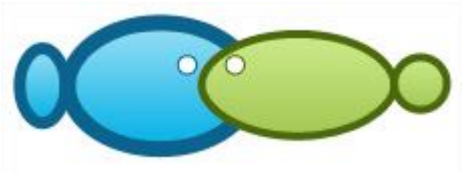

The morphological characters of Nile tilapia (*Oreochromis niloticus*) produced with bull testicle powder extract application

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Abstract. Tilapia cultivation using the male monosex system is to increase production which can be done by masculinization using natural hormones such as bull testicle powder extract (BTPE). The purpose of this study was to evaluate the morphological ratio of Nile tilapia (*Oreochromis niloticus*) treated using BTPE. The study employed a Complete Randomized Design with BTPE immersion treatment dosages of 3 mL L^{-1}, 5 mL L^{-1}, and 7 mL L^{-1}, a positive control (methyltestosterone), and negative control (without immersion). Each treatment was done triplicate. BTPE was applied by immersion of 150 four-day-old Nile tilapia seed with an average weight of 0.01 g for 8 hours. The fish were kept up to 60 days old. The morphological characters measured were: the standard length (SL), trunk length (TRL), body height (BH), tail spine height (TSH) and total length (TL). The ratios of these measures (SL:TL, TRL:TL, BH:TL, TSH:TL) were also evaluated. Measurements were made using a 0.1 cm accuracy ruler and were taken at age 60 days by randomly collecting 15 fish from each repeat unit, totaling 225 sample fish. The results showed different dosages of the hormone did not affect tilapia morphology up to age 60 days ($p > 0.05$). The highest average ratio of SL:TL was found in the methyltestosterone (79.98±0.74%) and 5 mL L^{-1} BTPE (79.09±0.81%) treatments; TRL:TL in the 5 mL L^{-1} BTPE (59.29±0.83%) followed by methyltestosterone (58.93±0.74%). The average ratio for BH:TL was almost the same in all the treatments ranged from 27.80±0.48 to 28.48±0.35%. The average ratio for TSH:TL was ranged from 10.58±0.19 to 10.96±0.23% in all the hormone treatments and 10.16±0.22% in the treatment without hormones. The hormone application did not affect morphological character ratios in Nile tilapia, and generally, the average morphometric character ratio in the fish against the total length (TL) after treatment with 5 mL L^{-1} BTPE and methyltestosterone was higher than that of the other treatments.
Key Words: hormone, Nile tilapia, sex reversal, masculinization, morphology.

Introduction. Nile tilapia (*Oreochromis niloticus*) is an important freshwater aquaculture commodity. In freshwater aquaculture, it is reported to be the second most important cultivated fish group after carp, *Cyprinus carpio* (Waite et al 2014). Nile tilapia is also one of the popular fish with increased worldwide consumption (Ogello et al 2014).

The growth of cultivated fish is one of the determining factors in aquaculture production level (Carman et al 2009; Ajiboye 2015). The growth rate of male Nile tilapia is twice faster than that of the female (Fuentes-Silva et al 2013; Felix et al 2019) raised in either a mixed-sex system or in separate sexes in a floating net (Dunham 2004), making male Nile tilapia preferred by Nile tilapia farmers (El-Greisy & El-Gamal 2012). Some advantages of the male monosex Nile tilapia aquaculture include that it enables the fish to grow uniformly, they could reach large sizes, and it reduces spawning (Ajiboye 2015; Francis & Esa 2016; Abo-Al-Ela 2018). The growth performance of monosex male Nile tilapia is also better than that of the mixed-sex (Githukia et al 2015).

The development of Nile tilapia aquaculture is directed more toward the male monosex system (Gabriel 2019) such as through sex reversal programs to improve the production of Nile tilapia. Monosex male Nile tilapia can be produced in a few methods, including by using hormones. Various hormones are used for masculinization such as the synthetic hormone 17α-methyltestosterone;

however, the use of synthetic hormones nowadays is not recommended because they are less safe for food (Hoga et al 2018). One step that can be done is by exploring natural sources of the hormone testosterone such as bull testicles. The use of this natural material, bull testicles, as a source of the hormone has been done by several researchers (Muslim et al 2011; Irmasari et al 2012; Iskandar et al 2014; Huda et al 2018; Yustiati et al 2018).

The use of natural hormones is expected to have no adverse effects on the Nile tilapia. One of the effects that can be evaluated from the use of this hormone is changes/differences in the morphological character of fish treated with hormone application. Morphological characters can be evaluated using the morphometric method. Morphometry is the measurement of certain body parts of the fish's anatomical structure (a measuring method). Elawa (2004) defined morphometry as markings that describe the shape of the fish's body. The morphometric characters often used include the total length, the standard length, the fork length, the body height and width, the fin height and length, and eye diameter (Lagler et al 1977). A few studies have evaluated morphological characters in Nile tilapia such as Muhotimah et al (2013) and Ayuningtyas et al (2015). The purpose of the present study was to evaluate the morphological ratio of Nile tilapia (*Oreochromis niloticus*) produced by application of the natural material bull testicle powder extract (BTPE).

Material and Method

Experimental fish and BTPE application. The study was conducted from May 2019 to August 2019. Hormone treatment was performed under controlled environmental conditions at the Ompo Freshwater Fish Hatchery and Cultivation Development Centre, Soppeng, South Sulawesi. Morphological measurements were carried out in the Fish Physiological Laboratory of the Department of Aquaculture of the Pangkep State Polytechnic of Agriculture, South Sulawesi, Indonesia. The BTPE application was done by immersion of 150 Nile tilapia seed aged 4 days weighing an average of 0.01 g for 8 hours. The dosages of BTPE used were 3 mL L^{-1}, 5 mL L^{-1}, and 7 mL L^{-1}. As the positive control, fish were immersed in the hormone MT and the negative control was without hormone application.

Maintenance of the Nile tilapia seed post-application. After the immersion process, the Nile tilapia seed were kept in 15 water nets sized 2 x 5 m^2. During the maintenance period, the seed were fed natural feed 3 times a day. The seed were kept for 60 days.

Morphological measurements. Evaluation of the ~~4~~ morphological characters referred to the study by Hassanien et al (2011) and Sarmento el al (2018) which included the standard length (SL), trunk length (TRL), body height (BH), tail spine height (TSH) and total length (TL). The measurements were taken using a ruler with 0.1 cm accuracy. The fish samples were placed on waterproof paper with their heads facing left. The morphological measurements were taken at the age of 60 days by randomly collecting 15 fish from each repeat unit, creating a total of 225 sample fish.

Design. This study used a Complete Randomized Design with treatments using the natural hormone BTPE at dosages of 3 mL L^{-1} (Treatment I), 5 mL L^{-1} (Treatment II), and 7 mL L^{-1} (Treatment III), for the positive control methyltestosterone (MT) was used and the negative control was without immersion (WI). Each treatment was conducted in triplicates.

Data analysis. The morphometric character ratio was calculated from the results of the morphometric character measurement results (SL, TRL, BH, TSH) divided by the TL. The measurement data were analyzed using the SPSS software Version 26. Differences in the performance between Nile tilapia treatments were determined using one-way ANOVA and if there was found a significant effect, it was followed up using Duncan's at a

significance rate of $p < 0.05$ to determine the best dosage. Descriptive analysis was used to compare the morphological character ratio between treatments.

Results

The morphological characters ratio. The statistical analysis showed that masculinization of Nile tilapia using the BTPE hormone application treatment did not affect the morphological measurements of Nile tilapia up to 60 days of age ($p > 0.05$). Descriptively, however, in general, the average morphological character ratio for Nile tilapia against the total length (TL) for treatment using 5 mL L^{-1} BTPE and methyltestosterone were higher than that of the other treatments.

The highest average character ratio for SL:TL was found in the treatment using methyltestosterone, followed by the treatments using 5 mL L^{-1} BTPE at 79.98±0.74% and 79.09±0.81% respectively, while the lowest was found in the treatment using 3 mL L^{-1} BTPE at 77.83±0.72% (Figure 1). However, the highest average character ratio for TRL:TL was found in the treatment using 5 mL L^{-1} BTPE followed by methyltestosterone, and the lowest was in the treatment using 3 mL L^{-1} BTPE at 59.29±0.83%; 58.93±0.74% and 56.08±0.83%, respectively (Figure 2). This showed that the SL in Nile tilapia treated with an application of methyltestosterone was greater than those treated with an application of 5 mL L^{-1} BTPE, but they had the same TRL.

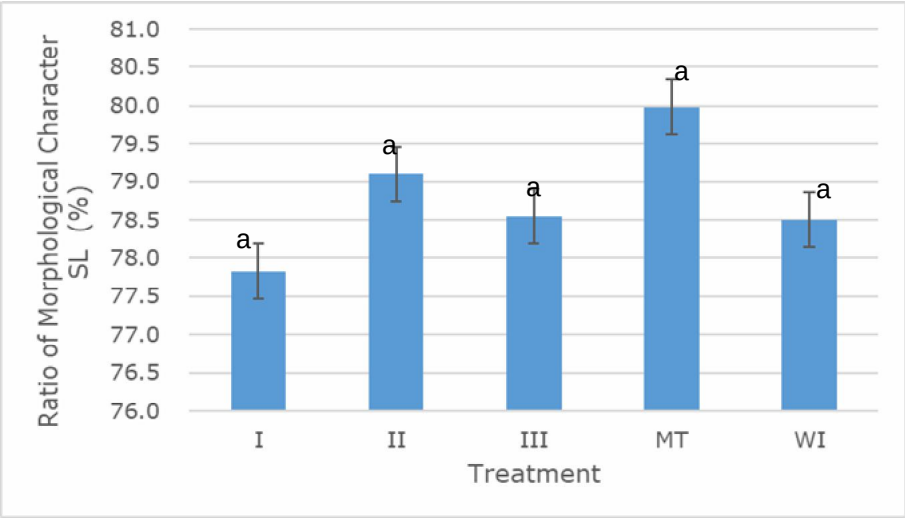

Figure 1. The average ratio between the standard length (SL) and the total length (TL) characters in the Nile tilapia for each treatment.

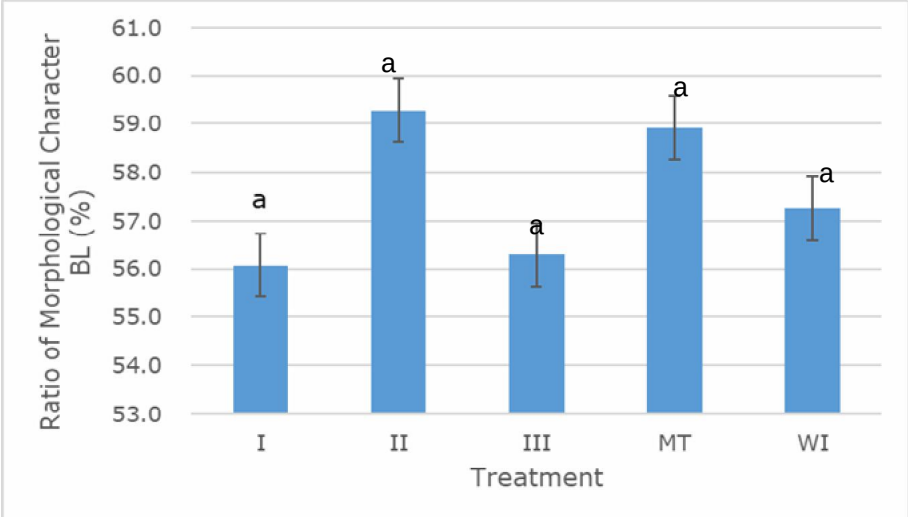

Figure 2. The average ratio between trunk length (TRL) and the total length (TL) characters in the Nile tilapia for each treatment.

The average ratio for BH:TL was nearly the same in all the treatments ranged from 27.80±0.48 to 28.48±0.35%, which means that the fish's body height was almost the same in all treatments (Figure 3). The average ratio for TSH:TL was ranged from 10.58±0.28 to 10.96±0.23% in all the hormone application treatments and 10.16±0.22% in the treatment without any hormone application (Figure 4).

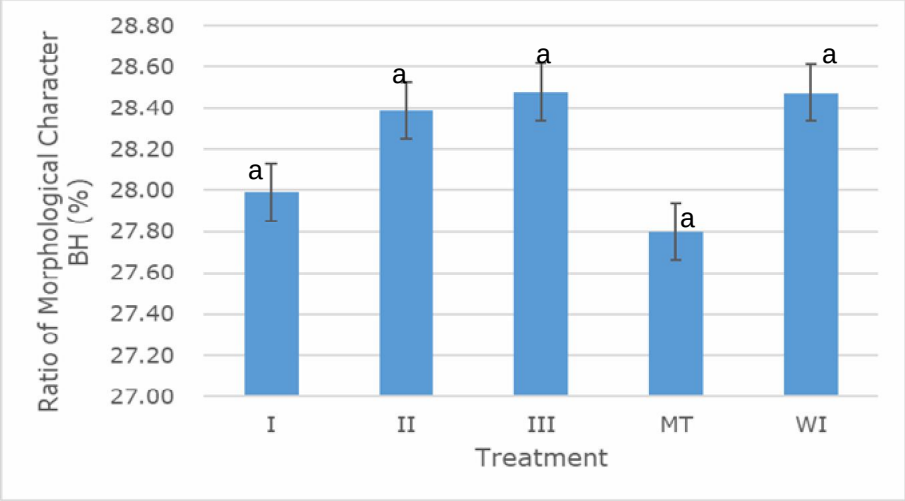

Figure 3. The average ratio between the body height (BH) and the total length (TL) characters in the Nile tilapia for each treatment.

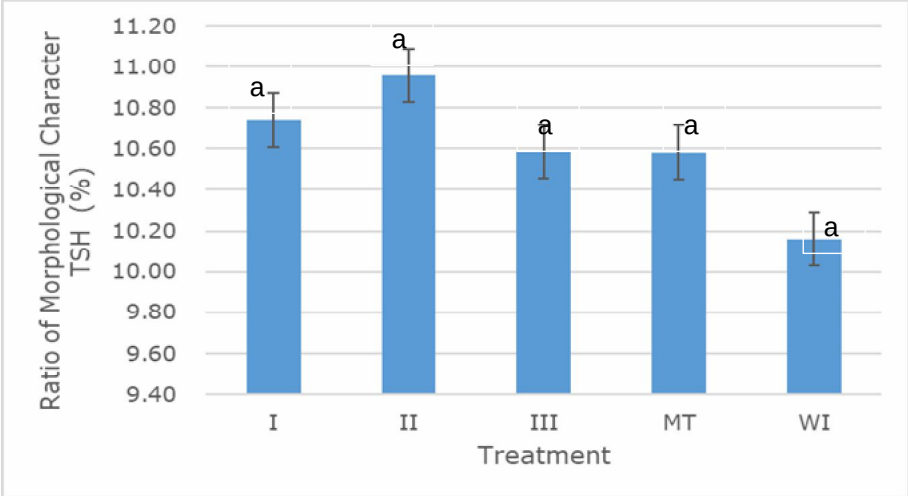

Figure 4. The average ratio between the tail spine height (TSH) and the total length (TL) characters in the Nile tilapia for each treatment.

Discussion. The standard length (SL) of the Nile tilapia treated using methyltestosterone was greater than that of the fish treated using 5 mL L^{-1} BTPE; however, the trunk lengths (TRL) in both treatments were the same. This showed that the size of the Nile tilapia heads in the treatment using methyltestosterone was longer than that of the fish treated using 5 ml L^{-1} BTPE. Therefore, fish treated using 5 mL L^{-1} BTPE had a greater edible portion. The morphological character where the trunk was longer and the head was smaller, also known as the edible portion, is an important point in the production of consumption fish. The edible portion, in this case, the trunk length (TRL) which is consumed, had a larger portion than the head, which will be discarded.

The average ratio of BH:TL was the same in all the treatments, as with the TSH:TL ratio, except in the treatment without any hormone application. This indicated that the tail spine height (TSH) in Nile tilapia without any hormone application was shorter than that of the fish given the hormone treatments.

The average ratio of the morphological characters against the total length (TL) did not show any distinctive variations. The fairly varied character variations were only

found in the SL and TRL characters. Meanwhile, in the TSH character, there were only 2 different values, and in the body height character, there was hardly difference or variation. Therefore, the morphology of the Nile tilapia in this study appeared to have similar body shapes and had the same height.

The ratio of the morphological characters among the fish treated with the hormones was not unlike those without the hormone application. The lack of body size difference was possibly because, at the age of 60 days, Nile tilapia has not reached the growth differentiation phase. Popma & Masser (1999) stated that male Nile tilapia grow twice as fast as females. This indicated a possibility for a difference in growth, in this case, the body size of the fish treated with hormones compared to fish not treated with any hormones if the fish were kept longer (Heriati 2012), at least until they reached the weight of 50 g (Felix et al 2019).

The three BTPE hormone dosages were within the range that is tolerable for Nile tilapia; therefore, they did not cause morphological changes in the Nile tilapia produced. Besides morphological changes, inappropriate hormone dosages could cause death (mortality) as stated by Hunter & Donaldson (1983) who found that the administration of a dosage that is too high or too low could result in sterility and abnormalities in gonad development, increased mortality, and a paradoxical phenomenon due to the pressures toward growth (Pandian & Sheela 1995).

BTPE is suitable for application in producing monosex male Nile tilapia, where the advantages of BTPE are that it is made of a natural material that contains organic materials (nutrients) useful for the fish. BTPE also does not cause physiological pressure on the fish that might cause death and is also environmentally friendly.

Conclusions. The results of this study demonstrated that the application of the hormones did not affect the morphological characters of the Nile tilapia at the age of 60 days. Generally, the average ratio of the Nile tilapia's morphometric character against the total length (TL) in the fish treated with 5 mL L^{-1} BTPE and methyltestosterone was greater than that of the other treatments.

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References

Abo-Al-Ela H. G., 2018 Hormones and fish monosex farming: a spotlight on immunity. Fish and Shellfish Immunology 72:23-30.

Ajiboye O. O., 2015 The efficacy of the steroid hormone, 17 α-methyltestosterone and semi flow-through culture system as growth promoters and reproduction inhibitors of Nile tilapia (*O. niloticus*) fed two feed type. International Journal of Aquaculture 5(20):1-8.

Ayuningtyas S. Q., Zairin Jr. M., Soelistyowati D. T., 2015 [Sex reversal of red tilapia using 17α-methyltestosterone-enriched feed and increased temperature]. Jurnal Akuakultur Indonesia 14(2):159-163. [in Indonesian]

Carman O., Saputra A., Alimuddin, Maskur, Herdianto D., Aliah R. S., Sumantadinata K., Yuniarti T., 2009 [Performance of sex reversed, genetically male and YY Nile tilapia (*Oreochromis niloticus*) in first nursery phase]. Jurnal Riset Akuakultur 4(1):33-38. [in Indonesian]

Dunham R. A., 2004 Aquaculture and fisheries biotechnology: genetic approaches. CABI Publishing, Cambridge, USA, 372 pp.

Elawa A. M. T., 2004 Morphometrics: application in biology and paleontology. Springer-Verlag, Berlin, Heidelberg, 264 pp.

El-Griesy Z. A., El-Gamal E. A., 2012 Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17α-methyltestosterone with respect to the degree of sex stability after one year of treatment. Egyptian Journal of Aquatic Research 38(1):59-66.

Felix E., Avwemoya F. E., Abah A., 2019 Some methods of monosex tilapia production: a review. International Journal of Fisheries and Aquatic Research 4(2):42-49.
Francis N. O., Esa Y. B., 2016 A review of production protocols used in producing economically viable monosex tilapia. Journal of Fish Aquatic Sciences 11(1):1-11.
Fuentes-Silva C., Soto-Zarazúa G. M., Torres-Pacheco I., Flores-Rangel A., 2013 Male tilapia production techniques: a mini-review. African Journal of Biotechnology 12(36):5496-5502.
Gabriel N. N., 2019 Review on the progress in the role of herbal extracts in tilapia culture. Cogent Food & Agriculture 5(1):1619651.
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 niloticu*s L.) reared in earthen ponds. Croatian Journal of Fisheries 73:20-25.
Hassanien H. A., Kamel E. A., Salem M. A., Dorgham A. S., 2011 Multivariate analysis of morphometric parameters in wild and cultured Nile tilapia (*Oreochromis niloticus*). Journal of the Arabian Aquaculture Society 6(2):237-250.
Heriyati E., 2012 [Sex reversal of Nile tilapia using honey and analysis of aromatase gene expression]. Magister Thesis, Bogor (ID), IPB University. [in Indonesian]
Hoga C. A., Almeida F. L., Reyes F. G. R., 2018 A review on the use of hormones in fish farming: analytical methods to determine their residues. CyTA – Journal of Food 16(1):679-691.
Huda R. N., Susilowati T., Yuniarti T., 2018 [Application of bull testicle flour containing rGH in artificial feed to sex ratio, growth and survival of Nile tilapia (*Oreochromis nioticus*)]. Pena Akuatika 17(2):59-69. [in Indonesian]
Hunter G. A., Donaldson E. M., 1983 Hormonal sex control and its application to fish culture. In: Fish physiology. Vol. 9. Reproduction. Part B Behaviour and fertility control. Hoar W. S., Randall D. J., Donaldson E. M. (eds), Academic Press, New York, pp. 223-291.
Irmasari, Iskandar, Subhan U., 2012 [Effect of cow testicle flour extract with different concentration on masculinization of Nile tilapia (*Oreochromis* sp.)]. Jurnal Perikanan dan Kelautan 3(4):115-121. [in Indonesian]
Iskandar A., Zairin Jr. M., Arfah H., 2014 [The effectiveness of BTME (bull testes meal extract) in sex-reversal of tilapia through immersion technique]. Jurnal Sains Terapan Edisi IV 1(1):27-34. [in Indonesian]
Lagler K. F., Bardach J. E., Miller R. R., Passino D. R. M., 1977 Ichthyology. Second edition. John Wiley and Sons, Inc., New York, 506 pp.
Muhotimah, Triyatmo B., Priyono S. B., Kuswoyo T., 2013 [Morphometric and meristic analysis of tilapia (*Oreochromis* sp.) strain larasati F5 and parents]. Jurnal Perikanan (Journal of Fisheries Sciences) 15(1):42-53. [in Indonesian]
Muslim, Zairin Jr. M., Utomo N. B. P., 2011 [Masculinization of Nile tilapia (*Oreochromis niloticus*) by administration of bull testes meal]. Jurnal Aquakultur Indonesia 10:51-58. [in Indonesian]
Ogello E. O., Musa S., Aura C. M., Abwao J. O., Munguti J., 2014 A critical appraisal of feasibility of tilapia production in earthen ponds using biofloc technology, a review. International Journal of Aquatic Sciences 5:21-39.
Pandian T. J., Sheela S. G., 1995 Hormonal induction of sex reversal in fish. Aquaculture 138(1-4):1-22.
Popma T. J., Masser M., 1999 Tilapia: life history and biology. SRAC Publ. No. 283:4.
Sarmento N. L. A. F., Martins E. F. F., Costa D. C., Mattioli C. C., da Costa Julio G. S., Figueiredo L. G., Luz M. R., Luz R. K., 2018 Reproductive efficiency and egg and larvae quality of Nile tilapia fed different levels of vitamin C. Aquaculture 482:96-102.
Waite R., Beveridge M., Brummet R., Castine S., Chaiyawannakarn N., Kaushik S., Munkung R., Nawapakpilai, Phillips M., 2014 Improving productivity and environmental performance of aquaculture. World Resources Institute, Working Paper, 60 pp.

Yustiati A., Bangkit I., Zidni I., 2018 Masculinization of Nile tilapia (*Oreochromis niloticus*) using extract of bull testes. IOP Conference Series: Earth and Environmental Science 139:012008.

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