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Concentration and time of feeding with 17-α-methyltestosterone oil diluted and incorporated to the feed for masculinization of Nile tilapia

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ABSTRACT. Tilapia masculinization can be induced by oral administration of α-methyltestosterone (MT), which is commonly dissolved in ethanol to be added to the feed. However, there are many benefits in using alternative vehicles, such as oil. The incorporation time, vehicles quantity, safety for handlers, fish and the environment are favorable factors. In fry fed for 35 days under temperature control, we found that masculinization rate was similar in both incorporation vehicles of MT (oil or ethanol) in the concentrations studied (30 and 60 mg MT kg^{-1} feed). In an experiment, using hormone oil dissolution and oral administration at 30 mg MT kg^{-1} feed, it was observed that the longer the administration time, the lower the coefficient of variation in the masculinization rate. Therefore, administration for 32 days showed the lowest variability in the masculinization rate (99.8 ± 0.5 %), compared to 24 (98.5 ± 3.0 %), 16 (97.0 ± 6.0 %) and 8 (89.0 ± 8.8 %) days. The field experiment confirmed the results obtained in the lab. We concluded that the oil can be used as MT vehicle and we recommend to dispense it at the lowest hormonal concentration (30 mg MT kg^{-1} feed) for 32 days for tilapia masculinization.

Keywords: sex reversal; hormone dilution; vehicle; hormone incorporation; *Oreochromis niloticus*.

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Introduction

Nile tilapia (*Oreochromis niloticus*) is regarded as one of the most important aquaculture species that must be studied for the improvement of the several available production systems, since it is within the capacity of the consumers around the globe in terms of nutritional value and prices (Amer, El-Nabawy, Gouda, & Dawood, 2021; Food and Agriculture Organization [FAO], 2018). The presence of female tilapia in the production systems is undesired, since it can lead to overpopulations of unevenly sized fish in the production units, due to the deviation of growth energy for gonadal development and reproduction, thus tilapia females do not grow as valuable as males (Pandian & Sheela, 1995; Homklin, Ong, & Limpiyakorn, 2012; El-Greisy & El-Gamal, 2012; El-Sayed, 2006). In addition, its rapid proliferation harms the final production because of competition for food and oxygen, causing heterogeneity of the population (Popma & Green, 1990). Therefore, higher yields and more uniformly-sized fish are obtained in production by the use of all-male tilapia.

Several techniques have been used to produce monosex tilapia, such as manual sexing (El-Sayed, 2006), hybridization (Hickling, 1960), genetic manipulation (Mair, Abucay, Abella, Beardmore, & Skibinski, 1997), heat treatment (Angienda, Aketch, & Waindi, 2010), and hormonal administration (Yamamoto, 1953; Shelton, Rodrigues-Guerrero, & Lopezmacias, 1981; Wassermann & Afonso, 2003). However, oral administration of feed incorporated with 17-α-methyltestosterone (MT) have been considered the most effective, practical, and economically feasible method for the production of all-male tilapia (Green & Teichert-Coddington, 1994; Popma & Green, 1990).

The masculinization protocol relies on several factors including route of administration, duration, and time of treatment, concentration, and general characteristics of the commercial hormones (Piferrer, 2001). In tilapia masculinization with oral administration of MT, the recommendations range from 30 to 60 mg MT kg feed, in periods of 30 to 60 days (Celik, Guner, & Celik, 2011; Clemens & Inslee, 1968; Jensi, Marx, Rajkumar, Shakila, & Chidambaram, 2016; Mainardes-Pinto, Verani, Campos, & Silva, 2000; Rima, Rahman, & Sarker, 2017; Singh, Saini, & Sharma, 2018; Yamazaki, 1983), in four or more daily feedings (Meurer, Bombardelli,

Paixão, Silva, & Santos, 2012). The field recommendation by the scientific community and practiced by most of tilapia hatcheries is that the hormone is first diluted in ethanol and then incorporated into the feed by mixing manually or in proper mixing equipment such as mixers. Following, the feed is exposed to air for ethanol evaporation (Guerrero III, 1975).

A hormonal product was recently launched on the market for incorporation in tilapia feed (in the sexual reversion phase) in oil (30 mL per kilo of feed), at 30 mg MT kg^{-1} feed. The commercial product seems to have advantages in the sense of not requiring the handling of large amounts of ethanol (half a liter per kilo of feed). In addition, the use of oil eliminates the risk of the presence of ethanol in the feed, which can cause liver damage, reducing health and survival of fingerlings (Valentim-Zabott et al., 2008). However, this product is diluted by 50% with lactose in order to help its incorporation in feed. To better understand the effect of dissolving pure MT with oil, we analyzed the masculinization rate and performance of Nile tilapia with both vehicle of MT incorporation (oil and ethanol), in different concentrations and time of administration under controlled temperature (laboratory conditions).

Material and methods

Trial 1: Solvents and concentrations experiment

The experiment was conducted at the Aquaculture Laboratory of Embrapa Western Agriculture, Dourados, MS. Nile tilapia larvae, GIFT strain, came from a commercial hatchery. The larvae (average weight 0.012 g) were distributed in twenty fiber tanks of 300-L each (400 fish per tank). The water flow was constant at 0.8 L $min.^{-1}$, and an individual stone diffuser per tank was used to ensure dissolved oxygen saturation above 90%.

Water quality parameters were measured in the morning at 07:30 am (once a day), before feeding the fish. The dissolved oxygen content (6.84 ± 1.05 mg L^{-1}) and water temperature (26.7 ± 0.1°C) were monitored daily (n = 76) by an oximeter (YSI 55/12 FT, YSI Incorporated, Yellow Spring, OH, USA). The pH (7.8 ± 0.2), salinity (0.01 %), and conductivity (0.287 ± 0.057 mS cm^{-1}) were measured once a week (n = 4) using a water quality meter (U-5000 Multiparameter, Horiba Ltd., Kyoto, Japan). The concentration of total ammonia (0.129 ± 0.148 mg L^{-1}), nitrite (0.083 ± 0.092 mg L^{-1}), and nitrate (1.800 ± 0.300 mg L^{-1}) were measured (n = 4) using a colorimetric kit (Alfakit Ltda., Santa Catarina, Brazil). Values shown were within the range considered normal for the species (El-Sayed, 2006).

The experimental design was completely randomized, with five treatments and four replicates. The treatments were two concentrations of synthetic hormone 17-α-methyltestosterone (30 and 60 mg kg^{-1}), two hormone incorporation vehicles (commercial ethanol 96 GL and soybean oil), and the control (hormone-free diet).

The 17-alpha-methyltestosterone (MT) was weighed at the test concentrations (60.0 or 30.0 mg) and diluted in oil (16 mL of oil per kg of feed) or ethanol (0.44 L of ethanol per kg of feed). The mixture of the diluted hormone in one kilo of the commercial powdered feed (55% Crude protein, CP) was processed manually until obtained lumps-free feed. The experimental feed containing the hormone diluted in oil was ready for use, while the feed diluted with ethanol was distributed in metallic trays lined with brown paper, forming a thin layer of feed for efficient drying. The trays were placed in an oven with forced air circulation at 50°C for 24 hours for ethanol evaporation. All feeds were kept under refrigeration (4°C) and protected from light until used. The proximate composition of experimental diets was estimated following standard protocols (Association of Official Agricultural Chemists International [AOAC], 2012). Dietary gross energy was analyzed using a bomb calorimeter (IKA-C 6000 Isoperibol). The proximate composition of all experimental diets is shown in Table 1.

Table 1. Treatments and analyzed proximate composition (dry matter basis) of experimental diets.

	Control	Ethanol (60 mg MT kg^{-1} feed)	Ethanol (30 mg MT kg^{-1} feed)	Oil (60 mg MT kg^{-1} feed)	Oil (30 mg MT kg^{-1} feed)
Dry matter (%)	90.6 ± 0.1	94.2 ± 0.1	93.8 ± 0.1	91.3 ± 0.3	92.9 ± 3.5
Crude protein (%)	53.6 ± 0.0	55.8 ± 0.1	55.6 ± 0.0	53.3 ± 0.2	52.9 ± 0.3
Crude fiber (%)	2.2 ± 0.1	2.3 ± 0.4	2.2 ± 0.4	2.0 ± 0.1	2.0 ± 0.2
Ether extract (%)	8.1 ± 0.9^{ab}	7.2 ± 0.2^{b}	7.1 ± 0.4^{b}	9.0 ± 0.5^{a}	9.1 ± 0.2^{a}
Ash (%)	14.5 ± 0.1	13.7 ± 0.1	13.8 ± 0.1	13.9 ± 0.1	13.7 ± 0.2
Crude energy (MJ/kg)	4552 ± 64	4659 ± 51	4596 ± 5	4686 ± 56	4670 ± 63

Note: Values are mean ± SD of three replicates per experimental diets.

Fish were hand-fed eight times a day (8:00 am, 9:00 am, 10:00 am, 11:00 am, 1:00 pm, 2:00 pm, 3:00 pm and 4:00 pm) for 28 days. The daily feeding rate (15% of biomass) was adjusted once a week, when 10% of fish from a random replicate of each treatment were weighed. After completion of hormonal treatment, fish were individually counted and weighted per tank. The animals (average weight) remained in the same tanks, receiving pelleted feed (0.8 – 1.5 mm; 36% CP) until apparent satiety, twice a day (8:00 am and 4:00 pm). After a 35-day growth period, 100 fish in each replicate were anesthetized with eugenol (1:50,000 v/v) and euthanized by spinal cord transection (Owatari et al., 2022) to evaluate the efficiency of the treatments on masculinization. Thus, sex ratios were determined by microscopic analysis (100 magnification) of gonadal squash. Ovaries contain oocytes characterized by their exocytosis, previtellogenic or vitellogenic features. Male gonads were clearly distinguished due to their lobular configuration (Kubitza, 2000; Nakamura & Nagahama, 1989; Phelps & Popma, 2000).

Performance variables were calculated, as follows: Weight gain (WG, %) = 100 × [final body weight (g) – initial body weight (g)]; Feed conversion ratio (FCR) = feed intake (g) / weight gain (g); Survival rate (S, %) = 100 x (number of fish at the end / number of fish at the beginning).

Trial 2: Period of 17-α-methyltestosterone administration (30 mg MT kg^{-1} feed) diluted with oil

The study was conducted for 66 days. Nile tilapia larvae (initial average weight 0.014 g) were distributed in a completely randomized design, consisting of five treatments with four replicates and 500 fish per replicate. Twenty circular fiber boxes (300-L) were used in a system with temperature control and oxygen supply. The treatments comprised five periods of MT administration (0, 8, 16, 24 and 32 days).

The hormone (30 mg MT kg^{-1}) was diluted with oil and incorporated into the feed following the same procedure as in the previous experiment. The fish were fed eight times a day (8:00 am, 9:00 am, 10:00 am, 11:00 am, 1:00 pm, 2:00 pm, 3:00, and 4:00 pm). The daily feeding rate was adjusted according to the growth of the animals (20 and 15% of biomass, in the first and other weeks, respectively), in the same way as Trial 1.

During the experimental periods, average water temperature, oxygen dissolved, pH, salinity, conductivity, nitrite, nitrate, and total ammonia were 27.9 ± 0.1°C, 6.24 ± 0.7 mg L^{-1}, 7.2 ± 0.2, 0.02%, 0.231 ± 0.028 mS cm^{-1}, 0.0 mg L^{-1}, 0.031 ± 0.028 mg L^{-1}, and 0.002 ± 0.002 mg L^{-1}, respectively. After a 32-day feeding period, the fish were weighted, counted, and returned to the same tanks. The same procedure as in the previous experiment was conducted to determine performance variables and sex rates.

Trial 3: field validation

Larvae of *O. niloticus* (n = 8,000) were transferred five days after hatching to two hapas (2 m^2) set in earthen pond (1,000 m^2) in March-April, 2021. The pond was equipped with an aerator and there was continuous water inlet (8% renewal rate/ day). The average water temperature and oxygen dissolved were 26.5 ± 0.5°C and 6.63 ± 0.4 mg L^{-1}, respectively. Feeds containing MT were prepared using the hormone (30 mg MT kg^{-1}). In this process, for the preparation of 25 Kg of feed, MT was diluted in oil (16 mL of oil per kg of feed) and the mixture was incorporated into eight kilos of feed by hand. This feed was added to the 17 kg remaining, using a sieve for a homogeneous incorporation. Finally, it was mixed mechanically for five minutes in an adapted concrete mixer. After this procedure, it was ready for use.

Fish were fed at 20-15% body weight daily, seven days per week, for the entire treatment period. A daily feed was divided into eight portions given at 08:00 am, 9:00 am, 10:00 am, 11:00 am, 1:00 pm, 2:00, 3:00, and 4:00 pm. Fish (approximately 400 fish per period) were collected on days 8, 16, 24 and 32 after starting hormonal treatment. Collected fish were stocked in other four hapas (2 m2) for growth, being separated by treatment. All hapas were placed in a pond (3,000 m2) which was not used for masculinization. Fish from each hapa were harvested once reached about 10 g. It was used the same methodology for the analysis of sex (n=100/treatment) as used in other trials.

Statistical analysis

The effects of hormone treatment on the weight gain, survival rate, feed conversion ratio, and sex ratios were tested for normality using a Shapiro–Wilk's test and fulfilling the assumptions for normality; data were analyzed by variance analysis (ANOVA). Differences among treatments were identified using Tukey test (p=0.05). All statistical analyses were performed in RStudio software (R Core Team, 2021).

Ethics and legal aspects

All procedures performed in this study were in accordance with the Ethical Principles in Animal Research and approved by the Committee for Ethics in Animal Experimentation of Embrapa Western Agriculture and Embrapa Pantanal (CEUA / CPAP) (Protocol no. 005/2018).

Results and discussion

In testing solvents and concentrations of MT administration, feed conversion ratio, and survival rate of the different treatments were not significantly different from each other (Table 2). The control group (hormone-free diet) had the lowest masculinization rate (51.5%), while the masculinization rate did not differ between hormone concentrations and solvents used (Table 2). The treatments affected the weight gain; the lowest weight gain was obtained with 60 MT kg^{-1} feed diluted with oil (Table 2). However, no clear relationship was found between the concentration and the solvent that justified the obtained results. Therefore, the differences found may have other sources of variation, in addition to the treatments.

Table 2. Weight gain (WG), feed conversion ratio (FCR), survival rate (SR), and male frequency (MF) of tilapia at different concentrations of administration and vehicles of 17-α-methyltestosterone (MT) for 28 days.

Hormone Concentration (mg MT kg^{-1} feed)	MT Vehicle	Variable[1]			
		WG	FCR	SR	MF
		%		%	%
0 (Control)	-	5972 ± 982^{ab}	1.40 ± 0.33	81.0 ± 9.0	51.5 ± 6.1^{b}
30	Ethanol	8356 ± 2082^{a}	1.20 ± 0.27	85.0 ± 17.8	98.8 ± 2.5^{a}
30	Oil	5588 ± 1866^{ab}	2.31 ± 1.86	68.3 ± 11.6	99.2 ± 1.0^{a}
60	Ethanol	6057 ± 380^{ab}	1.30 ± 0.24	73.3 ± 16.2	95.6 ± 2.6^{a}
60	Oil	4628 ± 813^{b}	1.37 ± 0.30	84.5 ± 16.8	97.3 ± 2.2^{a}

Note: [1]Mean (± standard deviation) of four replicates. Values with different superscripts indicate significant differences as determined by Tukey's test ($p < 0.05$).

The results indicated ethanol can be replaced by oil to dissolve 17-α-methyltestosterone in the both tested concentrations (60 and 30 mg MT kg^{-1} feed), for Nile tilapia fry (*Oreochromis niloticus*) masculinization. In Brazil, most of commercial tilapia hatcheries and juvenile farmers commonly use 60 mg MT kg^{-1} feed diluted in ethanol. The use of different solvents can influence the masculinization rate (Varadaj & Pangian, 1991), but this influence was not verified in the present study. This fact probably occurred due to the quality of the MT incorporation process, in which the oil was uniformly adsorbed in the feed and absorbed by the fish, without showing apparent losses of oil and, consequently, of the hormone in the water. Therefore, we presume that the concentration of 30 mg MT kg^{-1} feed was enough to induce a masculinization rate equal to ethanol, and above 98%.

Weight gain and survival rate have no difference among the periods of MT administration (Table 3). Based on the results, 30 mg MT kg^{-1} feed administrated for 32 days promoted better feed conversion ratio compared to control group. Frequencies of male in 8, 16, 24 and 32 days were significantly higher than the control group (Table 3). It was observed that the MT administration (30 mg MT kg^{-1} feed) for a period of 32 days showed greater accuracy of masculinization, as it presented a lower coefficient of variation (Figure 1).

Table 3. Weight gain (WG), feed conversion ratio (FCR), survival rate (SR), and male frequency (MF) of tilapia at different times of 17-α-methyltestosterone administration (30 mg MT kg^{-1} feed) after a 32-day feeding period.

Period of MT administration	Variable[1]			
	WG	FCR	SR	MF
(days)	(%)		(%)	(%)
0	9116 ± 825	1.41 ± 0.03^{a}	54.2 ± 4.2	61.0 ± 4.1^{b}
8	9057 ± 1115	1.48 ± 0.25^{ab}	49.4 ± 12.6	89.0 ± 8.8^{a}
16	7807 ± 861	1.37 ± 0.28^{ab}	58.2 ± 28.4	97.0 ± 6.0^{a}
24	8671 ± 1203	1.24 ± 0.06^{ab}	51.5 ± 3.9	98.5 ± 3.0^{a}
32	8237 ± 1110	1.19 ± 0.08^{b}	60.3 ± 13.8	99.8 ± 0.5^{a}

Note: [1]Mean (± standard deviation) of four replicates. Values with different superscripts indicate significant differences as determined by Tukey's test ($p < 0.05$).

In general, a concentration of 60 mg kg^{-1} of feed is used for 28 days in Nile tilapia fry, treated with MT administered orally and feed added in ethanol (Beardmore, Mair, & Lewis, 2001; Bombardelli, Hayashi, & Meurer, 2004; Celik, Guner, & Celik, 2011; Ferdous & Ali, 2011; Popma & Green, 1990). However, lower concentration administration (14 mg MT kg^{-1} feed) was recommended by Phelps and Okoko (2011) to obtain

α 95% males after 28 days of feeding. In addition, no difference was observed between 30 and 60 mg MT kg^{-1} feed, on the frequency of Nile tilapia males treated for 45 days (Mainardes-Pinto et al., 2000). In *Tilapia mossambica*, according to Nakamura (1975), methyltestosterone at 50 mg kg^{-1} feed, given for 19 days, is capable of inducing a complete masculinization.

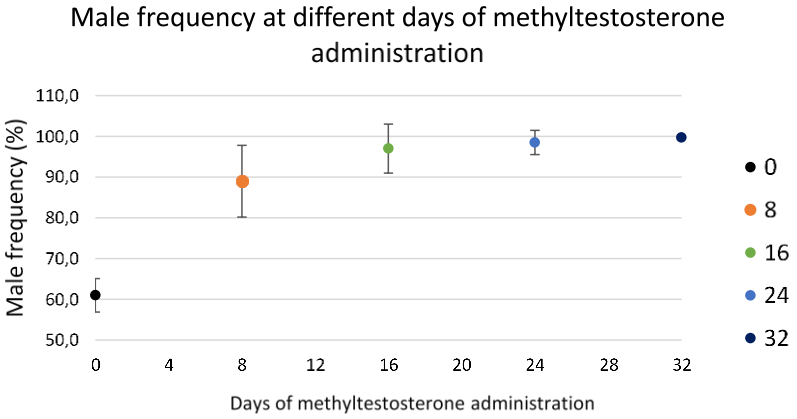

Figure 1. Male frequency in each timing of 17-α-methyltestosterone administration (0-control, 8, 16, 24, and 32 days) at a dose of 30 mg MT kg^{-1} feed. Values are mean of each hapa (n = 100). Bars represent the standard deviation.

In the present study, the administration periods of 16, 24, and 32 days induced a masculinization rate above 95% using MT diluted with oil at 30 mg MT kg^{-1} feed, and it was observed that the longer the administration time, the lower the coefficient of variation in the masculinization rate. *Tilapia nilotica* fed with MT (30 mg kg^{-1} feed) for 59 days showed 100% sex reversal (Tayamen & Shelton, 1978). According Vinarukwong, Lukkana, and Wongtavatchai (2018), in Nile tilapia, the percent of phenotypic males increased with the longer duration of hormone treatment. Considering the higher variability in the masculinization rate could lead to a greater population of fish with uneven sizes in production units, we recommend a 32-days treatment period using MT diluted with oil at 30 mg MT kg^{-1} feed. This is in accordance with Macintosh, Varghese, and Rao (1985), who stated that although sex reversion of *Oreochromis mossambicus* can occur using only 21 or 28-day treatment periods and a daily feeding rate of 3% body weight, it is clearly advantageous to use a higher feeding rate and/or to extend the length of treatment to more than 30 days.

The absence of significant differences in weight gain of hormone-treated fish compared to untreated fish in both experiment is in discordance with observations in other studies (Ahmad, Shalaby, Khattab, & Abdel-Tawwab, 2002; Khalil, Hasheesh, Marie, Abbas, & Zahran, 2011; Marjani, Jamili, Mostafavi, Ramin, & Mashinchian, 2009; Mateen & Ahmed, 2015; Rima, Rahman, & Sarker, 2017; Soltan, Hassaan, El-Nagaar, & Wahead, 2013). Faster growth of males (Bardach, Ryther, & McLarney, 1972), positive anabolic effect of the hormone (Yamazaki, 1976), and activation of other endogenous anabolic hormones enhancing growth (Lone & Matty, 1980) are observed in hormone-treated fish, which promoted better performance compared to untreated fish. However, the effect of hormone in fish growth seems to be controversial; androgens may positively or negatively affect the health and growth of fish (Abo-Al-Ela, 2018; Lone & Matty, 1980; Zanoni, Leal, Caetano Filho, Oliveira, & Ribeiro, 2013). In this study, no adverse effect due to hormone use was observed in Nile tilapia. On the contrary, in the experiment of period MT administration diluted with oil, the FCR decreased significantly, indicating that 30 mg MT kg^{-1} feed administration for 32 days promoted FCR. Similar result concerning food conversion efficiency was obtained with Nile tilapia (Hasanuzzaman et al., 2021).

Survival rate was not affected by concentrations, vehicles, and time of MT administration. These results are in line with the previous study, which demonstrated MT administration did not reflect negatively on tilapia survival rate when compared to group that did not receive any MT (Ahmad et al., 2002; Mainardes-Pinto et al., 2000; McGeachin, Robinson, & Neill, 1987; Soltan et al., 2013; Rima, Rahman, & Sarker, 2017). The low survival rate observed in the present study may be due to the individual fish characteristics (Celik, Guner, & Celik, 2011), and to the establishment of hierarchies in feeding among the fish (Vera Cruz & Mair, 1994).

Regarding the composition of the diet, the addition of oil increased the ether extract and, on the contrary,

the use of ethanol decreased the ether extract from the diet. This is because ethanol is a solvent and, depending on the MT incorporation procedure, can extract lipids from the diet. Although the variations obtained in the ether extract were not enough to influence the gross energy, lipids are a source of essential fatty acids, important for the healthy development of fish (Qiang et al., 2017). Therefore, the possibility of changes of essential nutrients from the original formulations must be considered and evaluated in further studies.

Under field conditions, masculinization rate in fish was over 90% when administrated for 16, 24, and 32 days (30 mg MT kg^{-1} feed) (Figure 2).

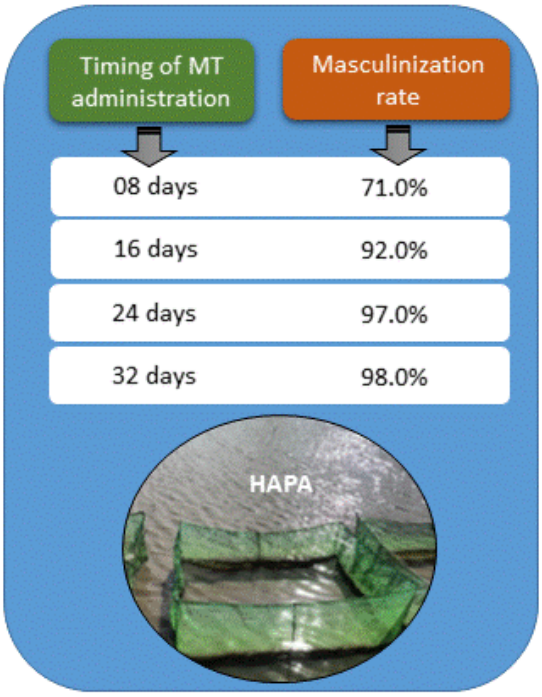

Figure 2. Tilapia masculinization at different days of 17-α-methyltestosterone administration (30 mg MT kg^{-1} feed diluted with oil) under field condition.

Based on the results of laboratory experiments and field validation, it is possible to dissolve MT (100% pure) with pure oil to produce monosex Nile tilapia. Since masculinization rate and tilapia performance at 30 mg MT kg^{-1} feed showed no difference to 60 mg MT kg^{-1} feed, the lowest concentration is considered the ideal, in order to reduce risks to workers, water and the environment. In the field, the evaporation of ethanol is commonly carried out at room temperature, taking around two to three days. In this sense, the standard process of incorporating MT with oil (with criteria to guarantee the homogeneous mixture of the defined amount of MT in the feed) also brings benefits with regard to the reduction of time in the incorporation of the diet with MT, in addition to using less vehicle. Reducing the amount of hormone and vehicle, preparation time, and labor, results in lower masculinization costs, adding one more advantage to the use of oil. Under favorable temperature conditions for tilapia, the 32 days of MT feeding period is recommended to achieve better feed conversion of Nile tilapia and greater precision in the masculinization rate. This information may favor many farmers of male tilapia juveniles.

Conclusion

Feeding Nile tilapia for 32 days with a diet containing soybean oil (16 ml oil kg^{-1} feed) as a vehicle for the hormone 17-α-methyltestosterone (30 mg MT kg^{-1} feed) proved to be efficient in the process of sex reversal.

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References

Ahmad, M. H., Shalaby, A. M. E., Khattab, Y. A. E., & Abdel-Tawwab, M. (2002). Effects of 17 α–methyltestosterone on growth performance and some physiological changes of Nile tilapia fingerlings (*Oreochromis niloticus* L.). *Egyptian Journal of Aquatic Biology and Fisheries*, *6*(2), 1-23. DOI: https://doi.org/10.21608/EJABF.2002.1736. Retrieved from https://ejabf.journals.ekb.eg/article_1736_26204 bb4b0cf682d0f9b1bf4ebd16ca3.pdf

Abo-Al-Ela, H. G. (2018). Hormones and fish monosex farming: A spotlight on immunity. *Fish and Shellfish Immunology*, *72*, 23-30. DOI: http://dx.doi.org/10.1016/j.fsi.2017.10.038

Amer, A. A., El-Nabawy, E.-S. M., Gouda, A. H., & Dawood, M. A. O. (2021). The addition of insect meal from *Spodoptera littoralis* in the diets of Nile tilapia and its effect on growth rates, digestive enzyme activity and health status. *Aquaculture Research*, *52*(11) 5585-5594. DOI: https://doi.org/10.1111/are.15434

Association of Official Agricultural Chemists International [AOAC]. (2012). *The Official methods of analysis* (19th ed.). Gaithersburg, MD, USA: AOAC.

Angienda, P. O., Aketch, B. O., & Waindi, E. N. (2010). Development of all-male fingerlings by heat treatment and the genetic mechanism of heat induced sex determination in Nile tilapia (*Oreochromis niloticus* L.). I*nternational Scholarly and Scientific Research and Innovation*, *4*. DOI: https://doi.org/10.5281/zenodo.1328738

Bardach, J. E., Ryther, J. H., & McLarney, W. P. (1972). *Aquaculture: the farming and husbandry of freshwater and marine organisms* (p. 350-384). Canada and USA: John Wiley and Sons Inc.

Beardmore, J. A., Mair, G. C., & Lewis, R. I. (2001). Monosex male production in finfish as exemplified by tilapia: applications, problems, and prospects. *Aquaculture*, *197*(1-4), 283-301. DOI: https://doi.org/10.1016/S0044-8486(01)00590-7

Bombardelli, R. A., Hayashi, C., & Meurer, F. (2004). Aplicação de métodos diretos e indiretos para produção de populações monossexuais na tilapicultura. *Arquivos de Ciências Veterinárias e Zoologia UNIPAR*, *7*, 57-68. Retrieved from https://ojs.revistasunipar.com.br/index.php/veterinaria/article/view/545/484

Celik, I., Guner, Y., & Celik, P. (2011). Effect of orally 17α-methyltestosterone at different doses on the sex reversal of the Nile tilapia (*Oreochromis niloticus*, Linneaus 1758). *Journal of Animal and Veterinary Advances*, *10*(7), 853-857. DOI https://doi.org/10.3923/javaa.2011.853.857

Clemens, H. P., & Inslee, T. (1968). The production of unisexual broods by *Tilapia mossambica* sex-reversed with methyl testosterone. *Transactions of the American Fisheries Society*, *97*, 18-21. DOI: https://doi.org/10.1577/1548-8659(1968)97[18:TPOUBB]2.0.CO;2

El-Greisy, Z. A., & Gamal, A. E. (2012). Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17a- methyltestosterone with respect to the degree of sex stability after one year of treatment. *Egyptian Journal of Aquatic Research*, *38*, 59-66. DOI: https://doi.org/10.1016/j.ejar.2012.08.005

El-Sayed, A.-F. M. (2006). Nutrition and Feeding. In A.-F. M. El-Sayed, *Tilapia culture*., Wallinggford, IA: CABI International

Food and Agriculture Organization [FAO]. (2018). *Aquaculture department, the state of world fisheries and aquaculture* (p. 2430). Rome, ITA: FAO.

Ferdous, Z., & Ali, M. M. (2011). Optimization of hormonal dose during masculinization of tilapia (*Oreochromis niloticus*) fry. *Journal of the Bangladesh Agricultural University*, *9*(2), 359-364. Retrieved from https://www.banglajol.info/index.php/JBAU/article/view/11052

Green, B. W., & Teicherto-Coddington, D. R. (1994). Growth of control and androgen-treated Nile tilapia, *Oreochromis niloticus* (L.), during treatment, nursery and grow-out phases in tropical fish ponds. *Aquaculture Research*, *25*(6), 613-621. DOI: https://doi.org/10.1111/j.1365-2109.1994.tb00725.x

Guerrero III, R. D. (1975). Use of androgens for the production of all-male *Tilapia aurea* (Steindachner). *Transactions of the American Fisheries Society*, *104*(2), 342-348. DOI: https://doi.org/10.1577/1548-8659(1975)104<342:UOAFTP>2.0.CO;2

Hasanuzzaman, H. M. D., Rana, S., Khanom, D. A., Nahid, S. A. A. (2021). Effect of hormonal masculinization on growth performance of tilapia (*Oreochromis niloticus*). *Bangladesh Journal of Veterinary and Animal Sciences*. 9(1), 52-58. Retrieved from https://bjvas.com/wp-content/uploads/2021/07/BJVAS_2021-1007.pdf

Hickling, C. F. (1960). The Malacca tilapia hybrids. *Journal of Genetics*, *57*, l-10. Retrieved from https://www.ias.ac.in/article/fulltext/jgen/057/01/0001-0010

Homklin, S., Ong, S. K., & Limpiyakorn, T. (2012). Degradation of 17-methyltestosterone by *Rhodococcus* sp. and *Nocardioides* sp. isolated from a masculinizing pond of Nile tilapia fry. *Journal of Hazardous Materials*, 221-222, 35-44. DOI: https://doi.org/10.1016/j.jhazmat.2012.03.072

Jensi, A., Marx, K. K., Rajkumar, M., Shakila, R. J., & Chidambaram, P. (2016). Effect of 17 α-methyl testosterone on sex reversal and growth of Nile tilapia (*Oreochromis niloticus* L., 1758). *Ecology, Environment and Conservation*, *22*(3), 1493-1498. Retrieved from https://core.ac.uk/download/pdf/228378609.pdf

Khalil, W. K., Hasheesh, W. S., Marie, M. A. S., Abbas, H. H., & Zahran, E. A. (2011). Assessment the impact of 17α-methyltestosterone hormone on growth, hormone concentration, molecular and histopathological changes in muscles and testis of Nile tilapia; *Oreochromis niloticus*. *Life Science Journal*, *8*(3), 329-343. Retrieved from http://www.lifesciencesite.com/lsj/life0803/055_6655life0803_329_342.pdf

Kubitza, F. (2000). *Tilápia*: tecnologia e planejamento na produção comercial. Jundiaí, SP: Divisão de Biblioteca e Documentação.

Lone, K. P., & Matty, A. J. (1980). The effect of feeding methyltestosterone on the growth and body composition of common carp (*Cyprinus carpio* L.). *General and Comparative Endocrinology*, *40*(4), 409-424. DOI: https://doi.org/10.1016/0016-6480(80)90004-0

Macintosh, D. J., Varghese, T. J., & Rao, G. P. S. (1985). Hormonal sex reversal of wild-spawned tilapia in India. *Journal of Fish Biology*, *26*(2), 87-94. DOI: https://doi.org/10.1111/j.1095-8649.1985.tb04245.x

Mainardes-Pinto, C. S. R., Verani, N. F., Campos, B., & Silva, A. L. (2000). Masculinização da tilápia do Nilo, *Oreochromis niloticus*, utilizando diferentes rações e diferentes concentrações de 17 α-Metiltestosterona. *Revista Brasileira de Zootecnia*, *29*(3), 654-659. DOI: https://doi.org/10.1590/S1516-35982000000300003

Mair, G. C., Abucay, J. S., Abella, T. A., Beardmore, J. A., & Skibinski, D. O. F. (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). DOI: https://doi.org/10.1139/f96-28

Marjani, M., Jamili, S., Mostafavi, P. G., Ramin, M., & Mashinchian, A. (2009). Influence of 17-alpha methyl testosterone on masculinization and growth in tilapia (*Oreochromis mossambicus*). *Journal of Fisheries and Aquatic Sciences*, *4*, 71-74. DOI: http://dx.doi.org/10.3923/jfas.2009.71.74.

Mateen, A., & Ahmed, I. (2015). Androgen sex reversal, subsequent growth and meat quality of Nile tilapia (*Oreochromis niloticus*), *Pakistan Journal of Agricultural Sciences*, *52*, 199-202. Retrieved from http://pakjas.com.pk/ papers/2407.pdf

McGeachin, R. B., Robinson, E. H., & Neill, W. H. (1987). Effect of feeding high levels of androgens on the sex ratio of *Oreochromis aureus*. *Aquaculture*, *61*(3-4), 317-321. DOI: https://doi.org/10.1016/0044-8486(87)90160-8

Meurer, F., Bombardelli, R. A., Paixão, P. S., da, Silva, L. C. R., & Santos, L. D. (2012). Feeding frequency on growth and male percentage during sexual reversion phase of Nile tilapia. *Revista Brasileira de Saúde e Produção Animal*, *13*(4), 1133-1142. Retrieved from https://www.bvs-vet.org.br/vetindex/periodicos/revista-brasileira-de-saude-e-producao-animal/13-(2012)-4/feeding-frequency-on-growth-and-male-percentage-during-sexual-reversio/

Nakamura, M. (1975). Dosage-dependent changes in the effect of oral administration of methyltestosterone on gonadal sex differentiation in *Tilapia mossambica*. *Bulletin of the Faculty of Fisheries Hokkaido University*, *26*(2), 99-108. Retrieved from http://hdl.handle.net/2115/23550

Nakamura, M., & Nagahama, Y. (1989). Differentiation and development of Leydig cells, and change of testosterone levels during testicular differentiation in tilapia *Oreochromis niloticus*. *Fish Physiology and Biochemistry*, *7*, 211-251. DOI: https://doi.org/10.1007/BF00004709

Owatari, M. S., Silva, L. R., Ferreira, G. B., Rodhermel, J. C. B., Andrade, J. I. A., Dartora, A., & Jatobá, A. (2022). Body yield, growth performance, and haematological evaluation of Nile tilapia fed a diet supplemented with *Saccharomyces cerevisiae*. *Animal Feed Science and Technology*, *293*, 115453. DOI: https://doi.org/10.1016/j.anifeedsci.2022.115453

Pandian, T. J., & Sheela, S. G. (1995). Hormonal induction of sex reversal in fish. *Aquaculture*, *138*(1-4), 1-22. DOI: https://doi.org/10.1016/0044-8486(95)01075-0

Phelps, R. P., & Okoko, M. (2011). A non-paradoxical dose response to 17α-methyltestosterone by Nile tilapia *Oreochromis niloticus* (L.): effects on the sex ratio, growth and gonadal development. *Aquaculture Research*, *42*(4), 549-558. DOI: https://doi.org/10.1111/j.1365-2109.2010.02650.x

Phelps, R. P., & Popma, T. J. (2000). Sex reversal of tilapia. In B. A. Costa-Pierce, & J. E. Rakocy (Eds.), *Tilapia aquaculture in Americas* (2nd ed.). Louisiana, EUA: World Aquaculture Society. Retrieved from https://freshwater-aquaculture.extension.org/wp-content/uploads/2019/08/Sex_Reversal_of_Tilapia.pdf

Piferrer, F. (2001). Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture*, *197*(1-4), 229-281. DOI: https://doi.org/10.1016/S0044-8486(01)00589-0

Popma T. J., & Green B. W. (1990). *Sex Reversal of Tilapia in Earthen Ponds*. Research and Development Series n. 35. Auburn, AL, USA: International Center for Aquaculture, Auburn University. Retrieved from https://aurora.auburn.edu/bitstream/handle/11200/1099/0204FISH.pdf?sequence=1

Qiang, J., He, J., Yang, H., Sun, Y.-L., Tao, Y.-F., Xu, P., & Zhu, Z.-X. (2017). Dietary lipid requirements of larval genetically improved farmed tilapia, *Oreochromis niloticus* (L.), and effects on growth performance, expression of digestive enzyme genes, and immune response. *Aquaculture Research*, *48*(6), 2827-2840. DOI: https://doi.org/10.1111/are.13117

R Core Team. (2021). *R: A language and environment for statistical computing* (1.4.1106 ed.). Vienna, AUT: R Foundation for Statistical Computing.

Rima, N. N., Rahman, Md. M., & Sarker, Md. J. (2017). Optimization of 17-alpha methyltestosterone (MT) hormone dose during masculinization of Nile Tilapia (*Oreochromis niloticus*) fry. *Journal of Noakhali Science and Technology University*, *1*(2), 35-41. Retrieved from https://www.researchgate.net/publication/323801017

Shelton, W. L., Rodriguez-Guerrero, D., & Lopez-Macias, J. (1981). Factors affecting androgen sex reversal of *Tilapia aurea*. *Aquaculture*, *25*, 59-65. DOI: https://doi.org/10.1016/0044-8486(81)90099-5

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. Retrieved from https://www.fisheriesjournal.com/archives/?year=2018&vol=6&issue=3&part= D&Article Id=1598

Soltan, M., Hassaan, M., El-Nagaar, G., Mohammed, W., Abdelhamid1, A., El-Barbary, M., & Mabrouk, E. (2013). Effect of rearing temperature and hormone treatment on sex ratio, survival and body weight of Oreochromis niloticus fry. *Egyptian Journal of Aquatic Biology and Fisheries*, *17*(4), 13-23. DOI: 10.21608/ejabf.2013.2182Tayamen, M. M., & Shelton, W. L. (1978). Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). *Aquaculture*, *14*(4), 349-354. DOI: https://doi.org/10.1016/0044-8486(78)90017-0

Valentim-Zabott, M., Vargas, L., Ribeiro, R. P. R., Piau Jr, R., Torres, M. B. A., Ronnau, M., & Souza, J. C. (2008). Effects of a homeopathic complex in Nile tilapia (*Oreochromis niloticus* L.) on performance, sexual proportion and histology. *Homeopathy*, *97*(4), 190-195. DOI: https://doi.org/10.1016/j.homp.2008.08.007

Varadaj, K., & Pandian, T. J. (1991). Effect of solubilizing 17∝- ethynyltestosterone in three different solvents on sex reversal of Mozambique Tilapia. *The Progressive Fish-Culturist*, *53*(2), 67-71. DOI: https://doi.org/10.1577/1548-8640(1991)053<0067:EOSEIT>2.3.CO;2

Vera Cruz, E. M., & Mair, G. C. (1994). Conditions for effective androgen sex-reversal in *Oreochromis niloticus* (L.) *Aquaculture*, *122*(2-3), 237-248. DOI: https://doi.org/10.1016/0044-8486(94)90513-4

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. Retrieved from https://he01.tci-thaijo.org/index.php/tjvm/article/view/146572

Wassermann, G. J., & Afonso, L. O. B. (2003). Sex reversal in Nile tilapia (*Oreochromis niloticus* Linnaeus) by androgen immersion. *Aquaculture Research*, *34*, 65-71. DOI: https://doi.org/10.1046/j.1365-2109.2003.00795.x

Yamamoto, T. O. (1953). Artificially induced sex-reversal in genotypic males of the medaka (*Oryzias latipes*). *Journal of Experimental Zoology*, *123*(3), 571-594. DOI: https://doi.org/10.1002/jez.1401230309

Yamazaki, F. (1976). Application of hormones in fish culture. *Journal of the Fisheries Research Board of Canada*, *33*(4), 948-958. DOI: https://doi.org/10.1139/f76-122

Yamazaki, F. (1983). Sex control and manipulation in fish. *Aquaculture*, *33*(1-4), 329-354. DOI: https://doi.org/10.1016/0044-8486(83)90413-1

Zanoni, M. A., Leal, T. V., Caetano Filho M., Oliveira, C. A. L. de, & Ribeiro, R. P. (2013). Sex reversal of Nile tilapia (*Oreochromis niloticus*) Supreme variety, fingerlings submitted to different temperatures for sexual differentiation phase. *Semina: Ciências Agrárias*, *34*, 455-466. DOI: https://doi.org/10.5433/1679-0359.2013v34n1p455