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Production of all-male tilapia through hybridization between *Oreochromis niloticus* and *Tilapia zillii*

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Abstract
The candidate fish were collected from the Kainji lake basin and the integrated fish farm NIFFR. Thirty-six females and twelve males from each of *Oreochromis niloticus* and *Tilapia zillii* with the average weight and length of the females ranges from 14.8cm – 20.40cm and weighed 51.70g - 120.50g while male breeders 17.32cm – 23.62cm and weighed 98.60g – 180.00g. The result of the study reveals that the progeny of (♀TZ x♂ ON) gave higher percentage of male (73±1.53) and in addition the higher survival was found in (♀TZ x♂ ON).

Keywords: *All male, hybridization, Oreochromis niloticus and Tilapia zillii*

1. Introduction

Tilapia is one of the most three important group of commercial fish i.e. carps, Tilapia and Salmons [1, 2]. Tilapia is in fact the most widely cultured of many farm fish species in the world. Tilapia possess all the valuable characteristics desirable of a good cultured fish species, such as adaptability to environment, hardiness and acceptance of wide range feed; these attributes, along with the relatively low input cost, have made Tilapia the most widely cultured freshwater fish in the tropical and subtropical countries [3]. Although the potential for Tilapia cultured is high, the production in Africa and more importantly in Nigeria is very low. The drawback being the early maturity, uncontrolled production in ponds leading to increased competition for food and reduction in growth rate resulting in a phenomenon called stunning [4]. There are various methods and techniques for the control of prolific breeding in tilapia [5]. Monosex culture of all male populations, which exhibit faster growth rate and it's usually produced through androgynous hormones, sex reversal is the most preferred options and its use extensively in countries that produce large number of Tilapia e.g. China.

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is likely to be the most important cultured fish in the 21 century [6]. It grows and reproduces in a wide range of environmental conditions and tolerates stress induced by handling [7]. With the purpose of achieving more productivity in growing tilapia, *Oreochromis niloticus*, at the unit time, it is important to produce monosex culture that constitutes totally of males [6]. Male monosex tilapia cultures are preferred to females because of the differential growth in favor of males. In males, the metabolic energy is channeled towards growth and they benefit from anabolism enhancing androgens [8, 9]. In females, there is a greater reallocation of metabolic energy towards reproduction. Although monosex male population can be obtained by direct or indirect methods, oral administration of Oreochromis niloticus has been reported to be the most preferred method in commercial uses [10, 11].

According to [12], hybridization is the mating of genetically differentiated individuals or groups. This breeding technique is used by aquaculturists with the hope of producing aquatic organisms with desired traits. Commonly, the desired goal is to produce offspring that perform better than both parental species (hybrid vigor or positive heterosis). Hybridization may also be used to transfer other desirable characteristics from one group or species to another, and to combine valuable traits from two species into a single group.

2. Materials and methods

This study was conducted in the Biotechnology laboratory at the hatchery complex of the National Institute for Freshwater Fisheries Research (NIFFR) New Bussa, Niger state Nigeria.

The candidate fish were collected from the Kainji lake basin and the integrated fish farm NIFFR. Thirty-six females and twelve males from each of *Oreochromis niloticus* and *Tilapia zillii* were collected. The average weight of the females used ranged from 14.8cm – 20.40cm and their average weight ranged from 51.70g – 120.50g while for the male breeders the average length ranged from 17.32cm – 23.62cm and average weight ranged from 98.60g – 180.00g.

After tanks preparation outdoor, each male breeder is mated at random to three female breeders in a nested mating design to produce paternal half-sib families. This allows for the calculation of phenotypic and genetic parameters (i.e. heritability, phenotypic and genetic correlations), which are necessary for calculating breeders values. The experiment was replicated three times with each generic mating combination having male and female in the ratio of 1:3 in 2x2x1m^2 concrete tanks. The generic mating combination is as shown below:

1. ♀ *O. niloticus* x ♂ *O. niloticus*
2. ♀ T. *zillii* x ♂ T. *zillii*
3. ♀ *O. niloticus* x ♂ T. *zillii*
4. ♀ T. *zillii* x ♂ *O. niloticus*

2.1 Spawning and fry collection

After pairing female and male in the ratio of 3:1 in 2x2x1m^2 concrete tanks, feeding started immediately with feed containing 25% crude protein. The brood fish were fed to satiation two times daily for six days. The seventh day was without feeding in order to enable or allow to clearance of feed fed for six days. Spawning responses were observed third week of pairing with purebred being the first to respond after which the offspring were collected washed, weighed, counted and taken into the laboratory for indoor studies.

After six weeks of experimentation indoor, the fingerlings were taken into 2x2x1m^2 outdoor concrete tanks in three replicates and each containing 100 fingerlings. The pool weight (initial weight) in grams, total length in centimetre of 10% sample size which is 10 taken before stocking. Water quality parameters which include p^{H,} dissolved oxygen, turbidity, temperature and conductivity were also monitored during the period of the experiment.

Sampling was done monthly for three months to monitor the growth parameters (weight and length). At six month of age, manual fish sexing based on external observation was carried out according to [13] where males and females of each of *O. niloticus*, *T. zillii* and their diallelic crosses were separately counted. The ratio of male to female of each generic mating combination was also investigated to identify their percentage maleness. The data obtained was subjected to statistical analysis.

2.2 Fish manual separation

The fish was manually separated based on visual examination of the genital papilla of sizable fish (hand-sexing) using manual sexing materials such as Hand lens, Methylene blue (dye), Bowls, Nets. The sex of 25-gram tilapia fingerling was determined by examining the genital papilla located immediately behind the anus. In males the genital papilla has only one opening (the urinary pore of the ureter) through which both milt and urine pass. In females the eggs exit through a separate oviduct and only urine passes through the urinary pore. Placing a drop of dye (methylene blue or food coloring) on the genital region helps to highlight the papilla and its openings.

2.3 Fish growth performance

Mean weight Gain (MWG)

This was calculated as follows:

MWG = final mean weight(g) – initial mean weight(g)

Mean Length Gain (MLG)

MLG = final mean length(cm) - final mean length(cm)

Specific Growth Rate (SGR)

$$SGR\ (\%) = \frac{LnW_2 - LnW_1}{t} \times 100$$

Where: Ln= the natural log W_1 = initial weight of fish (g), W_2 = final weight of fish (g) and t = number of days of the experiment.

2.4 Survival rate and feed utilization parameters

Survival Rate

$$SR\ (\%) = \frac{\text{Number of fingerlings that survived}}{\text{initial number of fish stocked}} \times 100$$

Feed conversion Ratio (FCR)

$$FCR = \frac{\text{Weight of feed fed (g)}}{\text{Fish weight gain (g)}}$$

2.5 Data analysis

Student T-test was used to compare the sex ratios and other parameters measured. Some parameters measured include: Initial and final body weight (g), mean weight gain (g), Specific growth rate (SGR %/day) and Feed conversion ratio (FCR). Data generated from growth and nutrient consumption were subjected to Analysis of variance (ANOVA) to determine the significant difference between means using S.A.S version 9.2 (2007) where significance differences were observed, the means were separated using Duncan's multiple range test (DMRT) (Duncan1955) [14]

3. Results

Table 1: Sex ratio of Nile Tilapia, Red belly Tilapia and their diallelic crosses

Treatment	Sex	Mean±SD	t-test	p-value
ONXON	Male	48±2.00	-2.449	0.070
	Female	52±2.00	-2.449	0.070
TZXTZ	Male	42±2.00	-12.394	0.000*
	Female	58±1.00	-12.394	0.001*
ONXTZ	Male	54±0.00	4.619	0.010*
	Female	46±3.00	4.619	0.044*
TZXON	Male	73±1.53	23.495	0.000*
	Female	27±3.00	23.495	0.000*

Note: Means with the same superscripts on the same row are not significantly different (p>0.05)

Table 2: Growth performance and survival rate of *Oreochromis niloticus, Tilapia* zillii and their diallelic crosses.

Growth parameter	Genotype			
	ONXON	TZXTZ	ONXTZ	TZXON
Initial weight (g)	104.31±63.07[b]	12.81±1.58[a]	29.54±0.60[a]	53.62±19.70[ab]
Initial length (cm)	3.87±0.28[c]	1.90±0.15[a]	2.87±0.40[b]	2.79±0.62[b]
Final weight (g)	590.50±3.30[b]	474.99±58.87[a]	602.67±89.79[b]	581.62±26.39[b]
Final length(cm)	7.46±0.72[b]	4.75±0.38[a]	6.70±0.38[b]	7.24±0.29[b]
Length gain (cm)	3.59±0.5[ab]	2.85±0.36[a]	3.83±0.42[ab]	4.45±0.88[b]
PWG(g)	520.36±292.00[a]	2600.68±153.24[a]	1629.45±2.57[a]	719±28±407.37[a]
MWG(g)	420.10±83.48[ab]	334.76±62.22[a]	481.34±9.82[b]	333.59±48.81[a]
SGR (g)	6.70±0.24[ab]	6.45±0.20[a]	6.86±0.20[b]	6.45±0.17[a]
FCR	011±0.04[b]	0.06±0.03[a]	0.08±0.05[ab]	0.10±0.01[ab]
SR (%)	77[a]	75[a]	78[a]	68[a]

Note: Means with the same superscripts on the same row are not significantly different (*p*>0.05)

The highest value of mean weight gain (MWG) and specific growth rate (SGR) were found in the crosses of ♀ON x ♂ON and ♀ON x ♂TZ but there are no significant differences from the hybrid ♀TZ x ♂ON (*p*>0.05). There is significant difference from the purebred of ♀TZ x ♂TZ. However, there is no significant differences among all the crosses in terms of pool weight gain (PWG) although, hybrid of ♀TZ x ♂ON recorded the highest value of 2403.27±3799.12 (*p*>0.05).

The best and highest mean value of survival rate (SR) was achieved by the purebred of (♀ *O.N* x ♂ *O. N*), and does not differ significantly from that of the hybrid ♀TZ x ♂ON but differ significantly from purebred ♀TZ x ♂TZ and hybrid ♀ON x ♂TZ (p≤0.05).

The best and highest mean value of FCR is achieved by the purebred of (♀ *O. niloticus x* ♂ *O. niloticus*), and differ (P≤0.05) significantly from those of the reciprocal hybrid and purebred.

4. Discussion

Hybridization has played an important role in monosex culture of tilapia. All- or nearly all-male broods can be produced in interspecific hybridization of female *O. niloticus* with male *O. aureus*. The yield of male progeny has varied from 52 to 100% in interspecific hybridization between the two species [5]. Pruginin *et al.* [15] reported that the proportion of males in single pair crosses between *O. niloticus* females and *O. aureus* males was found to vary between 50 and 100%.

The results of the present study are consistent with the findings of many authors, since male percentage was 73%. In a few cases, 95 to 100% male hybrids have been produced [15]. The result of this studies showed that the cross of (♀*O. niloticus* x ♂*T. zillii*) gave the highest male to female ratio which is in agreement with [16] (77.05%) and [17] (varied 52-100%) The success or failure in all-male tilapia populations by interspecific hybridization depends on the interaction of three components: a major determinant locus, a minor polygenic component and temperature during early fry phase [18]. Interspecific hybridization was successfully obtained in many fish and shellfish genera or families as a means of improving economic traits [19-21]. Hybridization between some species of tilapias such as Nile tilapia and Blue tilapia resulted in the production of predominantly male offspring [20].

The survival rate in this study was high in the genetic combination of *Oreochromis niloticus, Tilapia zillii* and their diallelic crosses (68-78), it was observed to be highest in the cross of ♀ON x ♂TZ (78.00±8.72). The differences observed among the genetic mating combination progeny might be no doubt due to species differences. Studies have shown that *Oreochromis niloticus* have the ability to survive for a long time without being fed, but it will be losing weight. Similarly, some authors reported their observations for different Tilapias and culture system as thus, *Oreochromis randalii* (86- 96%) in Mexico [22] have reported to have higher survival rate which was almost similar to the observed result in the present study. More so, [23] raised *Oreochromis niloticus* in earthen ponds and found survival rate to be 96.3-97.2%; [24] obtained survival rate of 96.5-99.5% in concrete tanks; [24] in fibre glass tanks observed survival rate of 99.2-100%; [24] in pond system found survival rate of 92.4% and [25] in glass aquaria found survival rate of 96-100%. The higher survival rates reported by these authors may have been as the result of the differences in the culture systems used. The survival rate observed for the crosses in this work is less or lower or similar to the survival rate reported by [26] (72.7-87.7%); [27] (69.3%) *Oreochromis niloticus*, while *Tilapia zillii* in Egypt have been reported to have the lowest survival rate ranging from 43.3 – 46.7% [25], which correspond with the findings of the present study.

The highest value of mean weight gain (MWG) and specific growth rate (SGR) were observed in the crosses of ♀ON x ♂ON and ♀ON x ♂TZ but there are no significant differences from the hybrid ♀TZ x ♂ON (*p*>0.05). More so, there is significant difference from the purebred of ♀TZ x ♂TZ. However, there is no significant differences among all the crosses in terms of pool weight gain (PWG) although, hybrid of ♀TZ x ♂ON recorded the highest value of 2403.27±3799.12 (*p*>0.05).

However, the observed results on weight gain in this present study are higher than that reported by [28] for tilapia fed on coffee pulp (45g); Hossain *et al*. 2003 used aquaria systems (56.76g); [29] in concrete tanks (48.77) and [30] in fiberglass tanks (49.33g). The differences might be due to the type of the diets used to feed the fishes and different feeding regimes.

Also comparing the present work with works reported by others on Nile Tilapia, the findings from this study agrees with the results on the specific growth rate reported by [31], who used different inclusion levels of duckweed as protein source in the fish diet, [23] who supplemented the fish with pelleted and unpelleted distillers grains in cages and [32] who fed the fish with diets containing graded levels of green algae Ulva meal (*Ulva rigida).*

The observation from this study showed that the cross of ♀TZ x ♂ON ranked second after ♀ON x ♂ON in growth performance. The mean weight gain, specific growth rate and pool weight gained was higher in ♀TZ x ♂ON among other genetic crosses and this makes them suitable as species that can complement ♀ON x ♂ON as a very good candidate for aquaculture. The observation of this work shows that the cross of ♀TZ x ♂TZ had the lowest growth performance. This is similar to the findings of [33]. Since the feed given in the present study were similar to all fish species, the differences in growth rate observed for these genetic crosses might be caused by difference in their genetic makeup.

A hybrid with selected or favored characteristics of each parent is one of the goals of animal husbandry. When a hybrid has a characteristic superior to both parents, it is said to have hybrid vigour or positive heterosis, which is the ultimate breeding goal. In the species studied, the hybrids of ♀ON x ♂TZ produced perform significantly better than their parents in terms of Growth performance and their survival rates were higher; therefore, they would probably hold attraction for aquaculture. *Oreochromis niloticus* is still the best choice of species for Aquaculture production. This study agrees with previous studies by [34], who stated that the most important species among cichlids is Nile Tilapia, *Oreochromis niloticus*, because they grow fast, they are easy to feed, they are resistant to poor water quality and disease and can easily reproduced [35].

5. Conclusion

The results of this study showed that the cross (hybrid) of ♀ *T. zillii x* ♂ *O. niloticus* gave the highest number of males and the cross (hybrid) ♀ *O. niloticus x* ♂ *T. zillii* gave the best growth performance when compared between *Oreochromis niloticus, Tilapia zillii* and their diallelic crosses. Furthermore, combining these two qualities, the hybrids of can be considered and developed as a suitable candidate for aquaculture production.

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