Utilization of Cepoka (Solanum torvum) Eggplant Fruit Extract on Catfish (Clarias sp.) Seeds in Female Sex Difference.

Hadijah[1*], A. G. Tantu[1], A. Supriyanto[1]

[1]Department of Aquaculture, Faculty of Agriculture, Bosowa University, 90231, Makassar.
* Corresponding author E-mail: hadijahmahyuddin@gmail.com

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Abstract

This research was conducted in September-November 2019 at the Biopharmaca Laboratory of the Medical Faculty of Hasanuddin University Makassar and at the Artificial Feed Production Laboratory, Bosowa University Makassar. The aim of the study was to determine the length of immersion in the cepoka eggplant fruit extract (*Solanum torvum*) against the change in male to female sex in catfish (*Clarias* sp.) seeds. The results showed that the length of immersion of catfish seeds in the Cepoka eggplant fruit extract had no effect on the change in the sex of males to females of catfish seeds. Likewise, the length of immersion in the Cepoka eggplant fruit extract did not affect the survival of catfish seeds.

Key words: extract, catfish, cepoka eggplant

Introduction

The development of freshwater cultivation is increasingly being promoted, especially freshwater cultivation, which on average tends to still apply extensive cultivation patterns. Freshwater aquaculture intensification mainly aims to meet the increasing needs of the community for animal protein derived from fish (Rohmawati, et al., 2010). The success of fish farming, of course, really depends on the provision of sufficient and good quality seeds and in accordance with the objectives of cultivation (Rosalina, 2015).

In the future, it is expected that the supply of fishery products from cultivation will be greater than from fishing. Thus, fish farming is a source of economic growth that must be realized through a competitive, sustainable, and just cultivation system (Fatuchri, et al., 2002).

Catfish (*Clarias* sp.) is a type of freshwater fish that has been cultivated commercially by the people of Indonesia. In addition to maintaining the species, cultivation activities need to be increased to meet market demand and community nutritional needs (Yulinda, 2012). Along with the high level of public consumption of catfish, it makes business opportunities more open. Starting from the hatchery business, enlargement to processing business. The things that encourage people to cultivate catfish are that they can be cultivated in limited land and water sources with high stocking density, cultivation technology is easily mastered by the community, marketing is relatively easy, and the required business capital is relatively low (Puddin, et al., 2015).

In the catfish farming business, there are two major activities that must be increased simultaneously, namely hatchery and rearing. These two activities cannot be separated in the process,

because hatchery activity is the initial activity in cultivation. Without seeding activities, other activities such as nursery and rearing will not be carried out (Musa, 2016). According to (Lindawati et al., 2015), catfish has various advantages over local catfish so that currently catfish is a very popular commodity and can bring huge profits. Some of the advantages include growing faster, being able to reach a larger size, having more egg content, and being tolerant of additional feed of various types.

According to Wahyudy et al., (2018) to support the success of fish farming, one of the determining factors is the availability of seeds that meet the requirements in terms of quality, quantity, and continuity. The seeds that are available in large quantities but of low quality will only burden the enlargement farmers because the results are not balanced with the quantity of feed given. Meanwhile, good quality seeds but limited in number will not increase the production of the enlargement business, because there will be a serious shortage of seeds. Raising single male fish tends to increase production, because the mating process will not occur, so that energy from feed is fully used for growth (Sabrina, et al., 2018). In general, there are several ways to change the sex or breeding of catfish and increase the percentage of individual females in the fish population, namely separating males and females by manual selection, but they are less efficient because they are wasteful of time and energy. The second way is to cross-breed (hybridize) between species, but it is less practical and takes a long time to produce 100% male catfish. The third way is chromosome manipulation, which can only be done by geneticists and takes a long time, and requires a high degree of accuracy and a large cost. For the farmer level, this method cannot be applied except in collaboration with research institutions that have already done this.

The newest technique for producing female fish seeds is sex reversal. In most fish it is possible to reverse sex by administering steroid androgen hormones through feed or immersion (Carman et al., 2008). One of the important factors for the success of sex reversal is the age of the catfish larvae immersed in a steroid hormone solution of cepoka eggplant fruit extract. This is closely related to the percentage of the number of larvae that managed to be formed into female. Research on the optimal age for catfish larvae carried out so far has not been determined with certainty (Kasim, et al., 2014). Sex reversal technology is a monosexual production technique that applies hormonal engineering to change the sexual character from female to male (masculinity) or from male to female (feminization). Furthermore, Junior et al. (2007) states that the application of sex reversal for sexual intercourse can be done by using the synthetic hormone 17α-methyltestosterone orally (through feed), immersion (in embryo, larva or parent stage), and injection (implantation). The use of the hormone 17α-methyltestosterone is reported to have a negative impact, namely a carcinogenic effect (causing cancer) when applied to fish consumption and causing environmental pollution that affects food safety and environmental sustainability (Sudrajat and Sarida, 2006) in Mubarokah, et al., 2019). One of the efforts to avoid the use of synthetic hormones is to conduct research on steroids that are safer to use. Hormones that have been used to date are using biological hormones using extracts from plants, one of which is the extract of Cepoka eggplant fruit, the advantages of this plant are that it can reduce stress on fish (Rahmadiah ,, et al., 2019). The availability of females is an important factor, because in its development, female fish seeds have great advantages to spur faster fish production, shorter harvest periods, and add to the economic value of fish farmers (Danriani, 2018)

Cepoka eggplant is one type of plant that has the efficacy of traditional medicine and has the

potential to be cultivated. The chemical content contained in cepoka eggplant is in the fruit, leaves and roots of plants. The fruit and leaves contain steroid alkaloids, namely solasodine, salosonin, chlorogenin and various vitamins (Sirait, 2009). The positive effect of using cepoka eggplant fruit as raw material for male to female sex reversal in catfish (*Clarias* sp.) seeds has not been widely studied, so this research needs to be done.

Methodology

Preparation of Containers

The research was conducted in 2 stages, namely the preparation stage and the implementation stage. The immersion container used is 12 plastic 3 liter volume, and 12 maintenance containers are 10 liter volume units. All research containers before use are washed and dried in the sun for 24 hours. The immersion container contains 1 liter of water each, while the maintenance container is filled with 8 liters of water. All containers filled with water are equipped with aeration to supply oxygen and a heater to keep the water temperature stable. The water used for hormone immersion and seed maintenance is fresh water that has been deposited for 48 hours, then put into the experimental media.

Hormone Solution Preparation

Cepoka eggplant fruit prepared weighed 1.1 kg (dry). Prepare 11 liters of 70% alcohol which will be used to soak the cepoka eggplant fruit. Enter the cepoka eggplant fruit and 70% alcohol in a glass jar for 3 days until the color turns clear brown. Filter the soaking results of the cepoka eggplant using filter paper. Cepoka eggplant fruit that has been filtered can be put into the evaporator for 10 hours.

The results of the extraction in solid form are stored in a petri dish and then weighed using an analytical balance weighing 100 g. The next step is that the extraction product is dissolved into 1 liter of 70% alcohol. Then shake it until it dissolves. After the hormone dissolves, we can take 0.3 ml each in a jar containing 1 liter of water, then homogenize it evenly for 24 hours.

Implementation Stages

Immersion Process

After being acclimatized, 15 catfish larvae aged 5 days were put into a container containing 1 liter of water which had been mixed with the extracted solution of Cepoka eggplant and soaked according to the treatment. As for the length of soaking the catfish seeds in the Cepoka eggplant extract solution according to the treatment. During the immersion period of catfish seeds, this hormone solution is still aerated. After the soaking period is complete according to the treatment, then the catfish seeds are transferred to the rearing container.

Maintenance Process

After soaking in a hormone solution, the seeds are removed from the solution, then put in a rearing container and maintained for 2 months or until the seams can be seen. During the maintenance, catfish seeds were given natural food in the form of Artemia for 2 weeks and artificial feed F999 until the end of the study. The feed was given ad libitum with a frequency of three times a day at 08.00 AM, 13.00 PM and 18.00 PM.

Research design

This research was conducted experimentally using a completely randomized design (CRD) with 4 treatments and 3 replications. The treatment in this study was the long soaking time of the catfish seeds in a solution of Cepoka eggplant fruit extract, namely treatment A: soaking time 25 hours. B: soaking time 30 hours, C: soaking time 35 hours, D: without soaking (control).

Test Parameters

1. Sex Ratio

The success of sex ratio calculated using the Junior et al. (2007) formula is as follows:

$$\text{Percentage of female fish} = \frac{\text{Number of female fish}}{\text{The number of samples}} \text{ x } 100$$

2. Survival Rate

The survival rate is calculated using the Effendie (1997) formula as follows:

$$\text{Survival Rate} = \frac{\mathbf{Nt}}{\mathbf{No}} \text{ x } 100\%$$

where:

Nt = number of fish at the end of the study (individu)

No = number of fish at the beginning of the study (individu)

3. Water Quality Parameters

Water quality parameters are parameters that support the survival that are measured including temperature and pH. Measurement of temperature and pH is carried out every day in the morning at 07.00 AM and in the afternoon at 18.00 PM.

Data analysis.

The research data were analyzed by using the Analysis of Variance (ANOVA). If the results have a significant effect, then proceed with the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Percentage of Female Catfish (%)

In this study, it can be seen that the difference in the length of time soaking the steroid hormone extract of Cepoka eggplant fruit in catfish seeds shows the percentage of female catfish in various treatments is presented in Table 1.

Table 1. Average percentage of female catfish in each treatment

Treatment	Percentage of female catfish seeds (%)
A (soaking time 20 hours)	79[a]
B (soaking time 25 hours)	78[a]
C (soaking time 30 hours)	83[a]
D (without soaking/control)	70[a]

Note: the same superscript letter indicates not significant treatment (P> 0.05)

From Table 1, it shows that the long immersion treatment in steroid hormones from cepoka eggplant fruit extract had no significant effect (P> 0.05) on the percentage of female catfish seeds. These results are consistent with research conducted by Suryanto and Budi (2007), that the provision of MT hormone in sex reversal at different ages of African catfish larvae does not have a significant effect on the success of male sex change and fish growth rate. Although statistically there is no significant difference, there is a tendency that the older the larvae are, the lower the percentage of males is obtained. This is in line with what was stated by several previous researchers that the older the larvae were immersed in natural MT resulted in a higher number of males (Junior et al., 2007).

In treatment A and B, the percentage of female catfish obtained in this study was higher than the previous study, possibly influenced by several factors, including (Pranata, et al., 2017). Cepoka eggplant fruit extract which contains solasodine compounds is a steroid hormone, so that the increased immersion time of the hormone in the body of catfish can lead to the formation of sex. Generally the hormones dissolved in the immersion vessel enter at the same time as the entry of fluids into the body, then proceed to the bloodstream and reach the target on the gonads. Meanwhile, steroid hormones are thought to be difficult to enter by diffusion into the body of catfish larvae and even fail to circulate in the circulatory system. Kurniati and Jumanto (2017) state that the work of hormones does not have to go through the blood, but can act by diffusion through the cell membrane around where the hormone circulates to the target organ and interacts directly with the receptors in catfish. The mechanism of action of hormones in the dipping method is diffusion through the skin, gills and digestive organs (Sukiya and Putri, 2016).

According to Farida, et al. (2018) the time of hormone treatment is highly dependent on the time interval for sex differentiation, namely the phase of gonadal development is still unstable. In this phase, the development of the gonads has not shown a tendency of sex determination towards male or female. Ariyanto et al. (2016) stated that the technique of giving hormones in sex reversal can be done by means of implants, injections, immersion, and through feed. In this study, it was carried out with differences in the length of soaking time. The technique of giving hormones shows that the percentage of female fish is less than optimal. This occurs because usually not all fish are directly sex-differentiated so that the effectiveness of hormones to affect sex differentiation is also reduced. According to Wihardi et al. (2014) stated that a certain paradoxical effect symptom will have the opposite effect as expected. This was stated by Yulinery, et al. (2009) stated that the effect of osmosis on immersion is still in the physiology of the fish itself but has not worked effectively. Meanwhile, Santoso (2018) states that natural steroids will have a more effective effect on the target organs if they are given by injection.

In treatment C (30 hours immersion time) showed 83% produced the highest female catfish. The

results of this study are due to the solasodine content that is absorbed by the fish body through a diffusion process with a long soaking time for 30 hours to get maximum results so that the process in the fish body affects FSH (Folicle Stimulating Hormone) which disrupts gonadotropins so that it can suppress the process of male emergence, so obtained the highest percentage of female catfish. This was also stated by Wihardi, et al. (2014) that the solasodine content in the extract of the leaves of the cepoka eggplant stalk (*Solanum torvum*) soaked in goldfish is diffused and secreted through the bloodstream, this steroid hormone stimulates the development of granulosa cells that form receptors for increasing FSH (Folicle Stimulating Hormone).), solasodine has the effect of estradiol as a liver stimulant which can inhibit the secretion of FSH (Folicle Stimulating Hormone) by the pituitary which will inhibit the balance of Gonadotropins so that it suppresses changes in the phenotype male to female phenotype (feminization).

Treatment D (without immersion / control) the lowest percentage of female catfish is 70%. This is because treatment D as a control did not receive the soaking treatment of Cepoka eggplant fruit extract so that the results obtained were also low. In D (control), the proportion of male fish obtained was smaller than that of female fish. The percentage of female fish produced is only 70%. This is because the female genetic factor in the parent is more dominant than the male genetic. In accordance with the opinion of Sembiring, et al. (2018) that if the parent with genetic characteristics that determine male sex is more dominant than female sex, it will have more male children than female children, and vice versa. According to (Junior et al., 2007) that in normal conditions without any interference, gonad development will take place normally. Furthermore, that under normal conditions, individuals will develop according to the phenotype expressed from the genotype. Individuals with genotype XX will develop into females, while individuals with genotype XY will develop into males at a ratio of 1: 1 without external influence. The same thing stated by Zamroni, et al. (2017) where genetically under normal circumstances fish will produce offspring with a male to female sex ratio of 50%: 50%.

Survival Rate

Observations during the study showed that the longer the immersion time, the lower the survival rate of catfish. The average survival rate of catfish seeds is presented in Table 2.

Table 2. The average survival rate of catfish seeds in each treatment

Treatment	Survival Rate (%)
A (soaking time 20 hours)	64[a]
B (soaking time 25 hours)	67[a]
C (soaking time 30 hours)	62[a]
D (without soaking/control)	69[a]

Note: the same superscript letter indicates not significant treatment (P> 0.05)

The results of the analysis of variance showed that the soaking time of the cepoka eggplant fruit extract did not significantly (P> 0.05) affect the survival rate of catfish seeds. The results showed that the administration of steroid hormones with different treatment of immersion time to the survival rate of catfish

seeds was relatively high in treatment D (without immersion) of 69%, treatment B (immersion for 25 hours) was 67%, treatment A (immersion 20 hours) by 64%, and the lowest was at treatment C (immersion for 30 hours) by 62%. The low survival of iele fish is caused by internal factors and external factors. Factors that can affect survival are disease and parasites and the physiology of the fish. Budiana and Rahardja (2019), state that internal factors are factors that are related to the fish itself, such as age and genetics which include heredity, the ability to use food and resistance to disease. Meanwhile, external factors are factors related to the living habitat environment which includes the physical and chemical properties of water and space to move.

The difference in the results obtained is influenced by soaking the catfish seeds with a solution containing alcohol which is used to dissolve the extraction results with water in the treatment. This result is not much different from the research conducted by Yulinery, et al. (2009) who obtained a survival rate of between 54-58% using an alcohol solution in dissolving sea cucumber flour extract in the betta fish process. According to Hadiroseyani, et al. (2007) although very small amounts of alcohol can cause death if immersion is carried out for a long time.

Density in stocking is very important to consider in fish farming because it will affect growth and can cause disease or even death (Widyarto, 2009). Competition for mobile space can affect fish growth, because with the stocking density in the container for each treatment, there may be competition in terms of opportunities to get feed. This situation causes the condition of the fish to be weak so that feed utilization is not optimal, this results in disrupted fish growth (Cholilulloh and Syauqy, 2018).

Good water quality will affect the survival and growth of fish. Meanwhile, the deaths that occur during maintenance are due to the fact that the space for movement is getting narrower so that it puts pressure on the fish. The impact of stress results in decreased fish immunity and even death (Dahlia, et al. 2019). In contrast to the opinion of Lestari and Dewantoro (2018), the important factors affecting the growth and survival of fish are the availability of food types and the presence of a good environment such as oxygen, ammonia, carbon dioxide, nitrate, hydrogen sulfide and hydrogen ions.

Water quality

The water plays a very important role as a living medium for fish, so in aquaculture, water quality is the main supporting factor in the maintenance of fisheries. Water change also determines water quality during maintenance. Water quality parameters observed every day are temperature and pH with the results of water quality measurements during maintenance can be seen in Table 3.

Table 3. Results of water quality measurements during maintenance

Parameters	Treatment				Advisability	References
	A	B	C	D		
Temperature	26 - 29	26 - 29	26 – 29	26 - 29	20 – 30 ^{0}C	(Lestari, 2018)
pH	5,6–6,8	5,6-6,8	5,6-6,8	5,6-6,8	6,5 - 8	(Mas'ud, 2018)

Water quality parameters observed during maintenance were temperature and pH. From the table above the temperature during maintenance ranges from 26-290C which is relatively the same between treatments. This temperature range is included in the optimal range for raising catfish seeds, where the proper range for catfish growth ranges from 25 - 320C (Lestari and Dewantoro, 2018). At a temperature of 180C-250C the fish still survive, but their appetite begins to decline. Meanwhile, temperatures below 120C tropical fish freeze to death (Djunaedi, et al., 2016).

The pH range obtained during the study period was between 5.5 - 5.8. The condition of the water's pH which is classified as acidic in this range is still said to be suitable for catfish growth, because catfish can live in waters with a wide pH range of 5-11 even though optimal growth occurs at a pH value of 7-8. In the pH range 5-11 catfish can still grow because tilapia has a high level of tolerance to environmental conditions. Lestari and Dewantoro (2018) stated that the pH of the water is influenced by the pH of the soil, especially if the water source around the maintenance area is still brackish, in this place the pH can reach a very low value because the sulfuric acid content in the subgrade is high. As stated by Mas'ud and Rahayu (2018), generally African catfish can live in waters with a pH range between 6.5-8.

CONCLUSIONS AND SUGGESTIONS

Based on the results of the study, it can be concluded that the soaking time of the steroid hormone cepoka eggplant fruit extract has no effect on the percentage of female catfish and the survival rate of catfish during the rearing period.

Based on the results of this study, it is suggested that the difference in the length of time soaking the steroid hormone cepoka eggplant fruit extract on the percentage of female catfish need to be further tested to get the optimal value. In addition, it is important to add information for farmers regarding steroid hormone administration.

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