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Efficacy of 17-alpha-methyltestosterone in the production of male fry of *Oreochromis niloticus* and study of their survival and growth in the first phase of breeding

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Abstract

Oreochromis niloticus is a species of tilapia in which males grow well compared to females. In order to contribute to the improvement of the breeding of this species, a study was carried out on the production of male fry by the incorporation of the hormone 17-alpha-methyltestosterone in the feed in the first phases of breeding. The survival and growth in concrete tanks of fry produced were studied during the first phase of breeding at a density of 25 fry m^{-3}. The results indicate that the use of 17-alpha-methyltestosterone at a dose of 60 mg Kg^{-1} of food produced 97.69% of males. Besides, during the first phase of breeding, the growth of fry fed with hormone feed appears to be good (0.80 ± 0.18 g d^{-1}) compared to that of untreated fry (0.52 ± 0.03 g d^{-1}). As for the survival of the fry, it was not influenced by the use of the hormone in the ration. These different results will prompt further studies on the most effective doses of the hormone and the most suitable breeding structures for the success of masculinization.

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Introduction

In Côte d'Ivoire, fish remains the primary source of animal protein. Its average consumption of 15.9 kg per capita and per year is almost 70% covered by imports (MIPRAH, 2014). The magnitude of these imports and the cost of foreign exchange prompted the government to intensify the development of the fisheries and aquaculture sectors. As regards aquaculture, enormous potentialities exist for its development, given the considerable natural assets (lagoons, rivers, lakes, reservoirs, etc.) available to the country. However, these potentialities are weakly exploited and the contribution of aquaculture to fish production remains insignificant (Failler *et al.*, 2014). The development of Ivorian fish farming faces many constraints, among which we can indicate the poor performance of fish cultivable species including tilapia *Oreochromis niloticus* which constitutes 90% of the species farmed in the country (MIPRAH, 2014). In addition, the continual laying spread over the whole year and the parental care provided by the female from the eggs to the fry leads to a high survival rate of the fry, which leads, in a closed environment and a situation of food competition, overcrowding and dwarfism (Hickling, 1963; Phillippart and Ruwet; 1982). In addition, many studies carried out on tilapia have shown that males show better growth performance than females (Hanson *et al.*, 1983; Amon *et al.*, 2013). Therefore, the search for males appears to be a necessity for the profitability of tilapia farms, especially since in these fish, the sex ratio is theoretically balanced in each offspring. Several practices have resulted in monosex male populations in tilapia. This involves hybridization between certain species of tilapia (Hickling, 1960), manual sexing (Lazard, 1980) and hormonal treatments (Baroiller, 1988). The present study proposes to produce male fry of *Oreochromis niloticus* by incorporating the hormone 17-alpha-methyltestosterone into the feed in the first phase of the feeding of this species.

Material and methods

Experimental environment

The present study was carried out in a fish farm (Poly-Elevages) located in Aboisso (south-eastern Côte d'Ivoire). On this farm, there are different breeding structures such as concrete tanks, ponds, cages and happas implanted in a pond. All these breeding structures are fed by a river whose water is filtered from settling tanks.

Biological material

The experiments focused on the Brazilian strain of *Oreochromis niloticus* species. Broodstock with a bodyweight of approximately 100 g for females and 190 g for males was used for the production of fry.

Technical material

The larvae were produced in happas measuring 3 × 2 × 1.5 m installed in a 1200 m² pond. As for the first phases of larval rearing, it took place in concrete tanks measuring 2 × 2 × 1m. During the experiment, temperature and dissolved oxygen of the water were measured using an oxy-meter model WTW OXI 330 and pH by using the pH-meter model WTW pH 90. The size and weight of the fish were determined respectively using an ichthyometer and an electronic scale model SARTORIUS with a maximum capacity of 6 kg and a precision of 0.01 g. Landing nets and plastic buckets were used respectively for fishing and transporting fish. The commercial granulated feed "SKRETTING" containing 30% protein was used for the broodstock. Concerning the fry, this same feed containing 45% protein in the flour form was used. To this material are added the hormone 17-alpha-methyltestosterone and ethanol (90%) which served for masculinization.

Reproduction of broodstock in happas and constitution of batches of fish

Six (6) happas were used for the reproduction of broodstock. Three (3) were used for the production of larvae intended for masculinization and the other three (3) for non-masculinized larvae. Thus, 20 broodstock (5 males + 15 females) were reproduced in each happa at a sex ratio of one male to three females. During the production of larvae which took place over a month, the broodstock was fed twice a day (8 am and 5 pm) using the concentrated feed "SKRETTING" at the daily ration of 4% of the biomass. Three larval

harvests were carried out. The first took place 14 days after the broodstock had started to reproduce. The other two were performed at 7-day intervals.

The harvest consisted of fishing the larvae with landing nets, which were then stored in concrete tanks. Three concrete tanks corresponding to the three harvests were used for the batches to be treated with the masculinizing hormone. As for the untreated larvae (controls), they were produced at the same time and under the same conditions as those intended for hormonal treatment. For the growth of the fry, six (6) other concrete tanks were used. Thus, three (3) batches of 100 fry per group (treated and untreated) with an initial average weight of around 4g were placed in the tanks at a density of 25 fish m^{-3}. These batches of fry were followed for 42 days to study their survival and growth.

Preparation and incorporation of the hormonal solution in the food

The synthetic sex steroid hormone 17-alpha-methyltestosterone (MT) was used in this study. The hormone was weighed using an electronic scale in aluminum foil. The hormonal solution was obtained by dissolving 60 mg of the hormone in 400 ml of ethanol (90%).

The food was subsequently sprayed with the hormonal solution at a rate of 60 mg kg^{-1} of food. The resulting hormonal food was spread out on a drying rack out of direct sunlight for two days to evaporate the alcohol. After drying, this food was put in a plastic bag and stored in a refrigerator at 4 ° C.

Application of the hormone feed to the larvae at the end of vitelline resorption and growth monitoring

Seven (7) days after the transfer of each batch into the concrete tanks, the larvae received daily in four meals (7 a.m., 10 a.m., 1 p.m. and 4 p.m.) the hormone feed at a rate of 25% of the biomass for one month and 20% of the biomass until the end of the experiment. The daily ration was adjusted every two weeks where a sample of 50 fries per tank was taken, measured and weighed individually to follow their growth. During the experiment, the pH, the dissolved oxygen and the temperature of the water were measured twice a day (6.30 a.m. and 3.30 p.m.). The water in the tanks was renewed every two weeks to avoid the effects of pollution due to the accumulation of food scraps. As for mortalities, they were recorded daily.

Determination of the number of fry and sexing

After forty-two (42) days of rearing, 100 fries were caught per tank to determine their average weight. Subsequently, all the fish from each tank were caught and divided into several batches. Each batch was weighed and the total weight of the fry was determined by adding the weights of each batch. To determine the number of fry, the total weight was divided by the average weight of the fry. As for sex, it was done by direct observation of the genital papilla.

Zootechnical parameters

At the end of the experiment, the zootechnical parameters were determined from the data collected. The calculation methods are presented as follows:

Survival rate (SR)

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

Daily weight gain (DWG)

DWG (g d^{-1}) = (Final body weight - Initial body weight) / Number of days

Linear Specific Growth Rate (LSGR)

LSGR (%d^{-1}) = [ln (final length) - ln (initial length) / Number of days] × 100

Weight Specific Growth Rate (WSGR)

WSGR (% d^{-1}) = [ln (final body weight) - ln (initial body weight) / Number of days] × 100

Feed conversion ratio (FCR)

FCR = Dry feed intake (g) / Body weight gain (g)

Masculinization rate (MR)

MR (%) = [Number of male fry / Number of fry produced] × 100

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) after prior verification of the homogeneity of the variances and the normality of the data to be analyzed. When significant differences were found, a Tukey HSD test was used for multiple comparisons at the 5% level of significance. All statistical treatments were performed using Statistica software version 7.1 for windows.

Results

Water quality

Water quality characteristics monitored throughout the study period are summarized in Table 1.

The parameters did not vary significantly from one tank to another during the 42 days of growth. The water temperature ranged from 27.73 to 30.01 °C, pH from 6.7 to 7 and dissolved oxygen 2.66 to 8.5 mg L^{-1}.

Table 1. Water quality parameters recorded in the experiment tanks.

Parameters	Batches	
	Treated	untreated
T (°C)	28.80 ± 0.11[a]	28.85 ± 0.18 [a]
pH	6.91± 0.01[a]	6.88 ± 0.02[a]
O_2 (mg L^{-1})	4.32 ± 1.06 [a]	4.35± 1.12[a]

Values are means ± SD. T = Temperature; pH = potential of Hydrogen; O_2 = dissolved oxygen. Values in the same row with same superscripts are not significantly different (p>0.05).

Number of fry produced and masculinization rate

The number of fry produced and the masculinization rate obtained after sexing are listed in Table 2. The number of fry produced in the batches treated with the hormone is 26 602 and 27 060 for the untreated batches. After treatment with 17-alpha-methyltestosterone, the number of males obtained was 25 988 (masculinization rate = 97.69%). As for the untreated batches, the number of males recorded after sexing was 13 553 (rate of males 50.08%).

Growth performance and survival rate

Data on the growth performance and survival rate of fry of *Oreochromis niloticus* are presented in Table 3. In general, the growth of fry treated with the hormone, after 42 days of rearing, is greater than that recorded in untreated fry. The daily weight gain and the weight and linear specific growth rates were respectively 0.80 ± 0.18 g d^{-1}, 5.22 ± 0.21% d^{-1} and 1.92 ± 0.01% d^{-1} at the level of the batches treated against 0.52 ± 0.03 g d^{-1}, 4.37 ± 0.01% d^{-1} and 1.39 ± 0.21% d^{-1} at the level of the untreated batches. The statistical comparison shows a significant difference ($p < 0.05$) in favor of the treated batches. In addition, the feed conversion ratio is low in the treated batches compared to untreated batches (1.80 ± 0.21 against 2.80 ± 0.81). Concerning the survival rates obtained during rearing, they were 96.91 ± 0.20% for the treated batches and 97.01 ± 0.10% for the untreated batches. No significant difference ($p > 0.05$) was observed between these survival rate values.

Discussion

In this study, the physico-chemical parameters of the water are within the limits recommended for tilapia culture. Indeed, according to Stickney (1986) and Faye *et al.* (2018), the most favorable pH values for growing tilapia are between 6.5 and 8.5. The lower and upper lethal temperatures obtained by Balarin and Haller (1982) are 8 ° C and 42 ° C, respectively. For Faye *et al.* (2018), a temperature of 27.7 ± 1.24 ° C is favorable for tilapia culture, which is in line with our results. Regarding dissolved oxygen, the mean values obtained are above the growth threshold value (2.3 mg L^{-1}) reported by Ross (2000). These parameters would therefore not influence the survival of *Oreochromis niloticus* fry.

Concerning hormonal inversion, our results are in agreement with those of Seck *et al.* (2018) who obtained an inversion rate of between 96 and 97% by feeding the fry using a hormone feed dosed at 60

mgKg^{-1} of feed. On the other hand, Barry *et al.* (2007) reported that more than 95% of tilapia fry were masculinized in 21-28 days when the larvae fed on 30-60 mg of 17- alpha-methyltestosterone per kilogram of feed. On the other hand, our results differ from those of Ouédraogo (2009) who obtained an inversion rate of 75.33% in concrete tanks. As for the control (untreated batches), the number of males is around 50%. This agrees with the results of Ouédraogo (2009).

Table 2. Masculinization rate of fry after hormonal treatment.

Parameters	Batches	
	Treated	untreated
Number of fry produced	26 602	27 061
Number of males	25 988	13 553
Masculinization rate	97.69%	50.08%

The growth results indicate that fry-fed hormone feed shows higher growth performance than untreated batches after forty-two (42) days of rearing. This agrees with Seck *et al.* (2018) who made the same observation. In addition, Little *et al.* (2003) showed that in some strains of treated *Oreochromis niloticus*, methyltestosterone resulted in a final size of 10.7% more than untreated fish. This would explain why in our study, the specific growth rates and the daily weight gain are higher with a lower feed conversion ratio at the level of the treated batches. These results would suggest anabolic effects of 17-alpha-methyltestosterone as observed by Pandian and Sheela (1995) and confirmed by El-Asaly (2015).

Table 3. Growth performance and survival rate of fry of *Oreochromis niloticus* reared for 42 days in concrete tanks.

Parameters	Batches	
	Treated	Untreated
IL (cm)	5.82 ± 0.21^{a}	5.90 ± 0.17^{a}
FL (cm)	13.04 ± 0.06^{a}	10.62 ± 0.21^{b}
IBW (g)	4.25 ± 0.07^{a}	4.39 ± 0.07^{a}
FBW (g)	38.18 ± 0.35^{a}	27.60 ± 0.67^{b}
DWG (g d^{-1})	0.80 ± 0.18^{a}	0.52 ± 0.03^{b}
LSGR (% d^{-1})	1.92 ± 0.01^{a}	1.39 ± 0.21^{b}
WSGR (% d^{-1})	5.22 ± 0.21^{a}	4.37 ± 0.01^{b}
FCR	1.80 ± 0.30^{a}	2.80 ± 0.81^{b}
SR (%)	96.91 ± 0.20^{a}	97.01 ± 0.10^{a}

Values are means ± SD. Values in the same row with same superscripts are not significantly different (p>0.05). IL = Initial lenght ; FL = Final lenght; IBW = Initial body weight ; FBW = Final body weight; DWG = Daily weight gain ; LSGR = Linear specific growth rate ; WSGR = Weight specific growth rate ; FCR = Feed conversion ratio ; TS = Survival rate.

In addition, the survival rates of the fry were practically similar in the different batches (treated and untreated). This result is in agreement with Asad *et al.* (2010) who showed that the hormone 17-alpha-methyltestosterone does not affect the survival of fish because it is rapidly excreted through the faeces and gills. The recorded mortalities could be of natural origin or be caused by the shocks received by the fish during the sampling operations.

Conclusion

At the end of this study, it should be noted that the use of 17-alpha-methyltestosterone at a dose of 60 mg kg^{-1} of feed made it possible to obtain a rate

masculinization of 97.69%. In addition, during the first phase of breeding, the growth of fry fed with hormone feed appears to be good compared to that of untreated fry. As for the survival of the fry, it was not influenced by the use of the hormone in the ration. These various results could prompt studies on the most effective doses of the hormone and the most suitable breeding structures for the success of masculinization.

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