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Evaluation of the efficacy of 17α-methyltestosterone incorporated into feed on the rate of masculinization in *Oreochromis niloticus* fry in the Upper Sassandra region (Ivory Coast)

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Abstract

This study, conducted at the fish hatchery of the Groupe Général AGRO owned by Mr. OUATTARA Yessorifiala Charles, located in GONATE, aims to produce high-quality, single-sex male fingerlings. The objective is to evaluate the dose of 17α-MT (methyl-testosterone) required for a high sex-reversal rate and to optimize the growth of male *Oreochromis niloticus* fingerlings by adding this hormone to their feed for 28 days. Forty thousand (40,000) male fingerlings were produced with a 95% success rate over three months. This study can be replicated using other methods of tilapia sex reversal. To ensure the sector's sustainable development, it is proposed to strengthen the technical and financial capacities of stakeholders.

Keywords: Evaluation; Feed; Fish Hatchery; Methyltestosteron; *Oreochromis niloticus*

1. Introduction

In Côte d'Ivoire, fish farming was introduced in the 1940s by the colonial administration [1]. It has grown over the years and is now a priority in the livestock sector. The country's natural assets for aquaculture production are characterized by its access to the Atlantic Ocean and the abundance of its inland waters. It is estimated that nearly 60% of the Ivorian territory is served by a permanent hydrographic network [2]. However, this significant potential remains largely untapped, and the contribution of fish farming to total fisheries production remains negligible. In fact, domestic fish production accounts for only 4% of the Ivorian population's estimated needs of 615,000 metric tons. The issue of meeting the population's needs is becoming increasingly concerning given population growth (25,123,000 inhabitants in 2020) and the importance of fish to Ivorian consumers (per capita consumption estimated at 24 kg/year/person [2]. On the other hand, if appropriate solutions to these constraints are found including through public-private partnerships this would enable domestic production to fully meet the demand for fish, prevent significant outflows of foreign currency, and guarantee sustainable jobs for the youth of Côte d'Ivoire. Given this multitude of constraints on the development of fish farming and the increasing intensification of fish farms, the production of *Oreochromis niloticus* fingerlings often becomes a critical issue for the continuity of commercial production and, consequently, for the profitability of the production system. This study aims to determine the effectiveness of the technique for producing single-sex male *Oreochromis niloticus* fry using the hormone 17α-methyl testosterone induced in the feed.

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2. Materials and methods

2.1. Presentation of Study area

The experiments were conducted at the fish hatchery of the GENERAL AGRO Group owned by Mr. OUATTARA Yessorifiala Charles, located in GONATE at geographic coordinates (6°54’20.538’’ N; 6°15’52.81776’’ W), in the Daloa department (Central-West, Côte d’Ivoire) . This region covers an area of 17,761 km^2. The Daloa department is bordered to the north by the Vavoua department, to the south by the Issia department, to the west by the Zoukougbeu department, and to the east by the Zuénoula and Bouaflé departments. According to the 2021 General Population and Housing Census (RGPH), this city alone has a population of 421,879 today (Figure 1).

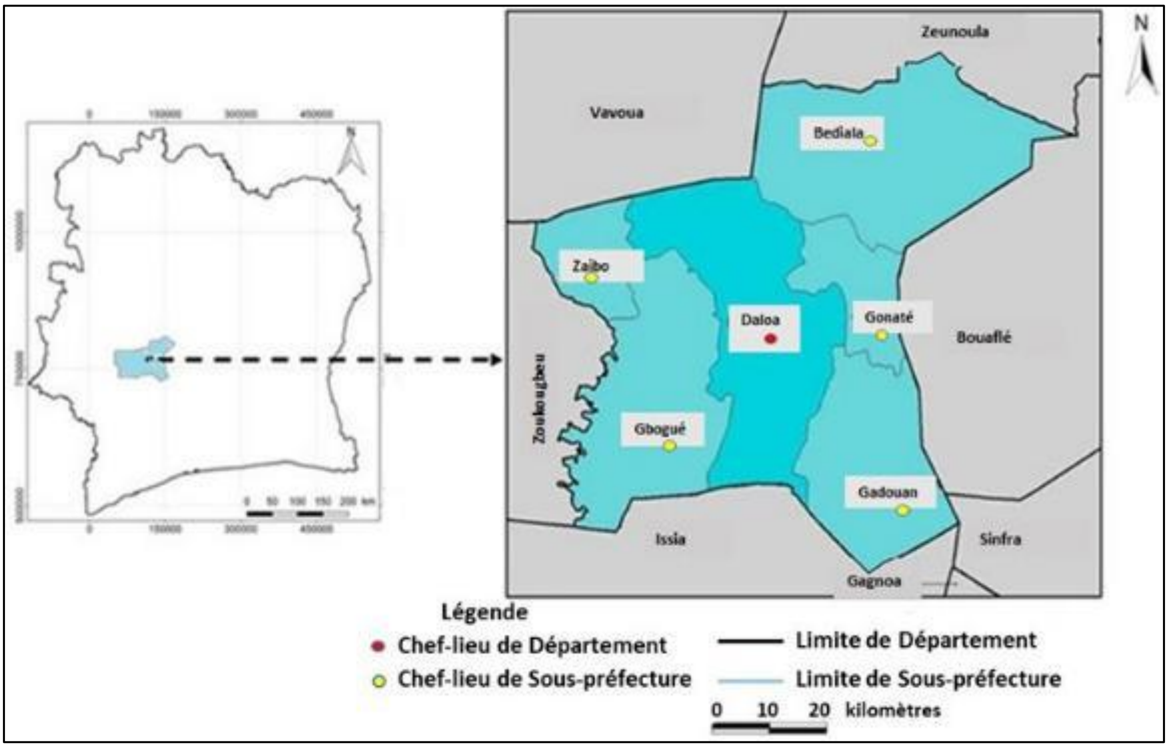

Figure 1 Location of the study site [3]

2.2. Material

This study required the use of the following technical equipment: a Garmin GPS (Global Positioning System) to record the station’s geographic coordinates, a notebook for taking notes and recording information collected in the field, 6 tanks, each with a capacity of 10 m^3, for rearing fry; and instruments for measuring physicochemical parameters, specifically a multi-parameter meter used to measure conductivity, pH, water temperature, dissolved oxygen, and total dissolved solids. RAANAN FISH FEED powdered fish feed with a protein content of 40% and a feed conversion ratio of 1.3, 17α-methyl testosterone, 95% ethanol, a WANT WT-B digital electronic scale, and a pH meter with a range of 0.00–14.00. The biological material consists of 40,000 fingerlings of the fish species *Oreochromis niloticus*

2.3. Methods

2.3.1. Preparation of Rearing Facilities

Various facilities are used for rearing tilapia larvae, including tanks, ponds, and even reservoirs from hydro-agricultural dams, which are supplied with water on a more or less continuous or intermittent basis. However, for the purposes of this study, we opted for concrete tanks. The tanks and small laboratory equipment were cleaned, disinfected, and left to dry for at least 48 hours before the larvae were placed in them. To conduct this study, we used: 6 breeding tanks of 10 m^3 capacity, for four treatments (control (1 kg), t1 (1 kg), t2 (1 kg), t3 (1 kg), t4 (1 kg)).

2.3.2. Water Quality Monitoring

The water supply comes from an on-site well via a properly designed piping system. Water quality is monitored by measuring physicochemical parameters. Temperature is measured using a thermometer three times a day (9 a.m., 1 p.m., and 5 p.m.). PH is measured once a day using a pH meter with a range of 0.00 to 14.00.

2.3.3. Feeding and Rearing Practices

A portion of the harvested fry is transferred to outdoor concrete tanks measuring 1 m x 1 m x 1 m, at a rate of 1,000 individuals per tank. These tanks were exposed to natural temperature and light conditions. The individuals treated with 17-alpha-methyltestosterone were distributed across two tanks. The control group was reared in one tank. The water temperature in the tanks was recorded twice daily at 7 a.m. and 5 p.m. and the water in the tanks was changed every two weeks. The mixed powdered feed corresponded to the following treatments:

- T1 : larvae fed a diet containing a dose of 50 mg of hormone,
- T2 : larvae fed a diet containing a dose of 55 mg of hormone,
- T3 : larvae fed a diet containing a dose of 60 mg of hormone,
- T4 : larvae fed a diet containing a dose of 65 mg of hormone.

Daily feed rations were manually distributed by hand four times a day (7 a.m., 11 a.m. 2 p.m., and 5 p.m.), taking care to ensure even distribution so that each tilapia larva had access to a sufficient amount of food [4, 5]. Weight and length measurements were taken every two weeks until the end of the experiment.

2.3.4. Preparation of the 17α-MT feed

The dose of 17α-MT for sex reversal in tilapia fry is 60 mg/kg for 21 days. The 17α-MT feed was prepared as follows [6]

- Measure 60 ml of buffer solution using a 100-ml graduated pipette ;
- Measure 500 ml of ethanol or denatured alcohol using a 1-liter graduated cylinder;
- Mix 60 ml of buffer solution with 500 ml of ethanol (or denatured alcohol) in a 1-liter graduated beaker to obtain the 17α-MT solution ;
- Weigh 1 kg of fine feed (Raanan feed with 40% protein is recommended);
- Wear rubber gloves (required) and a face mask;
- Thoroughly mix 1 kg of feed with 560 ml of 17α-MT solution in a plastic container ;
- Dry the mixture for 24 hours in a cool, dry place to allow the ethanol to evaporate;
- Store the 17α-MT feed in a cool place, away from light.

The powdered feed is weighed and added to this mixture until a homogeneous substance is obtained. This product is dried in a well-ventilated area away from sunlight for 48 hours, then stored in the refrigerator at 4 °C.

At the end of the trial, biological parameters were determined based on the collected data. The calculation methods are as follows:

2.3.5. Monitoring of zootechnical parameters [7,8,9,10]

(1) **Survival rate (SR)**

SR (%) = (Final number of fish / Initial number of fish) x 100

(2) **Average daily gain (ADG)**

ADG (g/day) = (Final average weight – Initial average weight) g / rearing duration (days)

(3) **Specific weight growth rate (SWGR)**

SWGR (%/day) = [ln (final average weight) – ln (initial average weight) / Number of days] x 100

(4) **Specific linear growth rate (SLGR)**

SLGR (%/day) = [ln (average final length) – ln (average initial length) / Number of days] x 100

2.4. Statistical analysis

STATISTICA 7.1 software was used to perform the various statistical analyses.

3. Results

3.1. Physicochemical parameters of the water

The average values obtained for the physicochemical parameters of the medium during the experiment are shown in Table 1. During the treatment period, with the exception of temperature, statistical analysis revealed no significant differences ($p < 0.05$) between the physicochemical quality of the water in the control aquariums and that in the aquariums subjected to treatment at 37 °C.

At the end of the experiment, the values for temperature, pH, total dissolved solids (TDS), dissolved oxygen (O_2), and conductivity (C) measured in the tanks showed no significant differences ($p < 0.05$). This indicates homogeneity in the physicochemical quality of the water in the closed-loop tanks. As for the outdoor tanks, the average values of these parameters obtained are closely linked to natural temperature conditions. The control group showed high values for these parameters. The values recorded during the experiment demonstrate the ecological tolerance ranges of the species O. niloticus (Table 1).

Table 1 Average values of the water's physicochemical parameters**.**

Treatments	Temperature (°C)	PH	Conductivity (µs /cm)	Dissolved oxygen (mg/l)	TDS (µS/cm)
T1	38.00 ±0.203	6.79±0.543	27.05 ± 5.063	5.79 ± 0.543	47.05±5.73
T2	35.54 ±0.64b	6.74±0.563	21.62 ± 5.7	5.74 ± 0.63	41.42± 4.7
T3	32.82 ± l.21c	6.20±0.43b	26,27 ± 5.903	6.20±0.53b	36.27±5.40
T4	30.85 ± 1.33c	5.81±0.39b	26.9 ± 5,93	4.81 ± 0.4b	56.9 ± 5.93
witness	29.20 ± 1.28d	7140.51c	87.65 ± 18.4b	6.14 ± 0.41c	87.65±16.4b

Values (mean ± standard deviation) in the same column marked with different letters are significantly different ($p < 0.05$). DS : Dissolved Solids; pH: Hydrogen Ion Concentration

3.2. Changes in zootechnical parameters of Oreochromis niloticus fingerlings

Table 2 shows relatively high weights, with a final weight in the T2 treatment group (1194.3 ± 129.83a) and a very low weight in the control group (847.3 ± 43.09b). No significant differences were observed between the mean weights obtained (at $p < 0.05$). After hormonal treatment, growth was faster, and the means obtained were similar: 16.19 ± 0.29ab,

16.76 ± 0.40a, 16.55 ± 0.38a, 16.57 ± 0.39a ($p < 0.05$). During tank rearing, the growth of the control groups remained below that of the treated groups (11.75 ± 0.32b) ; statistical analysis reveals a significant difference between the mean weights of the different treatments during this experiment ($p < 0.05$). Analysis of the weight gains obtained during this experiment indicates a significant difference ($p < 0.05$) between the performance of the hormone-treated groups and that of the control groups, regardless of age and heat treatment. Length gain rates ranging from 38.18 ± 1.27b to 43.43 ± 1.01a were obtained; a significant difference was observed between the mean values of the treated groups and those of the control groups. Regarding feed efficiency, the calculated feed conversion ratios did not indicate a significant difference ($p < 0.05$) between the treated groups and the control groups throughout the experimental period.

Table 2 Growth parameters and survival rates of tilapia after 28 days of treatment

	Tc	T1	T2	T3	T4
Initial weight (mg)	10.69 ± 0.01a	10.88 ± 0.03a	10.88 ± 0.03a	10.87 ± 0.03ab	10.85 ± 0.00b
Final weight (mg)	847.3±43.09b	1014.3±83.03ab	1194.3 ±129.83a	1122.3 ±113.14a	1126.3 ±118.85a
Growth rate	11.75 ±0.32b	16.16 ±0.29ab	16.76 ±0.40a	16.55 ±0.38a	16.57 ±0.39a
Daily weight gain	31.66 ±2.97b	35.84 ±2.97ab	42.27 ±4.64a	39.70 ±4.04a	29.84 ±4.24a
Weight gain (mg)	486.4±83.18b	1103.±73.03ab	1183.4 ±129.84a	1121.5 ±113.17a	1115.5 ±118.85a
% Weight gain	844 ±74.65b	2220 ±462.86ab	10275±1201.52a	102.30±1066.79a	1021.1±1045.43a
Initial length (mm)	0.85 ±0.01a	0.84 ±0.01a	0.65 ±0.02a	0.85 ±0.02a	0.86 ±0.01a
Final length (mm)	18.88 ±1.88b	34.02 ±1.26b	43.29 ±0.05a	41.43 ±1.46ab	41.05 ±0.51ab
Length gain (mm)	28.03 ±1.87b	38.18 ±1.27b	42.43 ±1.01a	40.58 ±1.48ab	38.19 ±0.51ab
Average length (mm)	1.36 ±0.07b	1.36 ±0.05b	1.52 ±0.04a	1.45 ±0.05ab	1.44 ±0.02ab

4. Discussion

The mean values for T1, T2, T3, T4, and the control group were 38, 35.54, 32.82, 30.85, and 29.20 °C, respectively. The effect of temperature appears more favorable than that observed in Lake Rudolph, where only 61% of males were recorded after 10 days of treatment at 36 °C [11]. However, the variation in temperature observed in these different populations could be attributed to the timing of the heat treatment. In contrast, the domesticated strains from Bouaké and Manzala, treated at 36.5 °C and between 35–37 °C for 28 days, respectively, showed male proportions ranging from 78% to 100%, indicating a positive response to heat treatment [12].

During the experiment, relatively low survival rates were observed. This could be attributed to the physicochemical production parameters mentioned in the results. Mortality rates were particularly high during the 28-day period of hormonal inversion. According to [13], mortality rates are generally high in the early stages of development, particularly during hormonal treatments, before stabilizing in later stages of ontogenesis. [14] study evaluated the impact of different doses of 17α-MT (30, 60, and 90 mg/kg of feed) on a control group during the first 28 days of feeding and revealed different results compared to our study, which tested doses of 50, 55, 60, and 65 mg of 17α-MT/kg of feed. Our results also confirmed the androgenic effect of 17α-MT on the development of male sexual characteristics, with 88% of males in the T1 group treated with 50 mg of 17α-MT, 82% in T2 (55 mg), and 80% in T4 (65 mg), while the control group showed a more balanced distribution (60 mg) of males and 48% females. However, it appears that certain doses of 17α-MT (notably 60 mg) have a more pronounced androgenic effect on *Oreochromis niloticus* fry compared to other doses. In general, the growth of fry subjected to hormonal reversal is quite impressive, reaching 200 g in just two months and fifteen days. [15] compared the growth performance of different strains of *Oreochromis niloticus* and found that, among all strains, treatment with 17α-MT resulted in a final size 10.7% larger than that of untreated fish. These results are consistent with the rapid growth of the tilapia, which reached 200 g. All of these male fish were placed in a happa to minimize the risk of cannibalism.

The mechanism by which temperature influences the process of sexual differentiation appears to be linked to its ability to alter the structure and function of proteins and macromolecules [16]. This can induce specific proteins that disrupt the normal differentiation cascade, notably by inhibiting the expression of the aromatase gene, either by interacting with the transcription factors of this gene or by directly repressing its activity [17]. The biochemical process by which high temperatures inhibit aromatase gene expression may be due to hypermethylation of its promoter, as gene expression requires low promoter methylation.

Excessive methylation of the aromatase gene promoter following exposure to high temperatures has been demonstrated in *Sparus aurata* [18], *Dicentrarchus labrax* [19], and *Cynoglossus semilaevis* [20]. Other studies have also shown that temperature can influence sexual differentiation during embryonic development by affecting the expression of sex-determining genes in the hypothalamus [21]. This results in the regulation of steroid secretions involved in sexual differentiation [22], thereby directing the expression of certain genes such as cypl 9 al and amh [23,24]. In this context, the brain aromatase, cypl 9al b, is thought to play a key role in the regulation of phenotypic sex [21,25,24]. It is also important to note that the inversion rate of a given population is strongly linked to the genetic specificity of their ancestry and is likely to be heritable [17,12,11,26,24]. The observed rates of masculinization can vary in either direction (increase or decrease) depending on the parental pairs used.

5. Conclusion

The study resulted in the production of 40,000 male inverted fry over a 6-month period. This production could have been improved if the hatchery had been equipped with a permanent aeration and filtration system for well water, a thermostat to regulate the water temperature in the conditioning tanks, generators to ensure a continuous supply of electricity, and a regular supply of high-quality feed. Similarly, the effectiveness of hormonal treatments varies depending on the environmental conditions in which the breeding facilities are maintained. Treatment effectiveness increases with control over environmental factors such as temperature, light, and water quality. Although the treatments did not result in 100% males, the rates achieved (88%) clearly demonstrate that the dose used is effective in inducing hormonal sex reversal.

Compliance with ethical standards

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Declaration of Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

Author's contribution

The first author collected, processed and drafted this article. The other authors read and corrected the manuscript. All the authors read and approved the final manuscript.

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