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EFFECT OF *TURRAEA HETEROPHYLLA* EXTRACT SMITH, 1819 ON MASCULINIZATION IN *TILAPIA OREOCHROMIS NILOTICUS* (LINNE, 1758) REARED IN A CONTROLLED ENVIRONMENT IN IVORY COAST

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ABSTRACT

In order to propose an alternative to the use of the hormone 17- α -methyltestosterone for sex reversal in Nile tilapia (*Oreochromis niloticus*), a masculinization study was conducted in a controlled environment using an extract of the plant *Turraea heterophylla* at the Nangui ABROGOUA University fish farm in 2024. Two diets were prepared using a commercial feed (ALIMPOI) containing a dose of *Turraea heterophylla* (30 g/kg) and 17- α -methyltestosterone (70 mg), compared to a control diet. Larvae with an average initial weight of 15 ± 0.01 mg were fed these diets in aquariums at a density of 50 larvae per aquarium for 21 days. The fish were then reared in tanks at a density of 30 fish/m³ (148 fish/tank) and fed their standard diet for 90 days. At the end of the experiment, the average final weights ranged from 46.12 ± 2.59 g to 67.94 ± 2.87 g, depending on the treatment. The best growth and feed conversion ratios were observed in the fish treated with hormones, followed by those treated with *Turraea heterophylla*. The 17- α -methyltestosterone treatment resulted in a 100% masculinization rate, compared to 86.83% for the *Turraea heterophylla* extract and 51.41% for the control group. A survival rate of 96.81 % was observed at the end of the rearing period. This study demonstrated that completely replacing 17 α -methyltestosterone with plant extracts would significantly reduce the production costs of single-sex fry.

Keywords: Sexual Reversal, *Turraea Heterophylla*, *Oreochromis Niloticus*, monosexual, growth

1 INTRODUCTION

In Côte d'Ivoire, the production of *Nile oysterfish* (*Oreochromis niloticus*) faces several obstacles, including: (i) the poor nutritional quality of local feed, (ii) the limited availability of quality imported feed, (iii) the high cost of imported feed, considered prohibitive by industry stakeholders, and, most importantly, (iv) the lack of quality fry. Addressing this last constraint necessarily involves the production of monosex male fry, obtained through the larval sex reversal technique (Lacroix 2004; Lazard 2009). However, in Africa, hormonal reversal using 17-alpha-methyltestosterone, although banned in Europe, Asia, and the United States, remains the most common approach (Baroiller & Toguyeni 1996; FAO 2014), due to its high masculinization rate. However, on this continent, the challenges lie in acquiring suitable exogenous feed for larval feeding, the availability and cost of the hormone 17-alpha-methyltestosterone, and environmental conditions, such as water quality, which strongly influence the masculinization rate and larval survival (Hung et al. 2001; Pangni et al. 2008). Furthermore, this method raises numerous concerns regarding its impact on the environment, the health of aquaculture workers, and that of consumers (Lazard, 2009). In response to these controversies, several authors (Ojha et al. 2016; Yeganeh et al. 2017; Tigoli et al. 2018) have conducted trials on the use of aphrodisiac medicinal plants as natural dietary supplements. Extracts from these plants contain various bioactive principles such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids, essential oils which have been reported to promote various activities such as anti-stress, growth and appetite stimulation, tonicity and immunostimulating and antimicrobial properties during fish production (Mutlen et al. 2019). These various studies have revealed promising results in terms of growth and masculinization rates. Among aphrodisiac plants, *Turraea heterophylla*, commonly known as gouro toothpick, is frequently used in Ivory Coast to treat sexual impotence. This plant is believed to exert inhibitory effects on the action of estrogens. Phytochemical screening of the extract of this Meliaceae has highlighted the presence of phytochemicals such as sterols, terpenes, alkaloids, coumarins, reducing sugars, polyphenols, steroidal saponins, and proteins (Boua et al. 2013). Given the aforementioned benefits of this plant for the masculinization of fry, it appears necessary to investigate the sexual reversal of larvae under controlled conditions. It is in this context that the present study was undertaken to evaluate the effects of treatment with *Turraea heterophylla* extracts on the masculinization of fry and the survival of *Oreochromis niloticus* larvae.

This study aims to experimentally test the incorporation of the medicinal plant *Turraea heterophylla* into the diet of Nile fish (*Oreochromis niloticus*) larvae in a controlled environment, in order to produce rapidly growing, monosex male fry. Specifically, the study involves incorporating crude *Turraea heterophylla* extract into the fish larvae's diet and evaluating its effectiveness on masculinization and fry growth in a controlled environment.

2 MATERIALS AND METHODS

2.1 Larval rearing structure

The experiment was conducted from April 15 to July 15, 2024 at the fish farm of Nangui ABROGOUA University. Three 12 m² happas were used for the production of larvae from male and female broodstock.

The experimental setup consisted of nine aquariums and three tanks. Each aquarium had a water volume of 14.5 liters (Length x Width X Height: 32.4 cm x 21.4 cm x 21 cm). Each aquarium was equipped with an air pump. All these structures were manually supplied with water from a 1 m³ storage and settling tank. The concrete tanks had a usable water volume of 5 m³. The rearing tanks were supplied with water from a 15 m³ settling tank via a PVC piping system and a SMART brand pressure booster.

2.2 Preparation of *Turraea heterophylla* extract

Turraea heterophylla plant used in this study was identified by the villagers and confirmed by the phytochemistry laboratory at Nangui Abrogoua University. Following this identification, a harvesting campaign of *Turraea heterophylla* roots was carried out in the forest in the Gohitafla department in west-central Côte d'Ivoire. For the preparation of the nutrient supplements, the *Turraea heterophylla* roots were chopped, dried in an open-air chamber, then ground (1 mm in diameter) using a locally made hammer mill and passed through a 0.5 mm mesh sieve. After this process, the extract was weighed according to the desired dosage and macerated for 48 hours in alcohol. After 48 hours, the nutrient supplement was weighed and mixed with the extract until a homogeneous powder was obtained. This mixture was then dried in a chamber to obtain a homogeneous, alcohol-free product.

2.3 Formulation of experimental foods

Three experimental diets corresponding to the different treatments were developed using a commercial feed (ALIMPOI) in granulated form, which was ground into a powder using a hammer mill. This feed contains 30% crude protein. These three experimental diets were used to feed the larvae during the experiments. The first diet was formulated from the initial feed containing 30% protein, mixed only with 96% ethanol and dried in the shade. This is the negative control

diet (AT). The second diet was prepared according to Rashid 2010. A hormonal solution was obtained by dissolving 70 mg of 17- α -methyltestosterone in 100 ml of 96% ethanol. The hormonal solution was sprayed onto 1 kg of feed and then dried in the shade for 24 hours. To evaporate the alcohol, the feed was thoroughly mixed and dried under the same conditions as the control. Diet (AH), containing 70 mg/kg of the hormone 17- α -methyltestosterone, was thus obtained. The third diet contained 30 g of crude extract of *Turraea heterophylla*. Specifically, 1 kg of feed was sprayed with the solution obtained after maceration. After mixing, the feed was dried in an air chamber for 24 hours to allow the alcohol to evaporate. After drying, the food was placed in well-covered plastic buckets. The three (3) test diets AT, AH, and AC were thus obtained.

2.4 Production of *Oreochromis niloticus* larvae

Oreochromis niloticus parent species were selected based on the observation of their genital papillae with the naked eye. This identification allowed for our study, 72 females and 36 males were selected for breeding. These broodstock were housed in three 12 m² tanks at a sex ratio of 1 male to 2 females, with a stocking density of 3 individuals/m². The fish were fed for 21 days at a ration rate of 3% of their total biomass using a commercial feed (ALIMPOI). This daily ration was distributed three times, at 9:00 AM, 11:00 AM, and 3:00 PM. The males had an average weight of 316 $\pm$ 6.25 g and the females 257 $\pm$ 7.29 g. Larvae were harvested three weeks after the broodstock were placed in the breeding tank. Larvae were selected using a 3 mm mesh net. Only larvae that passed through the net were retained for further use in the experiment. They had an average weight of 15 $\pm$ 0.01 mg.

2.5 Livestock management and monitoring

After harvesting, the larvae were randomly distributed among nine aquariums, forming three triplicate treatments with 50 fry per aquarium. The batches of larvae, with an initial average weight of 15 $\pm$ 0.01 mg, were fed ad libitum daily throughout the aquarium rearing period. The fish were fed every 30 minutes from 9:00 AM to 4:00 PM. After 21 days of aquarium rearing, the fry was counted, weighed, and transferred to three 5 m³ concrete tanks. The fish were fed four times a day (9:00 AM, 11:00 AM, 1:00 PM, and 4:00 PM) with a commercial feed containing 35% crude protein, at a feeding rate of 23% of the total biomass. Weight gain was assessed after one month of rearing at 7:00 AM. This monitoring allowed for a corresponding adjustment of the amount of feed distributed the following month. At the end of the 90th day of rearing, all fish were captured, manually sexed with methylene blue, and 30% of the fish randomly selected from each batch were individually weighed for statistical comparison tests.

2.6 Monitoring the quality of livestock water

Water quality monitoring of the aquariums was carried out by measuring the parameters pH, dissolved oxygen and temperature *in situ* every 2 days at 7 a.m., 12 p.m. and 5 p.m. The calibrated measuring instruments were switched on, then the different probes were immersed in the water and the desired function was selected in order to obtain the value of the parameter on the dial.

2.7 Determination of zootechnical performance parameters and inversion rates

The parameters taken into account for the comparison between the food treatments were calculated according to the formulas indicated below.

- Survival rate (SR): This is the percentage of individuals that survived at a time (t) of the experiment relative to the number of fish put in charge.
- Specific weight growth rate (SWGR): Expressed as a percentage per day (% D⁻¹), it represents the daily rate of weight growth.
- Average weight gain (AWG): This is the average weight gain over a given time interval.
- Average daily gain (ADG): This is the weight gain per unit of time. It represents the daily weight gain.
- Apparent feed conversion ratio (FCR): This expresses the quantity of feed in kg required to produce 1 kg of net live weight of fish. It is calculated assuming that all the feed provided has been consumed.
- Nutrient quotient (NQ) or feed conversion ratio (FCR): Is the indicator of the efficiency of the food.
- Protein Efficiency Ratio (PER): The protein efficiency ratio reflects the fish's ability to utilize and convert feed proteins into body mass.

2.8 Statistical analysis

The statistical analysis of data for the various zootechnical parameters studied—namely weight gain (WG), daily weight gain (DWG), protein efficiency ratio (PER), nutrient quotient (NQ), survival rate (SR), and water quality—was subjected to a one-way hierarchical classification analysis of variance (ANOVA) using R software version 4.1.3. The objective of the ANOVA was to determine whether there was a significant difference ($P < 0.05$) between the parameters obtained from the different feeding treatments used. For these comparisons, a significance level of 5% was used.

3 RESULTS

3.1 Larval stage in an aquarium

3.1.1 Physicochemical parameters in aquariums

Data relating to the water quality parameters of the aquariums are presented in **Table 1**. The temperatures recorded during the experiment ranged from 27 to 28 °C, with an average between 26.76 and 26.77 °C. Dissolved oxygen values ranged from 1.82 to 7.77 mg/L, with averages between 3.25 ± 0.85 mg/L. and 3.60 ± 0.78 mg/L. As for pH, values ranged from 7.61 to 10.80, with averages of 8.86 and 9.13. Overall, the average values of the parameters studied were similar across all aquariums. One-way analysis of variance (ANOVA-1) showed no statistically significant difference ($p > 0.05$) in water quality between the tanks.

Table 1: Physico-chemical parameter

	PHYSICOCHEMICAL PARAMETERS		
	AH	AC	AT
Temperature (°C)	26.76 ± 1.41^a	26.70 ± 1.20^a	26.77 ± 1.30^a
Dissolved oxygen (mg/l)	3.29 ± 0.56^b	3.25 ± 0.85^b	3.60 ± 0.78^b
PH	9.13 ± 1.24^a	9.13 ± 1.16^a	8.86 ± 1.43^a

The results were expressed as: Mean $\pm$ ECT (standard deviation) of two replicates. On each row, the values (Means $\pm$ ECT) associated with different letters were significantly different ($P < 0.05$) and those sharing at least one letter were not significantly different ($P > 0.05$).; AH: Food containing 70 mg of 17- α -methyl-testosterone; AC: Food containing 30g of *Turraea heterophylla*; At: Control food not containing hormones or *Turraea heterophylla*.

3.1.2 Aquarium growth parameters

Table 2: Growth performance of *Oreochromis niloticus* larvae in aquariums

	Treatment		
parameters	AH	AC	AT
Pi : Initial weight (mg)	15 ± 0.01^a	15 ± 0.01^a	15 ± 0.01^a
Pf: Final weight (mg)	398.33 ± 35.03^a	428 ± 43.11^a	439.87 ± 57.15^a
WG: Weight (mg/day)	383.33 ± 35.03^a	408 ± 43.11^a	424.87 ± 57.15^a
GPj(mg)	18.23 ± 3.25^a	19.42 ± 2.15^a	20.19 ± 2.85^a
SGR (%/day)	14.06 ± 1.34^a	14.57 ± 0.46^a	14.69 ± 0.68^a
NS: Nutrient ratio	2.12 ± 0.58^b	1.82 ± 0.16^b	1.81 ± 0.27^b
CEP	1.71 ± 0.48^b	1.86 ± 0.19^b	1.88 ± 0.25^b
SR: Survival rate (%)	99 ± 0.00^a	99 ± 0.00^a	98 ± 0.01^a

Results were expressed as: Mean $\pm$ SEQ (standard deviation) of two replicates. Within each row, values (Means $\pm$ SEQ) assigned by different letters were significantly different ($P < 0.05$), and those sharing at least one letter were not significantly different ($P > 0.05$). Pmf (mg): mean final weight, GP (mg): weight gain, GPj (mg/day): daily weight gain; TCS (%/day): specific growth rate, CEP: protein efficiency ratio; TS: survival rate; Qn: nutrient quotient.

The results for growth parameters (average final weight, daily weight gain, specific growth rate) and food processing (apparent feed conversion ratio), as well as the protein efficiency ratio and the survival rate of *Oreochromis niloticus* in the larval stage for 21 days, are recorded in **Table 2**.

For the parameter values Final weight (FW) (g), weight gain (WG) (g), daily weight gain (DWG) (g/day), specific growth rate (SGR) (%/day), and protein efficiency ratio (PER): no significant differences were observed between the other feeds pairwise ($P > 0.05$). Survival rates (SR) were 99%, 99%, and 98% for feeds AT, AC, and AH, respectively. After 21 rearing days, the mean final weights were 439.87 ± 57.15 mg, 428 ± 43.11 mg, and 398.33 ± 35.03 mg for feeds AT, AC, and AH, respectively. Similarly, the mean daily weight gain (DWG) values for the AH, AC, and AH diets were 18.23 ± 3.25 mg/day, 19.42 mg/day, and 20.19 mg/day, respectively. The calculated specific growth rates (SGR) ranged from 14.06 (AH), 14.57 (AC), and 14.69 (AT). The mean protein efficiency coefficients calculated over the 21 days ranged from 1.84,

1.82, and 1.95 for the AH, AC, and AT treatments, respectively. However, the one-way analysis of variance (ANOVA) did not show a significant difference between the treatments ($p > 0.05$).

3.2 Pre-growing phase in the pond

3.2.1 Physico-chemical parameters in the basin

Data relating to water quality parameters in the rearing structures (ponds) are presented in **Table 3**. The temperature was elevated in all ponds during the pre-growing phase, with average values ranging from 25.69 ± 1.66 to 25.85 .

± 1.57 °C (Table 3). The dissolved oxygen level and pH of the water in the basins varied respectively from 7.32 ± 0.65 to 8.07 ± 0.87 mg/L and then from 8.93 ± 0.90 to 9.27 ± 1.12 .

Table 3: Physico-chemical parameters in the basin

Physicochemical parameters			
	AH	AC	AT
Temperature (°C)	25.85 ± 1.57^a	25.79 ± 1.68^a	25.69 ± 1.66^a
Dissolved oxygen (mg/l)	7.39 ± 0.81^a	7.32 ± 0.65^a	8.07 ± 0.87^a
PH	8.93 ± 0.90^a	9.27 ± 1.12^a	9.15 ± 1.13^a

The results were expressed as: Mean $\pm$ ECT (standard deviation) of two replicates. On each row, the values (Means $\pm$ ECT) associated with different letters were significantly different ($P < 0.05$) and those sharing at least one letter were not significantly different ($P > 0.05$).; AH: Food containing 70 mg of 17- α -methyl-testosterone; AC: Food containing 30g of *Turraea heterophylla*; At: Control food not containing hormones or *Turraea heterophylla*.

3.2.2 Inversion results

Table 4 shows the treatment results obtained after 21 days of inversion and 60 days of rearing in tanks. The percentage of male fry treated with 17-alpha-methyltestosterone differed significantly ($p < 0.05$) from that of the AC group, as did the control group. A total of three hundred and forty-one (341) male fry were produced after two (3) months of rearing. The inversion success rate was 100% males for the AH treatment, 86.01% for AC, and 51.41% for the AT treatment. However, the female rate after inversion was low, at 13.99% for AC and 48.59% for AT. Pairwise comparison using Tukey's test showed a significant difference between AH and AC, and between AT and AC. The same analysis (Tukey's test) showed a difference between the AH and AT regimens.

Table 4: Proportions of inversion rates in fish

SETTINGS		AH	AC	AT	p-value
Initial workforce		147	148	148	$P > 0.05$
Manual sexing	Final sexing figures	144	143	142	$P > 0.05$
	Number of males	144	123	73	$p < 0.05$
	Number of females	0	20	69	$p < 0.05$
	Non-sexable individuals	0	0	0	-----
	Percentage of males	100%	86.01%	51.41%	$p < 0.05$
	Percentage of females	0%	13.99%	48.59%	$p < 0.05$
	Percentage of unsexed individuals	0%	0%	0%	-----
	Survival rate	97.88	96.62	95.94	$P > 0.05$

3.2.3 Growth parameters

The results of the growth parameters (average final weight, average daily weight gain, specific growth rate) and of food transformation (apparent feed conversion ratio, as well as the survival rate) are presented in **Table 5**.

After 60 days of rearing, the final mean weights were 68.36 ± 2.87 g, 47.97 ± 3.20 g, and 46.52 ± 2.68 g for the AH, AC, and AT treatments, respectively. It can be concluded that the growth of fish treated with the hormone was better than that of those treated with the Guru skin-on diet and the control. However, the fish treated with the hormone showed

superior zootechnical performance compared to those in the other treatments. Nevertheless, there was a significant difference between the treatments ($P < 0.05$). The mean daily weight gain (DWG) values for the AH, AC, and AT diets were 0.79 ± 0.09 g/day, 0.55 ± 0.06 g/day, and 0.54 ± 0.08 g/day, respectively. Regarding specific growth rates (SGR), the values ranged from $8.83 \pm 0.26\%/day$ (AH); $8.10\%/day$ (AC); and $8.00 \pm 0.21\%/day$ (AT), with a significant difference between treatments ($P < 0.05$). Mean feed conversion ratio (FCR) values varied from 1.64 (AH); 1.68 (AC); and 1.67 (AT). Survival rates (SR) were 97.88%, 96.62%, and 95.94% for the AH, AC, and AT diets, respectively, with a significant difference between these rates ($P < 0.05$).

Table 5: Growth performance of *Oreochromis niloticus* larvae in a pond

Food Processing			
Settings	AH	AC	AT
Pi : Initial weight (g)	0.42 ± 0^a	0.42 ± 0^a	0.40 ± 0^a
Pf : Final weight (g)	68.36 ± 2.87^b	47.97 ± 3.20^a	46.52 ± 2.59^a
WG: Weight gain (g)	67.94 ± 8.87^b	47.55 ± 5.20^a	46.12 ± 5.59^a
GPj (g/day)	0.79 ± 0.09^b	0.55 ± 0.06^a	0.54 ± 0.08^a
SGR (%/day)	8.83 ± 0.26^b	8.10 ± 0.18^a	8.00 ± 0.21^a
QN	1.64 ± 0.12^a	1.68 ± 0.07^a	1.67 ± 0.08^a
CEP	3.98 ± 0.57^a	4.27 ± 0.46^a	4.33 ± 0.53^a
SR(%)	97.88	96.62	95.94

Pmf (g): average final weight; GP (g): weight gain; GP (g/day): daily weight gains; TCS (%/day): Specific growth rate; CEP: Protein efficiency ratio; TS: survival rate; Qn: Nutrient quotient; AH: Food containing 70 mg of 17- α -methyl-testosterone; AC: Food containing 30g of *Turraea heterophylla*; AT: Control food not containing hormones or *Turra heterophylla*.

3.2.4 Weight gain

Figure1 shows the evolution of the average weight of *Oreochromis niloticus* fry subjected to treatments (AH, AC, and AT) during 90 days of rearing. After 30 days, the fish in the AH treatment showed greater weight gain than the other fish. In ascending order of weight gain, we have the fish in the AT treatment, then the fish in the AC treatment, and finally the AH fish. The final average weights of the fish in the AH, AC, and AT treatments were 68.36 g, 47.97 g, and 46.52 g, respectively. The tested diets had a significant effect on the fish's growth ($P < 0.05$), and this difference was observed between the treatments.

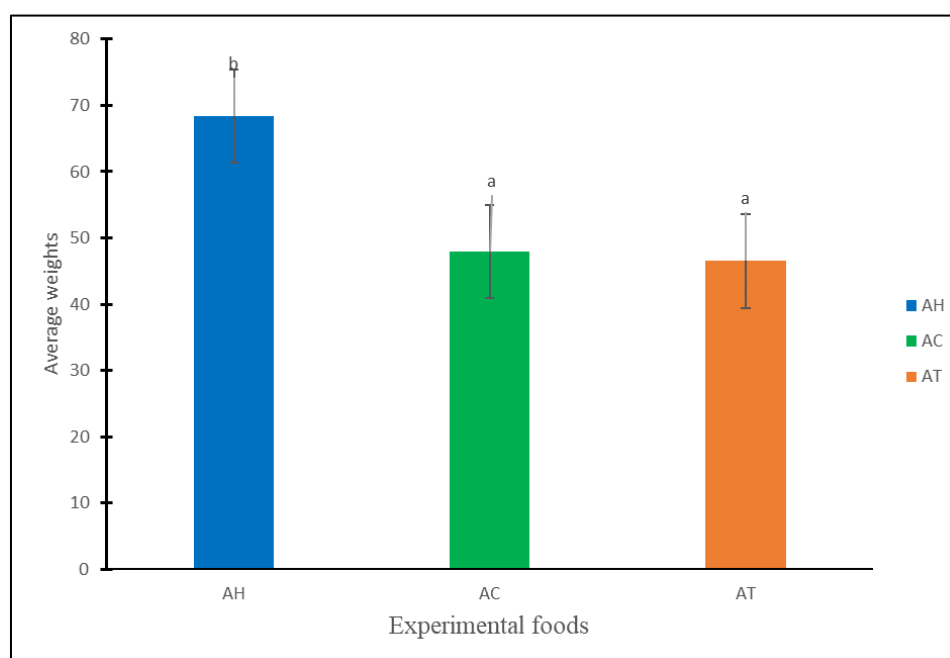


Figure 1: Variation in the average weight of *Oreochromis niloticus* as a function of treatment

4 DISCUSSION

The physicochemical parameters of the aquarium and pond water were within the ranges recommended by Bahnasawy et al. (2009) and Lazard (2009) (temperature ≥ 25 °C, dissolved oxygen ≥ 3 mg/l, pH: 6 to 9, and conductivity: 150 to 450 $\mu\text{S}/\text{cm}$). The average temperatures in the aquarium and pond were 26.74 °C and 25.77 °C, respectively, during this experiment. The pH variations (8.93 and 9.27 in the pond, then 8.86 and 9.13 in the aquarium) were well within the optimal limits for the growth of Nile tilapia (*Oreochromis niloticus*). As indicated by (Mélard 1999) (pH = 6 to 9, dissolved oxygen ≥ 3 mg/L and temperature $\geq 25^\circ\text{C}$). Nile tilapia exhibits a survival capacity in environments with extreme pH levels. However, the optimal pH recommended for its survival and rearing ranges between 7 and 8 (Huet et al. 1972). Dissolved oxygen concentrations are generally good, averaging 3.38 mg/l in aquariums and 7.59 mg/l in ponds. These values comply with European standards for fish farming water quality set by Directive 2006/44/EEC, which requires a dissolved oxygen concentration > 3 mg/l.

Survival rates obtained in rearing structures averaged 98.66% (aquarium) and 96.81% (pond). Similar survival rates for Nile tilapia have been reported in other studies. Specifically, Diabagate et al. (2024) recorded 95–97% survival, while Silue et al. (2024) reported values between 91–95% in aquariums. The results of this work are consistent with those obtained by Tigoli et al. (2018), Mutlen et al. (2019), and Coulibaly et al. (2023), who observed no negative impact on the survival of *Oreochromis niloticus* due to the incorporation of plant extracts into feed as a sex-reversing agent and as an alternative to the use of synthetic hormones. The recorded mortality cases could be related to handling. After treating tilapia fry with the different diets, we observed a high proportion of males (100%) in the diet containing 17- α -Methyltestosterone compared to the other diets, which yielded proportions of 86.01% for diet AC and 51.41% for diet AT. Overall, the control diet, which did not contain plant extract or the hormone 17- α -Methyltestosterone, produced a proportion similar to that observed with the diets containing both 17- α -Methyltestosterone and plant extracts. Among the three diets, diet AH had a significantly higher proportion of males ($p < 0.05$; ANOVA) than diets AC and AT. These results highlight the effects of both 17- α -Methyltestosterone and *Turraea heterophylla*. The high proportion of males obtained with the diet containing 17- α -Methyltestosterone is explained by the fact that the hormone used in this study is an artificial molecule derived from testosterone. Synthetic steroids are considered more effective than natural steroids for hormonal reversal in tilapia such as *Oreochromis niloticus* (Curtis et al. 1991). Regarding the test diets and the diet containing 30 g of *Turraea heterophylla* per kilogram, the results show that the percentage of males obtained with diet AC is lower than that of diet AH but higher than that of the negative control. This difference in the percentage of males observed could be explained by the fact that this diet contains natural steroids. The best proportion of males obtained in our study is higher than that reported by Ouedraogo et al. 2009 which obtained a proportion of 90%, 70.22% and 75.33% respectively in aquarium, in happa and in pond and Khemis & Maamri 2019 (65.3%). The difference between these two results could be explained by the incorporation rate of the hormone 17- α Methyltestosterone (30 $\mu\text{g}/\text{g}$). However, our results are lower than those of Mutlen et al. 2019, who obtained $92.66 \pm 2.08\%$ males using 17 α methyltestosterone at an incorporation rate of 60 mg/kg of feed. Similarly, Marjani et al. 2009 obtained a maximum proportion of males (98.09%) at an incorporation rate of 75 mg of 17 α methyltestosterone/kg of feed. Some studies, including those of Tigoli et al. 2020 obtained 100% males using 17- α methyl-testosterone at an incorporation level of 70 mg/kg of feed.

Turraea heterophylla root extracts to other results; these results are higher than those obtained by Tigoli et al. (2018), who achieved 65.75% with *Garcinia kola* nut extracts. However, it should be noted that the results obtained with *Turraea heterophylla* root extracts are similar to those of Tigoli et al. (2018), but higher than those of Sissao (2014), who obtained 78% males using high temperatures. Similarly, Coulibaly et al. (2023) obtained a maximum proportion of males (100%) with a diet containing *Sarcocephalus latifolius* fruit at an incorporation rate of 120 g/kg of feed; these proportions are higher than those obtained with *Turraea heterophylla* roots in our study. This difference could be explained by the difference in plant species used for each of the experiments.

Based on zootechnical parameters, the specific growth rates (SGR) obtained varied between $8.00 \pm 0.21\%$ / day and $8.83 \pm 0.26\%$ /day. These SGR recorded during this experiment are higher than those (2.79% /day; 2.92% /day) of Khemis. et al. 2019 who used hormonal and thermal treatment and those (4.02 ± 0.38 to $4.88 \pm 0.21\%$ /day) of Mutlen et al. 2019.

The nutrient quotients (NQ) obtained (1.64 ± 0.12 ; 1.68 ± 0.07 ; 1.67 ± 0.08) are similar to those reported by Mebanga et al. 2020 (1.638; 1.112; 1.119) in Cameroon during their work on the optimal dose of the hormone 17-alpha methyl-testosterone (17- α -MT) and the effect of the environment on the masculinization rates of *Oreochromis niloticus* tilapia fry, however they are lower than those of Coulibaly et al. 2023 (2.27 ± 0.07) for a diet containing the extract of the fruit of *Sarcocephalus latifolius* intended for *Oreochromis niloticus*.

Indeed, the highest final average weight, average daily growth, and feed conversion efficiency were obtained with the AH dietary treatment. This result appears to be linked to the anabolic effects of 17-alpha-methyltestosterone (Pandian

& Sheela 1995 and Flynn & Benfey 2007). Consequently, androgen-treated Tilapia fry generally exhibit faster growth than untreated individuals (Baroiller & Toguyeni 1996).

5 CONCLUSION

This study highlights the possibility of incorporating doses of *Turraea heterophylla* into the diet to induce sex reversal. The different rearing phases of the larvae revealed that the physicochemical parameters measured throughout the experiment appear perfectly suited to *Oreochromis niloticus*, as all recorded values fall within the optimal range. The sex ratio was determined by manual sexing of the fish obtained. The results lead to the conclusion that feeds containing 30 g/kg of *Turraea heterophylla* can be incorporated into the diet of *Oreochromis niloticus* to promote sex reversal. The masculinization rate recorded with the AC treatment is satisfactory, reaching 86.01%, with a commendable survival rate of 96.62% over a 90-day rearing period. This work demonstrates that incorporating *Turraea heterophylla* into the diet of *Oreochromis niloticus* larvae leads to significant sex reversal towards males, as well as a notable improvement in fish growth performance. Despite the high proportions of males obtained, these remain below the ideal requirement of a 100% male population.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no competing financial interests or known personal relationships that could have influenced the work presented in this article.

Statement of informed consent

Informed consent was obtained from all authors included in the study.

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