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Masculinization of Tilapia (*Oreochromis niloticus*) Using Dietary Purwoceng (*Pimpinella pruatjan*) Extract With Different Concentrations

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ABSTRACT
Male tilapia generally show a faster growth rate compared to female tilapia. Monosex tilapia farming typically targets an all-male population for more efficient growth. The use of 17α-methyltestosterone hormones has been restricted, creating a need for natural substitutes—one of which is *purwoceng* extract. This study aimed to determine the effectiveness of hormones and *purwoceng* extract in feed on the male sex ratio of 7-day-old tilapia larvae (average weight: 0.011 ± 0.001 g; average length: 0.80 ± 0.043 cm) reared for 60 days. The study was conducted using a completely randomized design consisting of five treatments with four replications. The five treatments were: negative control (KN) with no additions, positive control (KP) with the addition of 17α-methyltestosterone at 50 mg kg^{-1} feed, S1 with 0.35 g kg^{-1} of *purwoceng* extract, S2 with 0.70 g kg^{-1}, and S3 with 1.05 g kg^{-1}. The results showed that supplementation with purwoceng extract at 0.70 g kg^{-1} feed can increase the male sex ratio and specific growth rate, with no adverse effect on survival rate (SR). However, the percentage of males in the S2 treatment was lower than in the KP treatment. This indicates that purwoceng extract can increase the percentage of male tilapia, but is not more effective than 17α-methyltestosterone.

INTRODUCTION

The potential of tilapia in Indonesia has led the country to become the world's largest tilapia supplier with an Aquaculture Stewardship Council fisheries certificate in 2012 **(Hidayati *et al.*, 2020)**. Indonesian Directorate General of Aquaculture (IDGA) data show an increase in the volume of tilapia production in Indonesia in recent years. This can be seen in 2022 when the volume of tilapia production reached 1,356,654 tonnes and increased to 1,364,436 tonnes in 2023 **(IDGA, 2023)**. One of the main drawbacks of tilapia aquaculture is the problem of unwanted breeding and growth inhibition due to the presence of male and female tilapia in the same population. Tilapia are prolific breeders **(Mtaki *et al.*, 2022; Geletu & Zhao, 2023)**. Intensive tilapia farming also often leads to inbreeding, which can result in reduced fish growth, increased susceptibility to disease, and reproductive problems, especially if farming focuses on rearing **(Nguyen, 2016)**. In mixed-sex populations, uncontrolled spawning is a major problem in tilapia culture. Female tilapia can mature early and spawn frequently, even under farming conditions. As a result, fish in grow-out ponds divert considerable energy toward reproduction rather than somatic growth. This leads to stunted growth, uneven size distribution, and excessive recruitment, which complicates management and reduces overall productivity **(Bhatta *et al.*, 2012; Mekkawy *et al.*, 2017)**. This is commonly prevented by using monosex tilapia, allowing for more efficient use of resources and better growth performance **(Sah *et al.*, 2019)**.

Monosex tilapia farming is generally targeted at all-male populations. Male tilapia generally show faster growth rates compared to female tilapia or mixed-sex populations because energy that would otherwise be spent on reproduction is diverted to growth or somatic needs **(Lu *et al.*, 2022)**. The average growth of male tilapia reached 1.53– 1.69g day^{-1}, while female tilapia grew 0.83– 1.05g day^{-1} **(Rosmaidar *et al.*, 2014)**. The difference in growth rate is caused by different energy allocation. Female tilapia use more energy for reproduction than males, so males grow faster **(Angienda *et al.*, 2010)**. Faster growth rates can lead to shorter production cycles, higher yields, and increased profitability for tilapia farming operations, which would be much more beneficial to the farmer **(Sarker *et al.*, 2022)**.

According to **Prabu *et al.* (2019)**, although many techniques have been introduced to produce male tilapia, the sex reversal technique using hormones is still considered the most popular and economically viable practice. Sex reversal is the process of manipulating the endocrine system of fish through hormonal treatment techniques to induce a sex change. This process usually involves the administration of synthetic hormones to juvenile fish at critical developmental stages that trigger gonadal transformation and secondary sexual characteristics **(Soyano *et al.*, 2022)**. The sex-change process can occur before or during the sex differentiation phase, as these phases allow sex development to be directed as needed **(Deswira *et al.*, 2016)**.

Tilapia are naturally born with balanced amounts of androgen and estrogen hormones, so sexual differentiation has not yet occurred. This situation allows male hormone (androgen) or female hormone (estrogen) treatment to direct sex development as needed **(Fuentes-Silva, 2013)**. According to Law No. 18/2009 of the Republic of Indonesia, engineering in livestock or aquaculture is legal and permitted as long as it does not conflict with religious norms, does not harm the environment, animal welfare, or biodiversity. This engineering can be carried out because it aims to improve welfare and fulfill food needs, in accordance with applicable regulations in Indonesia.

According to **Wijayanti (2002)**, sex reversal can be done by two methods, namely immersion and oral. Sex reversal with feed is done by integrating hormones in fish feed by mixing to ensure uniform distribution. Fish will consume hormone-treated feed pellets orally, and the hormones are absorbed through the digestive tract into the bloodstream **(Stiefel & Stintzing, 2023)**. The sex reversal technique with feed is considered easier to perform than other methods because it does not require individual handling or direct administration of hormones to each fish **(Simó-Mirabet *et al.*, 2018; Asad *et al.*, 2023)**. **Budi *et al.* (2024)** clarified that the advantages of oral hormone administration, especially for small fish, are to avoid stress and potential mortality, which is particularly advantageous for small fish species and suitable for mass-scale production.

Hormonal manipulation is widely used for both small- and large-scale tilapia production in various countries, regardless of their level of development. Common steroids used to produce male tilapia include 19-norethyltestosterone, fluoxymesterone, ethyltestosterone, 17α-methyltestosterone (MT), mesterolone, androstenedione, trenbolone acetate (TBA), testosterone, 17α-ethynyltestosterone, dihydrotestosterone, and 17α-methyldihydrotestosterone **(Lalila *et al.*, 2015)**. MT hormones are the dominant and most widely used hormonal method to induce sex reversal in tilapia species due to their easy administration and remarkable effectiveness, with the potential to produce entirely male offspring when applied correctly **(Jensi *et al.*, 2016)**.

The use of MT hormone remains a polemic in aquaculture production due to concerns about its potential adverse effects on human health, such as carcinogenicity and endocrine system disruption. The hormone testosterone can effectively induce male gonad development if well absorbed, but there are absorption limitations in MT that can potentially cause residues in fish organs and pollute the environment. Testosterone is a short-acting hormone that is rapidly absorbed and metabolized in the digestive system through the liver by gut bacteria. The tilapia body overcomes this by synthesizing C17α-MT, which is not very effective due to its modified structure, raising toxicity concerns. This underlies the ban by the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia through decree No. KEP.52/MEN/2014, which classifies the hormone as prohibited for use in aquaculture due to its adverse effects **(Suseno *et al.*, 2020)**.

Natural ingredients that contain steroid hormones are one of the options for sex reversal. Natural ingredients are considered very effective and easy to obtain **(Tatalede *et al.*, 2019)**. Some natural ingredients that have been used in the masculinization process are purwoceng **(Putra *et al.*, 2011; Arfah *et al.*, 2013; Bertha *et al.*, 2016; Matondang *et al.*, 2018)**, honey **(Heriyati *et al.*, 2015; Abdullah, 2018)**, Java chili **(Wijaya *et al.*, 2017; Soelistyowati *et al.*, 2022)**, coconut water **(Maulana *et al.*, 2023)**, goat testicle meal **(Ramadhan *et al.*, 2024)**, and others. Tilapia masculinization research using honey through soaking with a dose of 15 mL L^{-1} for 10 h resulted in a male sex ratio of 86.7% **(Tomasoa *et al.*, 2021)**. In the research of **Mangaro *et al.* (2018)**, the administration of MT hormone in feed at a dose of 9% per kilogram produced 92.57% male individuals, higher than the immersion method for 6 h at a dose of 10 mg L^{-1}, which resulted in 85% male sex. Feeding mixed with cow testicle meal at a dose of 9% produced 77% males **(Hutagalung, 2020)**. In a study involving tilapia masculinization using purwoceng with a concentration of 20mg L^{-1} and an immersion method for 8h, 73.3% of the test population was male tilapia **(Awaludin *et al.*, 2019)**.

Purwoceng (*Pimpinella pruatjan*) is one of the potential alternatives, considering that it is a local plant containing phytosteroids with androgenic effects in its roots. The main phytosteroid found in purwoceng is stigmasterol, which accounts for 5.38% of its total phytosteroid content. This component is known to have properties similar to androgenic-like hormones **(Lestari *et al.*, 2018)**. Previous research has shown that administering purwoceng extract has been proven to increase vitality, as evidenced by increased levels of testosterone and luteinizing hormone (LH) **(Bertha *et al.*, 2016)**. In the latest research, **Tarigan *et al.* (2024)** stated that dietary supplementation of *Ulva reticulata* extract on male red tilapia (*Oreochromis* sp.) gave the same results. Chloroform extract in purwoceng has the most potent aphrodisiac activity among several other plant extracts **(Wahyuningrum *et al.*, 2016)**. Research by **Singh and Lal (2017)** showed a directly proportional relationship between nitric oxide (NO) content and sperm motility and viability related to maximization and steroidogenesis processes in fish. Therefore, researchers are interested in analyzing the potential application of purwoceng as an approach for monosex production and masculinization needs in tilapia.

MATERIALS AND METHODS

1. Preparation of purwoceng extract

All parts of the purwoceng plant were dried by hot air drying for one day at a temperature not exceeding 50˚C. The dried plants were cut into thin slices and ground using a blender to obtain powder (simplisia). A total of 100g of purwoceng powder was extracted using the maceration method, by soaking it in 0.5L of 96% alcohol as solvent for 24h, with stirring every 6h to maintain homogeneity. The solution was then filtered using a 60-mesh filter cloth. The extract was stored in an Erlenmeyer flask, while the residue was re-soaked in 0.5L of 96% alcohol for another 24h, with stirring every 6h. The second solution was filtered, and the filtrate was combined with the first extract in a 5L Erlenmeyer flask. The solvent was then removed by evaporation using a Büchi rotary evaporator (rotavapor) at 48˚C and 60rpm until a thick extract was obtained

2. Feed formulation

Commercial powdered feed (MS Prima) with 39% protein content was used and supplemented with purwoceng extract through the feed coating method. Purwoceng extract was dissolved in 100mL of water, after which carboxymethyl cellulose (CMC) was added as a binder at 5g kg^{-1} of feed. The mixture was used to coat the feed, which was subsequently air-dried to prevent mold growth. The coated feed was then ready to be provided to the experimental fish.

3. Fish and experimental design

Nirwana strain Nile tilapia (*Oreochromis niloticus*) larvae, aged 7 days (average weight 0.011±0.001 g; average length 0.80±0.043 cm), were obtained from Purwasari

Village, Ciomas, Bogor Regency. Fish fry were equally distributed into 20 tanks (volume 60L), with 120 fry per tank, corresponding to a stocking density of 2 fry L^{-1}. The experiment was conducted using a completely randomized design (CRD) consisting of five treatments with four replications each. Fish larvae were acclimatized and then reared for 60 days. During the first month, the larvae were fed to satiation five times daily at 08:00, 10:00, 12:00, 15:00, and 17:00 h, while in the second month feeding frequency was reduced to four times daily at 08:00, 10:00, 13:00, and 17:00 h.

4. The experiment measurements

4.1 Specific growth rate

The specific growth rate was determined at the end of the study using the formula described by **Zonneveld *et al.* (1991)**:

$$\text{SGR (\% day}^{-1}) = \frac{\text{In } W_t \text{ - In } W_0}{t} \times 100$$

Note:
W_t = final weight of fish (g)
W_0 = initial weight of fish (g)
t = duration of the experiment (days)

4.2 Survival rate

The survival rate was calculated according to **Effendie (2002)**:

$$\text{SR (\%)} = \frac{N_t}{N_0} \times 100$$

Note:
Nt = total number of fish at the end of the rearing period
No = total number of fish at the start of the rearing period

4.3 Male sex ratio

The sex ratio was determined by observing the histological structure of gonads under a light microscope using acetocarmine staining, following **Bhagawati *et al.* (2017)**. The male sex ratio was calculated as:

$$\text{Ml (\%)} = \frac{\text{mL}}{\text{sL}} \times 100$$

Note:
Ml = male sex ratio (%)
mL = total male fish (fish)
sL = total observed fish (fish)

5. Data analysis

The study was conducted under a completely randomized design (CRD). Data on survival rate, male sex ratio, and specific growth rate were analyzed using analysis of variance (ANOVA) at a 95% confidence level, with SPSS 27.0 software, followed by Duncan's multiple range test when significant differences were detected.

RESULTS

1. Male sex ratio

The effectiveness of using purwoceng extract on the masculinization process can be seen from the percentage of males observed after 60 days of rearing.

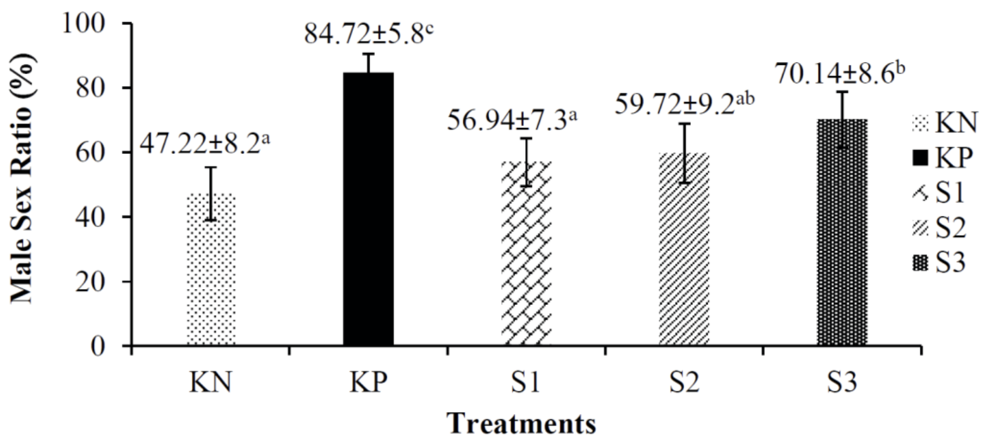

Fig. 1. Male sex ratio (Ml) of tilapia after 60 days of rearing using purwoceng extract in feed

Description: KN (no purwoceng extract supplementation); KP (MT 50mg kg^{-1} supplementation); S1 (purwoceng 350 mg kg^{-1} supplementation); S2 (purwoceng 700 mg kg^{-1} supplementation); S3 (purwoceng 1050 mg kg^{-1} supplementation). Different superscript letters indicate significant treatment effects ($P<0.05$).

The highest Ml value was found in the KP treatment with a value of 84.72±5.8% while the lowest value was found in the KN treatment with a value of 47.22±8.2%. The percentage of male tilapia in the purwoceng extract supplementation treatment has a higher value than the negative control but smaller than the positive control (Fig. 1).

2. Specific growth rate

The highest specific growth rate value was found in the KP treatment at 7.82±0.07%, while the lowest value was found in the KN treatment at 7.48±0.11%. The percentage of male tilapia in the purwoceng extract supplementation treatment in the feed had a higher value than the negative control but lower than the positive control (Fig. 2).

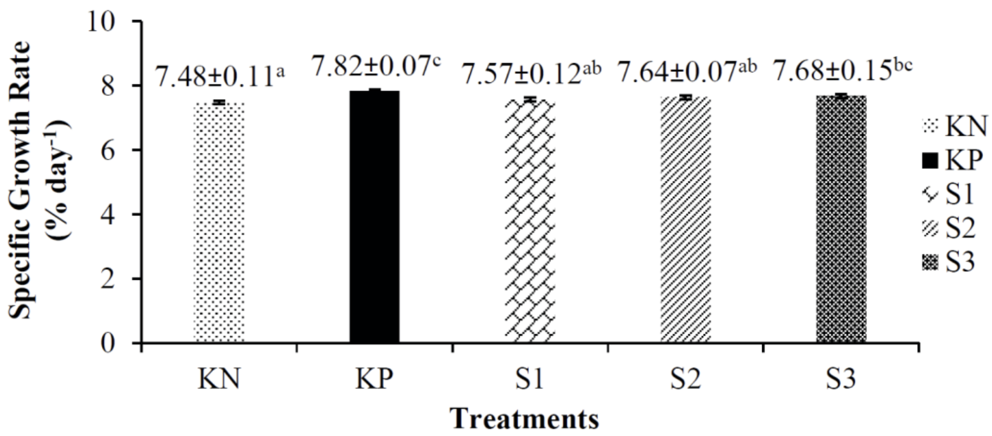

Fig. 2. Specific growth rate of tilapia (*Oreochromis niloticus*) after 60 days of masculinization using purwoceng extract through feed

Description: KN (no purwoceng extract supplementation); KP (MT 50mg kg^{-1} supplementation); S1 (purwoceng 350 mg kg^{-1} supplementation); S2 (purwoceng 700 mg kg^{-1} supplementation); S3 (purwoceng 1050 mg kg^{-1} supplementation). Different superscript letters indicate significant treatment effects (P<0.05).

3. Survival rate

The survival rate obtained can illustrate whether the treatment material has a positive or negative effect on the survival of the test animals. SR observed for 60 days showed results that were not significantly different (P> 0.05) with a 95% confidence interval. SR in the negative control treatment, positive control, S1, S2, S3, respectively, had values of 58.33; 60.63; 52.29; 55.21 and 55.63% (P> 0.05) (Fig. 3).

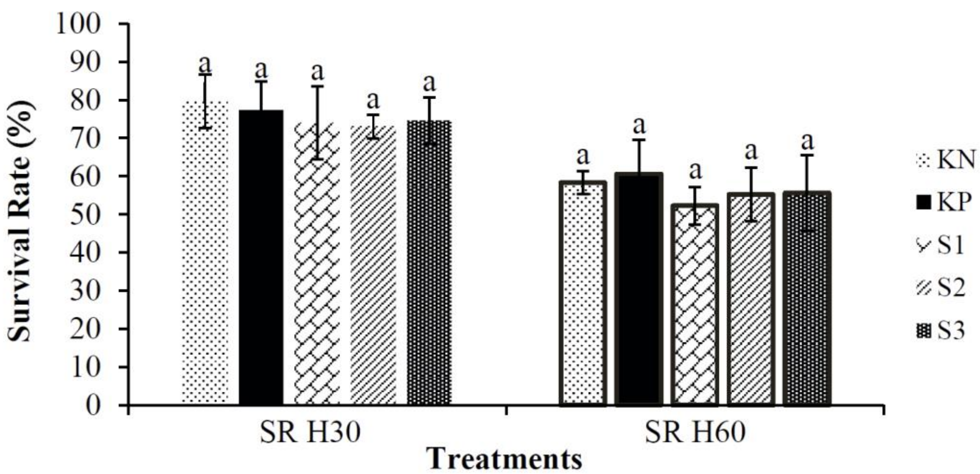

Fig. 3. Survival rate of tilapia (*Oreochromis niloticus*) after 60 days of maintenance on masculinization using purwoceng extract through feed

Description: KN (no purwoceng extract supplementation); KP (MT 50mg kg^{-1} supplementation); S1 (purwoceng 350 mg kg^{-1} supplementation); S2 (purwoceng 700 mg kg^{-1} supplementation); S3 (purwoceng 1050 mg kg^{-1} supplementation). Different superscript letters indicate significant treatment effects (P<0.05).

DISCUSSION

Purwoceng is a native Indonesian herbal plant that grows in mountainous areas. Purwoceng has properties as an aphrodisiac drug or increases sexual desire **(Nugraheni *et al.*, 2021)**. Purwoceng is one of the local plants that contain phytosteroids with androgenic effects. The main phytosteroid found in purwoceng is stigmasterol, which accounts for 5.38% of its total phytosteroid content. This component is known to have properties similar to androgenic-like hormones **(Putra, 2011; Lestari *et al.*, 2018)**. Some studies state that the use of purwoceng at certain doses can direct the sex of fish to be male. **Pradana *et al.* (2017)** stated that immersion of tilapia larvae for 10h in 20mg L^{-1} purwoceng extract produced 79.22% male tilapia. Based on the results obtained during the study, Fig. (1) shows the effect of MT supplementation and purwoceng extract in feed on the percentage of male tilapia. The treatment of purwoceng extract supplementation with doses of 0.35, 0.70, and 1.05g, respectively, produced 56.94, 59.72, and 70.14% male tilapia. The percentage of male fish was higher than the negative control treatment of 47.22% but lower than the positive control, which was 84.72%. ANOVA test results showed significantly different results among treatments ($P < 0.05$). The highest male sex ratio was found in the positive control treatment with MT supplementation at a dose of 50mg, followed by treatments S3, S2, and S1. These results indicate that there is an increase in the percentage of males proportional to the increase in the dose of purwoceng extract.

During the process of gonad development, the functional sex of fish can be influenced by several internal (genetic) and external (environmental) factors. Environmental conditions such as temperature, pH, and the addition of hormones from certain materials are external factors that help in the sex direction process **(Kautsari *et al.*, 2015)**. Temperature is an environmental parameter that affects the functional sex of fish in the sexual differentiation phase. Water temperatures above 32ºC induce sex change to males. Conversely, water temperatures close to 21ºC induce sex change to females **(Fuentes-Silva *et al.*, 2013; Lazaro-Velasco *et al.*, 2019)**. The results of the research on the percentage of males in the KN treatment resulted in a male sex ratio of 47.22% at a maintenance temperature of 25.8– 31.8ºC. This result is not much different from that recorded by **Wahyudin (2021)**, where at a temperature of 28.9–29.9 ºC the male sex ratio was 48.83%.

The increase in Ml value is due to steroid hormones contained in purwoceng extract stimulating sex direction into functional males. This is in accordance with **Tremblay and Van der Kraak (1998)**, who stated that the administration of steroid hormones in the differentiation phase can cause reversal of the sexual phenotype. Purwoceng extract contains stigmasterol compounds as much as 5.38% **(Putra, 2011)**. The dose of stigmasterol for each purwoceng extract supplementation treatment in S1, S2, and S3 treatments was 18.8, 37.66, and 56.4mg of stigmasterol compounds, respectively. Stigmasterol is one of the steroid hormones derived from plants that is androgenic so that it can increase testosterone levels in tilapia **(Sentosa *et al.*, 2023)**. Androgen receptors can bind to stigmasterol molecules because they have a chemical structure similar to cholesterol and testosterone molecules. This binding of stigmasterol molecules will affect the sex ratio and hormone levels of fish **(Tremblay & Van der Kraak, 1998; Hewitt *et al.*, 2008)**. Purwoceng root also contains furanocoumarin group compounds such as sphondin, isobargapten, bargapten, cytoserol, stigmasterol, and vitamin E that can increase testosterone **(Bertha *et al.*, 2016)**. The results showed that the percentage of Ml in purwoceng extract supplementation was lower than MT supplementation. The high Ml value in MT treatment is due to MT being an androgen with strong anabolic properties. MT has a methyl group in its chemical structure, making it difficult to degrade **(Barry *et al.*, 2007; Homklin *et al.*, 2011; Kusuma *et al.*, 2021)**.

Specific growth rate (SGR) is a measure that indicates the average daily weight gain rate of the test fish during the rearing period. Based on the results of the 60-day study, the specific growth rate values ranged from 7.42– 7.82% per day (Fig. 2). The highest specific growth rate during the study was found in the KP treatment with a value of 7.82%, while the lowest SGR value was found in the KN treatment with a value of 7.42%. ANOVA test results showed significantly different results across treatments ($P<0.05$). This indicates that the treatment of adding purwoceng extract does not negatively affect the growth of tilapia. Growth rate is generally influenced by internal and external factors. Internal factors that influence are heredity, age, and sex, while external factors are the environment, feed, disease, and cultivation media **(Aliyas *et al.*, 2016)**. The specific growth rate value of the positive control and purwoceng extract supplementation treatment was higher than the negative control. The difference in the value of SGR is thought to be caused by differences in the male sex ratio, which is significantly different. The results of this study are in accordance with those of **Githukia *et al.* (2015)**, postulating that monosex tilapia has a higher SGR value than mixed-sex tilapia. Monosex tilapia has an SGR value of 1.83%, while mixed-sex tilapia has an SGR value of 1.47%.

The growth of male and female tilapia in the larval stage generally has the same growth rate **(Putra, 2011)**. Differences in tilapia growth rates begin to occur during sexual differentiation. Female tilapia generally have a smaller growth rate than male tilapia. The average growth of male tilapia reached 1.53–1.69 g day^{-1}, while female tilapia grew 0.83–1.05 g day^{-1} **(Rosmaidar *et al.*, 2014)**. The difference in growth rate is caused by different energy allocation. Female tilapia use more energy for reproduction than males, so males grow faster **(Angienda *et al.*, 2010)**. The sex ratio can be directed to males or females before or during the sex differentiation phase **(Deswira *et al.*, 2016)**. Some studies have shown that the use of purwoceng at certain concentrations can change the sex of male fish. Directing the sex of tilapia larvae to males using purwoceng extract with 10h of immersion produced 79.22% male fish **(Pradana *et al.*, 2017)**.

The parameters of tilapia survival rate on the 30th and 60th days (Fig. 3), based on ANOVA tests, showed no significant difference. The survival rate of tilapia during 30 days of rearing ranged from 73.04–79.58%. The survival rate of tilapia during 60 days of rearing ranged from 52.29–60.63%. This indicates that purwoceng extract supplementation does not have a negative impact on the SR of tilapia either in the short term or long term (60 days). Fish SR is influenced by several factors such as environmental conditions, feed quality, stress levels, and larval conditions **(Marbun, 2014; Renita *et al.*, 2017)**. The large number of fish deaths during the study is thought to be due to fish experiencing stress so that they were unable to adapt to the new environment. According to the results of **Handayani (2012)**, tilapia reared for 14 days after transportation treatment of 500 fish per liter and 700 fish per liter had SR of 78 and 63%, respectively. The higher the density, the higher the stress level in post-transportation fish, making it difficult for fish to adapt to the new environment.

High fish stocking densities also have the potential to increase fish mortality. The higher the stocking density, the higher the competition between individuals in fighting for space. According to **Alfia *et al.* (2013)**, high stocking density will reduce the water quality of the rearing media and reduce fish appetite due to competition for space. SR on the 30th day of maintenance is still classified as good based on the SNI 7550:2009 of the first tilapia fingerlings, which is above 60%. Water temperature also plays an important role in fish survival. Temperature values observed during the study were between 25.8– 31.8ºC. Low water temperature makes mold easily attach to fish. Fluctuating water temperature during maintenance increases stress and mortality in fish **(Istiqomah *et al.*, 2018; Dewi *et al.*, 2022)**. The 60th-day SR value in each treatment from the highest to the lowest is KP 60.63%, KN 58.33%, S3 55.63%, S2 55.21%, and S1 52.29%. The percentage of tilapia survival rate of the research results is still relatively good. Based on the statement of **Mulyani *et al.* (2014)**, the survival rate of less than 30% is classified as unfavorable, 30–50% survival is classified as moderate, and survival of more than 50% is classified as good.

CONCLUSION

The sex orientation of tilapia fed *purwoceng* extract significantly affected the male sex ratio. The best results were observed in the 700mg kg^{-1} treatment, which produced a male percentage of 59.72%—a 26.47% increase compared to the negative control. These results suggest potential applications in hatchery procedures as a natural alternative to synthetic MT.

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