Effect of Honey Extender for Spermatozoa Dilution on Fertility and hatchability of Javaen Barb Fish (*Systomus orphoides*)

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Abstract. Javaen barb fish (*Systomus orphoides*) population was declined due to overfishing and environmental changes. Cultivation efforts have been made, but the high sperm concentration causes a decrease in fertility. Honey extender is used to dilute sperm, maintain buffer balance, and provide nutrition for sperm. The objective of this study was to obtain the optimum concentration of honey extender on the fertility and hatchability rate of Javaen barb fish sperm. The concentrations (0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1%) of honey were used. The ratio between sperm and the mixture of extenders (fish Ringer and honey) used was 1:10. Sperm and egg collection was done by stripping. Evaluation was carried out by observing of fertility and hatchability rate. The data was analysed using one-way ANOVA, followed by Tukey's test. Based on the results of the ANOVA test and Tukey's test, it was seen that there was a significant difference ($P < 0.05$) on the fertility and hatchability rate. The significant difference was found in hatchability rate, but not difference on fertility. Honey concentration 0.8% was the optimum concentration that produced the highest percentage hatchability (96,33 ± 6.27%) after dilution with honey extender.

Keywords: Extender · Fertility · Javaen barb fish · Spermatozoa · Systomus orphoides

1 Introduction

Javaen barb fish (*Systomus orphoides*) is a freshwater fish that is indigenous to Indonesia. [1]. The javaen barb fish found in the rivers of central Java [2]. The Javean barb fish is a consumer fish whose source of catch comes from nature. It was feared that overexploitation of Java barb fish would cause extinction [3]. The efforts that have been made such as tamed, artificial breeding, domesticated and cultivated [4, 5].

Javaen barb fish produce sperm with high concentration, high viscosity and low amount. High sperm concentration can inhibit sperm motility and cause a decrease in

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fertility rates. Sperm dilution with an extender was carried out to increase the yield of culture. The extender is used as a semen diluent to result a higher number of dilute sperm for artificial breeding purposes [5, 6]. Diluents that contain nutrients and energy are critical for sperm to maintain sperm motility and viability. The addition of supplements in extenders in sperm has been shown to improve the quality of spermatozoa [7].

Extender solution containing nutrients and energy are essential for sperm. Honey is the option to be a supplement extender. Based on research by Ayer *et al*., (2019), Honey has been shown to be effective as an extender supplement for sperm in increasing fish hatchability. Research by Tumanung used honey as an extender supplement to improve sperm quality such as motility, fertility, and hatchability of egg carp fish (*Cyprinus carpio L.*) [8].

The objective of this study was to determine the optimum concentration of honey extender at various concentrations (0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1%) for javaen barb fish (*S. orphoides*) sperm dilution. Honey supplementation is expected to improve sperm quality of javaen barb fish increase fertilize eggs and hatchability eggs after sperm dilution.

2 Materials and Methods

The research was carried out from January 2021 until April 2021, at the Installations for Freshwater Fish Genetic Resources, Research Institute for Freshwater Aquaculture and Fisheries Extension, Ministry of Marine Affairs and Fisheries, Cijeruk, West Java, Indonesia.

Stripping method on the abdomen of javaen barb fish conduct to collected sperm. Sperm were mixed with extender (fish Ringer + honey with various concentrations) with ratio 1:10. Fish Ringer was used as an extender solution consisting of NaCl, KCl, CaCl2.H2O, and NaHCO3. Treatment of sperm dilution with a honey extender was carried out using various concentrations (0%, 0.25, 0.4%, 0.6%, 0.8%, and 1%) [9, 10].

2.1 Spermatozoa Fertilization

Fertilization was done by mixing an egg sample using a Pasteur pipette as much as 1 drop of egg cells ± 0.05 g (110 to 170 eggs) with sperm, then into a plastic container filled with water to activate sperm cells [11]. The water change was done after 2 h, when the fertilization count is done to clean the remaining sperm. Water quality was maintained by replacing 50% of water every day [12]. Eggs that was successfully fertilized by sperm will be translucent white in color, while eggs that were not fertilized will be white [13]. The percentage of fertility is then calculated based on the formula of Lismawati et al. 2016.

2.2 Hatchability

The fertilized egg will develop into an embryo, while the unfertilized egg will die. Egg hatchability was determined based on the division between the number of eggs that hatched and the total number of successfully fertilized eggs. The hatchability of javaen barb fish eggs is calculated by the formula from Lismawati et al. 2016.

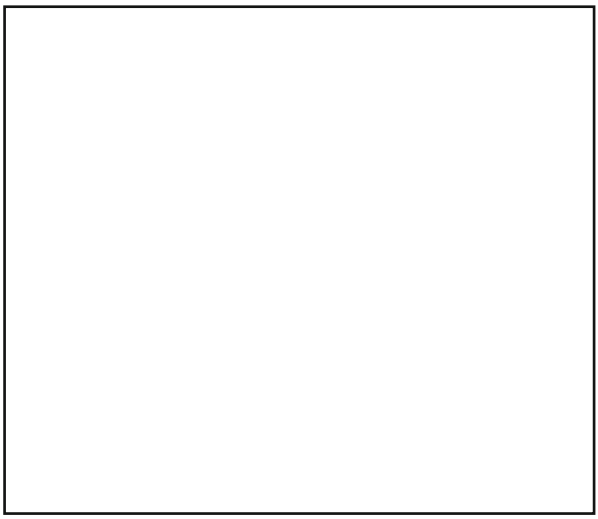

Fig. 1. Fertilization rate of javaen barb fish (*Systomus orphoides*) after dilution with honey extender

2.3 Data Processing

Software IBM SPSS Statistics 23 was using to calculated and data processed. Data tested with one-way ANOVA followed by Tukey Test [14].

3 Result and Discussion

3.1 Spermatozoa Fertilization

The result in Fig. 1 is shown the averages fertility of javaen barb fish (*S. orphoides*)**.** The one-way ANOVA test, showed that the fertility treatments were not significantly different. But, the hightest percentage fertility on honey concentration at 0.8% (96.31 ± 0.69%).

3.2 Hatchability Rate

The hatchability rate was determined using the ANOVA and Tukey's tests, it was showed that there was a significant difference ($P < 0.05$) on the percentage of hatchability rate of javaen barb fish spermatozoa after dilution. The concentration of 0.8% honey supplement in the extender is the optimum concentration that can maintain the percentage of hatchability (86.88 ± 6.53%). he averages fertility of javaen barb fish (*Systomus orphoides*) is shown in Fig. 2.

Fertilization percentage is one indicator of fish sperm quality. Determine the success rate of egg fertilization seen in color changes, fertilized eggs are transparent while unfertilized eggs are white. Lower percentage of fertilized due to decreases the motility of the spermatozoa and fertilization does not occur when the spermatozoa are immotile

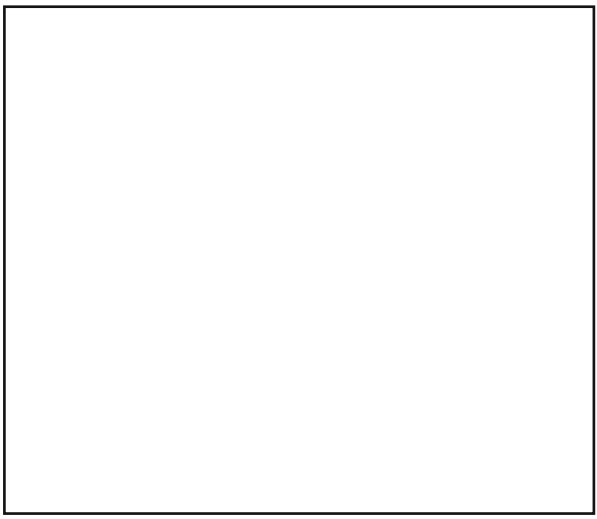

Fig. 2. Hatchability rate of javaen barb fish (*Systomus orphoides*) after dilution with honey extender

[15]. Based on the research on the percentage of fertility of Javaen barb fish (*Systomus orphoides*) after dilution with honey extender, the results were not significantly different. However, the highest percentage occurred with the addition of 0.8% honey at 96.31 ± 0.69%.

Based on the results of the research on egg hatchability of sperm after dilution with honey extender, the average value of egg hatchability for each treatment was found as follows: honey 0% (79.94 ± 4.73%), 0.2% (82.41 ± 0.32%), 0.4% (74.73 ± 6.32%), 0.6% (80.17 ± 1.57), 0.8% (86.88 ± 6.53%), and 1% honey (82.77 ± 1.77%). After that, it was tested with one-way ANOVA showing the results that were significantly different between 0.8% honey treatments ($P < 0.05$). After dilution with a honey extender, 0.8% honey treatment produced the best results for increasing hatchability of javaen barb fish (*S. orphoides*).

Environmental factors such as temperature are things that must be considered. The hatching rate of eggs is very dependent on temperature. Temperatures that are too high or that change abruptly will impede the hatching process. [16]. The changing weather conditions during the research period caused a significant increase and decrease in temperature. High temperatures will speed up metabolism and promote embryonic growth, but too high temperatures will inhibit embryo movement. On the other hand, a temperature that is too low will inhibit metabolism, which causes inhibition of organ growth in the embryo, and can harden the shell and make it impossible to break during hatching [17]. The time required from fertilization until the red eye fish eggs hatch in this study was 21–22 h at a temperature of 26.7–28 °C, and will become 24 h at a temperature of 25.1 °C.

This study shows that honey in the extender provides an energy source for spermatozoa until fertilization occurs. The addition of honey with various concentrations has an effect on the quality of spermatozoa. The composition of honey provides the benefits

of honey supplements in extenders, which is made up of simple sugars like fructose and glucose, organic acids, polyphenols, enzymes, amino acids, vitamins, and minerals. The composition of fructose and glucose reaches 75–85% of the total sugar in honey [18]. Honey also has antioxidant, anti-mutagen and antibacterial benefits, the viscosity of honey also has a buffering effect on the extender solution. Honey as extender supplement was shown to maintain and improve the quality of spermatozoa [8, 10].

4 Conclusion

In conclusion, sperm dilution with the addition of various concentrations of honey extender affects the percentage of fertility and hatchability of javaen barb fish (*Systomus orphoides*). After dilution with a honey extender, the optimum concentration of honey extender was 0.8%, which resulted in the highest percentage of fertility (96.31 ± 0.69) and egg hatchability (86.88 ± 6.53%).

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References

1. Direktorat Konservasi Kawasan Dan Jenis Ikan. Pedoman umum restoking jenis ikan terancam punah. Kementerian Kelautan dan Perikanan, Jakarta. 2015, pp. ix + 67 hlm.
2. S. Suryaningsih, M. K.H. Sagi, Nitimulyo, S. Hadisusanto. Beberapa aspek pemijahan ikan brek *Puntius orphoides* (Valenciennes, 1842) di Sungai Klawing Purbalingga, Jawa Tengah. Jurnal Iktiologi, Indonesia. 12(1), 2012, p. 35–48.
3. S. Suryaningsih, M. H.N. Sagi, Kamiso, S. Hadisusanto. Sexing pada ikan brek *Puntius orphoides* (Valenciennes, 1863) menggunakan metode truss morfometrics. Biosfera, 31(1), 2014, p. 8–16.
4. N. Setyaningrum, A. Nuryanto. Penjinakan dan budidaya ikan brek (*Puntius orphoides*) sebagai upaya menuju diversifikasi usaha tani ikan. Journal Ichtyology, 6(1), 2006, pp. 1–4.
5. S. Hadisusanto, S. Suryaningsih. Puntius orphoides Valenciennes, 1842: Kajian Ekologi dan Potensi untuk Domestikasi Biota. Jurnal Ilmiah Ilmu-Ilmu Hayati, 16(2), 2011, pp. 214–220.
6. N.K. Agarwal. Cryopreservation of fish semen. Dalam: Bhatt, J.P., M. Thapliyal & A. Thapliyal (eds). Himalayan Aquatic Biodiversity Conservation & New Tools in Biotechnology First edition, Chapter 8. Transmedia Publication, Uttarakhand, 2011, p. 104–127.
7. F.A. Chapman. A semen extender for the short-term storage of fish sperm. Fisheries and Aquatic Sciences, 2018, pp. FA193: 1–3.
8. O. S. Devi, T. Susilowati, R.A. Nugroho. pengaruh penambahan madu dengan dosis berbeda dalam media pengencer NaCl fisiologis terhadap Kualitas Sperma ikan tawes (*Barbonymus gonionotus*). Jurnal Sains Akuakultur Tropis, 3(2), 2019, pp. 21–30.
9. A. Sunarma, D.W.B. Hastuti, D.M. Saleh, Y. Sistina. Kombinasi efektif ekstender dan krioprotektan pada kriopreservasi sperma ikan nilem (*Osteochilus hasseltii* Valenciennes, 1842). Jurnal Perikanan, 10(1), 2008, pp. 76–84.

10. I. T.M. Sari, H. Alawi, Sukendi. Pengaruh Penambahan Madu Pada Media Pengencer NaCl Fisiologis Terhadap Kualitas Spermatozoa Ikan Baung (*Hemibagrus nemurus*) Selama Penyimpanan. Fakultas Perikanan dan Kelautan Universitas Riau, 2018, pp. 1–13.
11. J. Slembrouck, O.M.A.N. Komarudin, M. Legendre. Petunjuk teknis pembenihan ikan patin Indonesia, *Pangasius djambal*. Badan Riset Kelautan dan Perikanan, Jakarta, 2005, pp. vii + 142 hlm.
12. A.Iswantari, W. Cahyanti, F.P. Putri, D.A. Hedianto, V.A. Prakoso, A.H. Kristanto. Reproductive and larval performance of artificially spawned javaen barb *Systomus orphoides* from two populations. In IOP Conference Series: Earth and Environmental Science, 744(1), 2021, p. 012035
13. M. Iswahyudi, Marsoedi, M. Sri Widodo. Development of spotted barb (*Puntius binotatus*) eggs. Journal of Life Science and Biomedicine, 4(1), 2014, pp. 53–56.
14. J.H. Zar, Biostatical analysis. London: Prentice-Hall Inc, 1974pp. xiv + 620 hlm.
15. D. Urabi, F. Farida, T.P. Lestari. Pengaruh Penambahan Madu Pada Pengenceran Sperma Terhadap Motilitas Spermatozoa Ikan Baung (*Mystus nemurus*). Jurnal Ruaya: Jurnal Penelitian dan Kajian Ilmu Perikanan dan Kelautan, 7(2), 2019, pp. 47–54.
16. J. Hutagalung, H. Alawi, S. Sukendi. Pengaruh suhu dan oksigen terhadap penetasan telur dan kelulushidupan awal larva ikan pawas (*Osteochilus hasselti* CV) (Doctoral dissertation, Riau University), 2016, pp. 1–13.
17. N.R. Chattopadhyay. Induced fish breeding: a practical guide for hatcheries. Academic Press, India, 2016, pp. xli + 328 hlm.
18. R.I. El-Sheshtawy, D.A. El-Badry, A. Gamal, W.S. El-Nattat, A.M.A. Almaaty. Natural honey as a cryoprotectant to improve Arab stallion post-thawing sperm parameters. Asian Pacific Journal of Reproduction, 5(4), 2016, pp. 331–334.