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Comparative Effects of Doses of *Nauclea latifolia* and 17 Alpha Methyltestosterone on Masculinization and Growth Rates of Tilapia *Orechromis niloticus* Larvae in Senegal

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Abstract

One of the main obstacles to the development of aquaculture in Senegal is the availability of fry and juveniles in sufficient quantities. The objective of our study is to evaluate the effects of treatment with the hormone 17 alpha methyl testosterone (17-&-MT) and different doses of *Nauclea latifolia* root powder on the masculinization rates of tilapia *Oreochromis niloticus* larvae in Senegal. A total of 450 larvae of *Oreochromis niloticus*, with an average size of 0.012 g, were placed in 15 aquariums, each containing 30 individuals and fed one of the 5 diets in triplicate. These individuals were fed either a diet containing a plant extract at a dose of 180 mg (T180), 200 mg (T200) and 250 mg (T250) per kg of food, or a diet containing 60 mg/kg of the hormone 17-&-MT (TMT), or a diet without either the plant extract or the hormone (T0). Individuals were fed for 30 days with the treatment diets, and then with the industrial feed for the rest of the experiment. At the end of the study, the results of the masculinization rate were: 80.49%, 73.13%, 69.23%, 66.67% and 45.95% for T250; T200, TMT, T180 and T0 respectively. In summary, we can say that the 250 mg/kg dose provides better masculinization, followed by 200mg/kg and then 17 α MT. For the plant, the increase in the percentage of males is dose-dependent. These results also translate into better growth of individuals in this order. However, the relatively lower survival rates observed in batches treated with *Nauclea latifolia* extract indicate that these treatments could have a deleterious effect on the survival of *Oreochromis niloticus* larvae. These

results suggest the use of *N. latifolia* extract at a dose of 250 mg/kg feed for better production of male *O. niloticus* larvae without compromising their growth.

Keywords

Nauclea latifolia, Masculinization Rate, Growth Performance, *Oreochromis niloticus*

1. Introduction

Aquaculture is the fastest-growing feed sector and has great potential to accelerate the upward trend in fish farming. The need for quality fingerlings and feed is the main constraint of this sector. In Senegal, tilapia is the most farmed species. However, tilapia reach maturity very quickly (30 to 40 g) and begin to reproduce, leading to an increase in the number of fry and consequently preventing the growth of fish. Various techniques are used to overcome this problem, including monosexing. By producing high-quality monosex tilapia larvae with low environmental impact, fish farming will play a key role in the development of a sustainable food system.

Hormonal treatment is used in tilapia to obtain larvae of the same-sex. Several types of hormones are usually incorporated into the larval diet and given early in the larval stage over a sufficient period of time. Oral administration of hormones for tilapia sex reversal is generally safe and effective. However, traces of hormones in uneaten food and metabolites often remain a major environmental concern. For this reason, the use of hormones is sometimes criticized by the public.

The method of sexual reversal using the hormone 17 alpha methyl testosterone is known worldwide for its success in masculinization, due to its high percentage of male [1] [2]. Although used in several African countries, sustainability issues remain around this product. To answer these questions and reduce the cost of production, several natural products have been tested. Plant extracts contain phytochemicals capable of inhibiting estrogen biosynthesis and acting as aromatase inhibitors and estrogen receptor antagonists in gonadal germ cells, and can therefore be considered as potential means of sex reversal in fish [3]. Many plants have been successfully used to induce sterility in laboratory animals. Several authors have reported the effect of papaya (*C. papaya*) seed meal on sexual determination and fish growth [4]-[7]. Inclusion of *Mangifera indica* leaf powder in Nile tilapia diets at doses of 0.5 to 8.0 $g \cdot kg^{-1}$ for 56 days reduced the number of spawned hatchlings, with complete inhibition of oviposition observed at a dose ≥ 2.0 $g \cdot kg^{-1}$ of diet [8].

Notably, prolonged treatment of Nile tilapia with diets containing *Moringa oleifera* leaf extracts at 5% of total dietary protein, for 90 days, severely degenerated oocyte cytoplasm [9].

Nile tilapia fed a diet containing 1.0 - 8 g·kg^{-1} of crude ethanol-based *Azadirachta indica* leaf extracts reduced the number of hatchlings, with no oviposition at five weeks [8] [10] [11]. However, *A. indica* reduced Nile tilapia oviposition by only 76% at the highest inclusion of 8.0 g leaf powder kg^{-1} diet for 90 days [12].

Similarly, the inclusion of *Tribulus terrestris* seed powder (2 g per kilogram of feed) for 30 days achieved a masculinization rate of 91.5% in Nile tilapia [13]. Feeding Nile tilapia (*Oreochromis niloticus*) diet containing 2 to 8 g of *Aspilia mossambicensis* leaf extract per kg degenerated seminiferous tubules, reducing the number of hatchlings in experimental fish [12].

[14], in their studies, concluded that the plants *Nauclea latifolia* and *Tribulus terrestris* could be used as an ecological alternative method to produce a male monosex population in *Oreochromis niloticus*.

In this study, the extract of *Nauclea latifolia*, a medicinal plant, easy to find in Senegal, will be used. Research has shown that this plant contains alkaloids that are capable of stimulating testicular weight growth and spermatogenesis [14]. The use of this plant in aquaculture remains almost unknown in Senegal.

It is in this context that the present study was undertaken to evaluate the effects of treatment with the hormone 17 alpha methyl testosterone (17-&-MT) and different doses of *Nauclea latifolia* root powder on the masculinization rates of tilapia larvae *Oreochromis niloticus* in Senegal.

2. Material and Methods

2.1. Presentation of the Study Area

The experiment was carried out at the agricultural farm of the Gaston Berger University in Saint-Louis, Senegal (**Figure 1**). The farm is located in the district of Gandon (more precisely in Sanar) between the geographical coordinates 16˚13'N and 16˚18'W, twelve (12) kilometers from the city of Saint-Louis and about two kilometers from the water supply (Djeuss River). The farm was established in March 2007 and covers an area of 30 ha. The site has a sub-Saharan to Sahelian climate. It is characterized by two seasons: a dry season from October to July and a rainy season from August to September. Maximum temperatures, often recorded in April-May, are generally between 35˚C and 37˚C. The minimum temperatures recorded in January are around 16˚C. The site's soil is sandy to sandy-clay with a pH that is close to neutral, between 6.7 and 7.7.

2.2. Broodstock Selection and Maintenance

Broodstock were selected according to size and weight, in order to form a homogeneous batch and avoid aggression between individuals. Six (600) females were selected on the basis of the protuberance of their genital papilla and the emission of oocyte. Two hundred (200) male broodstock were selected on the basis of the development of the urogenital papilla and their health status. A sex ratio of one male to three females was observed. These broodstock were fed to satiety 3 times a day with artificial diet.

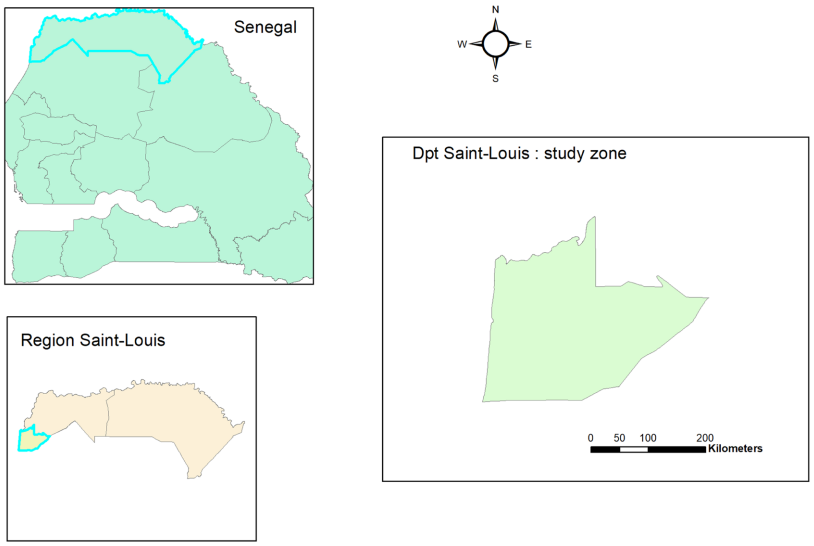

Figure 1. Study area map.

2.3. Larval Production

One week after loading the broodstock, observation sessions began on the edges of the pond for possible reproduction, until the larvae were observed. Harvesting of larvae from the mouths of incubating females and from the water column began two weeks after loading, using a mosquito net and a container (**Figure 2**; **Figure 3**). The larvae were then transported to the experimental room.

Figure 2. Larvae harvesting Methods.

Figure 3. Harvested larvae.

2.4. Harvesting and Preparation of the Plant Extracts

The plant used was harvested in Casamance, more precisely in the village of Sibogola, about 7 km from the district of Sindian. The roots were used, cleaned, sliced and chopped, then dried in the shade at room temperature (about 30 to 35˚C) for two weeks. Then, they are crushed in a mortar before passing through a sieve and then finely pulverized in the mill. The powder was extracted and stored in small bottles (Figure 4).

Figure 4. Treatment process for *N.latifolia* roots.

2.5. Preparation of Experimental Feeds

Five experimental diets corresponding to different treatments were developed to obtain 46% crude protein. Different ingredients were used in the preparation, including fish meal, peanut cake, corn flour, fish oil, vitamin-mineral complex and binder. The proportions of these ingredients are as follows: fish meal 36.96%, peanut cake 36.96%, corn flour 16.58%, fish oil 4%, vitamin premix 1.5%, mineral premix 2% and binder 2%.

The manufacture of the initial feed consisted of mixing the previously quantified ingredients to obtain a final product ready for use. During this process, we mixed the large quantities namely fish meal and peanut cake, then corn flour. After that, the small amounts of vitamins, minerals, and binder are mixed in another container. The latter is added to the first, followed by fish oil and water.

Two experimental diets served as controls for our experiments. The first (T0) consisted of the initial feed. The second (TMT) experimental feed was formulated from the initial feed to contain 17-alpha methyltestosterone. The hormone-treated feed was prepared according to the method of [15]. The hormone solution was obtained by dissolving 60 mg of hormone (17-alpha methyltestosterone) in 700 ml of 95% alcohol. The resulting solution was sprayed onto the feed at a rate of 60 mg of hormone/kg of feed. The mixture was thus mixed to facilitate the incorporation of the hormone solution into the feed. The mixture was air-dried in the shade for 24 hours to evaporate the alcohol. After drying, the feed was stored in containers and sealed tightly to preserve the hormone. The other 3 experimental diets were formulated from the initial diet to contain *Nauclea latifolia* extract at doses of 180 (T180), 200 (T200), and 250 (T250) mg/kg of diet. The different experimental diets containing the crude *Nauclea latifolia* extracts were prepared separately by dissolving them in 100 ml of distilled water and spraying them on one kilogram of control diet. The whole was air-dried in the shade for 24 hours.

All treated diets were stored in tightly sealed containers.

2.6. Experimental Procedure

The 0.012-gram larvae are introduced into fifteen (15) 300 cm^3 aquariums at a rate of 30 individuals per aquarium. The individuals are subjected to natural temperature and light conditions. The individuals thus introduced are fed to satiety with different foods 6 times/day for 30 days. The breeding water was renewed once every two days using a renewal and filling device that is not very stressful for the fish. Two large basins are filled one day before the renewal to allow time for the chlorine to evaporate. The system is oxygenated by an aerator and each morning and evening, the water parameters (temperature and pH) are recorded.

After one month of rearing, the feeding frequency is reduced to 4 times/day between 9 a.m. and 6 p.m. At this stage, an industrial food with 42% protein is distributed to all the aquariums. All individuals in each aquarium are weighed every two weeks during the 60 days of the experiment. The physicochemical parameters of the water are taken every day morning and evening. At the end of the experiment, the following zootechnical parameters (mean weight gain (AWG), specific growth rate (SGR), survival rate (SR), were calculated from the following formulas:

$$\mathrm{AWG} = \mathrm{fw} - \mathrm{iw} \tag{1}$$

$$\mathrm{ADWG} = \frac{\mathrm{fw} - \mathrm{iw}}{\mathrm{d}} \tag{2}$$

$$\mathrm{SGR} = 100 \times \frac{\log(\mathrm{wf}) - \log(\mathrm{wi})}{\mathrm{d}} \tag{3}$$

where:

AWG = average weight gain, expressed in gram (g);

ADWG = Average Daily Weight Gain expressed in gram per day (g/d);

SGR= Specific growth rate expressed in percent, gram per day (%.gd^{-1});

fw = final average weight;

iw = initial average weight;

d = duration of experiment in days.

2.7. Individual Sex Determination

From 60 days of age, individuals were not large enough to perform manual sexing. The microscopic observation method was performed on sixty-fifth days. All the fish in each aquarium were caught and put in ice. After dissection, the gonads were removed and a fragment placed between the slide and the lamellae and stained with methylene blue, crushed and then placed under a microscope in order to determine the sex on the basis of the gonadal structure. The percentage of males was also calculated using the following formula:

$$\text{Percentage of males} = \frac{\text{number of male obtained}}{\text{number of fish sampled}} \times 100 \tag{4}$$

3. Results

3.1. Variation of Physico-Chemical Parameters

The physicochemical parameters evaluated during this study are temperature and pH. The temperature varied from 24 to 30˚C. Measurements taken in the evening were always higher (27.04 - 30.8˚C) compared to those taken in the morning (24.09 - 27.88˚C). The pH values varied from 6.8 to 7.9 in the different treatments. The values obtained in the morning are identical to those recorded in the evening.

3.2. Zootechnical Parameters of Growth and Survival

A comparative analysis of control and control batches treated with *Nauclea latifolia* extract at different doses (180 mg/kg; 200 mg/kg; 250 mg/kg) shows a significant difference ($P < 0.05$) between treatments (Table 1). In fact, the different batches treated with *Nauclea latifolia* extract at a dose of 250 mg/kg and those treated at a dose of 200 mg/kg of feed had a significantly greater effect compared to the other treatments applied both in terms of Average Weight Gain (*i.e.* 3.38 ± 0.14g (T250) and 3.08 ± 0.87 g (T200) respectively), and the Specific Growth Rate (*i.e.* 10.08 ± 0.07 %g/d (T250) and 9.87 ± 0.48 %g/d (T200), respectively). The 180 mg/kg and methyltestosterone 60 mg/kg batches had a significantly greater effect ($P < 0.05$) compared to the T0 control group in terms of mean weight gain (2.75 ± 0.23 g (T180) and 2.78 ± 0.42 g (TMT)) and Specific Growth Rate (9.71 ± 0.15% g/d (T180) and 9.72 ± 0.27% g/d (TMT), respectively). No significant differences were observed between T250 and T200 treatments; as well as for the T180 and TMT (Table 1). T0 produced the lowest performances in terms of both Average Weight Gain (2.72 ± 0.31 g) and Specific Growth Rate (9.69 ± 0.20% g/d). The survival rate ranged from 68 to 86%. Control batches achieved significantly higher survival rates ($P < 0.05$) compared to batches treated with *N. latifolia* at 250, 200 and 180 mg/kg feed. The results show that the dose of *N. latifolia* extract does not significantly affect juvenile mortality.

Table 1. Zootechnical parameters for Growth and Survival.

	T250	T200	T180	TMT	T0
Initial number	30	30	30	30	30
Final number	20.5 ± 0.7	22.33 ± 1.15	22 ± 4.35	26 ± 1.14	24.33 ± 3.51
Survival rate (%)	68.33 ± 2.35^{b}	74.44 ± 3.84^{b}	73.33 ± 14.52^{b}	86.66 ± 4.71^{a}	81.11 ± 11.7^{a}
WG (g)	3.38 ± 0.14^{a}	3.08 ± 0.87^{a}	2.75 ± 0.23^{b}	2.78 ± 0.42^{b}	2.72 ± 0.31^{c}
ADWG (g/j)	0.06 ± 0.00	0.05 ± 0.02	0.04 ± 0.00	0.04 ± 0.01	0.05 ± 0.01
SGR (%. $g{\cdot}j^{-1}$)	5.94 ± 0.07^{a}	5.41 ± 0.48^{a}	4.83 ± 0.15^{b}	4.97 ± 0.27^{b}	4.77 ± 0.20^{c}

Numbers with the same letters in the same line are not significantly different ($P < 0.05$). T0 = Control treatment 1; TMT = Control treatment 2, methyltestosterone (60 mg/kg feed); T180 = *N. latifolia* treatment at 180 mg/kg feed; T200 = *N. latifolia* treatment at 200 mg/kg feed; T250 = *N. latifolia* treatment at 250 mg/kg feed. WG = average weight gain expressed in grams; ADWG= average daily weight gain expressed in grams per day; SGR = specific growth rate expressed in g, percent and per day.

3.3. Sexing of Individuals

The results show that the rates of masculinization varied from 47.95 to 80.49%. Batches treated with N.L extract at doses of 180; 200 and 250 mg/kg showed a significant difference ($P < 0.05$). The batch treated with T250 had the highest masculinization rates ($P < 0.05$), followed by the batches treated with T200 (Table 2). The lowest rates of masculinization are obtained with the T0 treatment. However, the T180 and TMT-treated batches achieved significantly higher masculinization rates ($P < 0.05$) than the T0 batch, and no significant differences ($P < 0.05$) were observed between the T180 and TMT-treated batches.

Table 2. Masculinization rate by treatment.

		Sex		
Treatments	Total individuals	M	F	% M
T0	73	35	38	47.95^{d}
T180	66	44	22	66.67^{c}
T200	67	49	18	73.13^{b}
T250	41	33	8	80.49^{a}
TMT	52	36	16	69.23^{c}

Numbers with the same letters in the same column are not significantly different ($P < 0.05$). T0 = Control treatment 1; TMT = Control treatment 2, methyltestosterone (60 mg/kg feed); T180 = *N. latifolia* treatment at 180 mg/kg feed; T200 = *N. latifolia* treatment at 200 mg/kg feed; T250 = *N. latifolia* treatment at 250 mg/kg feed. %M = percentage of masculinization.

4. Discussion

The results of our study showed that the physicochemical parameters (temperature and pH) did not change according to the treatments. This can be explained by the fact that the treatments were subjected to the same breeding conditions since the aquariums are the same size with the same amount of water, are the same as the frequency of water exchange. Water temperature is one of the main factors that influence the growth of tilapia in its various stages. Temperatures between 22 and 34˚C have been reported by various researchers as being suitable for tilapia cultivation. These results are of the same order as those reported by [16] [17]. These authors reported that the best growth performance for temperatures ranging from 24 to 28˚C. According to [18], the preferred temperature ranges for optimal growth of tilapia (*Oreochromis niloticus*) are between 28 and 32˚C.

[17] reported that highly acidic water with a pH below 5.5 limited fish growth and reproduction. This author, notes that the ideal pH range for freshwater aquaculture should be between 6.5 and 7.0, although a pH range of 6.1 to 8.0 is also considered satisfactory for fish survival and reproduction. [17] showed that a pH between 6.5 and 9.0 was optimal for tilapia growth. These results are in agreement with ours (t˚: 24 to 30˚C and pH: 6.8 to 7.9). These temperature and pH values presented are within the acceptable standards for the breeding of *Oreochromis*

niloticus.

The best growth performance was obtained with batches treated with *Nauclea latifolia* extract at a dose of 250 mg/kg and those treated with a dose of 200 mg/kg feed. Perhaps the latter have accumulated more androgen precursors compared to other treatments, as androgenic steroids play a stimulating role on muscle growth [14]. The high growth achieved by those fed diets containing more *N. latifolia* extract (T250 and T200) could be explained by the high proportion of males obtained by these diets. In addition, the better growth rates observed in relation to the increase in the concentration of L. latifolia extract are due to the fact that this plant is a testosterone activator.

These results are in agreement with those of [14], who in their studies, showed that the best growth performance batches were obtained with batches treated with Methyltestosterone at 60 mg/kg and *Nauclea latifolia* extract at 200mg/kg of feed. Other plant species rich in androgenic steroids have been shown to improve growth performance in several fish species. In their studies, [19] showed that *T. terrestris* extract had an effect on the growth and differentiation of the genitals due to the androgenic effect, which led to an increase in the growth rate in *O. niloticus*. Similarly, [20] demonstrated that different levels of *T. terrestris* extract (200, 400 and 600 $mg \cdot kg^{-1}$) in diets improved growth performance in *Oreochromis niloticus*. This result is consistent with studies by [21] in *O. niloticus* and those of [22] in *C. carpio*, which showed that extracts of papaya (*C. papaya*) leaves, skin, and seeds increased growth efficiency.

Survival did not affect the control groups (T0 and TMT). The batch treated with 17-α-methyltestosterone achieved a survival rate of 86.6%. This result had no effect on the survival rate of tilapia that had not received hormone (T0) treatment. Similar results were observed by [23]. Batches treated with *N. latifolia* doses at 250, 200 and 180 mg/kg feed had lower survival rates of 68.33% ± 2.35%, respectively; 74.44% ± 3.84%; 73.33% ± 14.52%. The relatively lower survival rates observed in the pools treated with *Nauclea latifolia* extract indicate that these treatments may have a deleterious effect on the survival of *Oreochromis niloticus* larvae. The results obtained with the pools treated with *Nauclea latifolia* extract are similar to those obtained by [14].

The results show that masculinization with *N. Latifolia* is dose-dependent. The batch treated with T250 had the highest masculinization rates ($P < 0.05$), followed by the batches treated with T200, and then the batch treated with T180. These results are in agreement with those of [14]. However, their masculinization rate obtained (92%) is higher than the values obtained in this study (80.49%). This difference could be due to the genetic variability inherent in each individual, which would have an impact on the response of the different treatments but also a problem of manufacture and storage of the food. In their experiment [24], *N. latifolia* root extracts produced (69.25% ± 8.91% males), which is lower compared to our result. This difference could be explained by the physiology of the plant which tends to concentrate saponins and flavonoids in the fruit during the

ripening season compared to other parts of the plant [25]. However, the masculinization rate obtained (100% males) with [26], at an incorporation level of 70 mg/kg of feed with the hormone 17-*α*methyl-testosterone is higher than ours (69.23% males). Similarly, [27] suggests the use of the hormone 17-*α*-MT at a dose of 55 mg per kg of feed at the hatchery for 100% male tilapia production. The amount of feed consumed by the larvae, the rearing environment, the duration of the treatment and the rearing conditions seem to be a determining factor in the rate of masculinization of tilapia. In his study, [28] points out that the masculinization rate of tilapia fry is high if the fry only ingests the feed with the hormone 17-*α*-MT during the period of sexual undifferentiation. It may be that the non-filtration of river water in our case could introduce a natural food into our experimental units.

5. Conclusion

This work is intended to be a modest contribution to a better understanding of the effects of *Nauclea latifolia* on masculinization and growth performance in *O. niloticus*. These results suggest the use of *N. latifolia* extract, a natural product, at a dose of 250 mg/kg feed for better production of male *O. niloticus* larvae without compromising their growth. This represents a sustainable alternative to the synthetic hormone 17*α* methyl testosterone. *N. latifolia* extract has the advantage of being available locally, relatively cheaper and accessible to fish farmers, unlike synthetic hormones. Further studies could be carried out to determine the specific bioactive compounds in *N. latifolia* responsible for the effects observed.

Authors' Contributions

All authors contributed to the management of the didactic and technical materials and to the writing of the manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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