (%) | Survival rate (%) | Larvae abnormality (%) | | P1 | 72.000±10.583a | 0,816±0,038a | 40,83±3,60a | 42,14±18,25a | 45,92±15,30a | 0,025±0,022a | | P2 | 97.333±2.309b | 0,942±0,014b | 75,67±2,52b | 70,59±1,22b | 80,00±10,00b | 0,015±0,013a | | P3 | 121.333±12.055c | 1,183±0,014c | 86,16±6,25c | 87,48±4,11c | 94,88±4,24c | 0±0a | |

The results of measuring water quality parameters during the study were presented in Table 3.

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| **Table 3.** Water quality parameters |
| |  |  |  | | --- | --- | --- | | No | Parameter | Value | | 1. | Temperature (oC) | 27-29 | | 2. | pH | 6.92-7.84 | | 3. | Dissolved Oxygen (DO) (mg/l) | 3.54-5.92 | | 4. | Ammonia (NH3)  (mg/l) | 0.008-0.078 | |

The results showed that the temperature ranged from 27-29oC, pH ranged from 6.92-7.84, DO ranged from 3.54-5.92 mg/l.

# DISCUSSION

This study indicated that the HCG could stimulate the ovulation process and fish spawning. The HCG hormone stimulated the release of activator plasminogen from granulosa folicle cells (Hsueh et al. 1988). After the increased secretion of activator plasminogen, plasminogen from follicular fluid and edema extracellular fluid was converted to plasmin. This plasmin would activate the latent collagenase in the collagen follicle wall which produced collagenase. This collagenase would break down collagen, resulting in the release of collagen telopeptides (Aziz 2018). There was no difference in the latent time in this study. The latent time of jelawat fish in this study was still within the appropriate timeframe because the stinging fish had a latency time of 4–12 hours (Suryana et al. 2015).

The data from Table 2 indicated a trend of increasing fecundity with increasing doses of the hormone HCG. These results were in accordance with previous studiy which concluded that there was an increase in fecundity due to the provision of the HCG hormone in the gonad maturation process (Badran et al. 2019). Based on the analysis of variance (ANOVA), it was found that the treatment of different HCG injection doses had a significant effect on the fecundity of the jelawat fish (p <0.05). The increase in the HCG hormone in the body would increase the vitellogenesis process, the yolk would increase in number and size, causing the volume of the oocyte to increase (Elekkanai et al. 2015).

Egg diameter in this study ranged from 0.8167-1.183 mm. Based on the ANOVA, it was found that the different treatment in the injection dose of HCG had a significant effect on the difference in the diameter of the jelawat fish eggs (p <0.05). The difference in the diameter of the fish eggs between treatments was due to the vitellogenesis process that occurred with the incorporation of vitellogenin proteins into egg yolk proteins, causing an increase in the size of the gonads of female fish until final maturation (Lubzens et al. 2010, Glasser et al. 2004).

The percentage of fertilization rate in this study showed value of 40.83-86.16%. The highest percentage of egg fertilization was in the P3 treatment with 86.16% and followed by P2 of 75.67%, and P1 of 40.83%. The higher the dose of the HCG hormone injected into the fish body would increase the fertilization of jelawat fish eggs. HCG could increase fertility rate through direct androgen release through the gonads to stimulate steroidogenesis (Guzmán et al. 2011). The high yield of jelawat fish egg fertilization was also due to the hormone given to the broodstock which could form perfect oocyte maturation so that it would increase egg maturity and boosted the percentage of fertilization (Satyani 2007).

The percentage of hatching rate in this study showed 42.14-87.48%. Based on the ANOVA, it was found that the different treatment in the injection dose of HCG had a significant effect on the hatching rate of jelawat fish eggs (p <0.05). The best percentage of hatching eggs was found in P3 with 87.48%. This showed that the HCG treatment 600 IU/kg in the jelawat fish was quite optimal. The low percentage of egg hatching in others was due to poor egg quality. HCG injection was able to stimulate and uniform the maturation of the gonads so that the eggs produced would increase oocyte quality, and consequently greater hatching rates (Kucharczyk et al. 2020).

The survival rate of jelawat fish larvae ranged 45.92–94.88%. Based on the ANOVA, it was found that the different treatments in the injection dose of HCG had a significant effect on the survival of the jelawat fish larvae (p <0.05). The highest survival rate was found in P3 of 94.88%, then P2 of 80.00% and P1 of 45.92%. The larval phase was a critical phase, especially when the yolk run out and the transition period began to take food from outside, so that in this phase the mortality rate was quite high. One of the factors that influenced the survival of the jelawat fish larvae during the egg incubation period and larval rearing was the quality of eggs and sperm. Good quality eggs and sperm would produce fish larvae with good quality. This is in accordance with the previous study (Kubcharczyk et al. 2020, Suryana et al. 2015) that HCG could improve egg quality so that it would increase the survival rate of the embryo.

The results of the abnormalities of jelawat fish larvae showed ranged 0-0.025%. Based on the ANOVA, it was found that the different treatment in the injection dose of HCG did not have a real effect on the abnormality of the jelawat larvae (p> 0.05). This was in accordance with previous studies that HCG would improve the quality of eggs by helping embryos development so that they could develop properly (Ljubobratović et al. 2019).

Water parameters value was still in optimal conditions for hatching eggs and rearing larvae of jelawat fish. These results were in accordance with Selawati et al. (2019), that the optimal value of water quality parameters for jelawat fish were temperatue of 25–32oC, pH 6.5–9.5 and DO 3-6 mg/l. Ammonia values ​​ranged from 0.008-0.078 mg/l and these value was still in optimal conditions for hatching eggs and rearing larvae of jelawat fish. This was in accordance with Rimalia (2014), the ammonia value in the hatchery and larvae rearing <0.3 mg/l. In fish Aquaculture, water quality played an important role and affected hatching and rearing of fish. Previous research stated (Putra and Mulah 2019) that fish eggs would grow well if they were in accordance with the required water parameter conditions.

It could be concluded that the injection of HCG was effective to increase the spawning and reproductive performance of jelawat fish with optimal results in the 600 IU/kg treatment. These treatments showed a 100% ovulation rate, 8 hours latency time, 121.333 eggs fecundity, 1.183 mm egg diameter, 86.16% fertilization rate, 87.48% hatching rate, 94.88% survival rate and larvae abnormality of 0.

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