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RESEARCH ARTICLE

# NATURAL PRODUCTION OF MONOSEX MALE NILE TILAPIA FRY USING CARICA PAPAYA EXTRACTS FOR SUSTAINABLE AQUACULTURE IN CÔTE D'IVOIRE

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## ABSTRACT

Production of Nile tilapia (*Oreochromis niloticus*) is constrained by early and uncontrolled reproduction in culture, leading to overpopulation and reduced growth performance. Although sex reversal using 17-α-methyltestosterone effectively produces male monosex populations, its use raises health, environmental, and regulatory concerns. In this context, *Carica papaya* emerges as a promising natural alternative. This study evaluated the efficacy of *Carica papaya* seed powders as a substitute for 17-α-methyltestosterone in sex reversal of Nile tilapia, as well as their effects on growth, survival, and water physico-chemical quality in two strains ("Bouaké" and "Brazil"). Sexually undifferentiated larvae were exposed for 28 days to five treatments: a negative control, a positive control (70 mg/kg of 17-α-methyltestosterone), and three diets containing *Carica* papaya (10, 30, and 50 g/kg), in triplicate. Data were analyzed using two-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Water physico-chemical parameters remained stable across treatments ($p > 0.05$), indicating no confounding environmental effects. *Carica papaya* powders induced significant masculinization, reaching 85-90 % males in the "Bouaké" strain and 78-82 % in the "Brazil" strain, compared with ≥ 96 % in the 17-α-methyltestosterone treatment. The 10 g/kg dose produced the best growth performance, comparable to the hormonal treatment, while higher doses significantly reduced growth. No significant adverse effects on survival or condition factor were observed. In conclusion, *Carica papaya* seeds constitute a credible natural alternative to 17-α-methyltestosterone for producing male monosex populations of *Oreochromis niloticus*, offering a relevant compromise between biological efficacy, environmental safety, and sustainability in semi-intensive aquaculture systems.



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# INTRODUCTION

Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) aquaculture plays a pivotal role in food security and the fisheries economy of tropical countries, particularly in Côte d'Ivoire. However, the early and excessive reproduction of this species under farming conditions leads to pond overcrowding, increased competition for resources, and reduced growth performance, ultimately resulting in fish of low commercial value[1]. Therefore, effective control of reproduction represents a key lever for improving productivity and sustainability in aquaculture systems[2]. Among the strategies developed to mitigate this proliferation, the production of all-male populations has emerged as the most efficient approach, owing to the faster growth rate and higher yield of males compared to females[3-4]. This strategy mainly relies on hormonal sex reversal through the administration of synthetic androgens, particularly 17-α-methyltestosterone, which enables the achievement of high masculinization rates[5-6]. Nevertheless, the use of 17-α-methyltestosterone raises serious health and environmental concerns due to its potential carcinogenic effects and its negative impacts on aquatic ecosystems[7-9]. These risks have led to regulatory restrictions in several countries[10], thereby reinforcing the need to develop natural, safe, and environmentally compatible alternatives in line with the principles of sustainable aquaculture. In this context, plant extracts have attracted increasing interest as potential masculinizing agents. Several plant species have demonstrated significant androgenic effects, yielding masculinization rates comparable to those obtained with 17-α-methyltestosterone[2]. Among them, *Carica papaya* (Linn.) stands out because of its wide availability in tropical regions and its richness in bioactive compounds, including benzyl isothiocyanate, β-sitosterol, and oleanolic glycosides, which are known for their endocrine-modulating properties[11]. Previous studies have reported masculinization rates of up to 80 % in various tilapia species following the administration of *Carica papaya* seed extracts. However, these studies remain limited by an incomplete understanding of the underlying mechanisms, a often partial assessment of associated zootechnical performance, and a lack of data under local farming conditions, particularly in West Africa. The present study aims to address these gaps by evaluating the effectiveness of incorporating *Carica papaya* extracts into the diet of *Oreochromis niloticus* fry for the production of all-male populations. Specifically, this study seeks to assess the resulting masculinization rate and to evaluate the effects on fish growth and survival under Ivorian aquaculture conditions.

# MATERIALS AND METHODS

**Experimental fish and sex reversal Agents:** The experiments were conducted using larvae from two strains ("Bouaké" and "Brazil") of Nile tilapia (*Oreochromis niloticus*), produced at the Wifish fish farm (latitude 5.81° N; longitude 5.69° W). Both plant-based and chemical sex reversal agents were used in this study. The plant material consisted of leaves of *Carica papaya*, whose botanical identification was confirmed by the National Floristic Center (CNF) at Félix Houphouët-Boigny University (UFHB), Abidjan-Cocody. The chemical products included 17-α-methyltestosterone and 96 % ethanol. The synthetic hormone was incorporated into the experimental diets to induce sex reversal, while ethanol was used as a solvent to facilitate the incorporation of both 17-α-methyltestosterone and *Carica papaya* powders into the feeds.

**Larval production and experimental design:** Larvae with an initial mean weight of 0,01 ± 0.02 mg were obtained from broodstock of the "Bouaké" and "Brazil" strains. The broodstock consisted of females with an average body weight of 110 ± 10 g and males weighing 206 ± 19 g, stocked at a sex ratio of 3:1 (three females per male). For each strain, 80 broodfish (20 males and 60 females) were stocked in 12,5 m² hapas installed in 1000 m² earthen ponds[12]. Two spawning hapas were used per strain. Every 14 days, larvae were harvested by reducing the hapa surface area, allowing larvae to aggregate at the water surface and be collected using a 1 mm mesh net[12]. Prior to restocking, females were individually examined and those still carrying eggs were removed. For each strain, 4,500 larvae were randomly distributed into fifteen (15) 1 m² hapas at a density of 300 larvae per hapa, corresponding to five (5) dietary treatments with three replicates each. Each treatment was conducted in a separate 1000 m² pond, resulting in a total of five ponds used for the experiment.

**Diet preparation:** Five experimental diets were prepared from a commercial feed containing 48 % crude protein, corresponding to five treatments per strain, including two control diets and three test diets. The basal feed was manufactured by Raanan Fish Feed (Table 1). The positive control diet was prepared following the protocol described by Rashid[13]. A hormonal solution was obtained by dissolving 70 mg of 17-α-methyltestosterone in 100 mL of 96 % ethanol. This solution was sprayed onto 1 kg of the basal feed, thoroughly mixed, and air-dried at room temperature in the dark to allow complete ethanol evaporation. The resulting positive control diet contained 70 $mg \cdot kg^{-1}$ of 17-α-methyltestosterone while maintaining a crude protein level of 48 %. For the negative control diet, the basal feed was sprayed with ethanol only and dried under the same conditions. For the test diets, fresh *Carica papaya* leaves were washed with distilled water, cut into small pieces, and air-dried at room temperature for seven days. The dried leaves were ground into powder and sieved through a 0,1 mm mesh. Powder samples (10, 30, and 50 g) were macerated separately in 50, 100, and 150 mL of 96 % ethanol, respectively, for 24 h at room temperature and protected from light. Each macerate was then sprayed onto 1 kg of the basal feed, thoroughly mixed, and air-dried in the dark at room temperature. The three test diets contained 10, 30, and 50 g/kg of *Carica papaya* powder, respectively, with a constant protein level of 48 %. Details of the experimental design are presented in Table 2.

**Feeding management:** Hormonal and plant-based treatments were applied for 28 days. During the first three weeks, larvae were fed daily at 100 % of their biomass, followed by 20 % during the fourth week. Daily rations were manually distributed at hourly intervals, totaling 10 meals per day between 07:00 and 16:00. The feeding rates applied were 11 %, 9 %, 7 %, and 6 % of total live weight during the first, second, third, and fourth weeks, respectively[12]. After the treatment period, fingerlings were fed at 13 % of body weight during the first post-treatment month, 9 % during the second month, and 6 % until the end of the rearing cycle. Feed was manually broadcast, with feeding frequencies of 10 meals per day during the first month, 9 meals during the second month, and 6 meals per day thereafter, between 08:00 and 18:00. Feeding rates were adjusted weekly based on fish growth.

**Measurement of water physico-chemical parameters:** Water quality parameters, including temperature, dissolved oxygen, pH, and transparency, were measured weekly in situ before feeding, between 07:00 and 08:00. Measuring devices were calibrated and powered on for at least 10 minutes prior to use. Probes were then immersed in the water to record temperature, dissolved oxygen concentration, and pH values displayed on the screen. Water transparency was assessed using a Secchi disk, which was lowered into the water until it completely disappeared and then slowly raised. The depth at which the disk became visible again was recorded as the transparency value.

**Zootechnical performance evaluation:** Weekly sampling was conducted on 25 % of the stocked population to monitor weight gain and adjust feeding rates based on total biomass. At the end of the 28 day treatment period, all fish were counted, and 30 individuals per hapa were randomly sampled to measure total length, standard length, and individual body weight. At the end of the post-treatment period (90 days), all fish were harvested and manually sexed. Sex determination was confirmed by gonadal observation following dissection. Based on these data, several zootechnical performance parameters were calculated, including sex ratio, survival rate, mean weight gain, daily weight gain, specific growth rate, feed conversion ratio, condition factor, and survival rate (Table 3).

**Statistical analysis:** Zootechnical parameters, including final weight, daily weight gain, specific growth rate, feed conversion ratio, condition factor, and survival rate, were analyzed using a two-way analysis of variance (two-way ANOVA). When significant differences were detected ($p < 0.05$), Tukey's honestly significant difference (HSD) test was applied for pairwise comparison of means. The *O. niloticus* strain ("Bouaké" vs. "Brazil") and the sex reversal treatment (17-α-methyltestosterone vs. *Carica papaya* powder) were considered as fixed factors in the two-way ANOVA. All statistical analyses were performed using STATISTICA software version 7.

# RESULTS

**Water physico-chemical parameters:** The physico-chemical parameters of the rearing water are presented in Table 4. No significant differences were observed among treatments for either the "Bouaké" or "Brazil" strains of *Oreochromis niloticus* (two-way ANOVA, $p > 0.05$). Water temperature ranged from 26,67 ± 1,67 °C to 29,33 ± 0,88 °C across treatments.

**Table 1. Biochemical composition of the basal commercial feed**

| Feed composition | Feed composition |
|---|---|
| Crude protein | 48 % |
| Crude lipid | 7,0 % |
| Calcium | 2,0 % |
| Phosphorus | 1,3 % |
| Ash | 8,0 % |
| Crude fiber | 2,5 % |
| Lysine | 2,4 % |
| Methionine + cystine | 1,9 % |
| Vitamin A | 30 000 UI/kg |
| Vitamin E | 340 UI/kg |
| Vitamin C | 600 mg/kg |
| Ingrédients | Vegetable oil, fish meal, fish oil, vitamins and minerals |

The pH values varied between 6,70 ± 0,12 and 7,43 ± 0,27. Dissolved oxygen concentrations ranged from 4,30 ± 0,30 $mg \cdot L^{-1}$ to 6,45 ± 0,44 $mg \cdot L^{-1}$. Water transparency values ranged between 45,67 ± 0,67 cm and 49,13 ± 0,70 cm. These results indicate stable and comparable environmental conditions throughout the experimental period.

**Sex reversal rate:** The proportions of males obtained in the "Bouaké" and "Brazil" strains treated with *Carica papaya* powders are summarized in Table 5.

**Table 2. Details of the experimental design**

| Fish strain | Experimental group | Treatment |
|---|---|---|
| "Bouaké" | Negative control | Commercial feed containing 48 % crude protein |
| | Positive control | 70 mg of 17-α-methyltestosterone per kg of commercial feed containing 48 % crude protein |
| | "10 g" group | 10 g of *Carica papaya* powder per kg of commercial feed containing 48 % crude protein |
| | "30 g" group | 30 g of *Carica papaya* powder per kg of commercial feed containing 48 % crude protein |
| | "50 g" group | 50 g of *Carica papaya* powder per kg of commercial feed containing 48 % crude protein |
| "Brazil" | Negative control | Commercial feed containing 48 % crude protein |
| | Positive control | 70 mg of 17-α-methyltestosterone per kg of commercial feed containing 48 % crude protein |
| | "10 g" group | 10 g of *Carica papaya* powder per kg of commercial feed containing 48 % crude protein |
| | "30 g" group | 30 g of *Carica papaya* powder per kg of commercial feed containing 48 % crude protein |
| | "50 g" group | 50 g of *Carica papaya* powder per kg of commercial feed containing 48 % crude protein |

**Table 3. Formulae used for the calculation of zootechnical performance parameters.**

| Parameters | Formula |
|---|---|
| **Sex ratio (♂:♀)** | Number of males / Number of females |
| **Survival rate (%)** | (Final number of fish / Initial number of fish) × 100 |
| **Weight gain (g)** | Final weight (g) - Initial weight (g) |
| **Daily weight gain (gd/day)** | Weight gain (g) / Rearing duration (days) |
| **Specific growth rate (%/day)** | [(ln Final weight - ln Initial weight) / Rearing duration (days)] × 100, where ln denotes the natural logarithm |
| **Feed conversion ratio (FCR)** | Quantity of feed distributed (g) / Fresh weight gain (g) |
| **Condition factor (K)** | [Fish weight (mg) / Standard length (mm)$^3$] × 100 |

**Table 4. Physicochemical parameters of the rearing water in larval culture systems of the "Bouaké" and "Brazil" strains of *Oreochromis niloticus***

| Strains | Treatments | Temperature (°C) | pH | Dissolved oxygen (mg·L$^{-1}$) | Water transparency (cm) |
|---|---|---|---|---|---|
| **"Bouaké"** | Negative control | 27,33 ± 0,88$^{a}$ | 7,10 ± 0,056$^{a}$ | 4,90 ± 0,76$^{a}$ | 46,67 ± 2,73$^{a}$ |
| | Positive control | 29,33 ± 0,88$^{a}$ | 7,03 ± 0,15$^{a}$ | 6,45 ± 0,44$^{a}$ | 49,03 ± 2,09$^{a}$ |
| | "10 g" group | 27,00 ± 1,00$^{a}$ | 7,10 ± 0,10$^{a}$ | 4,73 ± 0,89$^{a}$ | 47,60 ± 0,81$^{a}$ |
| | "30 g" group | 28,33 ± 1,20$^{a}$ | 6,70 ± 0,12$^{a}$ | 4,63 ± 0,68$^{a}$ | 47,20 ± 0,64$^{a}$ |
| | "50 g" group | 28,33 ± 0,88$^{a}$ | 6,93 ± 0,23$^{a}$ | 4,30 ± 0,30$^{a}$ | 49,13 ± 0,70$^{a}$ |
| "Brazil" | Negative control | 28,67 ± 0,33$^{a}$ | 6,87 ± 0,19$^{a}$ | 4,67 ± 0,88$^{a}$ | 48,87 ± 3,50$^{a}$ |
| | Positive control | 29,00 ± 0,58$^{a}$ | 7,23 ± 0,28$^{a}$ | 4,83 ± 1,09$^{a}$ | 47,33 ± 1,86$^{a}$ |
| | "10 g" group | 26,67 ± 1,67$^{a}$ | 7,43 ± 0,18$^{a}$ | 5,03 ± 0,61$^{a}$ | 46,33 ± 0,88$^{a}$ |
| | "30 g" group | 27,00 ± 1,00$^{a}$ | 7,37 ± 0,20$^{a}$ | 4,63 ± 0,91$^{a}$ | 45,67 ± 0,67$^{a}$ |
| | "50 g" group | 28,00 ± 1,53$^{a}$ | 7,43 ± 0,27$^{a}$ | 5,03 ± 0,55$^{a}$ | 47,33 ± 1,20$^{a}$ |

Negative and positive control groups correspond to diets containing 0 and 70 mg of 17-α-methyltestosterone per kg of feed, respectively. The "10 g", "30 g", and "50 g" groups correspond to diets supplemented with 10, 30, and 50 g/kg of *Carica papaya* powder, respectively. Values sharing at least one common superscript letter within a column are not significantly different (p > 0,05). Data are presented as mean ± standard deviation (SD).

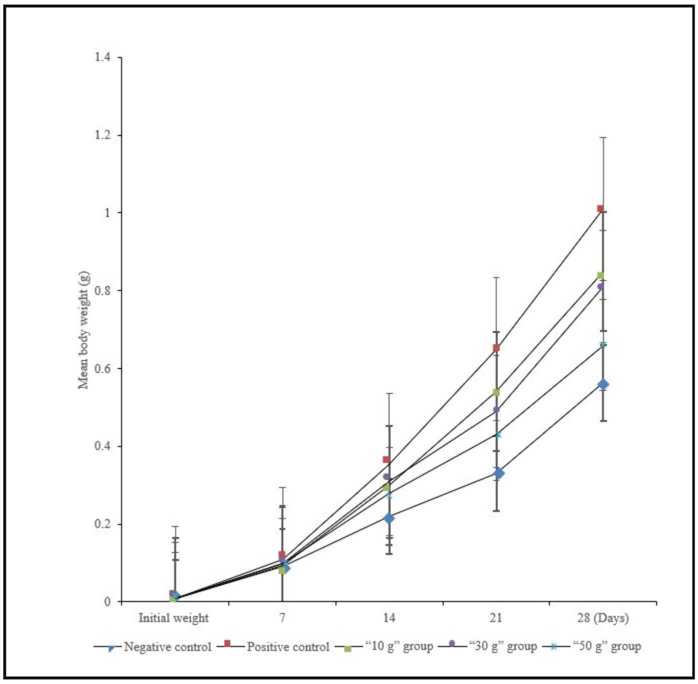


**Figure 1. Weekly body weight growth of "Bouaké" strain larvae of *Oreochromis niloticus* treated with *Carica papaya* powders. Negative and positive control groups correspond to diets containing 0 and 70 mg 17-α-methyltestosterone per kg of feed, respectively. The "10 g", "30 g", and "50 g" groups were fed diets supplemented with 10, 30, and 50 g/kg of *Carica papaya* powder, respectively**

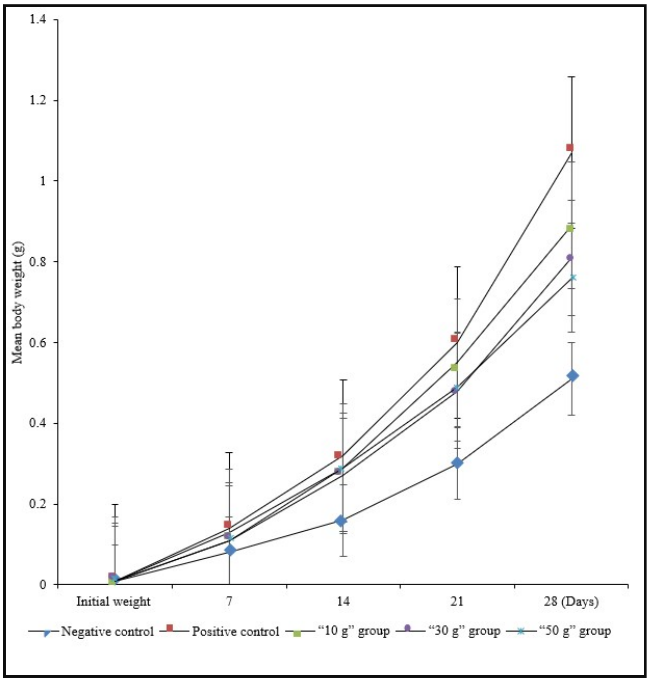


**Figure 2. Weekly body weight growth of "Brazil" strain larvae of *Oreochromis niloticus* treated with *Carica papaya* powders. Negative and positive control groups correspond to diets containing 0 and 70 mg 17-α-methyltestosterone per kg of feed, respectively. The "10 g", "30 g", and "50 g" groups were fed diets supplemented with 10, 30, and 50 g/kg of *Carica papaya* powder, respectively**

**Table 5. Male proportions in the "Bouaké" and "Brazil" strains of *Oreochromis niloticus* treated with *Carica papaya* powders**

| Strains | Treatments | Male proportion (%) |
|---|---|---|
| "Bouaké" | Negative control | 52,50 ± 1,89[a] |
| | Positive control | 96,22 ± 1,46[b] |
| | "10 g" group | 85,00 ± 2,52[cd] |
| | "30 g" group | 85,00 ± 1,53[cd] |
| | "50 g" group | 90,00 ± 2,08[d] |
| "Brazil" | Negative control | 49,50 ± 1,15[a] |
| | Positive control | 97,50 ± 0,50[b] |
| | "10 g" group | 78,33 ± 2,54[c] |
| | "30 g" group | 80,00 ± 2,02[c] |
| | "50 g" group | 81,66 ± 2,11[c] |

Negative and positive control groups correspond to diets containing 0 and 70 mg of 17-α-methyltestosterone per kg of feed, respectively. The "10 g", "30 g", and "50 g" groups correspond to diets supplemented with 10, 30, and 50 g/kg of *Carica papaya* powder, respectively. Values sharing at least one common superscript letter within a column are not significantly different ($p > 0.05$). Data are presented as mean ± standard deviation (SD).

In the "Bouaké" strain, the negative control exhibited the lowest male proportion (52,50 ± 1,89 %). In contrast, the positive control showed a significantly higher male proportion (96,22 ± 1,46 %) than all other treatments (Tukey's HSD test, $p < 0.05$). The *C. papaya* treatments resulted in intermediate male proportions. The 10 $g \cdot kg^{-1}$ and 30 $g \cdot kg^{-1}$ diets each induced 85,00 % males (± 2,52 and ± 1,53, respectively), while the 50 $g \cdot kg^{-1}$ diet yielded 90,00 ± 2,08 % males. However, these values remained significantly lower than those obtained with the hormonal treatment ($p < 0,05$).

**Final mean body weight:** Figure 1 shows that, in the "Bouaké" strain, larvae treated with 17α-methyltestosterone (positive control) exhibited the highest final body weight, whereas the lowest values were recorded in the negative control group. The *Carica papaya*-treated groups showed intermediate growth performance, with the 10 g/kg treatment yielding the highest weight, followed by the 30 g/kg and 50 g/kg treatments, respectively. Similarly, Figure 2 illustrates that, in the "Brazil" strain, the positive control group exhibited the highest growth, while the negative control showed the lowest. The *Carica papaya* treatments resulted in intermediate values. The 10 g/kg group displayed a final body weight close to that of the positive control, whereas the 30 g/kg group showed lower growth than the 10 g/kg group but higher than the 50 g/kg group. Overall, the "Brazil" strain exhibited slightly higher growth performance than the "Bouaké" strain.

**Growth performance of larvae:** Tables 6 and 7 present the growth performance parameters of larvae from the "Bouaké" and "Brazil" strains, respectively. Compared with the negative control, improved growth performance was observed in larvae fed *C. papaya*-based diets. In the "Bouaké" strain, the mean final weight in the negative control (0,56 ± 0,06 g) was significantly lower than that in the positive control (1,01 ± 0,03 g ; Tukey's HSD test, $p < 0,05$). Intermediate values were observed in the *C. papaya* treatments. Mean weights in the 10 g/kg (0,88 ± 0,04 g) and 30 g/kg (0,81 ± 0,03 g) groups were significantly higher than in the negative control ($p < 0,05$) but lower than in the positive control. The 10 g/kg group showed a significantly higher mean weight than the 50 g/kg group

**Table 6 : Zootechnical performance of "Bouaké" strain larvae of *Oreochromis niloticus* treated with *Carica papaya* powders**

| Parameters | Controls | | *Carica papaya* powders | | |
|---|---|---|---|---|---|
| | Négative | Positive | "10 g" group | "30 g" group | "50 g" group |
| **Initial weight (g)** | 0,01 ± 0,03[a] | 0,01 ± 0,03[a] | 0,01 ± 0,03[a] | 0,01 ± 0,03[a] | 0,01 ± 0,03[a] |
| **Final weight (g)** | 0,56 ± 0,06[a] | 1,01 ± 0,03[b] | 0,88 ± 0,04[bc] | 0,81 ± 0,03[bcd] | 0,66 ± 0,06[ad] |
| **Daily weight gain (g/day)** | 0,020 ± 0,002[a] | 0,036 ± 0,001[b] | 0,031 ± 0,001[bc] | 0,029 ± 0,001[bcd] | 0,023 ± 0,002[ad] |
| **Specific growth rate (%/day)** | 14,35 ± 0,40[a] | 16,48 ± 0,10[b] | 15,98 ± 0,17[bc] | 15,69 ± 0,14[bcd] | 14,94 ± 0,32[ad] |
| **Feed conversion ratio** | 2,78 ± 0,32[a] | 1,50 ± 0,04[b] | 1,73 ± 0,08[bc] | 1,88 ± 0,07[c] | 2,35 ± 0,22[d] |
| **Condition factor** | 2,54 ± 0,11[a] | 1,99 ± 0,05[a] | 2,50 ± 0,19[a] | 2,49 ± 0,15[a] | 2,27 ± 0,19[a] |
| **Survival rate (%)** | 90,00 ± 2,00[a] | 92,00 ± 1,15[a] | 92,00 ± 2,52[a] | 93,00 ± 1,53[a] | 94,00 ± 1,53[a] |
| **Rearing duration (days)** | 28[a] | 28[a] | 28[a] | 28[a] | 28[a] |

Negative and positive control groups correspond to diets containing 0 and 70 mg of 17-α-methyltestosterone per kg of feed, respectively. The "10 g", "30 g", and "50 g" groups correspond to diets supplemented with 10, 30, and 50 g/kg of *Carica papaya* powder, respectively. Values sharing at least one common superscript letter within a column are not significantly different ($p > 0.05$). Data are presented as mean ± standard deviation (SD).

**Table 7. Zootechnical performance of "Brazil" strain larvae of *Oreochromis niloticus* treated with *Carica papaya* powders**

| Parameters | Controls | | *Carica papaya* powders | | |
|---|---|---|---|---|---|
| | Négative | Positive | "10 g" group | "30 g" group | "50 g" group |
| **Initial weight (g)** | 0,01 ± 0,03[a] | 0,01 ± 0,03[a] | 0,01 ± 0,03[a] | 0,01 ± 0,03[a] | 0,01 ± 0,03[a] |
| **Final weight (g)** | 0,51 ± 0,05[a] | 1,07 ± 0,02[b] | 0,89 ± 0,04[bc] | 0,81 ± 0,05[c] | 0,76 ± 0,07[c] |
| **Daily weight gain (g/day)** | 0,018 ± 0,002[a] | 0,038 ± 0,001[b] | 0,031 ± 0,001[bc] | 0,029 ± 0,002[c] | 0,027 ± 0,002[c] |
| **Specific growth rate (%/day)** | 14,00 ± 0,37[a] | 16,69 ± 0,06[b] | 16,02 ± 0,16[bc] | 15,68 ± 0,22[c] | 15,44 ± 0,29[c] |
| **Feed conversion ratio** | 3,07 ± 0,32[a] | 1,42 ± 0,02[b] | 1,71 ± 0,07[bc] | 1,89 ± 0,11[cd] | 2,03 ± 0,16[d] |
| **Condition factor** | 2,40 ± 0,10[a] | 2,13 ± 0,14[a] | 2,70 ± 0,12[a] | 2,57 ± 0,09[a] | 2,33 ± 0,22[a] |
| **Survival rate (%)** | 92,67 ± 1,86[a] | 92,00 ± 1,15[a] | 94,00 ± 2,52[a] | 94,00 ± 1,15[a] | 93,00 ± 2,52[a] |
| **Rearing duration (days)** | 28[a] | 28[a] | 28[a] | 28[a] | 28[a] |

Negative and positive control groups correspond to diets containing 0 and 70 mg of 17-α-methyltestosterone per kg of feed, respectively. The "10 g", "30 g", and "50 g" groups correspond to diets supplemented with 10, 30, and 50 g/kg of *Carica papaya* powder, respectively. Values sharing at least one common superscript letter within a column are not significantly different ($p > 0.05$). Data are presented as mean ± standard deviation (SD).

A similar trend was observed in the "Brazil" strain. Male proportions were 49,50 ± 1,15 % in the negative control and 97,50 ± 0,50 % in the positive control. A dose-dependent increase in male proportion was observed in the *C. papaya*-treated groups, reaching 78,33 ± 2,54 % (10 g/kg), 80,00 ± 2,02 % (30 g/kg), and 81,66 ± 2,11 % (50 g/kg). All these values were significantly higher than the negative control ($p < 0,05$), but lower than those obtained in the "Bouaké" strain at equivalent doses and significantly lower than the hormonal control ($p < 0,05$).

(0,66 ± 0,06 g ; $p < 0,05$). In the "Brazil" strain, the final weight in the negative control (0,51 ± 0,05 g) was significantly lower than that in the positive control (1,07 ± 0,02 g ; $p < 0,05$). The 10 g/kg (0,89 ± 0,04 g), 30 g/kg (0,81 ± 0,05 g), and 50 g/kg (0,76 ± 0,07 g) treatments showed similar mean weights ($p > 0,05$), all of which were significantly higher than the negative control ($p < 0.05$). Only the 10 g/kg treatment showed a mean weight comparable to the positive control ($p > 0,05$).Across both strains, mean weight, weight gain, and specific growth rate followed similar trends.

**Feed conversion ratio:** The feed conversion ratio (FCR) varied according to treatment in both the "Bouaké" (Table 6) and "Brazil" (Table 7) strains. In the "Bouaké" strain, the lowest FCR was recorded in the positive control (1,50 ± 0,04), whereas the highest value was observed in the negative control (2,78 ± 0,32), with a significant difference between these treatments ($p < 0,05$). The *Carica papaya* treatments yielded intermediate values: 1,73 ± 0,08 (10 g/kg), 1,88 ± 0,07 (30 g/kg), and 2,35 ± 0,22 (50 g/kg). No significant differences were observed between the 10 g/kg treatment and either the positive control or the 30 g/kg treatment ($p > 0,05$). In the "Brazil" strain, the positive control again showed the lowest FCR (1,42 ± 0,02), while the negative control exhibited the highest value (3,07 ± 0,32). Intermediate values were recorded for the *C. papaya* treatments: 1,71 ± 0,07 (10 g/kg), 1,89 ± 0,11 (30 g/kg), and 2,03 ± 0,16 (50 g/kg). No significant differences were observed between the 10 g/kg treatment and either the positive control or the 30 g/kg treatment, nor between the 30 g/kg and 50 g/kg treatments ($p > 0.05$).

**Condition factor and survival rate:** Tables 6 ("Bouaké" strain) and 7 ("Brazil" strain) present the condition factor and survival rate values. For both strains, no significant differences were observed among treatments for either parameter ($p > 0.05$). In the "Bouaké" strain, the highest condition factor was recorded in the negative control (2,54 ± 0,11), while the lowest value was observed in the positive control (1,99 ± 0,05). In the "Brazil" strain, condition factor values ranged from 2,13 ± 0,14 (positive control) to 2,70 ± 0,12 (10 g/kg treatment). Survival rates in the "Bouaké" strain ranged from 90,00 ± 2,00 % (negative control) to 94,00 ± 1,53 % (50 g/kg treatment). In the "Brazil" strain, survival rates varied between 92,00 ± 1,15 % (positive control) and 94,00 ± 2,52 % (10 g/kg treatment).

# DISCUSSION

The present study aimed to evaluate the effectiveness of *Carica papaya* seed powder as a natural alternative to 17-α-methyltestosterone for the sex reversal of *Oreochromis niloticus*, as well as its effects on zootechnical performance, within the framework of sustainable aquaculture. The results demonstrate that *Carica papaya* based treatments induced significant masculinization of treated populations without causing major alterations in survival or rearing environmental conditions. The homogeneity of physicochemical water parameters across treatments, regardless of strain ("Bouaké" or "Brazil") and experimental diet (*Carica papaya* extracts or 17-α-methyltestosterone), indicates a stable rearing environment ($p > 0,05$). This stability rules out the influence of confounding abiotic factors on the observed biological responses. The recorded water temperatures (26,7-29,3 °C) fall within the optimal range for growth, survival, and sexual differentiation of *O. niloticus*[14] and, in accordance with[15], minimize the risk of unintended thermal masculinization. The relatively stable pH values (6,7-7,4), close to neutrality, correspond to favorable conditions for tilapia metabolism and osmoregulation[16] and ensure consistent bioavailability of plant-derived compounds[15]. Dissolved oxygen concentrations (4,3-6,5 mg $L^{-1}$), above the critical thresholds for the species, support normal aerobic metabolism and limit physiological stress that could interfere with the hypothalamic-pituitary-gonadal (HPG) axis. Finally, the uniform water transparency (45,7-49,1 cm) reflects low turbidity and moderate organic load, reducing physicochemical interactions likely to impair the effectiveness of plant extracts. Taken together, these findings confirm that the observed effects are primarily attributable to the dietary treatments.

Incorporation of *Carica papaya* seed powder into the diet of sexually undifferentiated larvae resulted in a significant increase in the proportion of males compared with the negative control, with masculinization rates reaching 85-90 % in the "Bouaké" strain and 78-82 % in the "Brazil" strain, depending on the applied dose. Although these values remain lower than those achieved with 17-α-methyltestosterone (≥ 96 %), they fall within a range considered functionally effective for limiting uncontrolled reproduction in culture systems[17-18]. These results are consistent with previous studies reporting masculinization rates between 60 % and 82 % in various tilapia species treated with papaya seed products[19-22]. The overall dose-dependent response observed in the present study confirms that the effectiveness of *Carica papaya* relies on a sufficient concentration of bioactive phytochemicals, while also suggesting the existence of an optimal threshold beyond which benefits are no longer proportional.
The differences observed between the "Bouaké" and "Brazil" strains indicate a potential influence of genetic background on sensitivity to plant-derived compounds. Such inter-strain variability has previously been reported in *Oreochromis niloticus* and may be related to differences in endocrine regulation, sexual differentiation plasticity, and expression of key enzymes involved in steroidogenesis[23-24]. These findings highlight the importance of locally adapted sex-reversal protocols. The masculinizing effects observed can be attributed to phytochemicals such as β-sitosterol, oleanolic acid glycosides, and benzyl isothiocyanate, which are known for their antifertility and endocrine-modulating properties[19; 25-26]. These compounds may inhibit aromatase activity, thereby reducing the conversion of androgens to estrogens and promoting testicular differentiation during the critical window of gonadal development[27-28]. They may also interact with hormone receptors or disrupt the HPG axis through feedback mechanisms involving GnRH, FSH, and LH[29-30]. The heightened sensitivity of undifferentiated gonads to endocrine disruptors thus explains the effectiveness of early dietary treatments[31].
Growth performance of fish treated with *Carica papaya* generally fell between that of the negative control and the hormonal control. The 10 g $kg^{-1}$ dose appeared to be the most favorable, yielding final body weights and specific growth rates close to those obtained with 17-α-methyltestosterone, particularly in the "Brazil" strain. These findings are consistent with previous reports indicating that low to moderate doses of phytochemicals may exert mild anabolic effects, possibly associated with a relative increase in circulating androgens[32]. In contrast, the reduced growth performance and increased feed conversion ratio observed at higher doses (30 and 50 g/kg) suggest antinutritional effects, likely due to the presence of tannins, saponins, and alkaloids, which are known to reduce nutrient digestibility and utilization[33-34]. Similar trends have been reported for other saponin-rich plants used in aquaculture[12; 35-36]. The absence of significant differences among treatments in terms of survival rate and condition factor indicates good overall tolerance of *Carica papaya* seed powder. These findings corroborate those of Ekanem and Okoronkwo[37], Hossam and Wafaa[38], and Omeje *et al.*[20], who also reported no major adverse effects on survival or health status of treated tilapias. This represents a major advantage over synthetic hormones, whose use raises health, environmental, and regulatory concerns[39].

# CONCLUSION

Under Ivorian conditions, characterized by semi-intensive aquaculture systems and the local availability of *Carica papaya*, the use of papaya seeds emerges as a promising and environmentally compatible alternative to 17-α-methyltestosterone. Although the masculinization rates achieved are lower than those obtained with the synthetic hormone, they are sufficient to significantly reduce uncontrolled reproduction and improve overall production performance. However, the variability in responses observed among strains and doses highlights the need for further studies to optimize protocols (dose, duration, and timing of application) and to assess long-term effects on reproduction, fish health, and product quality. Such research will contribute to strengthening the rational use of phytochemicals as sustainable tools for tropical aquaculture.

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**GLOSSARY OF ABBREVIATIONS**

**ANOVA:** Analysis of variance
**CNF:** National Floristic Center
**FCR:** Feed Conversion Ratio
**FSH:** Follicle-Stimulating Hormone
**GnRH:** Gonadotropin-Releasing Hormone
**HPG:** Hypothalamic-Pituitary-Gonadal
**HSD:** Honestly Significant Difference
**LH:** Luteinizing Hormone
**UFHB:** Université Félix Houphouët-Boigny
vs.: Versus

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