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Production of salinity tolerant Nile tilapia, *Oreochromis niloticus* through traditional and modern breeding methods: II. Application of genetically modified breeding by introducing foreign DNA into fish gonads

Samy Yehya El-Zaeem[1,2]*, Mohamed Morsi M. Ahmed[3,4], Mohamed El-Sayed Salama[1] and Hamad Abdel-Razek El-Maremie[5]

[1]Animal and Fish Production Department, Faculty of Agriculture, (Saba-Bacha), Alexandria   University, Alexandria, Egypt.
[2] Al-Jeraisy Chair for DNA Research, Zoology Department, Faculty of Science, P.O. Box 2455, King Saud University, Riyadh 11451, Saudi Arabia.
[3] Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Scientific Research & Technology Applications, Alexandria, Egypt.
[4]Biological Sciences Department, Faculty of Sciences, P.O Box 80203, King Abdul-Aziz University, Jeddah 21589, Saudi Arabia.
[5]Animal Production Department, Faculty of Agriculture, Omar Almokhtar University, Al-Bayda, Libya.

This study was conducted to produce a salinity tolerant Nile tilapia, *Oreochromis niloticus* through genetically modified breeding by introducing a fragmented purified DNA isolated from sea bream, *Sparus aurata* or Artemia, *Artemia salina* into the gonads. The results showed a significant improvement (P ≤ 0.05) in most of the growth performance and feed utilization parameters of genetically modified *O. niloticus* treated with sea bream-DNA and reared at different salinity levels up to 16 ppt compared to both genetically modified *O. niloticus* treated with Artemia-DNA and the control fish reared at the same salinity levels. Genetically modified *O. niloticus* treated with Artemia-DNA reared at 32 ppt, had displayed better traits results (P≤ 0.05) compared to the other fish within the same salinity level. Furthermore, genetically modified *O. niloticus* treated with sea bream-DNA showed a silver color covering all the body and no dark vertical bands. The results of the random amplified polymorphic DNA (RAPD) fingerprinting showed highly genetic polymorphic percentage (35.95%) among fish receiving foreign DNA and their control using different random primers. The results of the present work suggested that, hyper-saline genetically modified *O. niloticus* with higher growth rate can be produced using a feasible and fast methodology.

Key words: Salinity tolerance, *Oreochromis niloticus*, productive performance, genetically modified, DNA transfer.

INTRODUCTION

The shortage in freshwater in many countries and the competition for it in agriculture and other urban activities has increased the pressure to develop aquaculture in brackish water and sea water (El-Sayed, 2006). Tilapia are important species, especially for tropical aquaculture and euryhaline fish that can live and thrive in a wide range of salinity from fresh water to full sea water even though some species tolerate a wider range of salinity than others (Philippart and Ruwet, 1982; Guner et al., 2005; Kamal and Mair, 2005).

Among the species cultured commercially, the Nile tilapia *Oreochromis niloticus* is one of the most important freshwater finfish in aquaculture but is not considered

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*Corresponding author. E-mail: selzaeem@ksu.edu.sa, selzaeem@yahoo.com. Tel: +20103552398.

amongst the most saline tolerant species (Kamal and Mair, 2005). It grows fast but is less salt tolerant than blue tilapia *Oreochromis aureus* (Avella and Doudet, 1993; Hulata, 2001), mosambique tilapia *Oreochromis mossambicus* and red-belly tilapia *Tilapia zillii* (Stickney, 1986). Although, both *O. mossambicus* and *T. zillii* are highly salt-tolerant, they are not popular species for culture (Suresh and Lin, 1992); therefore, the first candidate that one may think of for aquaculture in brackish water and sea water is Nile tilapia.

Gene transfer relates to the process of introducing foreign DNA/RNA fragments into the nucleus or cytoplasm of gametes, zygotes, embryos or somatic cells using physical or chemical approaches allowing foreign genes to be reproduced and expressed in the host cells. These foreign DNA fragments may be originated from the host genome, related species or totally different species. Such a DNA fragment can be cDNA or genomic DNA but at least it must consist of: (1) The regulatory regions, such as enhancer, repressor, promoter or initiator; (2) the coding region for the production of protein; and (3) the untranslated regions, including terminator. After such a transfer, a gene fragment would then make the protein performs actively inside the cell of the host. When the fish are treated with this transfer technique, they would then feature nature and display the genetic traits encoded by the foreign genes, making it known as genetically or transgenic fish (Tsai, 2003).

A commonly used method to introduce foreign DNA, is by microinjection into the nucleus or cytoplasm of fertilized eggs. This method, however, requires some skill and involves some difficulties and is time consuming (Inoue et al., 1990; Sin et al., 1993). To avoid the difficulties accompanying microinjection, much more convenient methods are required, especially if such techniques are to be applied in aquaculture for fast breeding of commercially important species. The most common potential mass methods are: (1) The use of electroporation of fertilized eggs (Inoue et al., 1990; Inoue, 1992; Xie et al., 1993); (2) electroporated sperm (Muller et al., 1992; Symonds et al., 1994); (3) the use of sperm cells as vectors to introduce foreign DNA into fish eggs (Khoo et al., 1992); (4) the direct injection of foreign DNA into fish gonads (El-Zaeem, 2001; Lu, 2002).

Many species of genetically modified fish have been developed since the first genetically modified gold fish was reported (Zhu et al., 1985). The production of genetically modified fish has become a popular technique not only for producing desirable traits but also for studying mechanisms of developmental regulation of various genes and gene promoters like faster growth rate, disease resistance, cold or salinity tolerance, age at sexual maturity and flesh quality, metabolic modifications are important breeding goals. Many laboratories all over the world have turned to study the genetically modified fish to gain new farming strains (Shears et al., 1991; Sin, 1997; Maclean and Laight, 2000; El-Zaeem 2001, 2004a,

2004b; El-Zaeem and Assem, 2004, 2006; Assem and El-Zaeem, 2005). Compared with the traditional approaches such as the inter-specific hybridization, genetically modified breeding avoids the productive isolation between two different species. Since more manipulated genes are available for foreign DNA transfer, it is hopeful for the investigators to shorten the breeding period through directional genetic breeding (Wang et al., 2001).

This study aims at producing a salinity tolerance Nile tilapia, *O. niloticus* through genetically modified breeding, by introducing a fragmented purified DNA isolated from sea bream, *Sparus aurata* or Artemia, *Artemia salina* into the ovaries and testes of *O. niloticus* adult. The effects of introducing foreign DNA on growth performance, body composition, amino acid analysis, feed utilization and some reproductive characteristics of the offspring produced under different salinity levels were studied.

Materials and Methods

The experimental work was undertaken at two areas: (1) Fish farm and the laboratory of Breeding and Production of Fish, Animal and Fish Production Department, Faculty of Agriculture (Saba-Bacha), Alexandria University and; (2) Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI) Mubark City for Scientific Research and Technology Applications, Alexandria, Egypt.

Fish origin

The Nile tilapia used in this study descended from a randomly mating population at the Middle East Fish Farm, Tolombat Halk El-Gamal, El-Behera Governorate, Egypt.

Experimental design

Preparation of genomic DNA

High molecular weight DNA was isolated according to Baradakci and Skibinski (1994) method by reducing liver sample from sea bream, *S. aurata* and whole tissue sample of Artemia, *A. salina*. The extracted DNA was restricted by Eco R1 restriction enzyme type II. It digested DNA between guanine and adenine (Tsai et al., 1993). Then, a concentration of 10 µg/0.1 ml/fish was adjusted by extrapolating the dilutions for each type of DNA extracted using 0.1 x SSC buffer (El-Zaeem, 2001).

Injection of genomic DNA into fish gonads

Adult Nile tilapia, *O. niloticus* with an average live weight (93.00 ± 2.30 g/male and 67.00 ± 2.00 g/female), were chosen. Readiness of females to spawn was ascertained by examining the degree of swelling of the urogenital papilla (Hussain et al., 1991). Males were examined by stripping sperm (Wester and Foote, 1972). Two males and four females were injected directly with the foreign DNA into fish gonads using a hypodermic needle; besides the control group (two males and four females) carried out. To inoculate the adult fish, the needle was inserted into the openings of oviduct and sperm duct (El-Zaeem, 2001; Lu et al., 2002). Immediately after DNA treatments were carried out, each group of treated fish was

Table 1. The sequences and the annealing temperatures of the primers used.

Primer	Sequence 5`- 3`	Annealing Tm °C/s
1	CCA GCC GAA C	28/30
2	ACC GCC GAA G	28/30
3	AAA GCT GCG G	28/30
4	AGG GGT CTT G	28/30
5	ATG CCC CTG T	28/30
6	AGG CCC CTG T	28/30

stocked separately in fiber glass tanks (total volume 350 liter) supplied with dechlorinated water with an adequate aeration. Brood fish were fed twice daily pellet containing 26% protein up to satiation for six days a week.

Base generation (F$_0$)

Culture conditions

Base generation (F$_0$) offspring produced from genetically modified Nile tilapia and their control were collected, counted and weighed. Fry were transferred separately to glass aquaria (100 x 34 x 50 cm) at a density of 1 fish/10 L and randomly divided for subsequent different salinity treatments. The glass aquaria were supplied with fresh dechlorinated tap water and aeration. Water temperatures were maintained at 28.00 °C.

Saline water acclimation

Two salinity levels (16 and 32 ppt) were prepared by mixing fresh water with crude natural salt (Likongwe, 2002) obtained from El-Nasr Company for salt, Borg El-Arab, Alexandria, Egypt, beside a third group of freshwater used as control. Fry obtained from each treatment of DNA and their control were gradually acclimated to the respective salinities by raising the salinity at the rate of 4 ppt daily (Watanabe and Kuo, 1985). Moreover, a fourth group of each treatment of DNA and their control were transferred directly to 16 ppt. Water in each glass aquaria was partially changed once daily and totally every three days. Fry were fed three times daily with pellets containing 38% protein, to satiation, six days a week for 90 days. Then, all the fish were fed on diet containing 32% to satiation, six days a week up to the end of the experiment (135 days). Fish were weighed and counted biweekly. A refractometer (S/Mill-E, ATAGO Co., LTD) was used to measure salinity.

Quantitative traits measurements

The following parameters were determined: Initial and final body weight (g), daily gain (g/day), specific growth rate (SGR %/day), total body length (cm), condition factor (K), feed intake and feed conversion ratio (FCR), protein efficiency ratio (PER), protein and energy retention percent (PR% and ER%). Gross energy contents of feed were calculated from MacDonald's tables (MacDonald et al., 1973). Gross energy of fish was calculated from their chemical composition using the factor of 5.7 and 9.5 for protein and fat, respectively, according to Viola et al. (1981). Initial and final body composition analyses were performed for moisture, crude protein and lipid contents according to the standard AOAC (1984) methods.

In addition, a new modification of Lowry et al. (1951) method was used for the determination of total protein content (Tsuyosh and

James, 1978). The analysis and composition of total amino acids of fish muscular protein were determined on different samples using 119 CL amino acid analyzer. All amino acids values are expressed as gram percent of protein on dry bases.

At the end of the experiment, gonads were carefully removed, weighted and fixed in 10% formal saline solution. Pieces of fixed ovary were examined under binocular microscope to determine oocyte diameters. The oocyte diameters were divided into two groups; the first groups (0.24 to less than 0.8 mm) were small and transparent, while the remaining ova ranging between 0.8 and 2.0 mm in diameter were yolky. Gonadosomatic index was calculated as

GSI = Gonad weight·(100)/Body weight

Random amplified polymorphic DNA (RAPD) analysis

DNA was extracted from the liver tissue of the base generation (F$_0$) of genetically modified Nile tilapia and their control following the method described by Baradakci and Skibinski (1994). In this work, ten base long oligonucleiotide primers (Table 1) were used to initiate polymerase chain reaction (PCR) amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplification. The PCR amplifications were performed following the procedure of Williams et al. (1990, 1993). The reaction (25 µl) was carried out using 0.8 U of Taq DNA polymerase (Fanzyme), 25 pmol dNTPs and 25 pmol of random primer, 2.5 µl. 10X Taq DNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (Eppendorf[®]). The PCR programme included an initial denaturation step at 94 °C for 2 min followed by 45 cycles with 94 °C for 30 s. For DNA denaturation, annealing temperature of each primer, as indicated in Table 1, extends at 72 °C for 30 s and finally extends at 72 °C for 10 min. The samples were cooled at 4 °C. The amplified DNA fragments were separated on 1.5% agarose gel and stained with ethidium bromide. 100 bp DNA Ladder marker (bp 2642, 1500 to100) was used in this study. Moreover, to ensure that the amplified DNA bands originated from genomic DNA, and not from primer artifacts, negative control (without DNA source) was carried out for each primer/ treatment combination. The amplified pattern was visualized on an UV transilluminator and photographed by Gel Documentation system.

Statistical analysis

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

$$Y_{ijk} = \mu + T_i + S_j + (TS)_{ij} + B_k + e_{ijk}$$

Where, Y_{ijk}, Observation of the ijk[th] parameter measured; μ, overall mean; T_i, effect of ith type of DNA; S_j, effect of Jth salinity; $(TS)_{ij}$, interaction type of DNA by salinity; B_k, effect of Kth block; e_{ijk}, random error.
For body composition traits at the first analysis, data were analyzed by fitting the following model (CoStat, 1986):

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where, Y_{ij}, Observation of the ij[th] parameter measured; μ, overall mean; T_i, effect of ith species; e_{ij}, random error. Significant differences ($P \leq 0.05$) among means were tested by the method of Duncan (1955).

RESULTS AND DISCUSSION

The highest initial body weight (IBW) were achieved by

Table 2. Effect of different types of foreign DNA, and salinity levels on growth performance, survival % and GSI of *O. niloticus*.

Treatments	IBW g	FBW g	DG g/day	SGR%/ day	Condition factor (K)	Survival rate (%)	GSI
DNA source							
Control	0.06 ± 0.00[b]	43.86 ± 9.15[b]	0.32 ± 0.06[b]	4.82 ± 0.17[b]	1.91±0.03	85.83 ± 21.83	3.61 ± 0.66[b]
Sea bream-DNA	0.07 ± 0.00[a]	51.99 ± 9.53[a]	0.38 ± 0.07[a]	4.90 ± 0.15[a]	1.91 ± 0.02	86.66 ±11.22	4.04 ± 0.7[a]
Artemia-DNA	0.07 ± 0.00 [a]	45.34 ± 2.30[b]	0.33 ± 0.01[b]	4.82 ± 0.1[b]	1.88 ± 0.04	91.6 ± 10.36	3.73 ± 0.36[ab]
Salinity ppt							
Fresh water (FW)	0.07 ± 0.03	50.75 ± 5.50[a]	0.38 ± 0.04[a]	4.90 ± 0.1[a]	1.92 ± 0.02	95.55 ± 5.09[a]	4.11 ± 0.51[a]
16 ppt indirect (ID)	0.06 ± 0.00	50.24 ± 5.21[a]	0.37 ± 0.04[a]	4.92 ± 0.1[a]	1.88 ± 0.03	95.55 ± 3.85[a]	4.03 ± 0.15[a]
16 ppt direct (D)	0.07 ± 0.00	50.69 ± 5.60[a]	0.37 ± 0.04[a]	4.91 ± 0.1[a]	1.90 ± 0.03	94.44 ± 1.92[a]	4.09 ± 0.32[a]
32 ppt indirect (ID)	0.07 ± 0.00	36.58 ± 5.90[b]	0.27 ± 0.04[b]	4.66 ± 0.1[b]	1.90 ± 0.05	66.66 ± 12.01[b]	2.94 ± 0.29[b]
DNA X Sal.							
Control at FW	0.06 ± 0.00	48.66 ± 2.12[b]	0.36 ± 0.02[b]	4.91±0.02[abcde]	1.93 ± 0.08	96.66 ± 4.71	3.82 ± 0.40
S-DNA at FW	0.07 ± 0.00	57.03 ± 0.22[a]	0.42 ± 0.00[a]	4.95 ± 0.01[abc]	1.94 ± 0.14	89.99 ± 4.71	4.57 ± 0.66
A-DNA at FW	0.07 ± 0.00	46.54 ± 0.93[b]	0.34 ± 0.01[b]	4.83 ± 0.03[e]	1.89 ± 0.11	100 ± 0.00	3.95 ± 0.22
Control at 16 ID	0.06 ± 0.01	48.80 ± 0.50[b]	0.36 ± 0.01[b]	4.93 ± 0.05[abcd]	1.89 ± 0.06	100 ± 0.00	4.00 ± 0.14
S-DNA at16 ID	0.07 ± 0.00	56.02 ± 1.68[a]	0.41 ± 0.01[a]	5.00 ± 0.03[a]	1.91 ± 0.05	93.33 ± 0.00	4.20±0.14
A-DNA at16 ID	0.07 ± 0.00	45.90 ±1.55[b]	0.34 ± 0.01[b]	4.84 ± 0.05[de]	1.84 ± 0.08	93.33 ± 9.43	3.90±0.28
Control at 16 D	0.06 ± 0.00	47.83 ± 2.76[b]	0.35 ± 0.02[b]	4.89 ± 0.02[bcde]	1.88 ± 0.02	93.33 ± 9.43	3.99±0.29
S-DNA at16 D	0.07 ± 0.00	57.19 ± 0.01[a]	0.42 ± 0.01[a]	4.98 ± 0.03[ab]	1.88 ± 0.06	93.33 ± 9.43	4.38 ± 0.23
A-DNA at 16 D	0.07 ± 0.00	47.02 ± 0.17[b]	0.35 ± 0.01[b]	4.87 ± 0.03[cde]	1.94 ±0.08	96.66 ± 4.71	3.88 ± 0.31
Control at 32 ID	0.06 ± 0.00	30.14 ± 1.78[e]	0.22 ± 0.01[e]	4.57 ± 0.01[g]	1.94 ± 0.08	53.33 ± 9.43	2.62 ± 0.24
S-DNA at 32 ID	0.07 ± 0.00	37.70 ± 3.51[d]	0.28 ± 0.01[d]	4.68 ± 0.08[f]	1.91 ± 0.05	69.99 ± 4.71	3.00 ± 0.16
A-DNA at 32 ID	0.07 ± 0.00	41.87 ± 2.34[c]	0.31 ± 0.02[c]	4.73 ± 0.06[f]	1.84 ± 0.04	76.66 ± 4.71	3.19 ± 0.12

Means within each comparison in the same column with the different superscripts differ significantly (P ≤ 0.05).   Initial and final body weight (IBW and FBW) = body weight at start and end of experiment. Daily gain (DG) = (final weight - initial weight)/ number of days; specific growth rate (SGR%/day) = (Ln final weight – Ln initial weight) 100 /number of days; condition factor (K) = body weight (100)/cubic total length; GSI=Gonad weight*(100)/body weight.

genetically modified *O. niloticus* treated with sea bream and Artemia-DNA and differed significantly (P ≤ 0.05) from those of the control fish (Table 2). Yet, no significant differences were observed in IBW of *O. niloticus* reared at different salinity levels. El-Zaeem (2004a) reported that, IBW of the first and second generations delivered from fast growing genetically modified *T. zillii* significantly increased (P ≤ 0.05) compared to the control fish. The results of this study are consistent with these findings. The highest mean values of final body weight (FBW), daily gain (DG) and SGR were recorded by genetically modified *O. niloticus* treated with sea bream-DNA and these records were significantly higher (P ≤ 0.05) than those of the genetically modified *O. niloticus* treated with Artemia-DNA and control fish. In all fish groups, the highest mean values of FBW, DG and SGR were obtained for the fish reared up to 16 ppt and differed significantly (P ≤ 0.05) from those of the fish reared at 32 ppt.

Moreover, genetically modified *O. niloticus* treated with sea bream-DNA reared at different levels of salinities up to 16 ppt had significantly higher (P ≤ 0.05) FBW, DG and SGR than the genetically modified *O. niloticus* treated with Artemia-DNA and the control fish group. On the

other hand, at 32 ppt, the Artemia-DNA treated fish had significantly (P ≤ 0.05) higher growth parameters than the other treatments (Table 2). These differences may be due to the differences in the type of donor DNA (Ali, 1999; El-Zaeem, 2001, 2004a, b; Ali, 2002), since Artemia is more salt tolerant than sea bream and control group *O. niloticus*.

Generally, with increasing salinity of up to 32 ppt, the growth performance decreased. This may be attributed to the increase in energy cost of osmoregulation at high salinity level. Morgan and Iwama (1991), Toepfer and Barton (1992) and Grau et al. (1994) reported that, there is an increasing metabolic rate of osmoregulatory acti-vities at high salinity. Furthermore, Rao (1968) noted that, osmoregulation appears to use a high proportion of the available energy ranging from 20 to 50% of total energy expenditure, depending on the environmental salinity.

Despite the adverse effect of salinity on growth, the genetically modified *O. niloticus* showed higher growth performance than the control. This may be attributed to the effect of growth hormone. Rahman et al. (1998) and Meri and Devlin (1999) reported that, growth hormone gene in transgenic fish elevates growth hormone in their plasma from 10 to 13 folds and above 40 fold higher than

that of non-transgenic fish. Moreover, Martinez et al. (1996, 1999 and 2000), Pitkanen et al. (1999), Rahman and Maclean (1999), El-Zaeem (2001) and Mori et al. (2007) reported heavier weight of transgenic fish than the non-transgenic fish.

In addition, the data of the present work show that genetically modified *O. niloticus* treated with Artemia-DNA had higher growth performance at salinity 32 ppt than genetically modified *O. niloticus* treated with sea bream-DNA and control fish reared at the same level of salinity (32 ppt). This may be attributed to genetic factor. It is clear that tolerance of saline differs between organisms due to variation in their genetic material. Lavens and Sorgeloos (1987) reported that, Artemia populations are found in about 500 natural salt lakes and found at salinity $\geq$ 70 ppt and dies off at salinities close to NaCl saturation (250 ppt and higher). Moreover, the growth performance of genetically modified *O. niloticus* treated with Artemia-DNA was better than genetically modified *O. niloticus* treated with sea bream-DNA and control fish at salinity 32 ppt, but the same effect was not noticed at other salinity levels. This may be due to induced expression of the salt tolerance gene in Artemia at elevated salinities. Al-Zahaby et al. (2005) reported that the survival rate of genetically modified *Sarothoredon galilaeus* treated with *Clarias gariepinus* DNA was higher than control when fish were subjected to the high polluted levels.

No significant differences were observed for the condition factor of genetically modified *O. niloticus* treated with different types of DNA and salinity levels. The lowest survival rate (66.66%) was obtained by the fish reared at 32 ppt and differed significantly (P $\leq$ 0.05) from those reared at different salinity levels up to 16 ppt (Table 2). In this study, survival rate was significantly lower at 32 ppt (66.66%) than those of the lower salinities. This result is consistent with the finding reported by Watanabe et al. (1985), Mair (2002) and Robert (2003) for *O. niloticus* and *O. aureus* and Florida red tilapia.

The highest gonadosomatic index (GSI) was obtained by the genetically modified *O. niloticus* treated with sea bream-DNA, but did not differ significantly (P $\leq$ 0.05) from that of genetically modified *O. niloticus* treated with Artemia-DNA. Fish reared at 32 ppt had significantly (P $\leq$ 0.05) lower GSI than the others reared at freshwater or acclimated to 16 ppt or transferred directly to 16 ppt (Table 2).

The highest percentage of yolky ova (91%) was achieved by genetically modified *O. niloticus* treated with sea bream-DNA reared at fresh water. This percentage decreased to 88 and 54% with increased levels of salinity to 16 and 32 ppt, respectively. Moreover, genetically modified *O. niloticus* treated with different types of DNA surpassed the percentage of yolky eggs from 35% for control fish to 54 and 56%, for *O. niloticus* treated with sea bream and Artemia-DNA, respectively (data not shown). These results indicated that, salinity tolerant

genetically modified *O. niloticus* can be produced successfully. The better results from the present work may be attributed to the successful transfer and expression of foreign DNA (Wen et al., 1993; Ali, 2001; El-Zaeem, 2001, 2004a, 2004b; Hemeida et al., 2004; El-Zaeem and Assem, 2004, 2006; Assem and El-Zaeem, 2005).

At the beginning of the experiment, no significant differences in moisture, protein and lipid content were detected among genetically modified *O. niloticus* treated with different types of DNA and their control. By the end of the experiment, no significant differences were detected in moisture content among treatments. Yet, crude protein was significantly lower (P $\leq$ 0.05) in genetically modified *O. niloticus* treated with Artemia-DNA, showing lower means when compared to both genetically modified *O. niloticus* treated with sea bream-DNA and control fish. Moreover, the highest mean values of lipids content was achieved by control fish and differed significantly (P $\leq$ 0.05) from those of genetically modified *O. niloticus* treated with the two types of DNA (Table 3). The genetically modified fish had less body lipids than control which was a function of their greater energy demand and elevated metabolic rate.

Fish acclimated and reared at 16 ppt, had significant (P $\leq$ 0.05) higher moisture and crude protein contents compared to the fish reared at 32 ppt, but did not differ significantly (P $\leq$ 0.05) from those fish reared at fresh water and non-acclimated fish reared at 16 ppt. Chatakondi et al. (1995), Dunham et al. (2002) and El-Zaeem (2004b) reported that, the moisture and lipids content were lower while the protein content was higher in transgenic common carp and red-belly tilapia muscles compared with their control. Martinez et al. (2000), Lu et al. (2002), El-Zaeem (2004a, 2004b), El-Zaeem and Assem (2004) and Assem and El-Zaeem (2005) reported that, anabolic stimulation and average protein synthesis were higher in transgenic than that of non-transgenic fish. The results of the present work are consistent with these findings.

The highest mean values of feed intake was recorded for genetically modified *O. niloticus* treated with sea bream-DNA, which was significantly (P $\leq$ 0.05) higher than those of genetically modified *O. niloticus* treated with Artemia-DNA and control fish. The best or highest means of food conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR) and energy retention (ER) percentage were achieved by control fish, and differed significantly (P $\leq$ 0.05) from those of genetically modified *O. niloticus*. Moreover, fish reared at 32 ppt had significant (P $\leq$ 0.05) decrease of feed intake, compared with the others reared at various salinities up to 16 ppt. The best or highest records of FCR, PER and PR% were achieved by fish reared at fresh water and differed significantly (P $\leq$ 0.05) from those reared at different salinity levels. The highest mean of ER% was recorded for fish reared at fresh water, but did not differ signifi-

Table 3. Effect of different types of foreign DNA and salinity levels on body composition and feed utilization of *O. niloticus.*

Treatment	Moisture	% on dry matter basis		Feed intake	FCR	PR%	PER	ER%
		Protein	Lipid					
At the start								
Control	81.01 ± 0.15	56.00 ± 1.41	18.24 ± 0.51					
Sea bream-DNA	80.46 ± 0.48	56.75 ± 1.10	17.89 ± 0.46					
Artemia-DNA	81.11 ± 0.01	54.50 ± 0.70	18.38 ± 0.23					
DNA source								
Control	75.635 ± 0.42	57.28 ± 0.43[a]	24.68 ± 0.28 [a]	78.84 ± 13.20[c]	1.82 ± 0.11[b]	1.57 ± 0.1[a]	21.98 ± 1.02[a]	16.82 ± 0.78[a]
Sea bream-DNA	75.77 ± 0.68	56.98 ± 0.58 [a]	24.09 ± 0.08 [b]	97.21 ± 16.32[a]	1.88 ± 0.07 [a]	1.51 ± 0.06[b]	20.90 ± 0.61[b]	15.68 ± 0.66[b]
Artemia-DNA	76.07 ± 0.95	56.06 ± 0.45 [b]	24.11 ± 0.143[b]	86.92 ± 3.98 [b]	1.89 ± 0.14 [a]	1.49 ± 0.1 [b]	20.09 ± 1.60[b]	15.22 ± 1.06[b]
Salinity ppt								
Fresh water (FW)	76.03 ± 0.44[ab]	56.97 ± 1.26 [a]	24.31 ± 0.13	89.99 ± 12.41[a]	1.77 ± 0.05[c]	1.61 ± 0.04[a]	22.36 ± 0.62[a]	16.79 ± 0.45 [a]
16 ppt indirect(ID)	76.47 ± 0.45[a]	57.04 ± 0.80[a]	24.31 ± 0.50	93.00 ± 7.46 [a]	1.85 ± 0.10[b]	1.54 ± 0.1 [b]	20.66 ± 1.52[b]	15.92 ± 1.40[ab]
16 ppt direct (D)	75.91 ± 0.30 [ab]	56.89 ± 0.67 [a]	24.42 ± 0.45	94.75 ± 13.32 [a]	1.84 ± 0.10[b]	1.52 ± 0.1 [b]	20.86 ± 1.38[b]	15.85 ± 1.11[ab]
32 ppt indirect(ID)	74.9 ± 0.18 [b]	56.18 ± 0.44 [b]	24.13 ± 0.24	72.87 ± 13.49 [b]	1.99 ± 0.05 [a]	1.43 ± 0.04[c]	20.07 ± 0.83[b]	15.08 ± 0.50 [b]
DNA source								
Control at FW	75.78 ± 1.24	57.43 ± 0.32	24.46 ± 0.49	84.13 ± 6.41[bc]	1.73 ± 0.05 [b]	1.65 ± 0.05	22.94 ± 0.32	17.26 ± 0.28
S-DNA at FW	75.78 ± 1.10	57.32 ± 0.15	24.19 ± 0.04	104.25 ± 4.42[a]	1.83 ± 0.07 [b]	1.56 ± 0.05	21.70 ± 1.87	16.36 ± 1.38
A-DNA at FW	76.55 ± 0.95	55.18 ± 0.05	24.28 ± 0.04	81.58 ± 3.93[bc]	1.75 ± 0.05 [b]	1.62 ± 0.05	22.43 ± 1.10	16.75 ± 0.40
Control at 16 ID	75.97 ± 0.81	57.68 ± 0.76	24.90 ± 0.43	87.69 ± 0.43 [b]	1.80 ± 0.04 [b]	1. 58 ± 0.07	21.97 ± 0.11	17.25 ± 0.44
S-DNA at16 ID	76.66 ± 2.03	57.31 ± 0.47	24.04 ± 0.17	101.53 ± 1.06 [a]	1.81 ± 0.04[b]	1.57 ± 0.03	21.03 ± 1.25	16.10 ± 0.64
A-DNA at16 ID	76.82 ± 0.53	56.15 ± 0.07	24.00 ± 0.15	89.78 ± 0.45 [b]	1.96 ± 0.06[a]	1.45 ± 0.04	18.99 ± 0.96	14.38 ± 0.77
Control at 16 D	75.80 ± 2.26	57.36 ± 0.20	24.94 ± 0.25	84.35 ± 7.26 [b]	1.76 ± 0.05 [b]	1.610.05	22.44 ± 1.40	17.13 ± 1.01
S-DNA at16 D	75.66 ± 2.17	57.19 ± 0.04	24.12 ± 0.04	109.77 ± 7.11[a]	1.95 ± 0.08[a]	1.46 ± 0.05	20.31 ± 1.01	15.31 ± 0.77
A-DNA at 16 D	76.25 ± 0.12	56.12 ± 0.03	24.19 ± 0.04	90.15 ± 5.00[b]	1.81 ± 0.04 [b]	1.48 ± 0.09	19.85 ± 1.10	15.1 ± 0.83
Control at 32 ID	75.01 ± 0.29	56.66 ± 0.77	24.41 ± 0.41	59.19 ± 1.45[d]	1.97 ± 0.07 [a]	1.44 ± 0.05	20.56 ± 0.70	15.64 ± 0.50
S-DNA at 32 ID	75.00 ± 0.15	56.11 ± 0.14	23.99 ± 0.16	73.26 ± 4.19[c]	1.95 ± 0.07[a]	1.46 ± 0.0	20.55 ± 0.67	14.95 ± 1.42
A-DNA at 32 ID	74.69 ± 0.75	55.79 ± 0.77	23.99 ± 0.15	86.18 ± 7.18 [b]	2.06 ± 0.06[a]	1.38 ± 0.03	19.11 ± 0.73	14.66 ± 0.64

Means within each comparison in the same column with the different superscripts differ significantly (P ≤ 0.05). Feed conversion ratio (FCR) = dry feed intake/ gain; protein efficiency ratio (PER) = gain/ protein intake; protein retention percent (PR %) = protein increment (100)/ protein intake ; energy retention percent (ER %) = energy increment (100)/ energy intake.

cantly from those of acclimated and non-acclimated fish reared at 16 ppt.

In addition, feed intake of genetically modified *O. niloticus* treated with sea bream-DNA and reared at different salinity levels up to 16 ppt, improved significantly (P ≤ 0.05), compared to the genetically modified *O. niloticus* treated with Artemia-DNA and control fish at the same levels of salinities. Genetically modified *O. niloticus* treated with Artemia-DNA reared at 32 ppt, had surpassed feed intake significantly (P ≤ 0.05), compared to the genetically modified *O. niloticus* treated with sea bream-DNA and control fish reared at the same level of salinity (32 ppt). These differences may be attributed to the type of donor of DNA (Ali, 1999; El-Zaeem, 2001; El-Zaeem, 2004a, b; Ali, 2002), since the Artemia is more salinity tolerant than sea bream and control group of *O. niloticus*. The poorest FCR were obtained in all fish reared at 32 ppt, but did not differ significantly (P ≤ 0.05) from those of non-acclimated genetically modified *O. niloticus* treated with Artemia-DNA and acclimated genetically modified *O. niloticus* treated with sea bream-DNA reared at 16 ppt of salinity. The study results are consistent with findings obtained by Martinez et al. (2000) and El-Zaeem (2001, 2004b).

It is clear from the results that feed consumption of genetically modified *O. niloticus* treated by sea bream-DNA at different salinity up to 16 ppt, improved. This may be attributed to the effect of elevated growth hormone in fish plasma that resulted from those treated by sea bream-DNA. Rahman et al. (1998) reported that, growth hormone binds to specific cell receptors, which induces synthesis and secretion of insulin-like growth factors (IGF-1 and IGF-II), resulting in the promotion of somatic growth through improved appetite, feeding efficiency and growth rate (De la Fuente and Castro, 1998). Also, Oakes et al. (2007) reported that, the enhancement perfor-mance of transgenic Coho salmon was due to enhanced dietary intake. Many authors (Cook et al., 2000; Wu et al., 2003; Hallerman et al., 2007) noted that, growth hormone transgenic fish had feed efficiency better than non-transgenic. Besides, Ron et al. (1995) and Haroun (1999) reported that, tilapia in sea water utilize the feed more efficiently than in fresh water. Clark et al. (1990) noted that, maximum growth rate of Florida red tilapia in sea water occurred at satiation feed rate, on the other hand the feed conversion improved at lower feeding rate.

The highest value of amino acid was recorded for glutamate, while the lowest was observed for cystine. The results showed that, genetically modified *O. niloticus* treated with sea bream-DNA reared at different salinity levels up to 32 ppt, had higher amino acid contents compared to both the genetically modified *O. niloticus* treated with Artemia-DNA and control fish, except for threo-nine, valine and isoleucine (Table 4). Martinez et al. (1999) reported that, transgenic *Oreochromis hornorum* had higher levels of alanine andaspartic acid in muscle

tissue sample than that of non-transgenic *O. hornorum*. Also, Chatakandi et al. (1995) reported that, transgenic common carp (*Cyprinus carpio*) had higher levels of aspartic acid, cystine, glutamic acid, histidine and lysine in tissue sample than that of non-transgenic. The results of the present work support these findings, since the transfer of foreign DNA lead to change in the levels of muscle amino acids.

Genetically modified *O. niloticus* treated with either sea bream or Artemia-DNA and their control are presented in Figure 1. Genetically modified *O. niloticus* treated with sea bream-DNA was monitored to detect possible morphological alterations; they showed a silver color covering all the body and no dark vertical bands. Genetically modified *O. niloticus* treated with Artemia-DNA, had a red color covering around the head compared to the control group (Figure 1). Wen et al. (1993) reported that, total DNA iso-lated from Japanese phytophagous crucian carp (*Carassius auratus cuvieri*) liver was transferred into fertilized eggs of red carp (*Cyprinus carpio* red var). The melanin expression during embryonic development was chosen as a selective marker of transgenic fish. All fry that survived showed similar melanin expression to that of crucian carp which resulted from gene transfer. Ali (2002) also reported abnormal fish colors observed in genetically modified *O. niloticus* treated with pituitary glands DNA isolated from *Diplodus vulgaris*. The results of this work are consistent with these findings.

All DNA samples from genetically modified *O. niloticus* treated with different types of DNA and their control were examined using RAPD marker. Six random primers were used to determine DNA fingerprinting in genetically modi-fied *O. niloticus* treated with sea bream-DNA, Artemia-DNA and their control fish. The results showed that, no amplification was detected in the control reactions (without DNA source). All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions (Figure 2).

The results also showed that, the number of amplified bands detected varied, depending on the primers and DNA treatment. Highly genetic polymorphic percentage ranged from (10.00 to 66.66%) with an average of 35.95% using different random primers (Table 5 and Figure 2). It may be due to the differences in DNA molecule among normal and modified fish as a result of direct injection of foreign DNA isolated from sea bream and Artemia. Moreover, some fragments of foreign DNA may be randomly integrated into *O. niloticus* genomes. This integration could be functional or silent (Yaping et al., 2001). The results of the present work are consistent with the findings obtained from previous studies (El-Zaeem, 2001; Hemeida et al., 2004; Ali, 2002; Assem and El-Zaeem, 2005, El-Zaeem and Assem, 2006). Also, the sensivity of the RAPD marker played an important role in the detection of these differences (Ahmed et al., 2004; Ali et al., 2004; El-Zaeem et al, 2006; El-Zaeem

Table 4. Amino acids (g /100 g protein) of genetically modified *O. niloticus* treated with different types of DNA and salinity levels.

Treatment \ Amino acid	Control FW	Control 16 ppt-D	Control 16 ppt-ID	Control-32 ppt	Sea bream DNA-FW	Sea bream DNA16 ppt-D	Sea bream DNA16 ppt-ID	Sea bream DNA 32 ppt	Artemia DNA-FW	Artemia DNA-16 ppt-D	Artemia DNA-16 ppt-ID	Artemia DNA-32 ppt
Aspartate	9.13	9.15	9.14	9.00	9.20	9.21	9.24	9.18	9.10	9.00	9.11	9.00
Threonine	4.72	4.71	4.70	4.53	4.53	4.56	4.51	4.41	4.66	4.56	4.52	4.48
Serine	4.10	4.15	4.11	4.00	4.25	4.23	4.30	4.33	4.14	4.17	4.20	4.10
Glutamate	14.10	14.22	14.25	13.97	14.32	14.35	14.40	14.23	14.18	14.14	14.20	14.00
Proline	5.20	4.10	4.80	4.2	5.19	5.13	5.10	5.11	5.20	5.22	5.20	5.10
Glycine	4.22	4.19	4.22	4.12	4.55	4.60	4.61	4.55	4.49	4.53	4.55	4.48
Alanine	6.30	6.25	6.20	6.35	6.37	6.40	6.38	6.28	6.39	6.42	6.39	6.40
Cystine	0.39	0.33	0.38	0.40	0.70	0.72	0.74	0.73	0.51	0.56	0.53	0.52
Valine	5.31	5.20	5.22	5.17	5.10	5.13	5.12	5.10	5.20	5.17	5.21	5.00
Methionine	2.70	2.68	2.70	2.63	2.72	2.64	2.69	2.55	2.45	2.39	2.40	2.36
Isoleucine	7.66	7.50	7.53	7.40	6.98	6.88	6.77	6.66	6.90	6.88	6.78	6.70
Leucine	7.40	7.33	7.30	7.18	7.10	7.15	7.18	7.22	7.14	7.11	7.12	7.11
Tyrosine	3.23	3.30	3.29	3.32	3.41	3.45	3.51	3.53	3.54	3.55	3.54	3.47
Phenylalanine	3.68	3.72	3.69	3.71	3.80	3.91	3.80	3.83	3.70	3.74	3.78	3.70
Histidine	2.33	2.40	2.37	2.28	2.30	2.42	2.43	2.40	2.35	2.33	2.30	2.35
Lysine	7.25	7.18	7.20	7.19	7.60	7.56	7.60	7.61	7.41	7.39	7.40	7.24
Arginine	4.60	4.56	4.61	4.55	5.30	5.23	5.30	5.28	5.10	5.22	5.18	5.12

Table 5. The percentage of polymorphic bands among genetically modified *O. niloticus* treated with different types of DNA using different random primers.

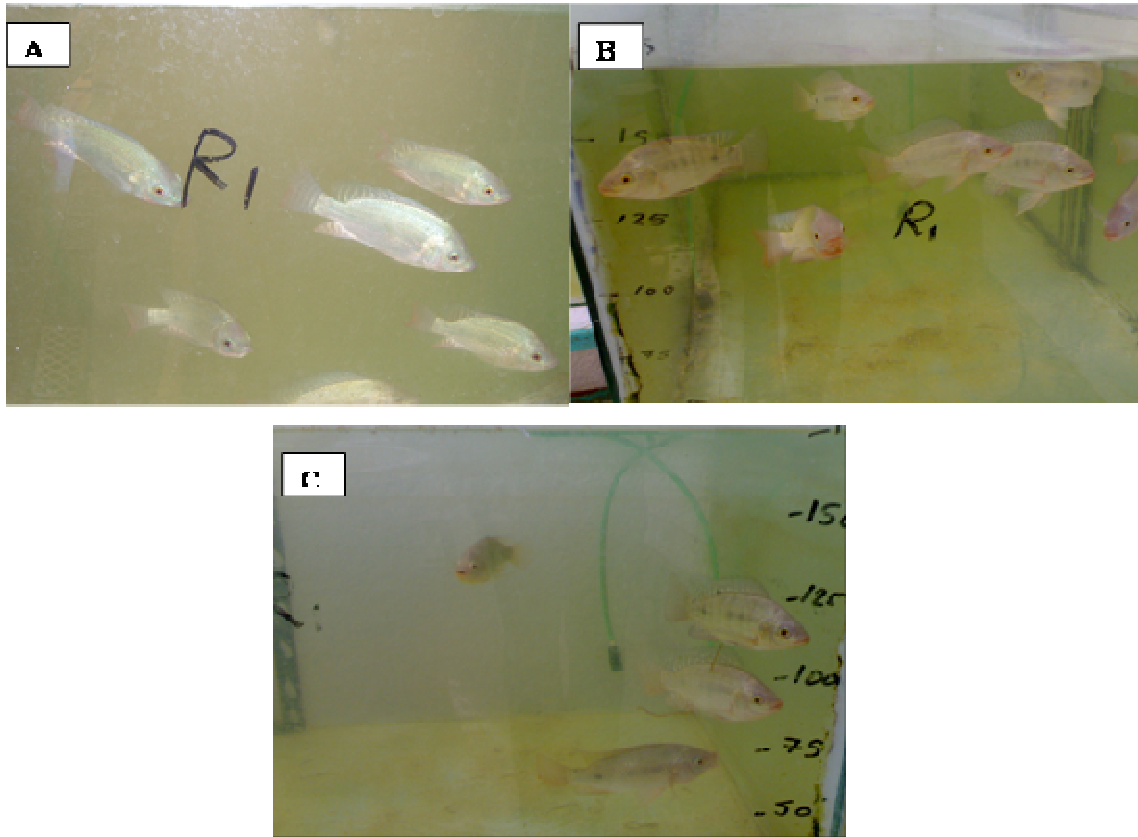| Primers no.	Total band	No. of polymorphic band	Polymorphic band %
1	10	1	10.00
2	14	5	35.71
3	10	3	30.00
4	5	2	40.00
5	6	4	66.00
6	12	4	33.33
Average	-	-	35.95

Figure 1. Genetically modified *O. niloticus* treated with: (A), Sea bream-DNA; (B), Artemia-DNA   and (C), their control.
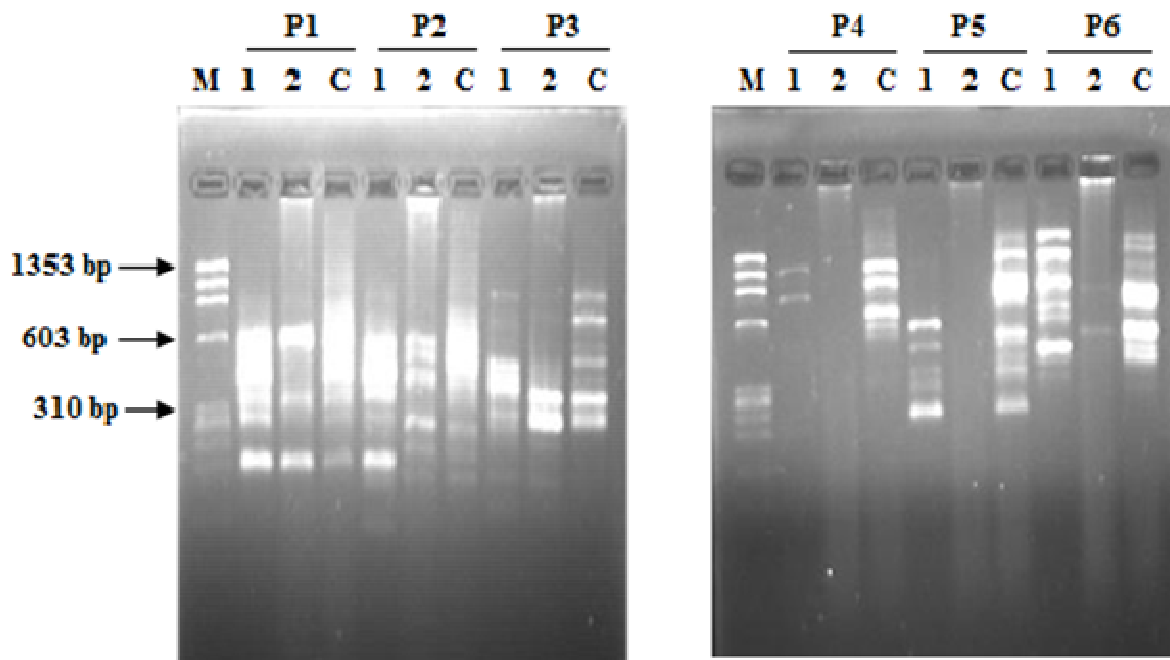

Figure 2. RAPD amplification products generated from genetically modified *O. niloticus* treated with sea bream-DNA, Artemia-DNA and their control fish, using six random primers. Lane M: ΦX174 DNA marker, lanes 1, 2 and C: *O. niloticus* treated with sea-bream DNA, Artemia-DNA and their control fish, respectively.

and Ahmed, 2006). The specific characterization of the RAPD method (random, uncharacterized multiple genome loci; dominant nature of markers; and possibility of migration of no-homologous bands) result in limitations based on RAPD analysis alone. Despite these limitations, the RAPD analysis can be used effectively for initial assessment of genetic variation among fish species (Barman et al., 2003). The main advantages of RAPD markers are the possibility of working with anonymous DNA, relatively low expense, fast and simple to produce (Hadrys et al., 1992; Elo et al., 1997; Ali et al., 2004).

The results of the present work suggested that, hypersaline genetically modified *O. niloticus* with higher growth rate which can be produced by the transfer of a foreign DNA isolated from sea bream and Artemia as a feasible and fast methodology compared to interspecific hybridization which is one classical breeding methods (El-Zaeem et al., 2010). According to these results, genetically modified *O. niloticus* treated with sea bream and Artemia-DNA surpassed most of the productive performance traits under different levels of salinity up to 32 ppt, compared to *O. niloticus*, *O. aureus* and interspecific crossbreeding of (♀*O. niloticus* X ♂ *O. aureus*) and (♀ *O. aureus* X ♂ *O. niloticus*) reared at the same levels. Furthermore, genetically modified *O. niloticus* treated with Artemia-DNA reared at 32 ppt of salinity had higher growth rate than that of genetically modified *O. niloticus* treated with sea bream-DNA and their control at the same level. These better results are due to the successful transfer and high expression of Artemia-DNA at 32 ppt.

Abbreviations

SGR, Specific growth rate; **PCR,** polymerase chain reaction; **RAPD,** Random amplified polymorphic DNA; **IBW,** initial body weight; **FBW,** final body weight; **DG,** daily gain; **FCR,** food conversion ratio; **PER,** protein efficiency ratio; **PR,** protein retention; **ER,** energy retention; **IGF,** insulin-like growth factors; **GSI,** gonadosomatic index.

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