# Production of genetically male tilapia through interspecific hybridization between *Oreochromis niloticus* and *O. aureus*

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## **Abstract**

This study was conducted to produce a high percentage of genetically male tilapia through interspecific hybridization between Nile tilapia, *Oreochromis niloticus* and Blue tilapia, *O. aureus* and evaluate sex ratio, productive performance and heterosis of the progeny produced. The results revealed that sex ratios of the progenies of ( $\subsetneq$  *O. aureus* ×  $\circlearrowleft$  *O. niloticus*) and ( $\supsetneq$  *O. niloticus* ×  $\circlearrowleft$  *O. aureus*) were 4.28: 1.00 and 3.59: 1.00 and differ (P<0.05; df =1) significantly from the expected sex ratios of 3.00: 1.00 and 1.00: 0.00, respectively. In addition, the productive performance traits of ( $\supsetneq$  *O. aureus* ×  $\circlearrowleft$  *O. niloticus*) had significant (P≤0.05) superiority in most of these traits. Moreover, although both hybrids displayed a positive or best heterosis for daily gain, SGR%/day, FCR and PER, the hybrid of ( $\supsetneq$  *O. aureus* ×  $\circlearrowleft$  *O. niloticus*) showed the highest or best heterosis.

**Keywords:** Genetically male, interspecific hybridization, Nile tilapia, Blue tilapia, productive performance, heterosis

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#### Introduction

Tilapias have great importance in world fisheries; besides, they are among the most important groups of food fishes in the world, next to the carps. Nile tilapia, *Oreochromis niloticus* accounted for a harvest of nearly 2.54 million tones in 2009 (FAO, 2011). Tilapia are easy to culture and reproduce with rapid sexual maturation at 6-7 months from hatch and also marketable at this age. Nile tilapia is also an excellent laboratory animal; consequently, it deserves to be studied (Maclean et al., 2002).

In aquaculture it is often preferable to produce monosex populations of fish due to growth differences between sexes, e.g. males tilapia grow faster than females (Chakraborty and Banerjee, 2010), whereas female salmonids and sparids grow better than males. A specific sex may produce a valuable product, such as caviar; and monosex populations help reduce unwanted reproduction that would result from mixed-sex populations, e.g. tilapia overpopulation and stunting (Kamaruzzaman et al., 2009; Bartley et al., 2001).

Hybridization between some species of tilapias (Cichlidae) such as Nile tilapia and the blue tilapia results in the production of predominantly male offspring and reduces unwanted natural reproduction in grow out ponds (Rosenstein and Hulata, 1994). This cross produces predominately males because of different sex-determining mechanisms in the two species: Nile tilapia has the XX, XY system with the male being heterogametic, whereas blue tilapia has ZZ, ZW with the heterogametic genotype being female (Wohlfarth, 1994).

Although the precise mechanisms by which sex is determined in tilapia, specifically O. niloticus, are not fully understood. Early hypotheses were based on the sex ratios observed in hybrid crosses of different species; the most comprehensive of these hypotheses is based on a theory of autosomal influence (Hammerman and Avtalion, 1979). However, so far, no theory based on hybrid sex ratios successfully explained all observed sex ratios, which may be highly variable in some crosses such as those between O. niloticus and O. aureus. Recent research concentrated on intraspecific sex ratios. Several authors presented evidence which indicated that O. niloticus has a predominantly monofactorial mechanism of sex determination with heterogametic XY males and homogametic XX females (Mair et al., 1991). However, this simple monofactorial hypothesis fails to explain some deviations from predicted sex ratios based on studies involving sex reversal and chromosome set manipulation. Hussain et al. (1994) hypothesized the existence of an autosomal sex modifying locus (with alleles SR and sr) epistatic to the gonosomal locus which induces female to male sex reversal when sr is homozygous. Baroiller et al. (2009) reported that sex determination of tilapia appeared to be largely controlled by the interaction of three components: a major determinant locus. minor polygenic component and temperature during early fry phase.

The main goal of this work was to produce a high percentage of genetically male tilapia through interspecific hybridization between Nile tilapia (*Oreochromis niloticus*) and Blue tilapia (*O. aureus*), compare the progeny sex ratios, productive performance characteristics, and estimate the heterosis.

### Materials and methods

The present study was carried out at the Experimental Fish Farm and the Laboratory of Breeding and Production of Fish, Animal and Fish Production Department, Faculty of Agriculture (Saba-Bacha), Alexandria University, Alexandria, Egypt.

Fish origin

The Nile tilapia and Blue tilapia were obtained from Middle East Fish Farm, El-Behera Governorate, Egypt.

Eighteen females and six males from each of O. niloticus (with an average live weight 69.50±3.12 and 97.50±1.90g) and O. aureus (with an average live weight 65.50±1.42 and 89.09±2.98g), respectively were chosen according to their ripeness. Three replicates from each of Nile tilapia (O. niloticus), Blue tilapia (O. aureus), ( $\bigcirc$ O. aureus X  $\bigcirc$ O. *niloticus*) or ( $\bigcirc$  *O. niloticus*  $\times$   $\bigcirc$  *O. aureus*) were stocked for natural spawning in separated concrete ponds (3x1x1.2m) at a rate of 4 breeders/m<sup>3</sup>. The sex ratio of the fish was 3 females: 1 male. Brood fish were fed twice daily on pellet diet contained 26 % protein at satiation for 6 days a week.

Post-hatching fry of *O. niloticus*, *O. aureus*, and their diallelic crosses were collected, counted and weighed, then transferred separately to laboratory experimental glass aquaria (100 liter volume) in three replicates for each. The fry acclimation to laboratory

conditions were counted and weighed. Each aquarium was supplied with dechlorinated tap water and adequate continuous aeration systems, cleaned once daily by siphoning and was replaced one-half to two thirds of their water volume. Fry were fed three times daily on pellet diet containing 38% protein to satiation, six days a week for 90 days.

At 90 days of age, fish sexing based on external observation was carried out according to (Popma1 and Masser, 1999). Males and females of each of *O. niloticus*, *O. aureus*, and their diallelic crosses were separately counted.

The following parameters were measured: initial and final body weight (g), daily gain (g/day), specific growth rate (SGR %/day), feed intake, feed conversion ratio (FCR) and protein efficiency ratio (PER). Initial and final body composition analyses were performed for moisture, crude protein and lipid contents according to Association of Official Analytical Chemists methods (AOAC, 1984).

Heterosis was expressed in percentage by the formula reported by Nguenga et al. (2000):

Heterosis (H%) =  $[(C_1 + C_2)/2 - (P_1 + P_2)/2] / [(P_1 + P_2)/2] \times 100.$ 

Where:

 $C_1$  and  $C_2$  are the mean daily gain, SGR %/day, FCR, PER, PR%, or ER% of crossbreeds, and  $P_1$  and  $P_2$  are the mean daily gain, SGR %/day, FCR, PER, PR%, or ER% of the purebreds.

Data of quantitative traits were analyzed using the following model (CoStat, 1986):

 $Y_{ijk} = \mu + T_i + B_k + e_{ijk}$ 

Where:

 $Y_{ijk}$ : Observation the  $ijk^{th}$  parameter measured;  $\mu$ : Overall mean;  $T_i$ ; Effect of  $i^{th}$  species;  $B_k$ : Effect of  $K^{th}$  block;  $e_{ijk}$ : Random error.

Significant differences ( $P \le 0.05$ ) among means were tested by the method of Duncan (1955).

All sex ratios of the produced progeny were tested according to Snedecor and Cochran (1980), using Chi-Squared test ( $\chi^2$  test; df = 1; P<0.05), against the expected sex ratios of 1:1 (for progeny produced from purebreds) or 3:1 (for progeny produced from the hybrid of  $\varphi$  Blue tilapia x  $\Im$  Nile tilapia) or 1:0 (for progeny produced from the hybrid of  $\varphi$  Nile tilapia x  $\Im$  Blue tilapia).

# **Results**

Data of Table 1 show all sex ratios of the produced progeny of *O. niloticus*, *O. aureus*, and their reciprocal hybrids. Sex ratios of the purebred were not significantly different from 1:1. Sex ratio of the hybrid of ( $\bigcirc$  *O. aureus* x  $\bigcirc$  *O. niloticus*) was 4.28:1.00 and differ significantly (P<0.05; df =1) from the expected sex ratio (3.00:1.00). Also sex ratio of the hybrid ( $\bigcirc$  *O. niloticus* x  $\bigcirc$  *O. aureus*) was 3.59: 1 and differ significantly (P<0.05; df =1) from

the expected sex ratio (1.00: 0.00). Data of Table 2 show growth performance and feed utilization of Nile tilapia, Blue tilapia and their reciprocal hybrids. The highest value of initial body weight (IBW) was achieved by O. niloticus, but did not differ significantly from those of the hybrids. The highest values of final body weight (FBW) and daily gain (DG) were obtained by the hybrid ( $\bigcirc$  *O. aureus*  $\times$   $\bigcirc$  *O.* niloticus), but did not differ significantly from that of the hybrid ( $\bigcirc$  *O. niloticus*  $\times$   $\bigcirc$  *O.* aureus). The highest values of specific growth rate (SGR%/day) and feed intake were found by the hybrid ( $\bigcirc$  *O. aureus*  $\times$   $\bigcirc$  *O. niloticus*), but did not differ significantly from those of the hybrid ( $\bigcirc$  O. niloticus  $\times$   $\bigcirc$  O. aureus) and purebred of O. niloticus. The best and highest mean values of FCR and PER were achieved by the hybrid of  $(\mathcal{Q} \ O. \ aureus \times \mathcal{A} \ O. \ niloticus)$ , and differ (P≤0.05) significantly from those of the reciprocal hybrid and purebreds.

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

Genotypes	Number of	Male	Female	Male: Female	
	harvested			Expected	Observed
Nile Tilapia (N)	663	335	328	1.00: 1.00	1.02: 1.00
Blue Tilapia (B)	543	278	265	1.00: 1.00	1.05: 1.00
♀ B x ♂ N	528	428	100	3.00:1.00	4.28:1.00*
$\supseteq \mathbf{N} \times \circlearrowleft \mathbf{B}$	584	450	134	1.00: 0.00	3.59: 1.00*

<sup>\*</sup> Sex ratio significantly different ( $\chi^2$  test; df = 1; P<0.05).

Table 2: Growth performance and feed utilization of Nile Tilapia, Blue Tilapia and their diallelic crosses

Genotypes	IBW (g)	FBW (g)	DG (g)	SGR%/ day	Feed intake (g)	FCR	PER
Nile Tilapia (N)	0.00183±0.0 <sup>a</sup>	26.78±2.91 <sup>b</sup>	0.30±0.03 <sup>b</sup>	10.65±0.10 <sup>ab</sup>	58.47±6.23 <sup>a</sup>	2.18±0.01 <sup>b</sup>	1.20±0.00°
Blue Tilapia (B)	0.00173±0.0 <sup>b</sup>	19.97±4.38°	0.22±0.05°	10.38±0.25 <sup>b</sup>	44.73±9.54 <sup>b</sup>	2.24±0.02 <sup>a</sup>	1.17±0.01 <sup>d</sup>
♀ B x ♂ N)	.00180±0.0 <sup>ab</sup>	32.93± 1.19 <sup>a</sup>	0.37±0.01 <sup>a</sup>	10.91±0.07 <sup>a</sup>	64.97±2.36 <sup>a</sup>	1.97±0.01 <sup>d</sup>	1.33±0.00 <sup>a</sup>
$\supseteq N \times   B)$	.00180±0.0 <sup>ab</sup>	28.62±0.44 <sup>ab</sup>	0.32±0.01 <sup>ab</sup>	10.75±0.02 <sup>a</sup>	58.67± 0.91 <sup>a</sup>	2.05±0.00°	1.28±0.00 <sup>b</sup>

Means having different superscripts within column are significantly different (P≤0.05).

Initial and final body weight (IBW and FBW) = body weight at start and end of experiment.

Daily weight gain (WG) = final weight - initial weight/ number of days.

Specific growth rate (SGR% / day) = (Loge final weight - Loge initial weight) 100 / number of days.

Feed conversion ratio (FCR) = dry feed intake/weight gain.

Protein efficiency ratio (PER) = weight gain/protein intake.

The highest significant (P≤0.05) mean of the moisture content at the beginning of the experiment was obtained by purebred of O. aureus, but did not differ significantly from that of the hybrid  $\bigcirc O$ . *niloticus*  $\times \bigcirc O$ . *aureus*. While, no significant differences were detected in moisture content at the end of experiment (Table 3). The highest significant ( $P \le 0.05$ ) mean of protein content at the beginning of the experiment was achieved by purebred of O. niloticus, but did not differ significantly from those of the hybrids  $\bigcirc O$ . *niloticus*  $\times \bigcirc O$ . *aureus* and  $\bigcirc O$ . aureus  $\times \bigcirc O$ . niloticus. The highest significant (P < 0.05) value of protein content at the end of the experiment was obtained by purebred of O. niloticus, but did not differ significantly from that of the hybrid  $\bigcirc O$ . aureus

x  $\Im O$ . *niloticus*. The lowest significant (P $\leq$ 0.05) mean of fat content at the beginning of the experiment was obtained by purebred of O. *aureus*, but did not differ significantly from that of the hybrid  $\Im O$ . *niloticus* x  $\Im O$ . *aureus*. While the highest significant (P $\leq$ 0.05) mean of fat content at the end of the experiment was found by the hybrid  $\Im O$ . *aureus* x  $\Im O$ . *niloticus*, but did not differ significantly from that of the hybrid  $\Im O$ . *niloticus* x  $\Im O$ . *aureus*.

Data of Table 4 show the heterosis values of interspecific hybridization between O. niloticus, O. aureus. Although both hybrids displayed a positive or best heterosis for daily gain, SGR %/ day, FCR and PER, the hybrid of  $\bigcirc O$ .  $aureus \times O$ . niloticus, showed higher or best heterosis.

Table 3: Body composition of Nile Tilapia, Blue Tilapia and their diallelic crosses

	Moisture %		Dry matter (%)				
Genotypes			Crude p	rotein %	Crude fat %		
	Beginning	End	Beginning	End	Beginning	End	
Nile Tilapia	78.09±0.82°	77.17±0.32	55.75±0.62 <sup>a</sup>	56.28± 0.24 <sup>a</sup>	19.15±0.22 <sup>a</sup>	20.36±0.47 <sup>b</sup>	
Blue Tilapia (B)	80.26±0.23 <sup>a</sup>	77.75±0.87	53.92±0.82 <sup>b</sup>	$54.99 \pm 0.74^{b}$	17.55±0.64 <sup>b</sup>	19.64±0.41 <sup>b</sup>	
Q B X  N	79.23±0.17 <sup>b</sup>	77.64±0.41	55.45±0.79 <sup>ab</sup>	55.57±0.38 <sup>ab</sup>	18.99±0.39 <sup>a</sup>	22.13±0.30 <sup>a</sup>	
$\supsetneq N \ x \ \circlearrowleft B$	79.43±0.25 <sup>ab</sup>	78.17±0.72	55.18±0.72 <sup>ab</sup>	55.09±0.34 <sup>b</sup>	18.32±0.30 <sup>ab</sup>	21.43±0.87 <sup>a</sup>	

Means having different superscripts within column are significantly different (P≤0.05).

Table 4: Heterosis (H%) values of the weight gain, SGR%/day, FCR and PER of the interspecific hybridization between Nile tilapia and Blue tilapia

Parameters	H% <sup>1</sup>	H% (♀B x ♂N)²	H% (♀N x ♂B)³
DG	32.69	42.31	23.08
SGR%/day	3.00	3.76	2.23
FCR	-9.05	-10.86	-7.24
PER	10.13	12.24	8.02

- 1- General heterosis
- 2- Heterosis according to the mean of performance of F1 from the hybrid of ( $\bigcirc$ B x  $\bigcirc$ N)
- 3- Heterosis according to the mean of performance of F1 from the hybrid of ( $\cite{P}N$  X  $\cite{O}B$ )

#### **Discussion**

Marengoni et al. (1998) reported that the crossbreeding between *O. niloticus* and *O. aureus* has become very popular and thus one of the most effective measures for producing allmale progenies of tilapia in Japan. All-male populations are desired for control of reproduction and uniform marketable crop in ponds. 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 (Mair et al., 1991). Pruginin et al. (1975) 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 these findings, since males percentage was 77.05%. In a few cases, 95 to 100% male hybrids have been produced (Hulata et al., 1983). 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 (Baroiller et al., 2009).

Interspecific hybridization was successfully obtained in many fish and shellfish genera or families as a means of improving economic traits (Dunham et al., 2001; Hulata, 2001; Pushparaj, 2010; Granier et al., 2011). Hybridization between some species of tilapias such as Nile tilapia and Blue tilapia resulted in the production of predominantly male offspring (Hulata, 2001). This hybrid combined well the advantageous characteristics of both species, being more cold tolerant than O. niloticus and less borrowing in the mud than O. aureus. It also has good salinity tolerance and faster growth as a result of production of predominately male offspring, thus males grow faster than females in many tilapia (Wohlfarth, 1994; Penman and McAndrew, 2000; Hulata, 2001). Moreover, the results obtained by El-Zaeem (2011), are consistent with these findings, thus the hybrid of  $\bigcirc O$ . *niloticus*  $\times \bigcirc O$ . aureus and  $\bigcirc O$ . aureus  $\times \bigcirc O$ . niloticus had significantly higher (P < 0.05) traits of growth performance and feed utilization than those of purebred of O. niloticus and O. aureus. In addition, the results in the present study are consistent with the findings observed by (Haroun, 1999; El-Zaeem et al., 2010). They reported that there are slightly differences in chemical composition among the hybrids of  $\bigcirc O$ . niloticus  $\times \bigcirc O$ . aureus,  $\bigcirc O$ . aureus  $\times \bigcirc O$ . niloticus and their purebred.

Heterosis refers to the phenomenon that first generation progeny of diverse species or populations exhibit greater trait performance, either in terms of biomass, development, or fertility, than the better of the two parents (Guy et al., 2009; Nielsen et al., 2010; Granier et al., 2011). The heterosis expression levels observed in hybrids were variable depending on the trait or the period considered, but these levels are quite high when compared with other studies dealing with heterosis in fishes. For instance, the percentage of heterosis in guppy ranged from -1.3% for body length to 42.2 % for salinity tolerance (Shikano et al., 1997; Nakadate et al., 2003), whereas they found a mass advantage of 88.2% at the yolk-sac resorbtion stage and 48.3% after 15 weeks of exogenous feeding in hybrids between Laval dams and domestic sires compared with the mean values of pure strains.

In this connection, El-Zaeem et al. (2012) reported that the hybrid of (♀ Red tilapia x Nile tilapia) reared at 16 and 32 ppt of salinity showed a higher positive heterosis compared to the hybrid of ( $\bigcirc$ Nile tilapia x  $\bigcirc$  Red tilapia) for weight gain and SGR% / day. Nguyen et al. (2009) estimated heterosis, direct additive genetic and general reciprocal effects from a complete diallel cross involving four strains of Red tilapia Oreochromis spp. from Malaysia, Scotland, Taiwan and Thailand. The average heterosis for body weight across the testing environments was low (4.2%) and the average of all crossbreds was not statistically different from the mean of pure strains. Similar trends were reported in the present study, since the positive heterosis values of daily gain, specific growth rate, FCR, and PER showed that a positive interaction has occurred between the parental genes found at different loci in the inter-generic hybrid genome as reported by Sheridan (1981). The phenotypic variance of a quantitative trait such as growth is governed by

the genetic variance, environmental variance and the interaction between the genetic and environmental variance (Tave, 1993). In addition, Granier et al. (2011) investigated the presence of heterosis using reciprocal intraspecific crosses between three genetically different strains, (ii) to verify the presence of cross-type effects on the occurrence of heterosis, and (iii) to estimate heterosis at different developmental stages. Their study revealed a complex pattern for heterosis of brook trout that varied according to developmental stage. In contrast to previous studies in fish, and particularly in salmonids where the presence of heterosis in hybrids of the first generation does not seem to be a general feature (Hulata, 2001; Bryden et al., 2004; Gunther et al., 2005; Pongthana et al., 2010), they observed occurrences of heterosis for different growth traits and cross types.

The results of the present study suggested that the hybrid of  $(\bigcirc O. \ aureus \times \bigcirc O. \ niloticus)$  with significant superiority of most of the productive performance traits and positive or best heterosis can be produced for commercial culture.

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