Interaction of dose and immersion time of soy extract on female percentage and survival performance of tilapia (*Oreochromis niloticus*)

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Abstract. The factors of dose and duration of treatment, along with potential interactions between dose and immersion duration, determine the success of obtaining functional female tilapia (XY) during the fish feminisation stage. This study aimed to evaluate the effect of soybean extract with different doses, immersion duration, and the interaction of dose and immersion duration on the success rate of producing female tilapia in the feminisation process, as well as its effect on survival. The application of soybean extract was carried out three times, namely when the tilapia fry were 8, 12, and 16 days old. The treatments applied consisted of two factors, namely dose and duration of immersion. Each factor consisted of several levels, so a randomised complete factorial design (RALF) was used. Dosage factor involved the application of soybean extract (A) consisting of a dose of 0.02 g L^{-1}, 0.03 g L^{-1}, 0.04 g L^{-1}, and immersion time factor using soybean extract (B) consisted of 6 hours, 10 hours, and 14 hours, as well as control (without dose treatment and immersion time). Each combination of factors was carried out in as many as three replicates, resulting in 30 treatment units. Evaluation of feminisation success and survival was conducted after 10 weeks of rearing. The results showed that the factors of soybean extract dose and immersion time had a significant effect ($p < 0.05$) on the percentage of female tilapia; however, the interaction between the two had no significant effect ($p > 0.05$). Similarly, the factors of soybean extract dose, immersion time, and their interaction had no significant effect on survival. The highest percentage of females (74.25±1.41%) was at a dose of 0.04 g L^{-1}. Immersion time of 6 hours resulted in the percentage of females of 72.01±1.61%, not significantly different from other immersion times (10 and 14 hours). The survival rate obtained was around 56-58% and was not different from the control treatment, indicating that the administration of soybean extract did not have a negative impact on the survival of tilapia fry. Soybean extract can increase the percentage of female fish without affecting the survival of tilapia fry, so it can be used as an alternative in the process of feminising fish.
Key Words: natural hormones, phenotypic, sex reversal, soybeans, synthetic hormones.

Introduction. Nile tilapia (*Oreochromis niloticus*) is a freshwater fish with various advantages, one of which is its fast growth rate, where male fish grow faster than female fish (Singh et al 2017). Therefore, male monosex system cultivation is more profitable than mixed-sex cultivation (Githukia et al 2015; Teng et al 2020). Based on these considerations, there is a need to produce male tilapia. Several methods are used to produce monosex male tilapia, including genetic manipulation (Mair et al 1997), hybridisation (Hickling 1960), and hormone administration (Shelton et al 1981; Wassermann & Afonso 2003).

Common technologies developed to obtain male monosex tilapia are masculinisation using hormones and the use of super male (YY) broodstock. Masculinisation of tilapia using hormones from natural ingredients has been carried out by Attia et al (2023) using gokshura (*Tribulus terrestris*), Maulana et al (2023) using coconut milk, Wahidah et al (2021), and Wahidah et al (2023a) using bull testicle powder extract. In addition to the use of hormones from natural ingredients, farmers currently obtain male monosex tilapia seeds using super male (YY) parents obtained from the results of genetic manipulation with chromosome engineering. Chromosome engineering to produce male monosex tilapia begins with feminisation using hormones to produce functional females (XY). Feminisation studies that induce sex reversal in XY functional females have been conducted by Hashem et al (2019) and Lázaro-Velasco et al (2019).

The application of synthetic hormones in feminisation has been carried out on various fish species. For example, 17β-estradiol has been applied to salmon (*Salmo trutta*) (Voorhees et al 2023), Mexican snapper (*Centropomus poeyi*) (Vidal-López et al 2019), and tilapia (*Oreochromis mossambicus*) (Hekimoglu et al 2019). In addition, the hormones 17α-etinilestradiol (Juárez-Juárez et al 2017) and diethylstilbestrol (Ogira et al 2018) are also used in tilapia (*Oreochromis mossambicus*). In addition to these types of hormones, which are synthetic, feminisation can also use herbal ingredients such as *Solanum torvum* in climbing perch *Anabas testudineus* (Rahmania et al 2022), soybean isoflavone extract in Japanese eel (*Anguilla japonica*) (Turan & Yigitarslan 2019), and soy isoflavones in rainbow trout (*Oncorhynchus mykiss*) (Inaba et al 2022). The use of synthetic and herbal ingredients in fish feminisation gives different results depending on the success rate of feminisation or the resulting sex.

Sex ratio can be influenced by several factors in sex direction using hormones (Stelkens & Wedekind 2010). The effectiveness of hormones to direct fish sex depends on the type of hormone, hormone dose, fish species, age, time, and duration of treatment (Budd et al 2015). In addition to these factors, there may be an interaction effect of these factors that play a role in producing the resulting sex ratio.

Several studies have evaluated more than one factor that plays a role in fish sex direction, including Abd El-Azeem (2023), by treating different diethylstilbestrol hormone concentrations and duration of administration (days) on tilapia masculinisation. Asad et al (2021) evaluated the factors of 17α-methyltestosterone hormone concentration and immersion time (hours) and their interaction on carp masculinisation.

However, there is little information on the factors that play a role in determining the success of feminisation in tilapia, such as hormone dosage, start time, duration of administration, and the interaction of these factors. There is also little information on their effects on tilapia survival. Therefore, this study aimed to evaluate the dose and duration of immersion of Nile tilapia fry as well as the interaction of dose and duration of immersion on the success of feminisation using soybean extract and its effect on survival.

Materials and Method

The production of soybean extract. The production of soybean extract used a maceration method on 500 g of soybean flour in methanol at a ratio of 1:10 (soybean: methanol) for 48 hours; the extract mixed with the solvent was filtered and evaporated at 40°C.

Test fish samples. Gonadally mature Sultana strain Nile tilapia broodstock, with morphometric characters as evaluated by Amrullah et al (2023) and Wahidah et al (2023b), were spawned naturally with a male:female ratio of 1:3. Spawned tilapia fry were applied to soybean extract with different doses and immersion durations. Tilapia fry aged 8 days with an average weight of 0.01 g were reared in a 60 L container filled with 40 L of water. The density of fry per container was 40 fry L^{-1}, so there were 160 fry per container. During fry rearing, feeding and water quality management were carried out. The feed given at the beginning of rearing was silk worms, followed by commercial feed (40% protein content) at a dose of 5% of biomass weight per day. The frequency of feeding was twice a day. After 4 weeks, the larvae were transferred to hapa for rearing for 6 weeks.

Application of soybean extract. The application of soybean extract was carried out three times, namely when tilapia fry were 8, 12, and 16 days old. The treatments applied consisted of two factors, namely dose and duration of immersion. Each factor consisted of several levels, so a randomised complete factorial design (RALF) was used. The dosage factor of soybean extract (A) consisted of doses of 0.02 g L^{-1}, 0.03 g L^{-1}, and 0.04 g L^{-1}, and the immersion time factor using soybean extract (B) consisted of 6 hours, 10 hours, and 14 hours, and a negative control (without soybean extract treatment, specifically no dose and no immersion time respectively). Each combination of factors was carried out in as many as 3 replicates, thus obtaining ((3 x 3) x 3) + 3 = 30 treatment units.

Observed parameters. The variables observed in this study included the percentage of female fish (XX and XY) and survival of tilapia after 10 weeks of rearing. The percentage of female fish was calculated based on their proportion to the total number of fish at the end of the observation, while survival was measured by the number of fish still alive at the end of the period.

Data analysis*.* Data results were expressed as mean±SD. Data on female percentage and survival were analysed using SPSS Version 22 software (SPSS Inc., Chicago, IL, USA). Differences in performance between tilapia fish treatments were determined using one-way ANOVA, and if a significant effect was found, continued with the Duncan test with a significance level of $p < 0.05$ to determine the best dose and immersion time as well as the interaction between the two factors.

Results

Female percentage*.* Based on the results of the analysis, the treatment of dose and duration of immersion had a significant effect ($p < 0.05$), but the interaction between dose and duration of immersion from the application of soybean extract on tilapia seeds had no significant effect ($p > 0.05$) (Table 1).

Table 1

Effect of variables (dose, duration of immersion, and interaction of dose and duration of immersion) on the percentage of feminised tilapia fry

Treatment	*Females (p-value)*	*Male (p-value)*
Dose (D)	0.000*	0.000*
Immersion time (P)	0.009*	0.009*
D × P	0.272	0.272

Note: * indicates a significant effect ($p < 0.05$).

The range of female tilapia percentage values produced in the dose group was 70.09±1.02 to 74.25±1.41% (Table 2).

Table 2

Proportion of females and males (mean±SD) of tilapia fingerlings as a result of soybean extract application at different doses

Dosage (g L^{-1})	*Females (%) (mean±SD)*	*Males (%) (mean±SD)*
Control	53.43 ± 1.13^{a}	46.57 ± 1.13^{a}
0.02	70.09 ± 1.02^{b}	29.91 ± 1.02^{b}
0.03	72.66 ± 1.48^{c}	27.34 ± 1.48^{c}
0.04	74.25 ± 1.41^{d}	25.75 ± 1.41^{d}

Note: Different superscripts on the same column indicate significant differences between treatments.

The treatment of tilapia fry immersion with soybean extract resulted in a female proportion ranging from 71.66±1.98 to 73.33±2.62% (Table 3). Based on Table 3, the proportion of females and males of tilapia fry as a result of soybean extract application with different immersion durations showed that immersion for 6, 10, and 14 hours increased the proportion of females significantly compared to the control.

Table 3

Proportion of females and males (mean±SD) of tilapia fingerlings as a result of soybean extract application with different immersion durations

Immersion time (hours)	*Females (%) (mean±SD)*	*Males (%) (mean±SD)*
Control	53.43±1.13[a]	46.57±1.13[a]
6	72.01±1.61[b]	27.99±1.61[b]
10	71.66±1.98[b]	28.34±1.98[b]
14	73.33±2.62[b]	26.67±2.62[b]

Note: Different superscripts on the same column indicate significant differences between treatments.

The interaction of dose and duration of immersion of tilapia fish seeds with soybean extract ranged from 69.72±0.66 to 75.28±1.52%, and the control was 53.43±1.13% (Table 4).

Table 4

Proportion of females and males (mean±SD) of tilapia fingerlings as a result of soybean extract application with different dose and immersion time interactions

Dosage (g L^{-1})	*Immersion time (hours)*	*Females (%) (mean±SD)*	*Males (%) (mean±SD)*
Control	Control	53.43±1.13	46.57±1.13
0.02	6	70.29±0.33	29.71±0.33
	10	69.72±0.66	30.28±0.66
	14	70.27±1.83	29.73±1.83
0.03	6	72.10±0.90	27.90±0.90
	10	71.42±0.54	28.58±0.54
	14	74.45±0.27	25.55±0.27
0.04	6	73.63±0.99	26.37±0.99
	10	73.83±1.48	26.17±1.48
	14	75.28±1.52	24.72±1.52

Survival rate. Based on the results of the analysis of the treatment of dose, duration of immersion, and the interaction between dose and duration of immersion, the application of soybean extract on tilapia fish seeds did not affect the survival rate of tilapia fish seeds ($p > 0.05$) (Table 5).

Table 5

Effect of variables (dose, duration of immersion, and interaction of dose with duration of immersion) on the survival of feminised tilapia

Treatment	*Survival rate (p-value)*
Dose (D)	0.661
Immersion time (P)	0.900
D × P	0.856

The survival value in the dose and immersion time groups was around 57% (Figures 1 and 2). The interaction of dose and duration of immersion of tilapia fish seeds with soybean extract ranged from 56.88±1.09 to 58.13±0.63%, and the control was 57.71±0.36% (Table 6).

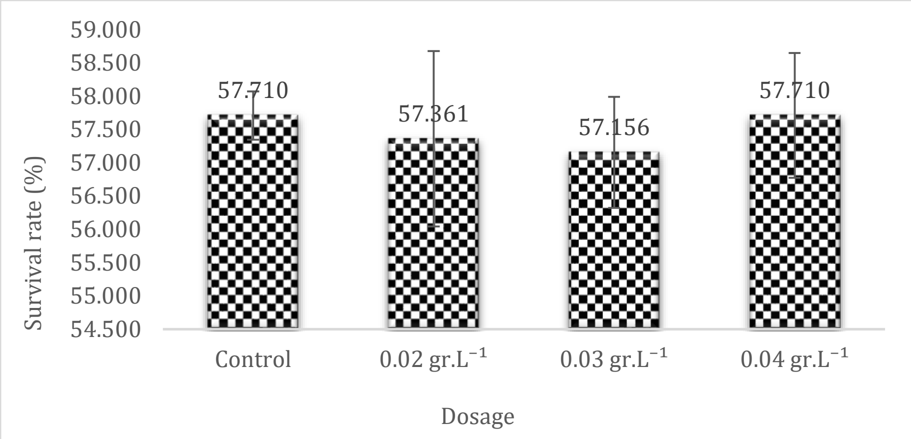

Figure 1. Survival rate (%) of tilapia fry from the application of soybean extract with different doses.

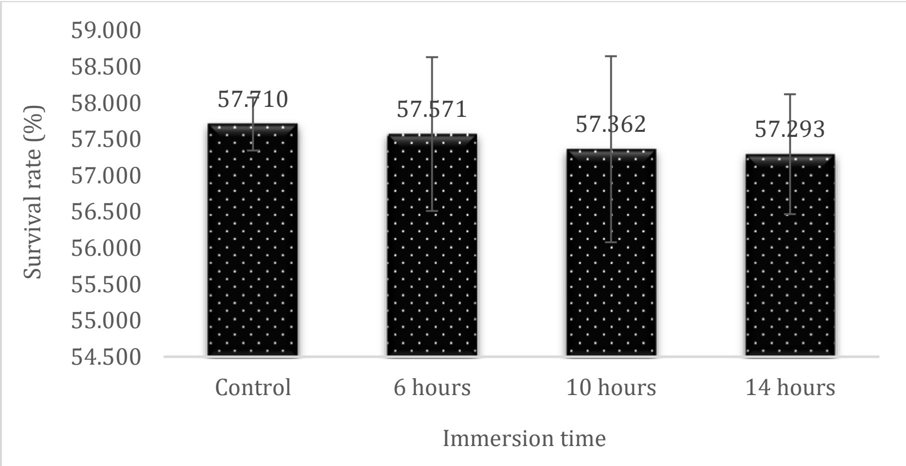

Figure 2. Survival rate (%) of tilapia fingerlings as a result of soybean extract application with different immersion times.

Table 6

Survival rate (%) of tilapia fingerlings as a result of soybean extract application with different doses and lengths of immersion

Dosage ($g\ L^{-1}$)	*Immersion time (hours)*	*Survival rate (%) (mean±SD)*
Control	Control	57.71±0.36
0.02	6	57.50±1.25
	10	57.08±1.91
	14	57.50±1.25
0.03	6	57.50±0.63
	10	56.88±1.09
	14	57.09±0.96
0.04	6	57.71±1.58
	10	58.13±0.63
	14	57.29±0.36

Discussion. This study used the immersion method and was carried out in a discrete manner, namely at the age of 8 days of tilapia fry, continued again at the age of 12 days, and finally at the age of 16 days. The advantage of using the immersion method is that less material is used, as Piferrer (2001) and Pandian & Kirankumar (2003) stated that immersion uses fewer hormones than feeding. However, immersion is much more complex, as various treatment durations and doses are usually required for fish at each different life stage (Pandian & Kirankumar 2003).

Using this method, the results showed a significant effect of dose and immersion time on the percentage of female tilapia produced ($p < 0.05$), while the interaction of dose and immersion time had no significant effect ($p > 0.05$). The use of soybean extract with various doses and lengths of immersion time showed a positive effect on the resulting female sex ratio. The 0.04 g L^{-1} dose group showed the highest percentage of post-treatment females (74.25±1.41%), followed by 0.03 g L^{-1} and 0.02 g L^{-1} in sequence. The three-dose treatment groups were significantly different. This value was higher than that obtained by Turan & Yigitarslan (2019), which was 69.00±0.82% using a soybean isoflavone extract dose of 0.2 g L^{-1}. This showed a significant correlation between the increase in dose and the female sex direction rate.

At the same time, the length of immersion time showed a significant effect compared to the control. Among the three groups, the length of immersion was not significantly different; 14 hours of immersion produced the highest number of females (73.33±2.62%), followed by 6 hours (72.01±1.61%), and not significantly different from 10 hours (71.66±1.98%). This study also revealed no significant effect of the interaction of dose and immersion time on sex reversibility.

The highest ratio of female tilapia was obtained in each group treated with soybean extract compared to the control group. This indicates that each group treated with soybean extract produced a female/male ratio that deviated significantly from the normal 1:1 ratio. An increase in the female population was obtained by utilising higher doses. Meanwhile, no hormone concentration was found to be exceptional in increasing the male population percent.

The value obtained is lower than the use of synthetic steroids as obtained by Abd El-Azeem (2023) on the feminisation of tilapia, with a hormonal treatment of 200 mg kg^{-1} feed with 28 days of administration obtained (85.51%) females and the highest at 40 days of administration (86.54%). Furthermore, Mangaro et al (2018) and Singh et al (2018) conducted masculinisation on tilapia fry aged 14 days soaked in 17 methyltestosterone solution with different concentrations (200 μg L^{-1} - 10 mg L^{-1}) and duration (2 hours - 3 days) and obtained a high percentage of male population (> 90%). A higher percentage (95%) in carp masculinisation was obtained by Asad et al (2021) by evaluating the factors of 17α-methyltestosterone hormone concentration and immersion duration and their interactions. The highest results were obtained in the interaction of the concentration of 450-600 μg L^{-1} with an immersion time of 72 hours.

In this study, the immersion period for tilapia fry was conducted at 8, 12, and 16 days of age and was able to induce a feminisation rate above 70%. Based on the values obtained, it is known that the higher the dose, the higher the ability to induce feminisation in tilapia. It is different for the duration of immersion, where the longer duration does not show the significance of directing to the female sex. And this condition is different from that obtained by Vinarukwong et al (2018) on tilapia masculinisation, where the percentage of phenotypic males increases with the longer duration of hormone administration in tilapia.

In the aspect of survival, the results showed that the provision of soybean extract, either the dose factor, the length of immersion, or the interaction of both, had no significant effect on the survival of the tilapia produced. The use of soybean extract did not cause a decrease in survival, as obtained by Marín-Ramírez et al (2016) using diethylstilbestrol and Juárez et al (2017) on the use of 17α-ethinylestradiol. This condition may be due to the fact that the material used is natural, so it does not affect the physiological process of tilapia, which allows the fish to adapt after immersion in soybean extract.

Descriptively, the percentage value of survival in this study (±57%) both in the control, dose group, and immersion duration group differs very little in their interaction (56-58%). This value is not much different from the climbing perch (17-54%) obtained by Maidie et al (2015) on a household scale and 38-55% by Susila (2016) on a limited container scale. This suggests that these values are within the typical range for confined-scale rearing. The survival rates obtained in this study reflect the rearing conditions over a 10-week period starting from 1-day-old larvae. This period is quite lengthy and involves fish at a very young stage, which can lead to high mortality rates due to the extreme vulnerability of Nile tilapia larvae or fry. Therefore, the survival rates observed do not indicate an effect of the treatment but rather illustrate the natural susceptibility of Nile tilapia at this developmental stage. This is supported by the survival rate in the control group, which tends to be similar to that in the treatment groups, indicating that the treatment did not significantly affect survival.

When compared to the results of research with the application of natural materials, the value obtained is not much different from that obtained by Helmizuryani et al (2017) using cow's milk, soy milk, and a mixture of cow's milk and soy milk (51.11-61.11%) in feminised climbing perch, *Anabas testudineus* reared for 60 days. However, this value was lower than the survival (93.75-94.58%) in tilapia fry treated with soybean isoflavone extract (Turan & Yigitarslan 2019) reared for 4 months.

Compared to the use of synthetic materials, Chakraborty et al (2011) obtained survival (90%) with the administration of 17-α methyltestosterone. Botero et al (2011) obtained a greater range (24-83%) with similar hormones. Furthermore, Marín-Ramírez et al (2016) obtained a decrease in survival close to 50% in groups given diethylstilbestrol for 20 days at concentrations of 100 to 400 mg kg^{-1} except at a concentration of 300 mg kg^{-1}. Although the survival obtained in this study (56-58%) was not as high as that reported by Chakraborty et al (2011) with the use of 17-α methyltestosterone (90%), these results remain competitive when compared to Botero et al (2011) who reported a highly variable survival range (24-83%) with the same synthetic hormone. Furthermore, the results of this study were more stable compared to those of Marín-Ramírez et al (2016), which reported a decrease in survival close to 50% with the use of diethylstilbestrol. An additional advantage of using soy extract as a natural hormone is its minimal impact on the environment and fish health, making it a more sustainable alternative to synthetic hormones.

Based on this description, the use of natural and synthetic materials can have both positive and negative impacts on survival. However, with proper handling and treatment, the negative effects can be minimized. One of the reasons for using soy extract as an alternative material for sex direction is that it does not cause adverse effects on survival. The use of soybean as a feminising agent has also been studied by Turan & Yigitarslan (2019) on rainbow trout, which showed that soybean extract did not affect survival. Survival rates remained high and uniform across all treatments (93.75±3.31 - 94.17±2.60%) as well as in the control (94.58±2.60%), reinforcing the assertion that soy is a safe alternative ingredient in fish feminisation.

The survival of fish fry is also influenced by the age of the organism being treated. In this study, the age of the tilapia fry used was 8 days post-hatching. At this age, the fry are more capable of dealing with exposure to other materials contained in the medium compared to the younger age of tilapia fish, which are generally used in sex reversal. Fish with a younger age are more susceptible to the influence of other materials contained in their living media compared to fish with older ages.

Conclusions. Soybean extract applied gradually to tilapia fry at 8 days of age, followed by 12 days of age, and finally 16 days of age, had a significant effect on female sex direction in tilapia feminisation. The dose of 0.04 g L^{-1} was the best dose to produce a female percentage of 74.25±1.41%. The immersion time of 6 hours was the most effective among the other immersion time groups (10 and 14 hours). The interaction between dose and immersion time had no significant effect on the percentage of females or the survival of tilapia. Soybean extract does not have a negative impact on the survival of tilapia fry. The use of soya extract as a natural ingredient can be applied as an alternative to synthetic hormones to support the production of male monosex tilapia.

Acknowledgements. The research team would like to express its thanks to the Academic Directorate of Vocational Higher Education, Directorate General of Vocational Education, Ministry of Education, Culture, Research and Technology for funding the implementation of research on the "Vocational Product Research" scheme in the 2023 budget year, based on Decree Number: 0793/D4/AL.04/2023 and Agreement/Contract Number: 130/PL.22.7.1/ SP-PG/2023.

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

References

Abd El-Azeem R. M., 2023 Feminization and molecular sexing of the Nile tilapia (*Oreochromis niloticus*, L) through sex reversal hormone diethylstilbestrol and molecular markers. Egyptian Journal of Aquatic Biology and Fisheries 27(3):121-130.

Amrullah, Ardiansyah, Dahlia, Wahidah, Dwinhoven I., 2023 Morphology of female tilapia (*Oreochromis* sp.) broodstock at hatchery centers in South Sulawesi. IOP Conference Series: Earth and Environmental Science 1273:012019.

Asad F., Qamer S., Ashraf A., Ali T., Shaheen Z., Akhter S., Nisar A., Parveen A., Cheema N., Mustafa G., 2021 Masculinization in common carp (*Cyprinus carpio*) by androgen immersion: the interaction effect of hormone concentration and immersion time. Brazilian Journal of Biology 81(2):285-290.

Attia E. I., Yones A. M., Metwalli A. A., Saleh H. H. E., 2023 Efficacy of gokshura (*Tribulus terrestris*) on sex reversal and growth performance of the Nile tilapia (*Oreochromis niloticus*) fries reared in prolonged water exchange. Egyptian Journal of Aquatic Biology and Fisheries 27(4):95-110.

Botero M., Pineda J., Gallego N., 2011 [Exposure of red tilapia (*Oreochromis* spp.) eggs to 17 alpha-methyltestosterone and the sex ratio of the fish offspring]. Revista Colombiana de Ciencias Pecuarias 24(1):38-47. [in Spanish]

Budd A. M., Banh Q. Q., Domingos J. A., Jerry D. R., 2015 Sex control in fish: approaches, challenges and opportunities for aquaculture. Journal of Marine Science and Engineering 3(2):329-355.

Chakraborty S. B., Mazumdar D., Chatterji U., Banerjee S., 2011 Growth of mixed-sex and monosex Nile tilapia in different culture systems. Turkish Journal of Fisheries and Aquatic Sciences 11:131-138.

Githukia C. M., Ogello E. O., Kembenya E. M., Achieng A. O., Obiero K. O., Munguti J. M., 2015 Comparative growth performance of male monosex and mixed sex Nile tilapia (*Oreochromis niloticus* L.) reared in earthen ponds. Croatian Journal of Fisheries 73(1):20-25.

Hashem M. H., Abdel-Azim R. M., Abel-Rahman M. M., Mousa I. E., 2019 Brackish water for cultivation of *Oreochromis niloticus* with sex-reversed female by diethylstilbestrol. International Multidisciplinary Scientific GeoConference: SGEM 19(3.1):65-72.

Hekimoglu M. A., Koyakomanda K. C. K., Firat M. K., Süzer C., Engin S., Sayğı H., Özden O., Güleç F., Şaka S., 2019 Effects of 17ß-estradiol hormone on growth and sex differentiation in the Mozambique tilapia (*Oreochromis mossambicus*, Peters, 1852). Aquatic Sciences and Engineering 34(1):14-21.

Helmizuryani, Muslimin B., Khotimah K., 2017 [Feminization of climbing perch, *Anabas testudineus* (Bloch, 1792) through larvae immersion in milk solution and soy milk]. Jurnal Iktiologi Indonesia 17(2):123-132. [in Indonesian]

Hickling C. F., 1960 The Malacca tilapia hybrids. Journal of Genetics 57:1-10.

Inaba H., Iwata Y., Suzuki T., Horiuchi M., Surugaya R., Ijiri S., Uchiyama A., Takano R., Hara S., Yazawa T., Kitano T., 2022 Soy isoflavones induce feminization of Japanese eel (*Anguilla japonica*). International Journal of Molecular Sciences 24(1):396.

Juárez-Juárez V., Alcántar-Vázquez J. P., Antonio-Estrada C., Marín-Ramírez J. A., Moreno-De La Torre R., 2017 Feminization by 17α-ethinylestradiol of the progeny of XY-female Nile tilapia (*Oreochromis niloticus*). Effects on growth, condition factors and gonadosomatic index. Turkish Journal of Fisheries and Aquatic Sciences 17(3):599-607.

Lázaro-Velasco A. L., Isidro-Cristobal M. J., Alcántar-Vázquez P., et al, 2019 Effect of the combination of a cold-water temperature and exogenous estrogens on feminization, growth, gonadosomatic index and fat muscle content of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). Latin American Journal of Aquatic Research 47(1):52-64.

Maidie A., Sumoharjo, Asra S. W., Ramadhan M., Hidayanto D. N., 2015 [Development of climbing perch (*Anabas testudineus*) hatchery for household scale]. Media Akuakulture 10(1):31-37. [in Indonesian]

Mair G. C., Abucay J. S., Skibinski D. O. F., Abella T. A., Beardmore J. A., 1997 Genetic manipulation of sex ratio for the large-scale production of all-male tilapia *Oreochromis niloticus*. Canadian Journal of Fisheries and Aquatic Sciences 54(2):396-404.
Mangaro R., Sinjal H. J., Monijung R. D., 2018 [Masculinization by using method of the immersion and oral to genital change of Tilapia *Oreochromis niloticus*]. Jurnal Ilmiah PLATAX 6(1):117-122. [in Indonesian]
Marín Ramírez J. A., Alcántar-Vázquez J. P., Antonio-Estrada C., Moreno de la Torre R., Calzada-Ruiz D., 2016 Feminization of Nile tilapia *Oreochromis niloticus* (L.) by diethylstilbestrol. Growth and gonadosomatic index. Ecosistemas y Recursos Agropecuarios 3(7):51-61.
Maulana F., Sudrajat A. O., Permana A., Mulyani L., Arfah H., 2023 Male sex ratio of red tilapia *Oreochromis* sp. after soaking in different concentrations of coconut milk at larvae stadia. Jurnal Akuakultur Indonesia 22(2):187-199.
Ogira P. G., David L., Ogutu P., 2018 Feminization of *Oreochromis niloticus* using diethylstilbestrol. Researchjournali's Journal of Agriculture 5(9):1-4.
Pandian T. J. Kirankumar S., 2003 Recent advances in hormonal injection of sex-reversal in fish. Journal of Applied Aquaculture 13(3-4):205-230.
Piferrer F., 2001 Endocrine sex control strategies for the feminization of teleost fish. Aquaculture 197(1-4):229-281.
Rahmania W., Baihaqi A. U., Heryanto, Handajani H., 2022 Potentiality of *Solanum torvum* as a feminization agent on *Anabas testudineus* Bloch 1792: a review. Omni-Akuatika 18(1):34-47.
Shelton W. L., Rodriguez-Guerrero D., Lopez-Macias J., 1981 Factors affecting androgen sex reversal of *Tilapia aurea*. Aquaculture 25(1):59-65.
Singh E., Saini V. P., Sharma O. P., Ojha M. L., Jain H. K., 2017 Comparative growth performance of monosex and mixed sex red tilapia (*O. niloticus* L.). Journal of Entomology and Zoological Studies 5(6):1073-1075.
Singh E., Saini V. P., Sharma O. P., 2018 Orally administered 17 α methyl testosterone at different doses on the sex reversal of the red tilapia (*Oreochromis niloticus*). International Journal of Fisheries and Aquatic Studies 6(3):301-305.
Stelkens R. B., Wedekind C., 2010 Environmental sex reversal, Trojan sex genes, and sex ratio adjustment: conditions and population consequences. Molecular Ecology 19(4): 627-646.
Susila N., 2016 [Effect of stocking density on the survival rate of climbing perch larvae (*Anabas testudineus*) reared in container]. Jurnal Ilmu Hewani Tropika 5(2):72-75. [in Indonesian]
Teng J., Zhao Y., Chen H. J., Wang H., Ji X. S., 2020 Transcriptome profiling and analysis of genes associated with high temperature-induced masculinization in sex-undifferentiated Nile tilapia gonads. Marine Biotechnology 22(3):367-379.
Turan F., Yigitarslan K. D., 2019 Effect of immersion treatment of soybean isoflavones extract on sex reversal in the rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). Biharean Biologist 13(1):32-35.
Vidal-López J. M., Contreras-Sánchez W. M., Hernández-Franyutti A., Contreras-García M. D. J., Uribe-Aranzábal M. D. C., 2019 Functional feminization of the Mexican snook (*Centropomus poeyi*) using 17β-estradiol in the diet. Latin American Journal of Aquatic Research 47(2):240-250.
Vinarukwong N., Lukkana M., Wongtavatchai J., 2018 Decreasing duration of androgenic hormone feeding supplement for production of male monosex in tilapia (*Oreochromis* spp.) fry. The Thai Journal of Veterinary Medicine 48(3):375-383.
Voorhees J. M., Mamer E. R. J. M., Schill D. J., Adams M., Martinez C., Barnes M. E., 2023 17-estradiol can induce sex reversal in brown trout. Fishes 8(2):103.
Wahidah, Rusli A., Rajamuddin M. A. L., Amrullah, 2021 The morphological characteristics of Nile tilapia (*Oreochromis niloticus*) produced with bull testicle powder extract application. AACL Bioflux 14(3):1811-1817.

Wahidah, Rusli A., Rajamuddin M. A. L., Amrullah, Taufik I., 2023a Survival rate, morphology, and abnormalities in masculinized Nile tilapia *Oreochromis niloticus*. AACL Bioflux 16(6):3202-3208.

Wahidah, Rusli A., Hartinah, Amrullah, Dwinhoven I., 2023b Evaluation of body performance of tilapia (*Oreochromis niloticus*). IOP Conference Series: Earth and Environmental Science 1273(1):012055.

Wassermann G. J., Afonso L. O. B., 2003 Sex reversal in Nile tilapia (*Oreochromis niloticus* Linnaeus) by androgen immersion. Aquaculture Research 34(1):65-71.

Received: 21 August 2024. Accepted: 22 September 2024. Published online: 30 October 2024.

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How to cite this article:
Wahidah, Rusli A., Khatimah K., Amrullah, Ardiansyah, Hanafi A., Taufik I., 2024 Interaction of dose and immersion time of soy extract on female percentage and survival performance of tilapia (*Oreochromis niloticus*). AACL Bioflux 17(5):2376-2385.