Effect of dietary 17-alpha methyltestosterone and 17-beta estradiol on sex reversal of African catfish (*Clarias gariepinus*) juveniles

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Abstract. The present study investigated the effect of two different hormones (17α-methyltestosterone and 17β-estradiol) on the sex reversal of African catfish (*Clarias gariepinus*) juveniles. For each hormone type, a total of three treatments and a control were used having different dosages of 100 mg kg^{-1} (T1), 200 mg kg^{-1} (T2), 300 mg kg^{-1} (T3), and 0 mg kg^{-1} (T0), respectively. The study also gathered the percentage of survival for each hormone. Each hormone was mixed with feed pellet using the alcohol evaporation method. The result of the sex reversal treatment for 17α-methyltestosterone showed that the male population increased as the dosage of hormone increases. Additionally, the sex reversal treatment using 17β-estradiol showed that female populations increased as the dosage of hormones increases. Treatments 1, 2, and 3 of 17α-methyltestosterone showed a significantly higher percentage of males compared to the control having 70.56%, 90.24%, and 100% males respectively. On the other hand, treatments 1, 2, and 3 of 17β-estradiol showed a significantly higher percentage of females compared to the control gathering 76.11%, 92.02%, and 98.89% females respectively. Meanwhile, the highest percentage of survival rate in 17α-methyltestosterone was observed in the control (55±7.0%) which showed a significant difference with treatment 3 (40.33±2.52%) ($p < 0.05$) who gained the lowest survival rate. Treatments 1 and 2 showed no significant difference among other treatments. On the other hand, treatment 3 of 17β-estradiol with 68.33±2.08% gained the highest survival with a significant difference between treatment 1 (56±3.61%) and the control (55±7.0%) but no significant difference with treatment 2 (58.67±3.79%). The present study proved that the use of 17α-methyltestosterone and 17β-estradiol can successfully masculinize and respectively feminize African catfish.

Key Words: alcohol evaporation method, dietary hormones, feminization, masculinization.

Introduction. Sex reversal is widely practiced in aquaculture to produce monosex populations, which improve production efficiency and economic returns. In many cultured fish species, particularly Nile tilapia (*Oreochromis niloticus*), all-male populations are preferred because males generally exhibit faster growth, better feed conversion efficiency, and reduced energy allocation to reproduction, thereby minimizing problems associated with uncontrolled breeding and overpopulation (Paankhao et al., 2025). Hormonal masculinization using 17α-methyltestosterone (MT) remains one of the most effective methods for producing monosex populations in aquaculture (Paankhao et al., 2025). The success of hormonal sex reversal depends on several biological and management factors. These include the quality and uniformity of the hormone-treated feed, the amount of hormone consumed by the fry, feeding frequency, treatment duration, and the developmental stage of the fish at the onset of hormone administration (Paankhao et al., 2025). Appropriate stocking density, environmental conditions, and feed management also influence hormone intake and ultimately determine the effectiveness of masculinization (FAO, 2006; Paankhao et al., 2025). In addition, factors such as fry age, size uniformity, and proper seed production and harvesting practices contribute significantly to successful sex reversal because younger fish during the period of gonadal differentiation respond more effectively to hormonal treatment (FAO, 2006; Paankhao et al., 2025). These predisposing factors play a vital role in the success of sex reversal in fish. In African catfish (*Clarias gariepinus*), size variability could become a problem in sex reversing due to the fish's cannibalistic trait. Size variability might cause social dominance wherein larger individuals possibly predate smaller ones and might lessen the total intake of hormone-treated feeds. Before the sex reversal treatment, the feeding of African catfish fry plays a vital role in achieving uniform sizes or less variability in size. Tubifex worms are among the most commonly used live feeds for catfish fry because of their high nutritional value, which promotes faster growth, lesser variability in size, and higher survival rates than many alternative feeds (Simangunsong et al., 2024). Therefore, using the best feed for fry prior to the sex reversal treatment helps to attain a higher success rate. There are many advantages or benefits of monosex in aquaculture. Its primary benefit is the production of one sex that has a higher growth rate and higher survival rate than the other sex. Other benefits of sex reversal are reduced aggressive interactions, greater uniformity of size at harvest, and reduced unfavorable environmental impacts. But these benefits vary from species to species (Beardmore et al., 2001). The present study aimed to assess which dosages of 17α-methyltestosterone and 17β-estradiol will achieve the highest sex reversal rate by administering different dosages of sex hormones in the diet of African catfish juveniles.

Material and Method

Hormonal sex reversal. The experiment was laid out in a completely randomized design. There were two hormones used in the experiment (17α-methyltestosterone and 17β-estradiol) with different dosages of administration (0, 100, 200, and 300 mg kg^{-1} respectively). Each hormone has a total of three treatments and control (Table 1), all replicated thrice. The period of sex reversal for both hormones lasted for 35 days during February-March 2023 based on the study of Enuekwe & Okonji, (2019).

Table 1

Experimental treatments at different dosages of hormone per kilogram of feed

Treatments	*Dosage/kg of feed*			
17α-methyltestosterone	T0 (0 mg kg^{-1})	T1 (100 mg kg^{-1})	T2 (200 mg kg^{-1})	T3 (300 mg kg^{-1})
17β-estradiol	T0 (0 mg kg^{-1})	T1 (100 mg kg^{-1})	T2 (200 mg kg^{-1})	T3 (300 mg kg^{-1})

Experimental procedure. A total of 1,050 6-day-old African catfish fry weighing about 0.2 grams was used for the experiment. According to Paankhao et al (2025), hormonal sex reversal should be initiated before or during the early stage of gonadal differentiation, when the gonads remain undifferentiated and are most responsive to hormonal manipulation. The fry was collected, transferred, and stocked at the rate of 50 fry per 20-liter capacity aquaria (2.5 pcs L^{-1}). The fry was acclimatized for 24 hours before the experiment. A total of 21 aquaria were used (9 aquaria for each of the two hormones, for a total of 18 aquaria, and additional 3 aquaria for the control, with no hormone addition). The sex hormones (17alpha-methyltestosterone; 17beta-estradiol) were diluted with 1 liter of food grade 95% ethanol, sprayed, and hand-mixed evenly with crumbled shrimp larvae feed at 100, 200, and 300 mg kg^{-1} using the alcohol evaporation method of Guerrero, (1975). The feeds were air-dried for 24 hours before feeding to the fish. The feeds were then fed to the fry 3 times a day at 8:00 am, 12:00 pm, and 4:00 pm respectively. The fry in each treatment group along with its replicates was fed in saturation (*ad libitum*) daily. One hour after feeding, the excess feeds and other wastes were removed from the aquaria by siphoning. The sex reversal period lasted for 35 days. After this period, the fish were transferred to larger tanks and grown for 4 weeks until they reached the size of about 20 cm weighing around 60 grams where their sex can be distinguished phenotypically through their genital papilla. During this phase, the fish was fed in satiation 3 times a day using pre-starter feeds and boiled eggs occasionally. The success rate of each treatment for both hormones as well as the survival rate was gathered at the end of the experiment. The percentage of survival was computed using the formula below:

$$\text{Survival rate} = \frac{\text{Final number of individuals}}{\text{Initial number of individuals}} \times 100$$

Water quality parameters. The water quality parameters such as dissolved oxygen (DO), temperature, pH, and ammonia were monitored daily using YSI multiparameter at 8:00 am, 12:00 pm, and 4:00 pm respectively.

Male and female determination. When the fish reached the size of around 20 cm, they were individually examined to determine their sex. The fish were placed in a tub with the culture water added with 3 mL of 2-phenoxyethanol to calm the fish. Once the fish showed no or slow movement, they were manually examined by looking at their genital papilla. The male genital papilla is elongated and pointed (Figure 1) while the female genital papilla is oval and bulging (Figure 2). When the fish does not yet reach the size of around 20 cm or if the genital papilla is still underdeveloped, the fish remains in the culture tanks to grow for another week. The total number of males and females for each tank was then recorded.

Figure 1. Juvenile male African catfish genital organ.

Figure 2. Juvenile female African catfish genital organ.

Statistical analysis. The water quality parameters, sex reversal rate, and survival rate was analyzed using ANOVA for a completely randomized design using SAS On Demand for Academics. The comparison among means of variables with $p < 0.05$ was conducted using Tukey's honest significant difference (HSD) test.

Results

Sex reversal. The result of the sex reversal treatment showed that both male and female populations increased as the dosage of hormones increased (Figures 3 and 4). The control gathered 46.62% male and 53.38% female populations. Treatments 1, 2, and 3 of 17α-methyltestosterone showed a significantly higher percentage of males compared to the control having 70.56%, 90.24%, and 100% males respectively. On the other hand, treatments 1, 2, and 3 of 17β-estradiol showed a significantly higher percentage of females compared to the control gathering 76.11%, 92.02%, and 98.89% females respectively.

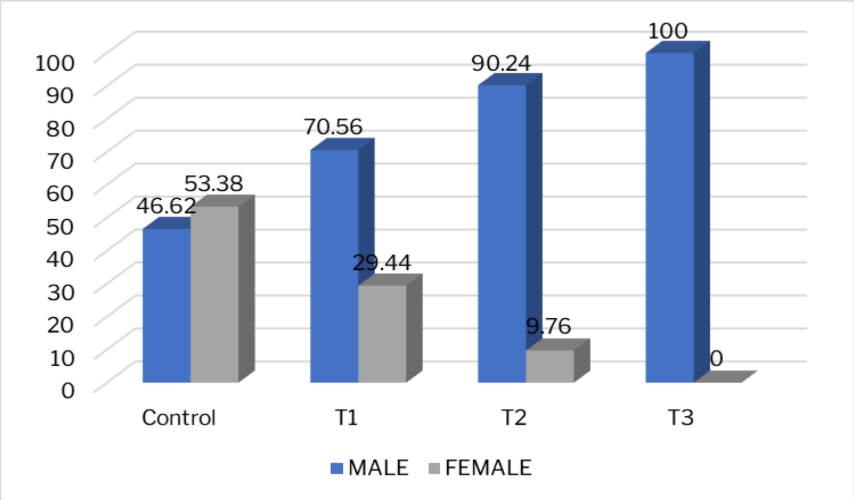

Figure 3. Sex-reversal rate of 17α-methyltestosterone on African catfish.

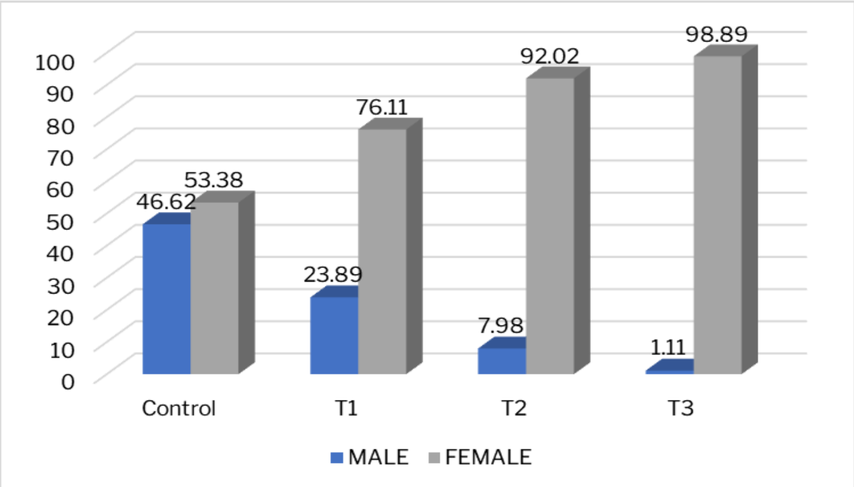

Figure 4. Sex-reversal rate of 17β-estradiol on African catfish.

Survival. The highest percentage of survival rate in 17α-methyltestosterone was observed in the control (55±7.0%) which showed a significant difference with treatment 3 (40.33±2.52%) ($p < 0.05$) who gained the lowest survival rate (Figure 5). Treatments 1 and 2 showed no significant difference among other treatments. On the other hand, treatment 3 of 17β-estradiol with 68.33±2.08% gained the highest survival with a significant difference between treatment 1 (56±3.61%) and the control (55±7.0%) but no significant difference with treatment 2 (58.67±3.79%) (Figure 6).

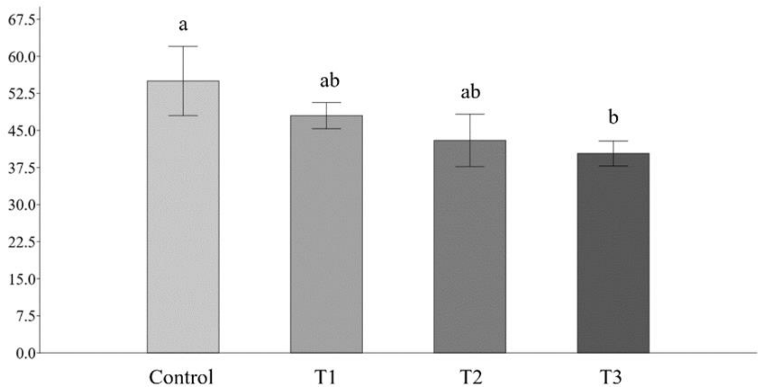

Figure 5. Percentage survival of sex-reversed African catfish using 17α-methyltestosterone.

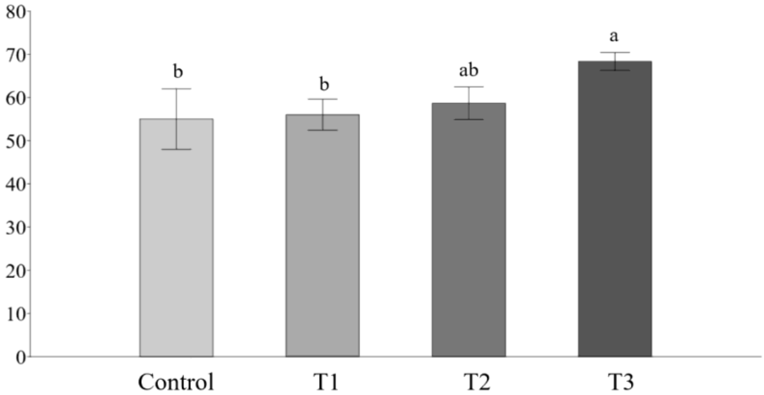

Figure 6. Percentage survival of sex-reversed African catfish using 17β-estradiol.

Water quality parameters. The means of the water quality parameters like temperature, pH, dissolved oxygen (DO), and ammonia were shown to be in the acceptable range throughout the experiment. The means of the treatments of temperature, pH, and DO show no significant difference from each other for both hormones (Tables 2 and 3). However, the mean ammonia of the control from 17α-methyltestosterone showed a significant difference from the mean of treatment 2 (Table 2). In treatments with 17β-estradiol, the control showed a significant difference with treatment 1 (Table 3). Except for that, other treatments showed no significant difference from each other for both hormones.

Table 2

Water quality parameters of hormone-treated African catfish reared for 35 days using 17α-methyltestosterone

Treatments	*Water quality parameters*			
	Temperature (°C)	*pH*	*DO (mg L^{-1})*	*Ammonia (mg L^{-1})*
Control	26.76±0.95	6.67±0.13	3.72±0.62	0.82±0.27^{a}
T1	26.66±1.04	6.69±0.13	3.74±0.60	0.68±0.29^{ab}
T2	26.69±0.99	6.70±0.13	3.72±0.53	0.65±0.31^{b}
T3	26.65±1.01	6.70±0.13	3.72±0.60	0.69±0.33^{ab}

Note: Different superscript in the same column indicates significant difference ($p < 0.05$).

Table 3

Water quality parameters of hormone-treated African catfish reared for 35 days using 17β-estradiol

Treatments	*Water quality parameters*			
	Temperature (°C)	*pH*	*DO (mg L^{-1})*	*Ammonia (mg L^{-1})*
Control	26.76±0.95	6.67±0.13	3.72±0.62	0.82±0.27^{a}
T1	26.67±1.00	6.69±0.13	3.62±0.52	0.64±0.34^{b}
T2	26.73±0.98	6.69±0.14	3.55±0.42	0.75±0.31^{ab}
T3	26.72±0.97	6.69±0.14	3.61±0.49	0.75±0.30^{ab}

Note: Different superscript in the same column indicates significant difference ($p < 0.05$).

Discussion. The use of 17α-methyltestosterone for male sex reversal at 100, 200, and 300 mg kg^{-1} of feeds for 35 days resulted in a 70.56%, 90.24%, and 100% male population respectively which is in agreement with the result of Enuekwe & Okonji, (2019) where they gather 81.95% sex reversal rate using 17α-methyltestosterone at 60 mg kg^{-1} of feeds to African catfish fry for 35 days. Unlike Nile tilapia, where dietary 17α-methyltestosterone has consistently produced very high masculinization rates (> 95%), African catfish generally require higher hormone dosages to obtain comparable male populations (Enuekwe & Okonji, 2019). On the other hand, the use of 17β-estradiol at 100, 200, and 300 mg kg^{-1} of feeds resulted in 76.11%, 92.02%, and 98.89% female populations respectively. These results were in agreement with the findings of Turan et al, (2022) who used an immersion method where 50 μg L^{-1} of 17β-estradiol dissolved in 30 liters of water resulted in a 96% female population. In addition, the study of Xiong et al., (2024) demonstrated that dietary administration of 17β-estradiol successfully produced all-female populations in Chinese longsnout catfish (*Leiocassis longirostris*), confirming the effectiveness of estrogen treatment for feminization when applied during the critical period of gonadal differentiation. The results of this study proved the effectiveness of 17α-methyltestosterone and 17β-estradiol for male and female sex reversal. However, the effectiveness of these hormones may be species-dependent and can be affected by different management conditions (Krol et al., 2014).

In terms of survival, the results indicated that female catfish have higher survival rates than male catfish. These findings were in agreement with the result of Siregar et al., (2021) who reported that 17β-estradiol could lower mortality which was in line with the decrease in cannibalism, thus, increasing the survivability of African catfish. The decrease in mortality which could be due to the decrease in cannibalism can be associated with a low level of aggression (Siregar et al., 2021). On the other hand, the low survivability of treatments with 17α-methyltestosterone can be associated with a higher mortality rate due to cannibalism that was presumably caused by a higher level of aggressiveness. This is supported by Forsatkar et al., (2013) and Zairin et al., (2016) who reported that androgen or male hormones like 17α-methyltestosterone can boost the aggressive behavior of Siamese fighting fish (*Betta splendens*).

In this experiment, the means of the water quality parameters were shown to be in the acceptable range throughout the study period. All parameters showed no significant interaction between treatments except in ammonia which showed a significant difference between treatments. Nevertheless, all the water quality parameters lie between the recommended range for catfish culture. In this study, the mean water temperature across all treatments and the control ranged from 26.65 to 26.76°C, which is within the recommended temperature range of 25-30°C for the optimal growth, metabolism, and survival of African catfish (FAO, 2006; Tan-Fermin et al., 2024). Likewise, the mean pH values remained within the optimal range of 6.5-8.5, which supports normal physiological processes, growth, and reproduction in freshwater fish (Boyd & Tucker, 2012; FAO, 2006). The dissolved oxygen (DO) concentrations in all treatments were maintained within the suitable range of 3-8 mg L^{-1}, which is considered adequate to sustain normal growth, hatchability, and fry survival in African catfish culture (Tan-Fermin et al., 2024; Kabeer et al., 2025). Similarly, the recorded ammonia concentrations remained below the recommended maximum threshold for freshwater aquaculture, indicating favorable water quality conditions for fish health and development (Boyd & Tucker, 2012; FAO, 2006). Nevertheless, in this experiment, the water quality did not negatively affect the results since all parameters fell within the desirable range.

Conclusions. The study proved that the use of 17α-methyltestosterone and 17β-estradiol can successfully masculinize and respectively feminize African catfish juveniles. The androgen 17α-methyltestosterone and estrogen 17β-estradiol can achieve a desirable sex reversal rate in African catfish juveniles at a dose of 300 mg kg^{-1} of feeds administered in the diet. In addition, female catfish showed to have higher survival rates than male catfish. The present study can be a basis for future research on the monosex culture of African catfish.

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Authors contributions. The research design, experimental implementation, data collection, data analysis, and manuscript preparation were conducted by EPE.

Conflicts of interest. The author declares that there is no conflict of interest.

Data availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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