Effect of Java Long Pepper Extract (*Piper Retrofractum*) on Spermatozoa Motility of Mustika Common Carp (*Cyprinus Carpio L.*)

B B Yeni¹, ² and A Abinawanto

¹ Master Degree Program Biologi, Universitas Indonesia, Jl. Profesor Doktor Sudjono, Depok 16424, Indonesia
² Departement Biologi, Universitas Indonesia, Jl. Profesor Doktor Sudjono, Depok 16424, Indonesia

*bela.lim440@gmail.com*

Abstract. Java long pepper is a plant has an aphrodisiac effect. The extract contains are cytosterol and piperine which capable of affecting there productive hormone for fish. The purpose of this study to find the optimum concentration of java long pepper extract on spermatozoa motility of mustika common carp. Java long pepper extract was injected by intramuscularly on the first of week with the concentration are 0.9ml kg⁻¹, 1.8ml kg⁻¹ and 3.6ml kg⁻¹ of body weight, respectively. SnGrH (ovaprim) with the concentration is 0.3mlkg⁻¹ bodyweight was administered as a positive control, whereas 0.3 ml of sodium solution was applied as a negative one. The result was observed in the second of week. Based on the ANOVA test showed that java long pepper extract has a significant effect on spermatozoa motility of mustika common carp (P<0.05). Further, according to the Tukey test that the highest spermatozoa motility rate was yield from a concentration 0.9 ml of java long pepper extract kg⁻¹ body weight (69.5±1.24%).

1. Introduction

Mustika common carp is the most superior strain cyprinidae family. This strain is resistant to koi herpes virus (KHV), which is usually attacks common carp affecting in mass deaths [1]. In the order to ensure continuous reproduction, therefore, the availability of parents should be maintained. This is done through proper cultivation by inducing gonad maturation using the synthetic hormone. The use of synthetic hormones. Steroid is the hormone for support breeding, the effectived one usually used 17 α-metiltestoteron [2]. The 17 α-metiltestoteron is prohibited so we need alternative hormone for support breeding. The java long pepper extract is one of the fitosteroid, furthermore, using the hormones derived from plants is considered safer and economical [3]. Based on previous studies, the doses of natural hormones produced by java long pepper extract were chosen as follow 0.9mL kg⁻1, 1.8 mL kg⁻1 and 3.6 mL kg⁻1 body weight, respectively. In this study also used 0.3mL kg⁻1 body weight of physiological 0.1% NaCl solution as a negative control, while ovaprim with a dose of 0.3mL kg⁻1 body weight as positive control.
2. Methods

Thirty males of three months old mustika common carp [Figure1] broods obtained from the Sukamandi Fish Research Institute, West Java were used as an experimental model. The body weight was approximately 130-225g.

![Figure 1. CyprinuscarpioL.](image)

Java long pepper was extracted from one kg of dried java long pepper fruit. The dried substance was grinded by a grinder, and was filtered by a 60 mesh filter. The residue was placed on a stainless steel macerator, and was added by 95%ethanolina ratio of 1:5, and was incubated at 32°Celsius overnight. The filtrate was the evaporated in order to separate the precipitate from the solvent. From one kg of java long pepper fruit extract was produced 55ml of concentrated java long pepper extract. Those extract was the diluted by physiological NaCl solution in a ratio 1:9, and was used for stimulating testis maturity of mustika common carp.

Injection were performed during the first week of study. The number of replications was determined by the Frederer formula, as follow, \((t-1)(n-1)>15\) where is the number of replications and is the number of treatments.

Sperm was collected by hand stripping method. Ten microliters of sperm was diluted by90μl of physiological NaCl solution. One micro liter of diluted sperm was placed on improved Neubauer counting chamber and was observed under a light microscope with 10x40 magnifications. Spermatozoa motility was analyzed in red blood cells area (figure 2).

![Figure 2. Improvred Neubauer counting chamber [4].](image)

The spermatozoa motility percentage was calculated by formulas as follow:

\[
\%\text{Motility} = \frac{\sum \text{motil spermatozoa} \times 100\%}{\sum \text{total spermatozoa}} \quad [5]
\]
3. Result and discussion

3.1. Result
The color of fresh sperm was milky white, while the pH was 7 and the volume of ejaculated sperm between 0.4-1.5ml. The fresh sperm evaluation result was demonstrated in table 1.

Table 1. The fresh sperm evaluation.

| N  | Treatment                        | Volume (ml) | PH  | Color       |
|----|----------------------------------|-------------|-----|-------------|
| 6  | Negative Control                 | 2.4         | 7.5 | Milkywhite  |
| 6  | A (0.9ml kg-1 body weight)       | 6           | 7.5 | Milkywhite  |
| 6  | B (1.8ml kg-1 body weighy)       | 3           | 7.5 | Milkywhite  |
| 6  | C (3.6ml kg-1 body weight)       | 4.2         | 7.5 | Milkywhite  |
| 6  | Positive Control                 | 2.7         | 7.5 | Milkywhite  |

Time of sampling after injection decided every day at 8.00 a.m. Two weeks after injection the fish can product sperm, so the sampling will do in that time. The result after two week injection can be seen in table 2.

Table 2. Spermatozoa Motility (%).

| Treatment                        | Motility (%)     |
|----------------------------------|------------------|
| Negative Control                 | 53.16 ± 1.11^a   |
| A (0.9ml kg-1 bodyweight)        | 69.5 ± 1.24^b    |
| B (1.8ml kg-1 bodyweight)        | 61.5 ± 1.17^ab   |
| C (3.6ml kg-1 bodyweight)        | 56.5 ± 1.05^a    |
| Positive Control                 | 53.16 ± 1.08^b   |

Difference letter in each column show significant difference (P<0.05).

3.2. Discussion
Evaluation of sperm motility by visual and objective [6]. Sperm motil usually move randomly in one room [7]. Based on the Anova test shown that java long pepper extract influence the percentage of spermatozoa motility (P<0.05). Further, the highest percentage of spermatozoa motility was demonstrated by the concentration 0.9 ml kg-1 body weight. According to the Tukey test (P<0.05). The lower yield (66%) was reported in percentage of spermatozoa motility of catfish after injected by java long pepper in a dose of 3.75 ml kg -1 body weight [8]. The percentage motility of fish spermatozoa was varied depend on the species [9]. Motility evaluated affected for fertilization rate, sperm that move actived can made highest fertilization rate [10]. The result of this study showed that the optimum concentration of java long pepper extract was 0.9 ml kg-1 body weight (figure 3).
4. Conclusion
The optimum result concentration of java long pepper extract for affecting sperm motility mustika common carp was 0.9 mL kg\(^{-1}\) weight body.

Acknowledgments
This research was supported by the Sukamandi Fish Breeding Research Centre (BRPI) in West Java, Indonesia.

References
[1] Mutiara 2013 The Effect of Addition Java Long Pepper (Piper retrofractum Vahl.) an Zinc (Zn) Against The Sum of Geminal cells Testis of Male White Rats (Rattus norvegicus) Journal of Medical Lampung University 2(1) 147—155
[2] Hanifa M A 2002 Induced Spawning of Spotted Murrel (Channapunctatus) a catfish (Heteropneustes fossilis) Using Human chorionic Gonadotropin and synthetic hormone (ovaprim) Veterinarski Arhiv 72(1) 51—56
[3] Ansari A H 2012 Repeated freeze thawing for assessment of semen freeze ability Emperaire, J. C., A. Audebert & E. S. Hafez (eds.). 2012. Homologous Artificial Insemination (AIH) (Springer: USA) 117—184
[4] Labce 2018 Examining CSF using the haemacytometer continued. 1hlm. https://www.labce.com/spg546396examiningcsfusingthehemocytometercontinued.aspx, diakses pada 6 Februari 2018, pk. 10.54 WIB.
[5] Elisdiana 2015 Induction of Gonad Ripening of Siamese Patin Fish (Pangasianodon hypotheralmus) (Sauvage,1878) Male for Using Java Long Peper extract (Piper retrofractum Vahl.) by feeding Journal of Indonesia Ichthyology 16(1) 35—44
[6] Rurangwa E D E, Kime F 2004 The measurement of sperm motility and factors affecting sperm quality in culture fish. *Journal of Aquaculture* **234** 1—28

[7] Contreras-Sanchez W M, Fitzpatrick M S, and Schreck C B 2001 Fate of methyl testosterone in the pond environment: Impact of mt-contaminated soil on tilapia sex differentiation. *Eighteenth Annual Technical Report. Effluents and Pollution Research* 83-86

[8] Fernandez C, Suare Y, Ferruelo A J, Gomez-Coronado D, and Lasuncion M A 2002 Inhibition of Cholesterol Biosynthesis by b22– Unsaturated Phytosterol via Competitive Inhibition of Sterol reductase in Mammalia Cells *Biochemical Journal* **366** 109–119

[9] Kahkesh F B, Feshalami M Y, Amiri F, and Nickpey M 2010 Effect of ovaprim, ovatide, HCG, LHRH-A2, LHRHA2+CPE and carp pituitary in benni (*Barbus sharpeyi*) artificial breeding. *Global veterinary* **5**(4): 209—214

[10] Dada A A, Adeparusi E O, and Alale O V 2010 Dietary dried *Kigelia africana* fruits meal as fertility enhancer in female *Clarias gariepinus*. *Agriculture and Biology Journal of North America* **1** 791-795