



## Effects of *Pimpinella pruatjan* powder extract on comet fish (*Carassius auratus*) larva masculinization

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**Abstract.** One of the efforts to increase the population of male fish is through masculinization. This could increase profit when males are more valuable. Masculinization a method to direct the sex of fish to become male during the sex differentiation period. An alternative material that can be used in the masculinization process is the purwoceng (*Pimpinella pruatjan*) plant. *P. pruatjan* contains phytosteroids, especially stigmasterol found in the root, which is capable of providing androgenic effects. The purpose of this study was to determine the effect of the dosage of *P. pruatjan* powder extract and to determine which dosage of *P. pruatjan* had the best results on the masculinization of comet fish (*Carassius auratus*) larvae. The data collected included the percentage of male and female sex and survival rates, different influence on the percentage of male sex (masculinization), and comet fish survival. The results showed that *P. pruatjan* extract with different doses had a significantly different effect on the percentage of male sex and the survival rate of comet fish. The dose of 25 mg L<sup>-1</sup> gave the best results on the percentage of male sex in comet fish (89.17%), and the control (0 mg L<sup>-1</sup>) had the best survival rate (95.33%).

**Key Word:** male and female sex, masculinization, purwoceng, survival rate.

**Introduction.** Comet fish (*Carassius auratus*) is one of the freshwater ornamental fish species that is in great demand, so that many are trying to cultivate it. The main advantage of comet fish is that it varies in color such as white, yellow, red or a combination of these colors. The color can increase the selling price of the fish. The fish may present beautiful and lighter colors, attractive shapes and movements, and are easy to maintain in an aquarium (Izzah et al 2020). Comet fish, especially male comets, have a higher price compared to females, so they are in more demand than females. The selling price of this fish reaches 0.35–0.7 USD (Rahardjo 2010).

The production, including reproduction, of male comet fish can be done in various ways. One of the efforts to increase the population of male fish is masculinization, directing the fish to become male during sex differentiation (Bulkini 2012). Commonly used masculinization methods include manipulating environmental factors by administering the hormone 17 $\alpha$ -methyltetosterone (Zairin 2002) and aromatase inhibitors (Utomo 2008). However, currently the use of synthetic hormone 17 $\alpha$ -methyltetosterone and aromatase inhibitors is not only relatively expensive, but also prohibited in aquaculture activities in Indonesia (KKP 2014). This is because 17 $\alpha$ -methyltetosterone is an environmental pollutant and it is also carcinogenic for humans (Tasdiq 2005). Therefore, it is necessary to use alternative natural materials as substitutes for synthetic materials used in masculinization.

One of the alternative materials that can be used for the masculinization process is the purwoceng plant *Pimpinella pruatjan*. *P. pruatjan* contains phytosteroids, especially stigmasterol found in the root, which is capable of providing androgenic effects. According to Darwati & Roostika (2006), the results of GCMS analysis showed that *P. pruatjan* contains the active ingredient stigmasterol, 5.38%. Stigmasterol has androgenic properties similar to male hormones that are thought to affect the sex ratio of fish (Zairin 2002).

The purpose of this study was to determine the effect of *P. pruatjan* powder extract in different doses, and to determine which dosage gave the best results on the masculinization of *Carassius auratus* larvae.

## Material and Method

**Time and place of research.** This research was conducted from April to July 2020. The maintenance of broodfish, feeding the broodstock to spawning, hatching larvae, immersing in purwoceng extract solution and rearing the larvae were carried out at the Regional Technical Service Unit of the Fish Seed Center of Ternate City, Indonesia. Observation of male and female sex percentages, as well as gonad histology observations were carried out at the Aquaculture Microbiology Laboratory, Faculty of Fisheries and Marine, Khairun University, Indonesia.

**Provision of test fish larvae.** Larvae were obtained from the spawning of comet fish from the Ternate City Fish Seed Center. 180 larvae were used in this study, being 6 days old.

**Implementation procedure.** In this study, different dosages of *P. pruatjan* extract were used. *P. pruatjan* was collected from the wild and ground with a blender. It was then accommodated in a plastic container and weighed the dose used on fish larvae. These treatments consisted of: treatment A - 0 mg L<sup>-1</sup> (control); B - 10 mg L<sup>-1</sup>; C - 15 mg L<sup>-1</sup>; and D - 25 mg L<sup>-1</sup>. Each treatment was repeated 3 times. The purwoceng powder extract used was dissolved first for 24 h in 95% ethanol, through an aeration process, until the ethanol evaporated. After that, the larvae were immersed in the purwoceng extract solution. Soaking was carried out for 24 h. After that, the larvae were transferred to different containers. 15 larvae were used per container. Maintenance was carried out for 8 weeks and the larvae were fed egg yolks for one week. Afterwards, the feed was replaced with artificial feed from the Takari brand. The feeding continued throughout the experiment.

**Data collection and analysis.** The data collected included: the percentage of male and female sex and the survival rate. Sex determination was done by observing morphological characters, especially color and genital opening. The sex percentage was calculated based on the formula used by Zairin (2002):

$$M\% = (\text{number of male fish} / \text{number of total fish}) / 100$$

$$F\% = (\text{number of female fish} / \text{number of total fish}) / 100$$

The survival rate was calculated using the formula used by Effendie (1997):

$$SR = (N_t / N_0) \times 100$$

Where: SR - survival rate (%); N<sub>t</sub> - number of fish at the end of the study; N<sub>0</sub> - number of fish at the beginning of the study.

A completely randomized design was used for the experiment. This is based on the assumption that all experimental units were considered homogeneous. The analysis of variance was used to determine significant differences between treatments (Steel & Torrie 1960). To determine the difference in the effect of each treatment, the LSD test was performed.

## Results and Discussion

**Percentage of males.** The increase in male comet fish sex percentage has occurred for fish treated with *P. pruatjan* powder extract. The percentage of males in comet fish based on different dosages of *P. pruatjan* extract can be seen in Figure 1.

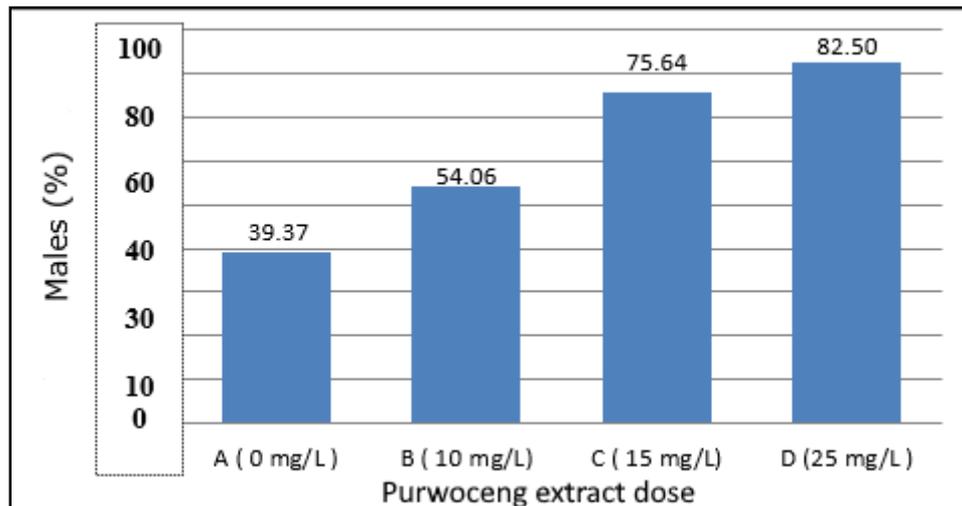


Figure 1. The percentage of males in comet fish based on different dosages of *P. pruatjan* extract.

The results in Figure 1 show that the highest percentage of males was in treatment D (82.50%), followed by treatment C (75.64%), treatment B (54.06%) and treatment A (39.37%). The analysis of variance showed that there were significant differences between treatments ( $p < 0.05$ ) in term of percentage of males produced, in favor of the treatments.

The LSD test results show that treatment D produced significantly different effects than treatment C ( $p < 0.01$ ). Treatments D and B differed significantly ( $p < 0.05$ ). Treatment D produced very significant differences compared to treatment A ( $p < 0.01$ ). Treatment C produced results very significantly different compared to those of treatment B ( $p < 0.01$ ). Treatment C produced very significant differences compared to treatment A ( $p < 0.01$ ). Treatment B produced very significantly different effects compared to the control ( $p < 0.01$ ). From these results, it can be seen that treatment D, with the highest dose of *P. pruatjan* produced the best results on the percentage of males.

The results of histological observations of male and female comet gonads using acetocarmine staining can be seen in Figure 2.



Figure 2. Male (left) and female (right) of comet fish gonads using acetocarmine solution (spermatocyte left, oocyte right).

**Survival rate.** The *Carassius auratus* survival rate during the study can be seen in Figure 3.

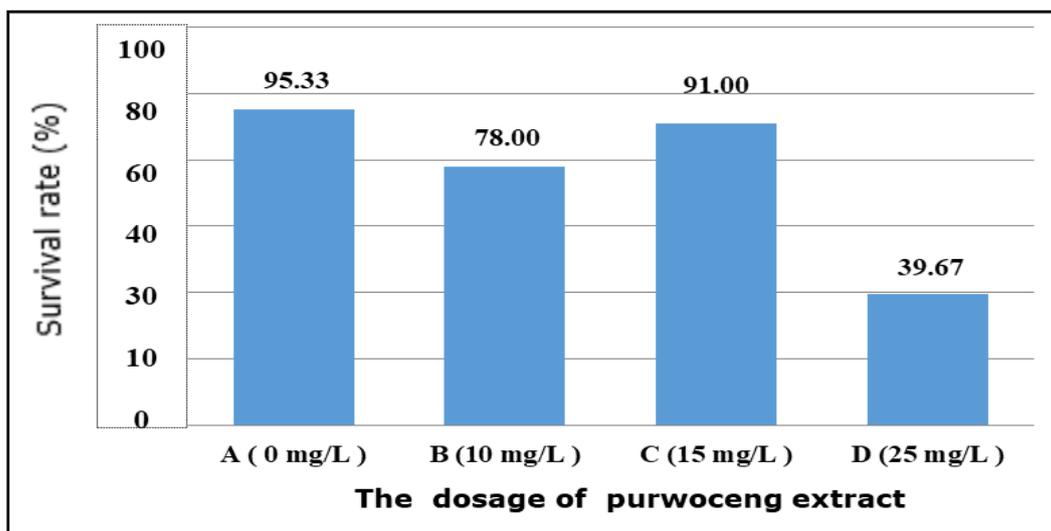


Figure 3. Comet fish survival rate.

Figure 3 shows that the highest survival rate was found in treatment A (control), namely 95.33%, then in treatment C (91%), while the lowest survival rate occurred in treatment D (39.67%).

The results of the analysis of variance show that the use of purwoceng extract in different doses has a very significant effect on the survival of comet fish. This shows that the experimental treatments affected very significantly the viability of comet fish. The LSD test results show that treatments A to C have a significant difference ( $p < 0.05$ ). Treatments A and B had a very significant difference ( $p < 0.01$ ). The results from the A and D treatments differed very significantly ( $p < 0.01$ ). From these results, it can be concluded that the control treatment had the best results regarding the survival of comet fish. Darwati & Roostika (2006) state that administering purwoceng extracts in the correct dose can increase fish immunity, increasing the survival rate. On the other hand, improper doses of purwoceng extract or high doses can cause the decrease of the survival rate. A very high dose of purwoceng extract will cause a high mortality rate, especially in the larval stage.

The high percentage of males obtained in this study was due to the content of the stigmaterol compound in the purwoceng extract. The stigmaterol compound has binding power at the receptor, affecting the endocrine system, which acts on target cells to carry out sex changes, thereby inhibiting the development of female gonads and resulting in male fish (Tremblay & Van Der Kraak 1998). Thus, a higher dosage of purwoceng extract will increase the percentage of males, as the results obtained in this study. The purwoceng extract used in this study produced significantly different results compared to the control, increasing the number of male fish.

The increase in the percentage of comet fish males was due to the administration of the appropriate dosage of purwoceng extract and in the right phase, namely the phase of sex differentiation. Zairin (2002) stated that administering purwoceng extract in the sex differentiation phase can result in an increase in the number of male fish. The absorption process of the purwoceng extract in the body of comet fish larvae occurs through diffusion, the extract entering the body through the pores, and then to target cells through the bloodstream (Arfah et al 2002). The mechanism of action of purwoceng extract to influence sex differentiation starts from the entry of the active stigmaterol compound. Stigmaterol transforms in steroid hormones, which enter cells across the plasma membrane by diffusion, interacting with specific receptors in the cytoplasm. They move then into the nucleus linked to receptors found on chromatin. Environmental factors can also cause sex changes in comet fish, especially water temperature. Changes in fish sex can occur at water temperatures around between 23-29°C (Zairin 2002).

Treatment A had the lowest male sex percentage (39.37%). This was due to the absence of purwoceng extract, so that the development of comet fish gonads took place

naturally. In addition, the determinants of female sex are more dominant than determinants of male sex. This condition is in accordance with Arfah et al (2002), who state that the difference in the number of fish larvae of different sex probably occurs due to unbalanced genetic determinants of sex.

The high survival rate in the control was caused because of the lack of pressure from the active ingredients produced by the purwoceng extract; so, physiologically, the energy obtained from the feed was utilized properly to maintain its survival and growth.

**Conclusions.** Based on the results of this study it can be concluded that the purwoceng extract with different doses had an effect on the percentage of male sex (masculinization) and the survival of comet fish. The purwoceng extract with a dose of 25 mg L<sup>-1</sup> produced the best results on the male sex percentage increase (89.17%), while the control treatment gave the best results on the comet fish survival rate (95.33%).

**Conflict of Interest.** The authors declare that there is no conflict of interest.

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