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**Research Paper**

# Reciprocal Crosses of Two Improved Strains of Nile Tilapia: Implications on Reproductive and Growth Traits

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***ABSTRACT:*** *Breeding programmes target harvest weight in most cases but reproductive traits also need to be considered since it is reproduction that brings the test organisms for selective breeding. This research used a reciprocal crossing system to evaluate the reproductive and growth traits of the progeny using two strains of Nile tilapia: chitralada and GIFT. A total of six females was allocated to each cross in a paring ratio of 2♀:1♂. Examination for mouthbrooding was done every three days for 13 weeks. Spawning was observed at varying rates across the crosses but all females spawned at least once. Swim-up fry were also collected and stocked in a recirculatory system indoors as they were being produced to determine their growth. This trial lasted for 8 weeks in each case. There was a significant difference ($p<0.05$) in female weight, clutch weight, relative fecundity and spawning index. Significant differences were not observed in absolute fecundity, standard length, egg weight and hatching rates ($p>0.05$). Body weight was higher in GIFT (1422.18g and 1508.68g) while clutch weight was higher in chitralada (27.00g and 27.65g). There was positive heterosis for clutch weight (5.46%) and egg weight (9.8%). Final weight was in favour of GIFT (3.57g) and crossbreeds involving GIFT (GT×CH=3.88g; CH×GT=3.49g). This further translate into better performance in daily growth rate and specific growth rate (SGR). Survival of fry during growth trials was not affected by cross ($p>0.05$). There was a positive heterosis for final weight (11.84%), DGR (18.18%) and SGR (1.65%). It is concluded that improvements that must combine reproductive traits and harvest weight should consider crossing of chitralada and GIFT*

***KEYWORDS:*** *breeding, clutch weight, egg, fecundity, spawning*

## I. INTRODUCTION

Among the freshwater aquaculture species farmed in Thailand, the Nile tilapia (*Orechromis niloticus*) is the most popular with a production volume of 217,928 tonnes in 2017 [1]. This freshwater fish species has been extensively developed by various agencies in the country as well as private sector farmers. There is considerable effort at genetic improvement of the species in Thailand [2, 3]. This has enhanced the production status of the species compared to other fish species under culture in the country.

Increased production of aquaculture products is linked to the availability of improved stock that can produce at various percentages more than the original stock [4]. When proper selection programmes are set up, the genetic gains at every generation can average 10% [5] but gains as high as 20% have been documented in Nile tilapia [3] (Uraiwan, 1988). In the execution of selective breeding of any species, the weight at harvest is the primary goal while other non-targeted traits such as reproductive capacity may be either enhanced [6] or depressed [7]. The foregoing implies that when crossbreeding is being carried out, both growth and reproductive traits ought to be monitored.

The Nile tilapia was first introduced to Thailand in 1965 [8]. The nomenclature of this strain became chitralada and it gained wide acceptance to become a major species for intensive aquaculture that is supporting the aquaculture industry [9]. The base stock of Nile Tilapia being culture in Thailand is only 38 individuals that emanated from the Royal Palace [10] with initial origins in Egypt [11]. Another species of tilapia, *O. mossambicus* had been introduced to Thailand much earlier than this from Malaysia in 1949 [12]. While feral populations of both species had established in natural water bodies of Thailand, the former was successful in establishing significant capture fisheries [10]. The Asian Institute of Technology, Thailand (AIT) maintained a unique chitralada strain, providing support to farmers in Thailand and elsewhere to develop thriving tilapia aquaculture industries. There is a plethora of strains of tilapia in Thailand including the chitralada, GIFT (developed by WorldFish), Big Nin (introduced from Indonesia), Nam Sai strain and the Super black tilapia (Manit Farm strain developed in a joint project with Akvaforsk (Norway) Genetics Centre) among others.

Some researchers have examined the reproductive traits of Nile tilapia originating either from selectively bred and crossbred varieties such as chitralada [13], specific details on fecundity and fertility [6] as well as the egg quality in clutches from various hatcheries [14]. However, the effects of crossing between different genetically improved varieties on reproductive traits is not well documented.

Selective breeding can directly affect the reproductive ability of animals [15] with increased negative impact resulting from inbreeding [16]. However, crossbreeding can be a useful tool in increasing both growth traits and reproductive traits [17]. Considering these, a preliminary analysis of improvement in reproductive traits as well as growth will give insight into the possible gains from selective breeding over many generations in an organised breeding programme. This study therefore, aims to provide information on reproductive traits and growth performance of progeny from reciprocal crosses between two improved strains of Nile tilapia: GIFT and chitralada.

## II. MATERIALS AND METHODS

**Broodstock and Spawning**

Two strains of Nile tilapia: chitralada 3 (Department of Fisheries - Bang Sai) and GIFT (Aquatic Animal Genetic Research and Development Institute, Klong 5 Pathumthani) were used in the experiment. The strains were coded as CH for chitralada and GT for GIFT. A reciprocal crossing system was adopted to produce four genetic groups: CH×CH, CH×GT, GT×CH and GT×GT (♀:♂). Mates were paired in 1m$^3$ net hapas distributed in two ponds of 20m$^2$ area each housing 12 cages respectively. There was a total of 6 cages per cross that housed three fish each using a sex ratio of 2:1. The broostock used were all 1+ years of age with morphometric characteristics including chitralada - ♀: 1113.6±83.4g; ♂: 1771.6±42.2g and Gift ♀: 1465.4±50.8g; ♂: 1534.6±60.0g.

The female fish were checked every three days for the presence of eggs in their mouth. When mouthbrooding was detected, the eggs were rinsed out of the mouth into a plastic bowl and the female was returned into the hapa. It took about 13 weeks for all the pairs to spawn and data was recorded on a continuous basis. The eggs were incubated in a 3L round bottom upwelling incubation unit with continuous water circulation. Water temperature in the incubation system was 26.5±1.0°C. Variables that indicated reproductive traits include: clutch weight per female (g), average oocyte weight (mg), absolute fecundity (number of eggs spawned), relative fecundity (number of eggs/female weight (g)), spawning index ([total egg weight (g)/female weight (g)]*100%), hatching rate ([number of hatched larvae per sample/total number of eggs and larvae in the sample]*100%), individual weight of the females (g), and standard length (cm) of females. Egg weight was determined as the average of eggs present in triplicate 1g egg samples. To estimate the absolute fecundity and relative fecundity the number of eggs in 1g sample was multiplied by the total clutch weight. Hatching rate was determined using a sample of 100 eggs from each of the females used per cross.

**Growth of swim-up fry**

To determine growth, 150 samples of the swim-up fry were obtained as they were produced and stocked in 1.1m x 0.8m x 0.5m indoor glass aquaria with self-re-circulation and aeration and reared for 8 weeks. Water level was maintained at tank height of 0.4m. Weight of the fish was determined weekly by collecting all fish and bulk weighing them using an electronic balance (UWE model DW-6000E). Survivors were counted in the process. Mortalities were recorded daily. Growth parameters determined include mean initial weight (MIW (g)), mean final weight (MFW (g)), average daily gain (ADG = ((final weight - initial weight)/56 days) (g.day$^{-1}$)) and specific growth rate (SGR = (((Ln final weight - Ln Initial weight)/56 days)*100) (%.day$^{-1}$)).

**Heterosis**

Heterosis for reproductive traits and growth was determined using the equation by Nguenga, et al. [18]:

$$[((((C_1 + C_2)/2) - ((P_1 + P_2)/2))/((P_1 + P_2)/2)] \times 100$$

Where $C_1$ and $C_2$ are the mean traits of the crossbreeds (CH×GT and GT×CH) and $P_1$ and $P_2$ are mean pure line traits (CH×CH and GT×GT).

**Feeding**

The broodstock were fed using a extruded tilapia feed (30% crude protein, 4.0% ether extract, 8% crude fiber and 88% dry matter) at 1% body weight per day using two feeding quotas at 8:00h and 16:00h. Fry were fed ad libitum using a powdered diet (35% crude protein, 6.0% ether extract and 4% crude fibre) for the first three weeks before being weaned unto 2mm extruded pellets (30% crude protein, 4.0% ether extract, 8% crude fiber and 88% dry matter).

**Water Quality**

Ammonia (mg.l$^{-1}$), Temperature (ºC), dissolved oxygen (mg.l$^{-1}$) and water pH in fry rearing tanks (Table 1) were determined using the YSI ProQuatro 606950 multiparameter water quality meter. Only temperature was monitored in the broodstock ponds. Temperature in the broodstock ponds ranged from 26.5 ºC to 28.2ºC during the spawning period.

**Table 1.** Mean water quality recorded against each cross in the rearing of swim-up fry.

| Cross | Temp (°C) | DO (mg.l$^{-1}$) | Ammonia (mg.l$^{-1}$) | pH |
|---|---|---|---|---|
| CH×CH | 26.00 ± 0.04 | 10.77 ± 0.13 | 0.14 ± 0.00 | 8.81 ± 0.13 |
| CH×GT | 28.00 ± 0.04 | 8.92 ± 0.13 | 0.07 ± 0.00 | 8.21 ± 0.10 |
| GT×CH | 28.96 ± 0.04 | 7.71 ± 0.09 | 0.05 ± 0.00 | 8.00 ± 0.09 |
| GT×GT | 26.95 ± 0.04 | 10.06 ± 0.12 | 0.11 ± 0.00 | 8.81 ± 0.09 |

**Data analysis**

The experiments were set up as a completely randomized design with four crosses (CH×CH, CH×GT, GT×CH and GT×GT) and six replicates (each female was an experimental unit). Data was subjected to analysis of variance followed by mean separation using Fisher's LSD at 5% level of significance. Data that were obtained as percentages (Spawning index and hatching rate) were arcsine transformed before the analyses. All analyses were performed using R v3.4.3 (R Core Team, 2017).

## III. RESULTS

There was a significant difference ($p<0.05$) in the body weight of the females across the four crosses. Standard length of the female broodstock were not significantly different across the genetic groups ($p>0.05$). The GIFT females weighed more than the chitralada females (Table 2). There was a significant difference in clutch weight ($p<0.05$) with the chitralada strain recording greater clutch weight than the GIFT strain. Egg weight was however not significantly different across the genetic groups ($p<0.05$). Absolute fecundity and hatching rates were not significantly different across the genetic groups ($P>0.05$). Relative fecundity and spawning index or gonado-somatic index were significantly different across the genetic groups ($p<0.05$) with better relative fecundity among the chitralada females than the GIFT females with a similar disposition for the spawning index as well.

**Table 2.** Means of reproductive performance traits in two strains of the Nile tilapia females (*Oreochromis niloticus*)

| Cross | CH×CH | CH×GT | GT×CH | GT×GT | p-value |
|---|---|---|---|---|---|
| Wt (g) | 1108.34 ± 174$^{b}$ | 1118.86 ± 14.4$^{b}$ | 1508.68 ± 92.50$^{a}$ | 1422.18 ± 45.20$^{a}$ | 0.020 |
| SL (cm) | 44.56 ± 0.80 | 45.11 ± 0.56 | 45.17 ± 0.47 | 42.85 ± 0.68 | 0.062 |
| CW (g) | 27.00 ± 1.73$^{a}$ | 27.65 ± 1.45$^{a}$ | 22.01 ± 1.92$^{b}$ | 20.09 ± 1.60$^{b}$ | 0.010 |
| EW (mg) | 5.14 ± 0.36 | 6.06 ± 0.40 | 4.92 ± 0.21 | 4.86 ± 0.36 | 0.073 |
| AF | 5268.50 ± 136 | 4624.67 ± 281 | 4531.67 ± 479 | 4274.00 ± 536 | 0.348 |
| RF | 5.14 ± 0.51$^{a}$ | 4.13 ± 0.23$^{ab}$ | 3.14 ± 0.52$^{b}$ | 3.07 ± 0.48$^{b}$ | 0.012 |
| SI (%) | 2.61 ± 0.28$^{a}$ | 2.48 ± 0.15$^{a}$ | 1.51 ± 0.20$^{b}$ | 1.43 ± 0.16$^{b}$ | 0.000 |
| HR (%) | 97.38 ± 0.76 | 95.92 ± 0.46 | 93.87 ± 1.15 | 95.43 ± 0.82 | 0.067 |

Wt = Weight (g); SL = Standard Length (cm); CW = Clutch Weight (g); EW = Egg Weight (mg); AF = Absolute Fecundity; RF = Relative Fecundity (eggs/g Wt.); SI = Spawning Index (%); HR = Hatching Rate (%)

There was a positive heterosis (5.46%) for clutch weight as well as a positive heterosis for egg weight (9.8%). Other reproductive traits recorded negative heterosis including absolute fecundity, relative fecundity, spawning index and hatching rate (Table 3).

**Table 3.** Heterosis for reproductive performance traits recorded from pairing of females of two strains of the Nile tilapia (*Oreochromis niloticus*)

| Clutch Weight | Egg Weight | Absolute Fecundity | Relative Fecundity | Spawning Index | Hatching Rate |
|---|---|---|---|---|---|
| 5.46 | 9.8 | -4.05 | -11.45 | -0.28 | -2.73 |

Growth of swim-up fry (Table 4) was significantly affected by the cross ($p<0.05$) considering differences in mean final weight, daily growth rate (DGR) and specific growth rate (SGR). Mean final weight was highest in the crossbreed GT×CH and least in the pure line cross CH×CH. The daily growh rate was higher in progeny from the cross GT×CH and least in progeny from the cross CH×CH as well. A similar trend is also observed in the specific growth rate (SGR) of the offspring. Survival rate was not significantly different across the crosses ($p>0.05$). Weekly progression in weight (Figure 1) shows an almost uniform growth among the progeny. However, progeny from the cross GT×CH outperformed the others with progeny of the cross CH×CH giving the least growth trend.

**Table 4.** Means of growth performance traits in progeny from reciprocal crosses of two strains of the Nile tilapia (*Oreochromis niloticus*)

| Cross | MIW (g) | MFW (g) | DGR ($g.day^{-1}$) | SGR ($\%.day^{-1}$) | Survival Rate (%) |
|---|---|---|---|---|---|
| CH×CH | 0.0019±0.0001 | $3.02 \pm 0.08^{c}$ | $0.05 \pm 0.00^{c}$ | $13.19 \pm 0.06^{c}$ | 74.56 ± 1.26 |
| CH×GT | 0.0019±0.0001 | $3.49 \pm 0.09^{b}$ | $0.06 \pm 0.00^{b}$ | $13.42 \pm 0.10^{b}$ | 75.67 ± 1.41 |
| GT×CH | 0.0018±0.0000 | $3.88 \pm 0.04^{a}$ | $0.07 \pm 0.00^{a}$ | $13.70 \pm 0.05^{a}$ | 76.22 ± 1.72 |
| GT×GT | 0.0019±0.0000 | $3.57 \pm 0.12^{b}$ | $0.06 \pm 0.00^{b}$ | $13.49 \pm 0.08^{ab}$ | 77.44 ± 1.13 |
| p-value | 0.720 | 0.000 | 0.000 | 0.001 | 0.540 |

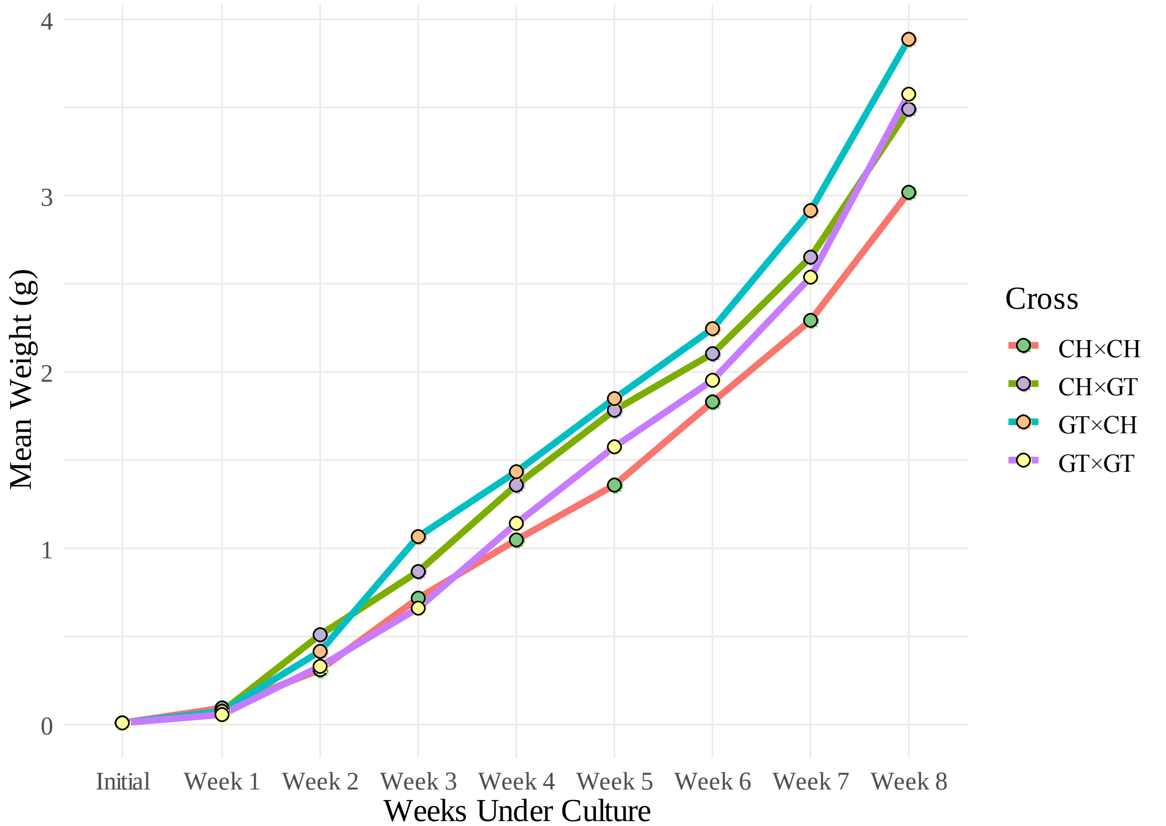


**Figure 1.** Temporal progression in weight of progeny from reciprocal crosses of two strains of Nile tilapia.

Hybrid vigour for growth traits (Table 5) were positive for final weight (11.84%), DGR (18.18%) and SGR (1.65%). There was however a negative heterosis for survival (-0.07).

**Table 5.** Heterosis for growth performance traits of progeny from reciprocal crosses of two strains of the Nile tilapia (*Oreochromis niloticus*)

| Final Weight | DGR | SGR | Survival Rate |
|---|---|---|---|
| 11.84 | 18.18 | 1.65 | -0.07 |

## IV. DISCUSSION

Temperature is an important modulator of reproduction in the Nile tilapia [19]. The temperature in the ponds housing the hapas ranged from 26.5 to 28.2°C, a range that falls well below 29°C that is the threshold for untoward effects on oocyte development in tilapia [20]. The fact that this experiment was conducted in the rainy season (March - July 2017) helped to hold temperature fluctuation at a minimal rate. Spawning among chitralada females occurred earlier (late March- early April) than GIFT females and this may explain the difference in spawning indices between these four genetic groups since higher temperatures exist in that period compared to the latter period of the rainy season. This indicates that the GIFT groups were more sensitive to the raised temperature at the beginning of the experiment. Earlier reports show that the chitralada strain spawns at about 30 to 45% rate when temperatures approach 30°C [13].

There were multiple spawns (2) from 10 GIFT females divided between the two crosses that involved them. Multilpe spawning in chitralada was less (9 females). The Nile tilapia is a prolific spawner with multiple spawns per year when adequate water temperature is present [21]. According to Trewavas [22], the female tilapia can spawn all year round (12 times) especially when temperature is above 20°C. This implies spawning can occur every 30 days. However, spawning has been reported to occur within 21 days at adequate temperature [13].

The size of the females (weight) was different across the cross combinations with the chitralada being smaller than the GIFT. This reflected further in the clutch weight and absolute fecundity such that the chitralada females gave a higher clutch weight than the GIFT. If smaller size is synonymous with young age, then it is easily concluded that the chitralada were younger and therefore exhibited better reproductive capacity [23]. The importance of this lies in the fact that selection for growth traits will not go in tandem with reproductive traits. Records of reproductive traits is also vital in any breeding programmes as a bridge between the reproductive traits and the target of the breeding programme [6].

The clutch weight was different across the genetic groups investigated in this study. The groups that had the female chitralada recorded higher clutch size than the GIFT females. There was however no difference between these groups with regard to egg weight, a situation that is further explained by higher absolute fecundity in the chitralada females compared to the GIFT females. Egg size is a consequence of diet [24, 25]. There seems to be some genetic × environment impact on the egg size of the broodstock since the fish came from different environments. Clutch weight and egg weight both gave positive heterosis an this indicates the affinity for improved performance in these traits as a result of crossing of the strains.

There was no influence of the strains on absolute fecundity. Almeida, et al. [13], reported lower absolute fecundities compared to the present study. However, this difference is due to the measurement of absolute fecundity itself. The previous research reported fecundity per spawn as against total spawn used currently. On the contrary, relative fecundity (RF) was affected by the strain. Absolute and relative fecundity in the Nile tilapia, are determined by both food and environmental conditions [26]. The relative fecundity reported in this study falls in the range of 1.1 to 5.7eggs/g female that was reported earlier by Valentin, et al. [23]. There was no hybrid vigour for absolute fecundity and relative fecundity suggesting that differences in egg number is blurred out by pooling eggs.

The ratio of egg weight to body weight as reflected by the spawning index was influenced by the tilapia strains. Spawning index is influenced strongly by season [27]. This makes variability in spawning index very high and is therefore often different at various periods of the year. There is need to factor in the influence of season in further studies that involve determination of spawning index. The negative heterosis for spawning index also indicates that the difference in weight and clutch weight is eliminated with the dimension of their ratio in the strains investigated.

The strain of tilapia has no effect on hatching rate. This implies that the level of genetic variability in the strains does not influence the phenotypic expression of hatch rate in embryos produced by the strains. Hatching rates are influenced strongly by incubation systems [28]. A previous report by Almeida, et al. [13] did not find any effect of strain on hatching rate.

Growth of the progenies from the crosses was dependent on the crosses carried out. There was better final weight in the crossbreed GT×CH than GT×GT and the cross CH×GT than the cross CH×CH. This suggests an interaction between the strains that improves growth. The trend of genetic interaction to improve final weight has also been reported by El-Zaeem [29] with crossing of *O. niloticus* and *O. aureus*. The trend where strain crossbreeding affects final weight was also translated into effects in daily growth rate and specific growth rate.

The positive heterosis for final weight, DGR and SGR shows the effect of crossbreeding on improving progeny performance relative to the pure line progenies. Since the survival rates were statistically uniform, the negative heterosis for survival rate is expected. The possible inverse relationship between harvest weight and reproductive traits [6] means that both traits need to be monitored at every generation of selective breeding.

## V. CONCLUSION

Reproductive traits varied across the strains and crosses carried out. Clutch weight was highest in chitralada strain compared to the GIFT strain. The highest spawning index was also observed in the chitralada strain. These results imply that the chitralada strain has advantage in reproductive traits. On the other hand, growth traits were better when crosses involved the GIFT strain. This also suggests that the GIFT strain holds the advantage in harvest weight. Therefore, future improvements that must combine reproductive traits and harvest weight should consider crossing of chitralada and GIFT.

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