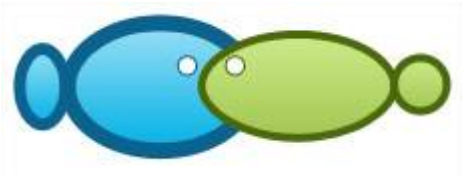

Survival rate, morphology, and abnormalities in masculinized Nile tilapia *Oreochromis niloticus*

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Abstract. The growth of male fish is faster than that of female fish at a certain age, thus masculinization should be carried out. One of the natural ingredients used in the masculinization process is bull testes. This study aimed to evaluate the survival rate, morphology and abnormalities of Nile tilapia (*Oreochromis niloticus*) in masculinization process using bull testes. The first phase of the study used a completely randomized design (CRD) with treatment by immersion using bull testicle powder extract (BTPE), bull testicle powder (BTP), methyltestosterone hormone (MT), and without hormone application (C), each treatment consisting of 4 replications. Each treatment unit consisted of 150 tilapia fry aged 4 days. Fish fries were reared for 60 days, fed 3 times a day and 20% water changes were carried out every 3 days. Quantitative morphological measurements consisted of total length (TL), standard length (SL), and head length (HL). Qualitative observations were carried out by observing abnormalities in morphological forms, including paired organs. At the end of Phase 1 of the study, evaluation of the survival rate, measurement of morphological characters, and observations of abnormalities were carried out. The best hormone application in Phase 1, BTP, was used to immerse 550 Nile tilapia fries aged 4 days at location 1 and 500 seeds at location 2, each with three replications with a maintenance period of 60 days. The results of Phase 1 the study showed that the average survival of tilapia fry with hormone application (BTPE, BTP, and MT) was 64.67%, 66.83%, and 69.50% respectively, while without hormone application (C) was 67.67%. The Phase 2 survival rate evaluation was carried out on the BTP application and the results were a survival rate range of 77-88% with an average of 84% at Location 1, and a range of 70-82% with an average of 75% at Location 2. The average value of the TL character of the MT application was 8.3 cm, higher than that in the BTPE and BTP application and without hormone application, which was 8.1 cm. The average value of SL in the C application was 7.5 cm, while BTPE and BTP each were 7.2 cm and the lowest was without hormone application, with a value of 7.1 cm. Likewise, the average HL in C application was 2.8 cm, the BTPE and BTP applications each had a value of 2.7 cm, while without hormone application was 2.6 cm. In the observations for abnormalities, no abnormal organs were found, including in paired organs. Therefore, both the application of hormones derived from bull testicles and methyltestosterone did not cause differences in morphology and did not cause abnormalities in the Nile tilapia.

Key Words: bull testes, masculinization, natural hormone, Nile tilapia.

Introduction. Nile tilapia (*Oreochromis niloticus*) has a different growth rate based on sex, where in general, the growth of male fish is faster than that of the female (Dagne et al 2013; Srisakultiew & Kamonrat 2013), due to the female's rapid maturation of the gonads that inhibits their growth. In addition, Nile tilapia readily spawn in the rearing mixed-sex hence the population increased rapidly, resulting in competition for food and space that causes nearly 50% of the tilapia to fail to reach the consumption size at harvest (Herrera & Cruz 2011). One method to overcome this problem in tilapia is to maintain a single-sex or monosex male tilapia population. Various methods are employed to produce male monosex seeds, including the use of hormones.

The hormone 17α-methyltestosterone is a synthetic hormone commonly used for male fish production. Methyltestosterone induced through feed and seed immersion technique on tilapia fry gave good results for reversing sex (Fuentes-Silva et al 2013). Furthermore, Zairin Jr. et al (2017) stated that 17α-methyl testosterone influenced the egg hatching rate, seed survival rate, and sex ratio of Nile tilapia.

Treatment with 17α-methyltestosterone on fish is a suitable and reliable method to achieve sustainable fish stocks (Megbowon & Mojekwu 2014). Although the effect of 17α-methyltestosterone on the success rate of fish masculinization is high and it is a commonly used hormone, the use of this type of hormone is not recommended because it is considered unsafe for human health and the environment; therefore, several studies have been carried out to find safer sources of hormones from natural ingredients. Awareness of the use of natural ingredients has triggered and encouraged the search for alternatives to synthetic chemicals, including from plant extracts (Gabriel 2019), such as *Aloe vera* (Gabriel et al 2017), *Mucuna pruriens* (Mukherjee et al 2015), and *Butea superba* (Kiriyakit 2014) as well as from animals such as bull testes (Adamu et al 2006).

Bull testicles have several advantages, including being easy to obtain, relatively inexpensive, large in size, and safer than using synthetic hormones. Therefore, information is needed regarding the effect of the application of natural ingredients of bull testes on the survival of tilapia seeds. Therefore, this study aimed to evaluate the survival, morphology, and abnormalities in the masculinization process of Nile tilapia using bull testicles.

Material and Method. The study was conducted from April to August 2021. Hormone treatment was performed under controlled environmental conditions at the Ompo Freshwater Fish Hatchery and Cultivation Development Centre, Soppeng, South Sulawesi. Morphological measurements and abnormalities were carried out in the Fish Physiological Laboratory of the Department of Aquaculture of the Pangkep State Polytechnic of Agriculture, South Sulawesi, Indonesia.

Phase 1

Hormone application. This was conducted using the immersion method for 8 hours on 150 tilapia fries aged 4 days, obtained from the natural spawning of Nile tilapia broodstock with a male:female brooders ratio of 1:3. The hormone application treatment consisted of bull testicle powder extract (BTPE); bull testicle powder (BTP); 17α-methyltestosterone (MT) and without hormone application (C). After the immersion process, the tilapia fries were kept in a 2 x 5 m^2 net for 60 days. The fries were fed 3 times a day. To maintain water quality, a 20% water exchange was carried out every 3 days. The fishnets were checked and cleaned every 2 weeks.

Morphological observation. The observations were conducted both quantitatively and qualitatively. Quantitative measurements were carried out by evaluating 3 morphological characters consisting of total length (TL), standard length (SL), and head length (HL). Measurements were made using a ruler with an accuracy of 0.1 cm, carried out on 5 fish taken randomly from each replication unit, totaling 80 sample fish. The fish samples were placed on waterproof paper with their heads on the left. Qualitative observations were carried out by referring to the Indonesian National Standard (SNI 2009) criteria regarding the Nile tilapia stocking class fries, including morphological abnormalities such as those in paired organs. Survival rate evaluation, measurement of morphological characters, and observation of abnormalities were carried out at the end of Phase 1 of the study.

Phase 2

BTP application. Phase 2 of the study was conducted based on the best results in Phase 1, treatment with bull testicles (BTP) which resulted in higher survival than BTPE, with the same morphological size. Thus, BTP was piloted in 2 locations. BTP was used to immerse 550 of 4-day-old tilapia fries at location 1 and 500 of 4-day-old tilapia fries at location 2. After the immersion process, the tilapia fries were reared in 5 x 10 m^2 fishnets. The fries maintenance method and duration in Phase 2 were the same as those in Phase 1 of the study. The survival rate evaluation was carried out at the end of Phase 2.

Design. Phase 1 of the study used a completely randomized design (CRD) with treatments using bull testicle powder extract (BTPE), bull testicle powder (BTP), MT hormone, and without hormone application (C). Each treatment was repeated 4 times.

Phase 2 of the research was carried out based on the best results of Phase 1, the BTP treatment was applied to two different locations, and each in triplicate. Parameters observed in Phase 1 were the survival rate of the fries during the rearing (Sivaramasamy et al 2016; Ismail et al 2019), morphological characters and abnormalities, whereas in Phase 2 only the survival rate was observed.

Data analysis. Differences in performance of the survival rate between tilapia treatments in Phase 1 were evaluated using one-way ANOVA and if a significant effect was found, Duncan's follow-up test was conducted at a significance level of $p < 0.05$. The morphological characters and abnormalities in Phase 1 and survival rate in Phase 2 were analyzed descriptively.

Results

The survival rate in phase 1. The survival rate of Nile tilapia varied post-application of BTPE and BTP, 64.67% and 66.83% respectively. The survival rate for the fry ranged from 64.67 to 69.5% (Figure 1). The results of analysis of variance showed that all the treatments had no significant effect ($p > 0.05$) on the survival of the tilapia.

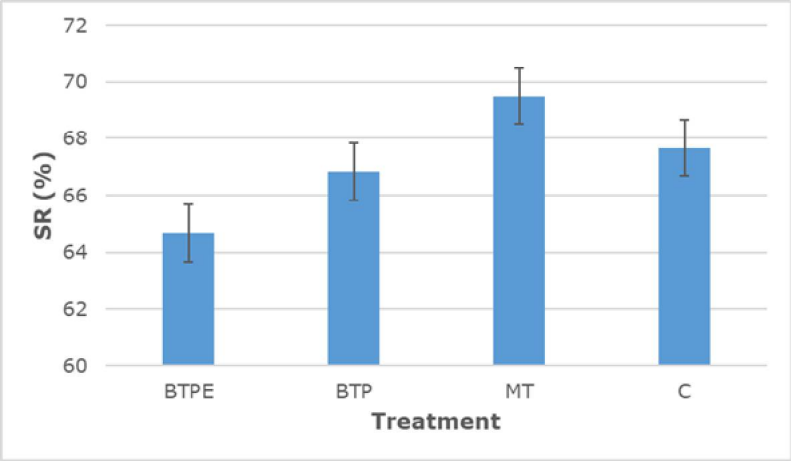

Figure 1. The Nile tilapia survival rate (%) for each treatment.

Morphology and abnormalities. The TL character in the MT application was 8.3 cm, longer than that of the BTPE, BTP, and without hormone application which were 8.1 cm each. The SL of fish in the MT applications was 7.5 cm, BTPE and BTP were 7.2 cm, each and the lowest was in the control (without hormone application) at 7.1 cm. Likewise, the average HL value was 2.8 cm in the MT treatment, BTPE and BTP were 2.7 cm, and the controls were 2.6 cm (Figure 2). Meanwhile, in observing abnormalities, there were no abnormal organs, including paired organs, in all treatments.

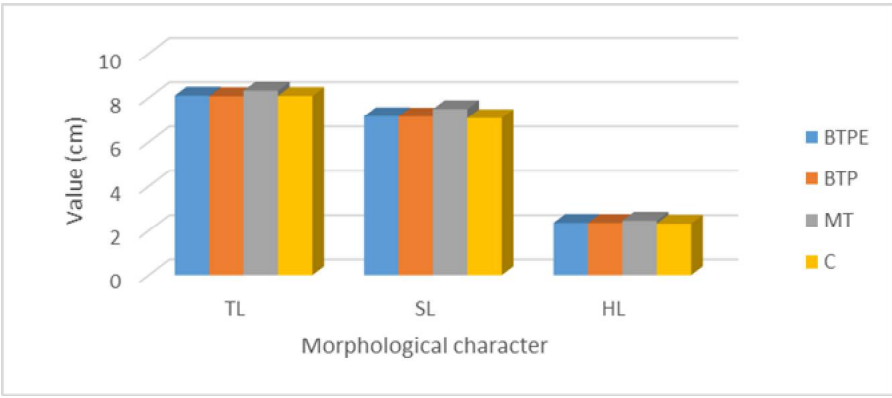

Figure 2. The total length (TL), standard length (SL), and head length (HL) characters in the Nile tilapia for each treatment.

The survival rate in Phase 2. The survival rate of the Nile tilapia given the BTP application after being reared for 60 days was 77-88% at location 1 and 70-82% at location 2 (Table 1). These values were higher than the results of the studies conducted by Iskandariah (1996), which was 45-68% using cow testicles, and Meyer et al (2008) which was 40.2% using fresh bull testicles, while by Murni (2005), 80% using oven-dried bull testicles and by Fashina-Bombata & Somotun (2008), 65% using goat.

Table 1

The survival rate (%) of the Nile tilapia in location 1 and location 2

Pond	*Location 1*		*Location 2*	
	SR (%)	*Average (%)*	*SR (%)*	*Average (%)*
I	77		82	
II	86	84	72	75
III	88		70	

Discussion. The survival rate of the Nile tilapia in Phase 1 of the study approached 70% with a maintenance period of 60 days, which is a common percentage of survival rates in pond maintenance. The survival rates between fish that had the hormone applied and those without hormone application were not significantly different. A similar thing was found in the survival of tilapia treated with imidazole (Deswira et al 2016). This indicated that bull testicles that have been processed into BTPE and BTP did not have a negative effect on the physiology of the Nile tilapia (fries stage) which were physically weak. These natural ingredients do not cause physiological stress on Nile tilapia fries as the use of 17α methyl testosterone which is a synthetic hormone that has a toxic effect when applied in high doses or due to the entry of synthetic hormones into the body of fish larvae (Hidayani et al 2016). This is an advantage of using BTPE and BTP in the production of male monosex Nile tilapia compared to using fresh bull testicles, synthetic hormones, or other synthetic chemicals.

The application of the hormone methyltestosterone in Phase 1 demonstrated a higher fish survival rate than the BTPE and BTP application, although it was not statistically significant. This is in line with the statement of Asad et al (2021) who reported 17α-methyltestosterone increased the survival rate and hatching rate of eggs in carp. Similar results were reported by Jensi et al (2016) and Rima et al (2017) regarding the effectiveness of this hormone.

The average survival rate of the Nile tilapia in Phase 1 ranged between 64.67 and 69.50% whereas that of Phase 2 ranged between 75 and 84%. These values are relatively lower than the results of laboratory tests obtained by Murni (2005) which were 80%, Triajie (2008) 83.33%, and Yusuf et al (2019) 88.00-88.67%. This is because the condition of the maintenance media or the environment during the maintenance period in the laboratory is more controlled compared to that of ponds where environmental conditions cannot be controlled in detail. However, the survival rates for phase 1 and phase 2 were higher than those in studies using synthetic materials including 60-70% using oral 17α-methyltestosterone (Adel et al 2007) and 42-88% using 17α-methyltestosterone and 17α-metildihydrotestosterone (MDHT) by immersion (Muslim et al 2011).

Currently, the use of natural materials for specific sex production is recommended. The natural ingredients in bull testicles that have been processed into BTPE and BTP are highly effective alternative hormones for the production of male Nile tilapia. In this study, the survival rate was quite high with the use of natural ingredients made from BTP. This is different from methyltestosterone which is a synthetic hormone with high effectiveness for the production of a monosex male population, but in terms of food safety, its use is not recommended. Synthetic hormones have harmful impacts on ecology and health (Papoulias et al 2020) and release harmful residues on fish and the environment (Pandian & Kirankumar 2003).

The survival rate is also influenced by biotic and abiotic factors. Biotic factors consist of the fish's age and ability to adapt to the environment, while abiotic factors

include food availability and the water quality of the living environment (Zooneveld et al 1991). In our study, the availability of food for Nile tilapia fries was sufficient and the optimum water quality conditions were maintained. Water quality was stable because the application of BTPE and BTP does not reduce water quality as in studies using fresh bull testicles with survival rates of 45-68% (Iskandariah 1996) and 40.2% (Meyer et al 2008). These values are lower than the results of the present study. The use of fresh bull testicles can affect water quality, causing the fish's appetite to decrease, and could even increase dissolved organic matter, forming toxic materials (ammonia) which could lead to fish mortality.

Morphological observations were carried out quantitatively and qualitatively. The results of quantitative observations showed that the application of BTPE, BTP, and methyltestosterone did not cause differences in the morphology of the Nile tilapia. Nile tilapia applied with the hormones had a TL, SL, and HL which tended not to differ much between treatments. This condition is in line with the results obtained by Wahidah et al (2021) who reported that the application of the BTPE hormone did not affect the ratio of morphological characters to TL of Nile tilapia.

The evaluation of the qualitative characteristics of 2-month-old Nile tilapia referred to the SNI criteria regarding Nile tilapia for stocking class (SNI 2009), namely: having a black body color, normal body shape, being able to move in the water on the surface and at the bottom of the container, exhibiting movements or behavior of clustering on the surface of the rim of the container, actively welcoming new water, and having very fast tail movements. The results of this study showed that the immersed in BTPE and BTP had met the SNI criteria. This also shows that there are no abnormalities in the Nile tilapia.

Conclusions. The application of BTPE and BTP for the masculinization of Nile tilapia did not affect the survival rate or morphological measurements and did not cause abnormalities in Nile tilapia. Natural ingredients derived from the bull testicles can be used as an alternative material for masculinizing tilapia to replace the use of synthetic materials.

Acknowledgements. We thank the Directorate of Resources, Directorate General of Higher Education, Ministry of Education and Culture, Research and Technology for the financial support for this research through the Higher Education Leading Development Research (Penelitian Pengembangan Unggulan Perguruan Tinggi, PPUPT) scheme, based on the Contract Number 191/E4.1/AK.04.TL/2021.

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

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Received: 24 October 2023. Accepted: 22 November 2023. Published online: 13 December 2023.
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How to cite this article:
Wahidah, Rusli A., Rajamuddin M. A. L., Amrullah, Taufik I., 2023 Survival rate, morphology, and abnormalities in masculinized Nile tilapia *Oreochromis niloticus*. AACL Bioflux 16(6):3202-3208.