Effect of turkey berry on feminimization of carp

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Effect of Turkey Berry (*Solanum torvum*) Leaf Extract on Feminization of Common Carp (*Cyprinus carpio*)

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Abstract: This study aimed to evaluate the effect of Turkey berry (*Solanum torvum*) leaf extract on feminization of common carp (*Cyprinus carpio*). Five days old post hatching larvae were used in this experiment. The Turkey berry leaf extract (1:1) were used in this experiment. This study used a completed randomized design which consisted of five treatment; P0 (0 mg.L^{-1} as a control), P1 (100 mg.L^{-1}), P2 (200 mg.L^{-1}), P3 (300 mg.L^{-1}), P4 (400 mg.L^{-1}), with three replications. The results showed that the immersion of Turkey berry leaf extract affected on feminization of common carp and increase the growth rate of fish were 82.20% and 0.98%, respectively at P3 (300 mg.L^{-1}).

Keywords: sex reversal, herb medical, phytosteroid, endocrine distruption

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1. Introduction:

Aquaculture development increases every year, including cultivation of common carp (*Cyprinus carpio*). According to FAO (2017), Karnai and Szücs (2018), the achievements of common carp production between 1985 and 2015 it reached 4.4 million tones (3.4%), which cover the third most significant fish spesies of the world's fish production and fisheries. In carp, the female are particulary valuable, since they growth faster than males (Wolfarth *et al.*, 1975). Moreover, there is an urgent need to sustainable aquaculture industry in common carp culture, such as produces all female.

Methods of producing all female common carp by sex reversal, it called feminization. The feminization process of fish can be carried out hormonally and induction of ginogenesis (Akbar and Hanafie, 2013). Hormonal uses steroid hormones such as 17α-methyltestosterone, 17β-estradiol (Alcántar-Vázquez *et al*. 2015; Singh *et al.* 2018), and aromatase inhibitor (Tsai *et al.* 2011). On the other hand, the optimal incubation can be produce a high feminization (Tseng *et al.* 2017).

Due to trade mark, the use of synthetic hormones can be stressful and carcinogenic. This is in line with the global trend of the people who take the slogan '*back to nature'* so that the demand for food use and the production of natural ingredients needs to be increased by using herbal drugs or phytoestrogens (Rohani *et al*. 2012). Phytoestrogens are other anti-nutrients found in Turkey berry (*Solanum torvum*), which may affect the development of reproductive

and sex differentiation in fish. El-Sayed *et al.* (2012) stated that the administration of phytoestrogens from soy flour (35% crude protein: 19 mJ.kg^{-1} isocalori) produced female tilapia by 77%.

The natural steroid hormone contained in the Turkey berry is solasodine. The mechanism of solasodine in sex reversal is to disrupt the balance of the gonadotropin hormone (Kaspul, 2007). Therefore, this study aimed to determine the effect of Turkey berry leaf extract as phytoestrogens on the feminization of common carp (*Cyprinus carpio*).

2. Materials and Methods

2.1. Preparation and Characterization of Turkey Berry (*S. torvum*) Extract

The characteristics of the Turkey berry have brownish green stems, filled with sharp thorns and downy hair, single-fingered leaves with a length of 6 to 30 cm, located alternately, wide ovoid shapes, pointed edges, pinched edges, light green, having tightly threaded hands and some having outboard spines, the flowers are white, in groups of 5-6 in one stalk, the pistils are yellow and the fruit is green when they are young and black when ripe (Andarwulan and Faradilla, 2012).

Before making a liquid extract, simplicia is made first. Making simplicia includes preparation of materials and drying. Preparation of ingredients, namely ingredients in the form of Turkey berry leaves separated from the stem, washed, cleaned and then drained. The drying process uses a dryer in the form of an oven. To facilitate the production process of herbal preparations an extraction process is carried out. The process of making extracts uses the maceration methods.

Characterization carried out included chemical characterization and proximate test. Chemical characterization in the form of qualitative steroid tests. The proximate test carried out included tests of water content, ash content, carbohydrate, protein, and fat.

2.2. Immersion of Fish Larvae

The fish used were larvae of common carp (*C. carpio*) aged five days after hatching obtained from the Fish Breeding Center (BBI) Bedegung, Muara Enim, South Sumatra. The process of immersion larvae in Turkey berry leaf extract is carried out in a 10 liter volume container. The use of extract stock is adjusted to the concentration of treatment. The larva used is 5 days after hatching. Each container contains 10 fish per liter. The duration of immersion is 24 hours and the media is equipped with aeration installation. After 24 hours, the larvae are transferred to the maintenance container. This study used a completely randomized design using 5 treatments and 3 replications with the treatment code is P. Treatment used were: Concentration of Turkey berry leaf extract 0 mg.L^{-1} (P0), 100 mg.L^{-1} (P1), 200 mg.L^{-1} (P2), 300 mg.L^{-1} (P3), 400 mg.L^{-1} (P4).

2.3. Rearing of Fish

Larvae reared in an aquaria (40 cm x 40 cm x 40 cm) which has been equipped with aeration installation in the middle of the media. The aquarium is filled with water with a volume of 40 liters of water. During 30 days of rearing, larvae are given natural food (naupli *Artemia* sp. and *Daphnia* sp.) and artificial feed (pellets, 40% protein). Naupli *Artemia* sp. obtained from hatching for 24 hours and it is given during immersion and for 7 days after immersion or when the larvae are 6 to 12 days old. For *Daphnia* sp. given for the next 16 days or when the larvae are 13 to 28 days old. Before the feed transition, a transition strategy was carried out on the 10th day by giving mixed feed between naupli *Artemia,* sp. and *Daphnia* sp. Initially the portion of naupli *Artemia,* sp. greater than the portion of *Daphnia* sp. until all replaced.

Furthermore, the feed transition was carried out on the 26th day until the portion of *Daphnia* sp. replaced with a pellet feed portion given on the 29th to 35th day. Natural feed is given in *ad libitum*, while artificial feed is given *at satiation* with the frequency of administration in the morning, afternoon and evening. Water quality measurements are carried out every week during rearing. Body weight measurements are carried out at the beginning and end of rearing.

2.4. Examination of Gonad Fish

Examination of gonads is carried out after 30 days of rearing or when the larvae are 36 days after hatching. The method used in gonad analysis is *Acetocarmine Squash Methods*. Asetocarmine is a coloring solution used to color the gonadal tissue on genital examination. Because the gonad tissue is too small, the staining is done by giving 1 drop of acetaminine solution to the inside of the body that has been finely chopped and placed in the glass object. After being left for 1 minute, the glass cover is placed slowly and observed under a microscope. The male gonads are point-shaped and the female gonads are round.

2.5. Statistical Analysis

Quantitative analysis was carried out on data on the percentage of female carp, survival rate, and final weights. The data is tabulated using *Microsoft Office Excel* 2007, analyzed by analysis of variance (ANOVA) through the Minitab 18.0 program with a confidence interval of 95%. If the analysis of variance shows that the results are significantly different, then a further test is conducted with Tukey. The results of the data in qualitative form consisted of the characterization of the Turkey Berry, as well as the quality of water presented in table form.

3. Results and Discussion

3.1. Characterization of the Turkey Berry

Turkey berry originating from the family *Solanaceae* presents a group of steroid alkaloids as solasodine ($C_{27}H_{43}O_2N_7$) which is an important source for steroid hormone synthesis (Moreira *et al*., 2010; Patel *et al*., 2013). Based on the results of the qualitative test of Turkey berry simplicia in Table 1, the steroid content in the fruit stalks was more than that of fruit and leaves. However, in Sirait (2009), it was explained that the solasodine content in leaves (0.84 mg) was more than fruit (0.10 mg). Based on the the quantitative test of Turkey berry simplicia were water (9.11%), ash (10.18%), fat (2.77%), protein (20.76%), and carbohydrates (57.17%). According to Kaspul (2007), steroid alkaloids are competitive against receptors *follicle stimulating hormone* (FSH). The functions of FSH as a mediator for binding to androgens in spermatogenesis. In biosynthesis, androgens require cholesterol as a precursor. Cholesterol is often called fat. The low percentage of fat synergizes with the work activities of solasodine so that it influences androgen biosynthesis in carrying out its functions to form masculinizing hormones to be hampered and to cause feminizing effects. So that why, in this study using stalk and leave of Turkey berry as a natural steroid.

Table 1. Qualitative test of simplicia of Turkey berry (*S. torvum*)

No	Sample	Qualitative test for steroids
1	Fruit	+
2	Stalks	+++
3	Leaves	++

Description: +: little; ++: moderate; +++: many

3.2. Female of Common Carp

Based on the results of statistical analysis in Figure 1, the dose of Turkey berry leaf and stalk extract stems significantly affected the percentage of female common carp ($p < 0.05$). The highest percentage of female common carp (82.22 ± 1.92%) at high doses of Turkey berry leaf and stalk extract (300 $mg.L^{-1}$), but if the dose is added (400 $mg.L^{-1}$) there is a decrease in the percentage of female common carp 78.31 ± 7.15%. This shows that there are symptoms of a paradoxical effect, as suggested by Piferrer (2001) that high doses for certain species will have the opposite effect and can result in high mortality.

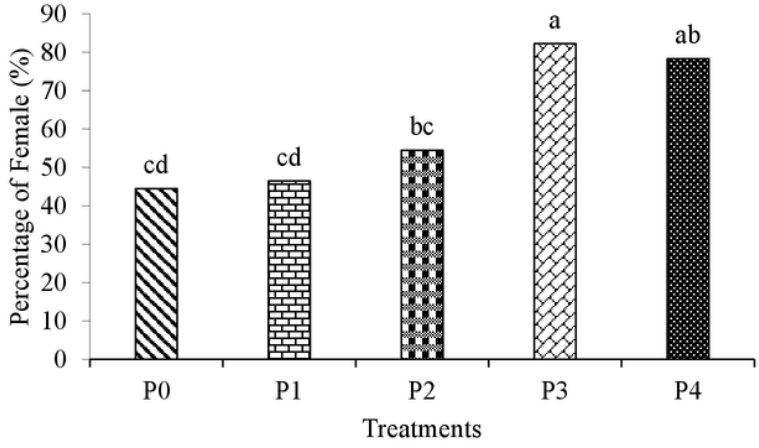

Fig 1 Percentage of female common carp

In the follow-up experiment, the use of Turkey berry leaves extract have been able to influence the hormonal system in the larva's body. Solasodine as a phytoestrogen in sex reversal is to disrupt the balance of the gonadotropin hormone (Kaspul, 2007). Ng *et al.* (2006), Green and Kelly (2009), and Simmler *et al.* (2013) added that the phytoestrogens can act as xenoestrogens at estrogen receptors or block estrogen's effects. It contains isoflavones that may mimic the effect of estrogen. Elakkanai *et al.* (2015) explain that neurosteroids play a vital role in governing the physiology of reproduction next to neuropeptides and neurotransmitters. The chemical messenger release into the blood by specific tissue such as pituitary gland. The hormones travel through the bloodstream to other tissues. The primary tissues involved in this hormonal cascade are the hypothalamus, pituitary gland, and gonad.

Most authors have reported feminization, for other fish species with various treatment, such as the optimum feminization protocol (90%) is feeding diethylstilbestrol at 1000 $mg.kg^{-1}$ to fry held at 1000 m^2 for a treatment duration of 10 days in tanks. Cruz and Mair (2000), estradiol-17β dose of 120 $mg.kg^{-1}$ in nile tilapia (*Oreochromis niloticus*) produces 88.5% females (Alcántar-Vázquez *et al.* 2011), the immersion of rainbow trout (*Oncorhynchus mykiss*) in ethynylestradiol-17α 400 $\mu g.L^{-1}$ for 2 hours produces 94.5% females (Razmi *et al.* 2011), phytoestrogen of soy bean meal with crude protein 45% produces 77% of females (El-Sayed *et al.* 2012), phytoestrogen of 0.5 $g.L^{-1}$ licorice root extract in guppy (*Poecilia reticulata*) produces 88% females (Turan 2017).

How to administer hormones is one of the determining factors for the success of sex differentiation (Arezo *et al.* 2014). Giving hormones can be done by immersion, oral and injection (Dunham, 2004). In this study, the application of Turkey berry leaf and stalks extract was done by immersion. The method of immersion has been able to direct the formation of females, although there are still some individuals who are intersex or hermaphrodite. It is

assumed that the larval response in receiving input of Turkey berry leaf and stalks extract from environmental media has not all been well accommodated. Interrelated factors are also fish species, genetics, hormone type, hormone dose, duration of treatment and time of treatment (Baroiller *et al.*, 1999; Phelps and Popma, 2000; Piferrer, 2001; Devlin and Nagahama, 2002; Dunham, 2004).

3.3. Survival Rate

Based on the results of statistical analysis in Figure 2, the dose of Turkey berry leaf and stalks extract did not significantly affect the percentage of female common carp larvae ($p > 0.05$).

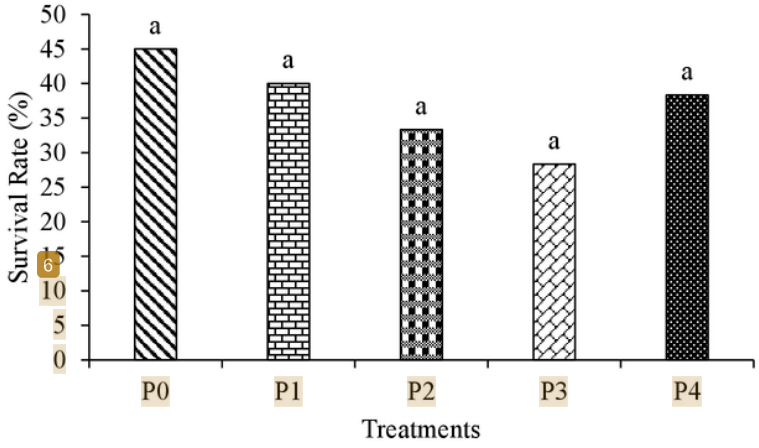

Fig 2 Survival rate of common carp larvae

The high mortality in the first week of maintenance is due to the age of larvae which are susceptible to changes in immersion media. In addition to media changes, the larvae handling factors when transferring larvae from immersion media to rearing media are also the cause of low survival of carp. This is explained by Mukti *et al.* (2009) that handling the wrong and too rough can result in stressed and weak larvae, so that the fish die easily. Acclimatization or adaptation of larvae and transfer of larvae to maintenance media also greatly affect larval mortality.

3.4. Final Growth

Based on the results of statistical analysis in Figure 3, the dose of Turkey berry leaf and stalks extract significantly affected the final growth of female common carp larvae ($p < 0.05$), but did not significantly influence the final growth of male common carp larvae and intersex larvae ($p > 0.05$).

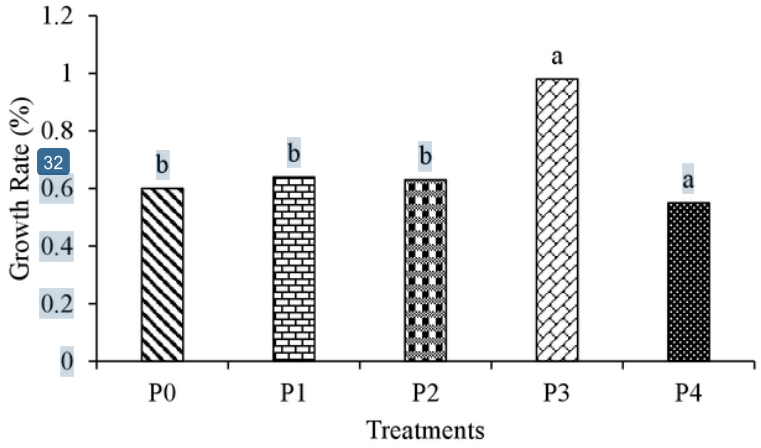

Fig 3 Growth rate of common carp

The given hormone dose should not be excessive because it can lead to low growth rates (Dunham, 2004). Piferrer (2001) also explained that the addition of excessive hormone doses can cause paradoxical and pressure effects on gonadal vessels which cause low growth rates. For groups *Salmonidae*, *Cyprinidae* and *Cichlidae* where at the optimum sub-dose growth increases sharply and reaches a peak at the optimum dose. However, this growth drops dramatically when it exceeds the optimum dose or super optimum. The results of the study also showed a tendency to increase the specific growth rate of female fish as the concentration of Turkey berry leaf and stalks extract was increased, then decreased again.

Besides being influenced by the dose of treatment, fish growth is also influenced by internal and external factors. Internal factors are gender and genetic growth. In carp, the female are particulary valuable, since they growth faster than males (Wolfarth *et al.,* 1975). While external factors include water quality (Hoar *et al.,* 1983; Phelps and Popma, 2000; Devlin and Nagahama, 2002) and nutrients, especially proteins (Muslim *et al.*, 2011).

3.5. Water Quality

The results of water quality during rearing of carp larvae are presented in Table 2. The water quality of rearing is still within the proper range.

Table 2. Water quality during rearing of common carp larvae

Parameters	Week of			
	1	2	3	4
Temperature (°C)	25-30	25-30	25-29	26-30
pH	6.5-7.0	6.7-7.0	6.7-7.1	6.8-7.1
Dissolved oxygen(mg.L^{-1})	4.4-6.5	4.9-6.0	5.0-6.7	5.2-7.3

External factors that influence the determination sex are temperature. Temperature affects metabolic activity in the body, in this case affecting the structure of proteins and other macro molecules in the body of the fish (Hoar *et al.,* 1983; Phelps and Popma, 2000; Devlin and Nagahama, 2002). Water temperature of 25 to 32 °C is considered suitable for fish culture (Boyd, 1998). The pH values ranging from 6.5 to 7.1 are still included in the range 6.5-9.0 (Swingle, 1967) which is good for fish growth and reproduction. The pH value affects carbon dioxide and alkalinity. The higher the value of alkalinity and the lower the free carbon dioxide. The toxicity of chemical compounds such as ammonia which is not ionized at high pH is toxic

(killing) and more easily absorbed into the body of aquatic organism. Aquatic organisms need oxygen to survive. Dissolved oxygen levels in water that can cause aquatic organisms to become stressed, hypoxia in the tissues, anorexia, unconsciousness, disease and parasites. Even in extreme conditions causes sudden and mass death. Boyd (1998) recommended suitable ammonia. Boyd (1998) recommended suitable ammonia-nitrigen as below 0.1 $mg.L^{-1}$, alkalinity more than 100 $mg.L^{-1}$ and free carbon dioxide ranged 1.0 to 10.0 $mg.L^{-1}$. Rothuis *et al.* (1998) added that the mean values of water temperature, pH, dissolved oxygen and ammonia-nitrogen concentration fluctuated from 28.40 to 34.30 °C, 6.46 to 6.79, 2.25 to 6.71 $mg.L^{-1}$, and 0.1 to 0.2 $mg.L^{-1}$ respectively.

4. Conclusion

Immersion of common carp larvae in Turkey berry leaf and stalks extract at a dose of 300 $mg.L^{-1}$ can increase the percentage of female common carp larvae (82.22±1.92%) and final weight of female common carp larvae (0.98±0.18 gr), but decrease survival rate (28.33±2.89%).

5. Acknowledgments

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