GENETIC EVALUATION OF GROWTH AND SURVIVAL AT 6 WEEKS OLD FRY OF EGYPTIAN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) BETWEEN IMPROVED ABBASSA AND COMMERCIAL MANZALA STRAINS IN A DIALLEL CROSSING

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SUMMARY

This study aims to evaluate growth performance and estimate additive genetic, heterosis, and general reciprocal effects for body weight, body length, condition factor, and survival of Nile tilapia (Oreochromis niloticus) fry at 6 weeks old after hatching using a complete diallel cross between the genetically improved "Abbassa" strain(A) and a commercially available strain Manzala(M). Body weight and total body length were observed significantly (P <0.001) higher in M_mA_f and A_mM_f crosses (2.87g & 5.55 cm; 2.78g & 5.33cm, respectively). The straight-bred A_mA_f weight and length were 2.64g, 5.18cm, respectively while the straight-bred M_mM_f showed the lowest growth parameters 2.42g and 5.04cm,respectively.Condition factor of A_mA_f, A_mM_f, and M_mM_f genotypes (1.89, 1.85, and 1.81 respectively) didn't differ significantly whereas the M_mA_f cross showed a significantly lower condition factor 1.67. The survival of the straight-bred A_mA_f was 0.98,which significantly (P <0.001) higher than A_mM_f, M_mA_f, and M_mM_f genotypes. Abbassa strain showed 5, 2.1, and 10% additive genetic effect above the overall mean in body weight, survival, and condition factor respectively. Heterosis estimates were positive and relatively high 11.7% and 6.2% for body weight and total body length while a negative heterosis estimates were observed for the condition factor and survival, being -6.7% and -2.6% respectively. Reciprocal effect was significant (P <0.001) for total body length and condition factor. The results of the current study suggest that body weight and total body length of the Nile tilapia fry at 6 weeks old after hatching could be improved through crossing schemes exploiting the heterosis effect however the crossing did not show any improvement in survival.

Keywords: additive variance, heterosis, reciprocal effect, Nile tilapia fry

INTRODUCTION

In Egypt, tilapia contributes about 60% of the total aquaculture production. Between 2003 and 2013 tilapia production showed a dramatic increase from 200,000 to 635,843 tons, representing an annual increase of 12%. Moreover, Egyptian Nile tilapia (*Oreochromis niloticus*) is being produced from many culture systems including ponds, cages, tanks, and large water bodies (GAFRD, 2004 and 2014). Such improvement on the Egyptian tilapia production aspects could be accompanied with a similar improvement in all production constrains such as production of balanced formulated feeds, adoption of advanced management techniques, development of improved strains, and potentially viable hybrids.

A genetic stock improvement programme for Nile tilapia has been conducted by World Fish in Egypt since 2002 in order to develop an improved tilapia strain, which has been named as the "Abbassa strain" (Rezk *et al.*, 2009). Ibrahim *et al.(2013)* concluded that the Abbassa strain is ready to release for tilapia industry in Egypt based on superior on-station performance compared to a widely and locally available commercial tilapia strain.

Egyptian tilapia hatcheries sell tilapia seeds to fish famers at 3-4 weeks after hatching with an average weight 0.2 - 0.5 g or they keep the fry for another 4-6 weeks before they sell them as fingerlings, weighing 1 - 5 g (Saleh, 2007 and Nasr-Allah *et al*., 2012). In addition, many studies reported that the stocking and harvest weights of Nile tilapia are positively and highly correlated (Abdel-Hakim *et al*., 2008 and Ammar, 2009).

The main objectives of this study were to evaluate growth performance and estimate line crossing parameters of improved Abbassa tilapia strain and a commercial Manzala strain within a complete diallel cross at 6 weeks old after hatching. This is done in order to determine the optimum utilization of these strains and crosses for the production of premium growth and survival of tilapia seeds.

MATERIALS AND METHODS

Origin of stocks:

This study included two sources of Nile tilapia: Abbassa improved strain, which has described in detail by Rezk *et al.* (2009) and Khaw *et al.* (2009) and commercial Manzala strain The Abbassa strain was obtained from World Fish Regional Center, Abbassa, Abu-Hamad, Sharkia, Egypt, and the Manzala strain was obtained from a commercial Nile tilapia hatchery, located at Manzala, Dakahlia, Egypt. Descriptive statistics of body measurement for the brood fish from both the sources, Abbassa and Manzala, are represented in Table (1). The study was carried out at a commercial fish hatchery at San El-Hagar El-Bharia, Sharkia, Egypt from 1st of June 2015 and lasted for 10 weeks.

Table 1. Descriptive statistics for body measurements of brood stock of two different Nile tilapia strains

	Males				Females			
Strain	Length	Weight	Depth	Girth	Length	Weight	Depth	Girth
Abbassa (A)	20.6	158.6	7.2	15.3	20.3	149.8	6.4	13.9
Manzala (M)	20.5	142.7	7.25	16.2	22.3	168.8	7.3	16.1

Mating design:

A complete diallel crossing was made to obtain all four possible combinations of genotypes. Three replicates were formed from each mating; each replicate consisted of two males and six females. The breeding groups were stocked in separate 1×2×1 m breeding hapas. A total of 12 breeding hapas were installed in 1000 m^2 earthen pond. After 14 days, swim-up fry were collected separately from each hapa and transferred to 1×2×1 m rearing hapas at a stocking density of 200 fry per m^2 for further rearing until 6 weeks after hatching (a.h.).

***Fish Management*:**

All hatchery ponds were in a static water system with a temperature range 24-29^{o}C during the study. Approximately 20% of the water was replaced daily with clean water to maintain a good water quality. Brood fish were fed two times a day with a pelleted commercial feed containing 35% crude protein @ 2% of their average body weights. The resulting progeny were fed on a compound commercial diet containing 45% crude protein and 8% fat and were manually fed *ad libitum*, four times a day during day light hours.

***Considered Traits*:**

Body weight and length**:** 30 fry per hapa were randomly chosen and individually weighed (g) and total length measured (cm) at six weeks old after hatching; Condition Factor (K):Condition factor was calculated for the 30 chosen fry per hapa according to the formula: K = (Weight (g) / Length (cm)^3) X 100 (Le Cren, 1951); and survival at six weeks old was treated as a binary trait, for each hapa the counted alive fish at the end of the experiment were coded as ‘1’, whereas the counted dead ones coded as ‘0’.

Statistical analysis:

For each trait, two different statistical models were used for the analysis. The first included the fixed effects of genotype (4 levels) and hapa nested within genotype (12 levels). The differences between the means of the genotypes were tested using Bonferroni test **(**Bonferroni, 1936).

To further partition of the difference between the four mating groups and in order to estimate the additive genetic, heterosis and general reciprocal effects a multiple regression model was used as follow:

$$Y_{ijk} = \mu + \sum k_i a_i + \sum k_{ij} h_{ij} + \sum t_i r_i + e_{ijk}$$

Where Y_{ijk} represents the observation on the k^{th} progeny produced from the i^{th} and j^{th} strain. k_i is the proportion of genes contributed by the k^{th} individual originating from the i^{th} strain (k_i= 0.0, 0.5 or 1.0 and Σk_i=1.0); a_i is the additive genetic effect of genes originating from the i^{th} strain; k_{ij} is the coefficient of the total heterosis effect for the cross between the i^{th} and j^{th} strains (k_{ij}= 0.0 or 1.0; $i \neq j$ and $ij \neq ji$ and Σk_{ij} =1.0); h_{ij} is the total heterosis effect for the cross between the i^{th} and j^{th} strains ($i \neq j$ and $ij \neq ji$); t_i is the coefficient of the general reciprocal effect for the i^{th} strain (t_i=0 for purebreds and −0.5 for male strain and 0.5 for female strain, for the crossbreds and Σt_i=1.0); r_i is the general reciprocal effect of the i^{th} strain; the set of coefficients for the general reciprocal effects assume that the additive effects of the genes of a given strain are similar regardless whether the genes are inherited through a male or female according to Gjerde *et al*. (2002) see Table 2; and $e_{ijk}$is the random residual associated with the individual observations on each fish. All analyses were conducted using SPSS (2013).

Table 2. Illustration of the coding for the coefficients of the additive genetic (a_i), total heterosis (h_{ij}) and general reciprocal (r_i) effects used in multiple regression model

Strain /cross	Overall mean	Additive genetic		Total heterosis	General reciprocal	
♂×♀	μ	a_A	a_M	$h_{AXM\ or\ MXA}$	r_A	r_M
A X A	1	1.0	0.0	0.0	0.0	0.0
A X M	1	0.5	0.5	1.0	-0.5	0.5
M X A	1	0.5	0.5	1.0	0.5	-0.5
M X M	1	0.0	1.0	0.0	0.0	0.0

As in Gjerde *et al*., 2002

RESULTS AND DISCUSSION

Basic analysis:

Analysis of variance revealed that the effect of genotype was significant (P <0.001) for all studied traits of Nile tilapia fry at 6 weeks old a.h. (Table3).The differences among genotypes were collectively highly significant according to the fixed effect model. The hapa had no significant effect on the growth, which might be due to the relative short time where the fish kept for in the hapas (6 weeks).

Analysis of variance (Table 4) shows that the body weight and total body length of 6 weeks old Nile tilapia fry were significantly higher in M_mA_f and A_mM_f crosses (2.87g, 5.55 cm and 2.78g, 5.33cm, respectively). Moreover, the straight-bred A_mA_f weight and length were 2.64g, 5.18cm, respectively while the straight-bred M_mM_fs showed the lowest growth performance (2.42g, 5.04cm, respectively). These results indicate that crossing of the Abbassa strain with commercial Manzala strain can improve the body weight and total body length of 6 weeks old Nile tilapia fry. Table 4 revealed that condition factor of A_mA_f, A_mM_f, and M_mM_f genotypes (1.89, 1.85, and 1.81 respectively)doesn't differ significantly while M_mA_f cross showed a significantly lower condition factor of 1.67. Survival of the straight-bred A_mA_f was a significantly higher (0.98) whereas the survival of M_mM_f, M_mA_f, and A_mM_f genotypes (0.94, 0.94, and 0.92 respectively) didn't differ significantly from each other. The survival for crossbreds was lower than pure strains, which led to slightly lower density in the crossbred hapas to enhance the growth for the crossbred. However, it was not possible to include the survival rate in each hapa as a covariate in the fixed effect model because it is confounded with the hapa effect. We dropped the hapa effect from the fixed effect model and fitted survival within each hapa as a covariate (results are not shown). The survival rate had no significant effect on the body weight, body length or condition factor.

Table 3. Analysis of variance for body weight (g), body length (cm), condition factor, and survival rate of different genotypes of Nile tilapia fry at 6 weeks old after hatching according to the fixed effect model

	Body weight			Body length			Condition factor			Survival		
Source of Variance	Df	SS	Sig	Df	SS	Sig	Df	SS	Sig	Df	SS	Sig
Genotype	3	10.5	***	3	12.6	***	3	2.3	***	3	1.05	***
Hapa(genotype)	8	6.6	Ns	8	2.08	Ns	8	0.3	Ns	8	2.6	***
Error	348			348			348			2388		
Corrected total	359			359			359			2399		

Asterisks or Ns across classification indicate the significance or absence of it as a source of variation; ***= significant at $p \leq 0.001$.

Table 4. Least squares means for body weight (g), body length (cm), condition factor, and survival rate from complete 2 X 2 diallel crossing of Nile Tilapia fry at 6 weeks old after hatching according to multiple regression model

Genotype	Body weight ±SE	Body length ±SE	Condition factor ±SE	Survival ±SE
A_m A_f	2.64^{ab}±0.066	5.18^{bc}±0.048	1.89^{a}±0.025	0.983^{a} ±0.009
M_m M_f	2.42^{b} ±0.066	5.04^{c} ±0.048	1.85^{a}±0.025	0.940^{b} ±0.009
A_m M_f	2.78^{a} ±0.066	5.33^{b} ±0.048	1.81^{a}±0.025	0.927^{b} ±0.009
M_m A_f	2.87^{a} ±0.066	5.55^{a} ±0.048	1.67^{b}±0.025	0.947^{b} ±0.009

ab…Within classification any two means having the same script are not significantly different using Bonferroni test $p \leq 0.05$.

Regarding the comparative performance of the experimental strains as pure breeding groups, Abbassa strain showed a noted superiority over the commercial strain Manzala concerning all traits including body weight (9%), total body length (2.7%), condition factor (4.4%), and survival (4.5%) at 6 weeks old a.h. (Table 4). Abbassa strain growth superiority could be due to that it was developed by genetic selection to improve growth for a number of generations. The current results were relatively in agreement with Ibrahim *et al.*, 2013who compared between Abbassa stain and another Egyptian commercial strain ie. Kafr El Sheikh. The study concluded that Abbassa strain showed a higher harvest weight (28%) than that of the Kafr El Sheikh strain and that both strains had a similar survival rate (approximately 80%) during the grow-out period.

Strain additive genetic effect:

Many studies reported variable significances of the additive genetic effect within a diallel crossing concerning growth performance of tilapia (Bentsen *et al*., 1998; Hussain *et al.* 2000a; 2000b; Maluwa and Gjerde 2006; Nguyen *et al.,* 2009;Pongthana *et al.,* 2010; Ngo *et al*., 2016), salinity and cold tolerance of tilapia (Gnaani *et al*., 2000; and Armas-Rosales 2006), growth and survival of rohu carp (Gjerde*etal*.,2002), and growth of fresh water prawn (Pillai *et al.*, 2011).Results of parameters estimates (Table 6) showed that the Abbassa strain had a positive additive genetic value of 5, 2.1, and 10% above the overall mean for body weight, survival, and condition factor respectively. However, the Abbassa strain also showed a negative additive genetic value for total body length at 6 weeks old a.h. with about-1.3% below the overall mean but this effect was not significant.

Heterosis:

Negative or low estimates of heterosis were reported in diallel crossing experiments that investigate growth performance and survival rate of tilapia (Bentsen *et al.,* 1998; Pongthana *et al.,* 2010; Ngo *et al*., 2016), Rohu carp (Gjerde *et al.,* 2002), and Giant freshwater prawn (Thanh *et al.,* 2010). In contrast highly significant heterosis estimates were obtained within other diallel crossing schemes *eg.*, Common carp (Bakos & Gorda 1995), Channel catfish (Argue, Liu & Dunham 2003), and Maluwa & Gjerde 2006 who reported a total heterosis of 15.3% for harvest body weight of *Oreochromis shiranus.* According to the obtained heterotic effects, alternative breeding strategies were recommended for simultaneous improvement of the target traits in each study. Analysis of variance (Table 5) revealed that heterosis was significant (P<0.001) in all studied traits including body weight, body length, condition factor, and survival of Nile tilapia fry at 6 weeks old after hatching. Parameters estimates (Table 6) shows that heterosis were relatively high 11.7% and 6.2% in body weight and total body length of 6 weeks old a.h., respectively. Such a significant effect of heterosis could be explained by genetic differences in allele frequencies between the experimental strains. The Abbassa strain has been kept and used for many generations by World Fish for a selective breeding program in a closed breeding nucleus and they were not mixed with other strains. Consequently, crossing of these two strains could increase the levels of heterozygosity in the crossbred and explain the heterosis effects observed in the current study. Furthermore, our results indicated that Abbassa and Manzala strains have a high nicking ability (also referred to as “combining ability”), which can be utilized in crossbreeding schemes to exploit high non-additive gene effects in the production of Nile tilapia fry. This indication can be optimized through applying reciprocal recurrent selection to maximize this effect. Table 6 shows that heterosis estimates were negative -6.7% and -2.6% for the condition factor and survival, respectively. The crossbreeding has improved the growth in the current study, however, it had a slightly negative effect on survival. The overall gain from body weight in the crossbred (11.7%) will compensate and exceed the loss in survival (-2.6%).

Reciprocal effects:

Results (Table 5) indicated that reciprocal effect was significant (P <0.001) for total body length and condition factor while it was non-significant for both body weight and survival of Nile tilapia fry at 6 weeks old. So, our results indicated that using either of these two strains as male or female parents in the planning of crossbreeding programs for the production of Nile tilapia fry would not affect the weight and survival of fry at 6 weeks old a.h.

Table 5. Analysis of variance for body weight (g), body length (cm), condition factor, and survival rate from complete 2 X 2 diallel crossing of Nile tilapia fry at 6 weeks old after hatching according to multiple regression model

	Body weight			Body length			Condition factor			Survival		
Source of variance	Df	SS	Sig.	Df	SS	Sig.	Df	SS	Sig.	Df	SS	Sig.
Additive A	1	0.35	Ns	1	0.12	Ns	1	0.7	***	1	0.08	Ns
He	1	8.01	***	1	9.59	***	1	1.36	***	1	0.37	***
Rec	1	0.39	Ns	1	2.1	***	1	0.88	***	1	0.12	Ns
Error	356	146.2		356	76.1		356	19.9		2396	114.7	
Corrected total	359	156.7		359	88.8		359	22.2		2399	115.8	

Asterisks or Ns across classification indicate the significance or absence of it as a source of variation; ***= significant at p≤0.001.

Table 6. Parameters estimates for body weight (g), body length (cm), condition factor, and survival rate from complete 2X2 diallel crossing of Nile Tilapia fry at 6 weeks old after hatching according to multiple regression model

	Body weight ±SE	Body length ±SE	Condition factor ±SE	Survival ±SE
Intercept	2.46 ± 0.83	5.15 ± 0.06	1.78 ± 0.03	0.95 ± 0.01
Additive A	0.12 ± 0.13	-0.07 ± 0.09	0.17 ± 0.05	0.023 ± 0.01
He	0.29 ± 0.06	0.32 ± 0.04	-0.12 ± 0.02	-0.025± 0.009
Rec	0.09 ± 0.09	0.21 ±0.06	-0.14 ± 0.03	0.02 ± 0.01

CONCLUSION

The current study showed that, body weight and total body length of the Nile tilapia fry at 6 weeks old a.h. could be improved using the non-additive genetic effects between the selection strain Abbassa and commercial strain Manzala. The crossing had a slightly negative effect on the survival however the enhanced growth of the crossbred was higher than the reduction in production because of the lower survival for the crossbred fry.

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التقييم الوراثى للنمو والبقاء على عمر ٦ اسابيع لزريعه البلطى النيلى من خلال الخلط بين سلالتى العباسه المحسنة والمنزلة التجارية

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تهدف هذة الدراسة تقييم أداء النمو و تقدير الآثر الوراثى التجمعى و قوة الهجين و الآثر المتبادل العام لكلا من وزن الجسم وطول الجسم وعامل الحاله والبقاء ليرقات بلطى نيلى على عمر ٦ اسابيع بعد الفقس بإستخدام خلط مزدوج كامل بين سلالة العباسة المحسنة (A) والسلاله التجارية منزلة (M) . اظهرت النتائج أن وزن وطول الجسم أعلى معنويا (P<0.001) فى خلطان M_mA_f و A_mM_f (٢.٨٧ جم- ٥.٥٥ سم و ٢.٧٨ جم – ٥.٣٣سم على الترتيب). أوضحت النتائج ان وزن وطول الجسم لسلالة العباسة A_MA_F كانوا ٢.٦٤ جم و ٥.١٨ سم على الترتيب فى حين سجلت سلالة المنزلة M_MM_F أقل وزن وطول (٢.٤٢جم و ٥.٠٤ سم على الترتيب). دللت النتائج على أن عامل الحالة لم يختلف معنويا بين كلا من A_mA_f، A_mM_f، M_mM_f (١.٨٩-١.٨٥-١.٨١ على الترتيب) فى حين كان عامل الحاله لناتج الخلط M_mA_f (١.٦٧) أقل معنويا(P<0.001). البقاء لسلالة العباسة A_mA_f كان (٠.٩٨)أعلى معنويا (P<0.001) عن التراكيب الوراثية A_mM_f، M_mA_f، M_mM. سجلت سلالة العباسة ٥ %، ٢.١ %،١٠% آثر وراثى تجمعى أعلى من المتوسط العام فى وزن الجسم والبقاء وعامل الحاله على الترتيب. قيم قوة الهجين كانت موجبة ومرتفعه نسبيا ١١.٧% و ٦.٢% لكلا وزن الجسم وطول الجسم فى حين كانت قيم قوة الهجين سالبة لكلا من عامل الحالة والبقاء -٦.٧ % و -٢.٦% على الترتيب. الآثر المتبادل كان معنويا (P<0.001) لكلا من صفتى طول الجسم عامل الحاله. أوضحت نتائج الدراسة أن وزن الجسم و طول الجسم ليرقات البلطى على عمر ٦ أسابيع بعد الفقس يمكن تحسينهم من خلال نظم الخلط لإستغلال تأثير قوة الهجين فى حين أن الخلط لم يظهر اية تحسين فى البقاء.