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*Full Length Research Paper*

# Evaluation of the productive performance characteristics of red tilapia (*Oreochromis* sp.) injected with shark DNA into skeletal muscles and maintained diets containing different levels of probiotic and amino yeast

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**This work aimed to study the effect of direct injection of shark (*Squalus acanthias* L.) DNA into skeletal muscles of red tilapia (*Oreochromis* sp.) fed at different additive levels (two probiotic levels; 0.3 and 0.5%, two amino yeast levels; 0.5 and 1.0% and a mixed of 0.3% probiotic and 0.5% amino yeast), on the productive performance. The results show that red tilapia injected with DNA had significant (P≤0.05) superiority of growth performance and feed utilization; besides the body composition was improved. In addition, the different levels of probiotic and amino yeast were more effective in stimulating most of the productive performance traits compared to the control group and the mixed of probiotic and amino yeast. The result indicates a possible easy and rapid way for improving red tilapia characteristics.**

**Key words:** red tilapia, shark DNA, direct injection, productive performance, probiotic, amino yeast.

## INTRODUCTION

A quick method and rapid way for introducing foreign DNA injected directly into the muscle tissue was reported by Wolff et al. (1990) and Ono et al. (1990) in adult mice, by Thomson and Booth (1990) in rat and by Hansen et al. (1991), Rahman and Maclean (1992), Tan and Chan (1997), Xu et al. (1999), El-Zaeem (2004), Hemeida et al. (2004), El-Zaeem and Assem (2004), Assem and El-Zaeem (2005), El-Zaeem (2012), and El-Zaeem et al. (2012) in fish. This procedure is useful because muscle injection is much easier than the others and very rapid results are obtained (Rahman and Maclean, 1992). The foreign DNA was presented extrachromosomally up to six months following injection (Wolff et al., 1990). Moreover, Sudha et al. (2001) reported that the expression of muscular injection of DNA was evident in several non muscle tissues, such as skin epithelia, pigment cells, blood vessel cells and neuron-like cells.

A series studies have focused on the use of shark DNA to boost immune responses in fish (El-Zaeem and Assem, 2004; Assem and El-Zaeem, 2005). Sharks contain high levels of immunoglobulin (IgM) proteins, which act as antibodies and help initiate immune responses to bacterial invasions. Although IgM can be found at high levels in shark (up to 50% of the serum proteins), it has been reported to be present at much lower levels in fish such as Atlantic salmon, Halibut (*Hippoglossus*

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*hippoglosus* L.), Haddock (*Melanogrammus aeglefinus* L.) and Cod (*Gadus morhua* L.) (2, 8, 13 and 20% of the serum proteins, respectively) (Marchalonis et al., 1993; Magnadottir, 1998). When shark (*Squalus acanthias* L.) DNA was injected into the skeletal muscles of Nile tilapia (*Oreochromis niloticus*) and red belly tilapia (*Tilapia zillii*) fingerlings, fish showed significantly higher levels of total antibody activity, serum total protein, and globulin (El-Zaeem and Assem, 2004; Assem and El-Zaeem, 2005). In addition, injected tilapia had significant growth enhancement and changes in proximate composition, with decreases in moisture and increases in both protein and lipid content. Injected fish showed high genetic polymorphism, indicating random integration of the shark genes into tilapia muscle DNA.

Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to control potential pathogens and to increase the growth rates and welfare of farmed aquatic animals (Gatesoupe, 1991, 1999; Wang et al., 2005; Wang and Xu, 2006; Wang, 2007). The beneficial effects of probiotics, such as improvement of feed utilization, modulation of intestinal microflora, enhancement of immune responses and antagonism to pathogens, have been demonstrated in a number of previous studies (Balcázar et al., 2006a,b; Irianto and Austin, 2002a,b; Kesarcodi-Watson et al., 2008; Merrifield et al., 2010a,b,c,d; Wang et al., 2008). Among the various benefits of probiotics, immunomodulatory activity is noteworthy in improving the overall health status of the host. However, there is limited research available for immunomodulatory activity of probiotics, especially for the long-term use of probiotics in fish diets. The most commonly used probiotics in aquaculture practices belong to lactic acid bacteria and Bacillus spp. (Wang et al., 2008).

Brewer's yeast, *Saccharomyces cerevisiae*, can be used as a probiotic (Chiu et al., 2010; Lara-Flores et al., 2003) and also as growth promoter (Abdel-Tawwab et al., 2008; Lara-Flores et al., 2003; Li and Gatlin, 2003, 2005) in aquaculture. Brewer's yeast (*S. cerevisiae*) has been used as a feed supplement for various animals. It contains various immunostimulating compounds such as β-glucans, nucleic acids, mannan oligosaccharides and other cell wall components (Li and Gatlin, 2003, 2005; Oliva-Teles and Gonçalves, 2001). It has been observed that *S. cerevisiae* can positively influence the non-specific immune responses (Ortuno et al., 2002; Siwicki et al., 1994) as well as growth performance (Abdel-Tawwab et al., 2008; Li and Gatlin, 2003, 2005; Noh et al., 1994; Oliva-Teles and Gonçalves, 2001; Rumsey et al., 1991; Taoka et al., 2006) of some fish species.

Therefore, the objective of this study was to evaluate the productive performance characteristics of red tilapia (*Oreochromis* sp.) injected with shark (*Squalus acanthias* L.) DNA (into skeletal muscles) and fed maintained diets containing different additive levels of probiotic and amino yeast.

## MATERIALS AND METHODS

### Fish origin

The red tilapia, *Oreochromis* sp. fry used in this study was a hybrid, descended of an original cross of female *Oreochromis mossambicus* x male *O. niloticus* and obtained from Marine Fish Hatchery, 21 Km, Alexandria, Egypt. Red tilapias were transported to the laboratory of Breeding and Production of Fish, Animal and Fish Production Department, Faculty of Agriculture (Saba-Bacha), Alexandria University, Alexandria, Egypt.

### DNA extraction

High molecular weight DNA was extracted according to Brem et al. (1988) method. Isolation of DNA was accomplished by reducing liver sample from shark (*Squalus acanthias* L.) to small pieces, which were then transferred to a microfuge tube and incubated overnight until the sample was digested in a buffer solution containing 50 mM Tris, 100 mM ethylenediaminetertracetic acid (EDTA, pH 8.0), 100 mM NaCl, 0.1% sodium dodecyl sulfate (SDS) and 0.5 mg/ml proteinase K. After incubation, samples were extracted twice for 15 to 20 min with one volume of phenol/ chloroform (1:1) and then again twice for 15 min with one volume of chloroform/isoamyle-alcohol (24:1). The aqueous phase was then precipitated with 2.5 volumes of 100% ethanol in the presence of 1/10 volume 3 M sodium acetate (pH 6.0). The pelleted DNA was washed with 70% ethanol and dissolved in 0.1x saline sodium citrate (SSC) buffer. The DNA concentrations were measured by UV spectrophotometry. The extracted DNA was restricted by Eco R1 restriction enzyme type II. It digested DNA between guanine and adenine according to Tsai et al. (1993).

### Experimental design

#### Culture condition

Red tilapias were acclimatized to laboratory conditions for two weeks. Fry with an initial body weight (2.9±0.11 g) were divided randomly to 12 groups and three replicates for each group, stocked at the rate of 10 fish per aquarium. The aquaria of dimensions 100 x 34 x 50 cm were supplemented with continuous aeration. Nearly half of the volume of water in the aquaria was changed daily by freshly stocked tap water and the aquaria were cleaned every day before feeding. Water temperature was maintained at 26°C by means of electric aquarium heaters. Fish were stocked at 1.0 fish/10 L and fed twice daily to satiation, six days a week. Fish were weighed at the beginning of experiment and then biweekly for eight weeks.

### Injection of foreign DNA *in vivo*

The DNA concentration of 40 µg/0.1 ml/fish (El-Zaeem, 2004; El-Zaeem and Assem, 2004; Hemeida et al., 2004; Assem and El-Zaeem, 2005) were prepared using 0.1x SSC buffer and injected into red tilapia muscles using a hypodermic needle. The injection was applied on six groups of red tilapia fingerlings, while the other six groups were left without injection as a control.

### Diets formulation and preparation

Six dietary treatments were tested in triplicate groups: the control diet with no dietary supplementation (C) and five other test diets which included; probiotic added at 0.3% (P1) and 0.5% (P2); amino

**Table 1.** Composition and proximate analysis of red tilapia (*Oreochromis* sp.) diets used during the growth period.

| Ingredient | C | P1 | P2 | A1 | A2 | P1+A1 |
|---|---|---|---|---|---|---|
| Wheat flour | 31 | 31 | 31 | 31 | 31 | 31 |
| Wheat bran | 18 | 18 | 18 | 18 | 18 | 18 |
| Soybean meal | 23 | 22.7 | 22.5 | 22.5 | 22 | 22.2 |
| Yellow corn | 14.2 | 14.2 | 14.2 | 14.2 | 14.2 | 14.2 |
| Fish meal | 11.5 | 11.5 | 11.5 | 11.5 | 11.5 | 11.5 |
| Bone meal | 2 | 2 | 2 | 2 | 2 | 2 |
| Vit. & Min. Mix* | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Probiotic[1] | 0 | 0.3 | 0.5 | 0 | 0 | 0.3 |
| Amino yeast[2] | 0 | 0 | 0 | 0.5 | 1.0 | 0.5 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| **Proximate analysis (%)** | | | | | | |
| Moisture | 10.63 | 10.73 | 10.71 | 10.83 | 10.96 | 10.78 |
| Protein | 23.84 | 23.82 | 23.84 | 23.70 | 23.69 | 23.83 |
| Fat | 7.31 | 7.30 | 7.29 | 7.29 | 7.21 | 7.24 |
| Fiber | 4.24 | 4.20 | 4.21 | 4.13 | 4.02 | 4.19 |
| Nitrogen free extract (NFE) | 44.07 | 44.07 | 44.04 | 44.18 | 44.30 | 44.10 |
| Ash | 9.91 | 9.88 | 9.91 | 9.87 | 9.82 | 9.86 |
| Metabolizable energy (kcal/100g) | 244.05 | 244.23 | 243.85 | 244.14 | 243.51 | 243.87 |

* Content/kg of vitamin and minerals mixture (P- Fizer, Cairo, Egypt). Vitamin A, 4.8 MIU; Vitamin D, 0.8 MIU; Vitamin E, 4.0 g; vitamin K, 0.8 g; vitamin B1, 0.4 g; vitamin B2, 1.6 g; vitamin B6, 0.6 g; vitamin B7, 20.0 mg; vitamin B12, 4.0 g; folic acid, 0.4 g; nicotinic acid, 8.0 g; pantothenic acid, 4.0 g; colin chloride, 200 g; zinc, 22 g; cooper, 4.0 g; iodine, 0.4 g; iron, 12.0 g; manganese, 22.0 g; selenium, 0.04 g.

[1] Probiotic; produced by Pura 2A. Company, Cairo, Egypt, containing: *Lactobacillus* sp. (*L. plantarum, L. fermentum, L. delbrueckii), Bacillus subtilis, Saccharomyces cerevisiae, Rhodopseudomonas palustris* and especially active additives. probiotic 1%, molasses 3%, water 96%.

[2] Amino yeast; produced by New Vetro vit 10 th. of Ramadan city, Egypt containing: B. Glucan 44.50 g, Rhammose (1500.00 mg), Xylose (3000.00 mg), D L- Methionine (10110.00 mg), lysine (2600.00 mg), monnanollgo saccharides (200.00 g.)

yeast added at 0.5% (A1) and 1.0% (A2); and mixed of probiotic and amino yeast 0.3% + 0.5% (P1+A1). Total protein content for all dietary treatments was adjusted by the parallel decrease of soybean meal with increase level of dietary supplementation as described in Table 1. Metabolizable energy was calculated according to feedstuff values reported by NRC, (1993). Dry ingredients were passed through a sieve (0.6 mm diameter hole) before mixing into the diets. Mixtures were homogenized in a food grinder mixer. Water was then blended into the mixture at the ratio of 50% for pelleted. The diets were pelleted using meat grinder with a 1.5 mm diameter.

#### Quantitative traits studied

The following parameters were measured; body weight (BG), weight gain (WG), specific growth rate (SGR %/day), percent body weight increases (%BWI), feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), protein and energy retention percent (PR% and ER%). Initial and final whole body composition analyses were performed using the standard methods (AOAC, 1984) for moisture (oven drying), for protein (macro-kjeldahl method) and lipid (ether extract method).

#### Statistical analysis

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

$$Y_{ijkl}=\mu+T_i+A_j+(TA)_{ij}+B_k+e_{ijk}$$

Where, $Y_{ijk}$ is the observation of the $ijk^{th}$ parameter measured; $\mu$ is the overall mean; $T_i$ is the effect of $I^{th}$ DNA; $A_j$ is the effect of $J^{th}$ additives; $(TA)_{ij}$ is the interaction DNA by additives; $B_k$ is the effect of $K^{th}$ block; and $e_{ijk}$ is the random error.

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

## RESULTS

Data presented in Table 2 show that the final body weight (FBW), weight gain (WG), percent body weight increases (% BWI) and specific growth rate (SGR %/day) of red tilapia (*Oreochromis* sp.) injected with shark DNA were significantly higher (P≤0.05) than those of the non-injected fish. Moreover, the highest value of FBW was obtained by the red tilapia fed the highest level of probiotic, but did not differ (P≤0.05) significantly from those fish fed the lowest level of probiotic and the lowest and highest levels of amino yeast. The highest WG was achieved by the fish fed the highest level of probiotic, but did not differ (P≤0.05) significantly from those of fish fed the lowest level of probiotic and the highest level of amino yeast. Red tilapia fed the lowest level of probiotic show higher mean of % BWI, but did not differ (P≤0.05) significantly from that of fish fed the highest level. The

**Table 2.** Effect of shark DNA injection and different additive levels on growth performance[1] of red tilapia (*Oreochromis* sp.).

| Treatment | IBW (g) | FBW (g) | WG (g) | %BWI | SGR %/day |
|---|---|---|---|---|---|
| **Type of DNA (T)** | | | | | |
| DNA | 2.92±0.10 | 14.87±1.76$^{a}$ | 11.9±61.74$^{a}$ | 409.97±59.84$^{a}$ | 2.90±0.20$^{a}$ |
| Non-DNA | 2.83±0.11 | 12.81±0.81$^{b}$ | 9.97±0.86$^{b}$ | 353.04±39.41$^{b}$ | 2.70±0.16$^{b}$ |
| **Type of Additive (A)** | | | | | |
| Control | 2.90±0.10 | 12.37±1.50$^{c}$ | 9.48±0.16$^{c}$ | 327.21±16.98$^{c}$ | 2.59±0.06$^{d}$ |
| P1 | 2.80±0.00 | 14.90±0.81$^{a}$ | 12.10±0.81$^{a}$ | 432.14±33.25$^{a}$ | 2.98±0.10$^{a}$ |
| P2 | 2.90±0.13 | 15.05±2.96$^{a}$ | 12.15±3.32$^{a}$ | 417.56±101.12$^{ab}$ | 2.91±0.31$^{ab}$ |
| A1 | 2.85±0.09 | 13.63±0.99$^{abc}$ | 10.78±0.94$^{b}$ | 378.06±33.51$^{bc}$ | 2.84±0.09$^{abc}$ |
| A2 | 2.90±0.10 | 13.93±1.20$^{ab}$ | 11.03±1.10$^{ab}$ | 379.34±29.21$^{bc}$ | 2.77±0.12$^{bcd}$ |
| P1+A1 | 2.90±0.10 | 13.16±0.77$^{bc}$ | 10.26±0.81$^{bc}$ | 354.70±40.57$^{c}$ | 2.70±0.14$^{cd}$ |
| **Interactions T x A** | | | | | |
| DNA x C | 2.90±0.10 | 12.45±0.15$^{b}$ | 9.55±0.05$^{b}$ | 329.65±13.64 | 2.60±0.04 |
| DNA x P1 | 2.80±0.00 | 15.50±0.70$^{ab}$ | 12.70±0.70$^{ab}$ | 453.57±35.36 | 3.06 ±0.09 |
| DNA x P2 | 3.00±0.00 | 17.90±1.10$^{a}$ | 14.90±1.10$^{a}$ | 496.67±51.85 | 3.19±0.11 |
| DNA x A1 | 2.90±0.10 | 14.44±0.22$^{ab}$ | 11.54±0.12$^{ab}$ | 398.27±13.57 | 2.87±0.04 |
| DNA x A2 | 3.00±0.00 | 15.10±0.30$^{ab}$ | 12.10±0.30$^{ab}$ | 403.33±14.14 | 2.89±0.04 |
| DNA X P1+A1 | 2.90±0.00 | 13.86±0.10$^{b}$ | 10.96±0.20$^{ab}$ | 378.34±27.81 | 2.80±0.08 |
| Non-DNA x C | 2.90±0.00 | 12.30±0.10$^{b}$ | 9.40±0.20$^{b}$ | 324.77±25.59 | 2.59±0.08 |
| Non-DNA x P1 | 2.90±0.00 | 14.30±0.30$^{ab}$ | 11.50±0.30$^{ab}$ | 410.72±15.15 | 2.91±0.02 |
| Non-DNA x P2 | 2.80±0.00 | 12.20±0.20$^{b}$ | 9.40±0.40$^{b}$ | 338.46±54.39 | 2.64±0.16 |
| Non-DNA x A1 | 2.80±0.20 | 12.82±0.78$^{b}$ | 10.02±0.78$^{b}$ | 357.86±39.39 | 2.82±0.12 |
| Non-DNA x A2 | 2.80±0.00 | 12.75±0.15$^{b}$ | 9.95±0.15$^{b}$ | 355.36±7.57 | 2.66±0.03 |
| Non-DNA X P1+A1 | 2.90±0.00 | 12.47±0.47$^{b}$ | 9.57±0.57$^{b}$ | 331.07±43.94 | 2.61±0.13 |

(1) Mortality rate was 0.0% for all fish injected and for the control.
Means having different superscripts within column in a main effect are significantly different (P≤0.05).P1 and P2, 0.3 and 0.5% of probiotic, respectively; A1 and A2, 0.5 and 1.0 % of amino yeast, respectively.
Initial and final body weight (IBW and FBW) = body weight at start and end of experiment.
Weight gain (WG) = final weight - initial weight.
Percent body weight increases (% BWI) = (final weight - initial weight) 100/ initial weight.
Specific growth rate (SGR%/day) = (Ln final weight - Ln initial weight) 100 / number of days.

highest value of SGR %/ day was recorded by red tilapia fed the lowest level of probiotic, but did not differ (P≤0.05) significantly from those of fish fed the highest level of probiotic and the lowest level of amino yeast.

Moreover, the highest record of FBW was obtained by red tilapia injected with shark DNA and fed the highest level of probiotic, but did not differ (P≤0.05) significantly from those of fish injected with DNA and fed the lowest level probiotic, the lowest and the highest levels of amino yeast and non-injected fish fed the lowest level of probiotic. Weight gain of red tilapia injected with shark DNA and fed with the highest level of probiotic showed a higher mean, but this mean did not differ (P≤0.05) significantly from that of all injected fish except for the control group and non-injected red tilapia fed the lowest level of probiotic.

At the end of the experiment, crude protein and crude fat of red tilapia injected with shark DNA were significantly (P≤0.05) higher than those of the non-injected group while, the moisture content decreased (P≤0.05) significantly by red tilapia injected with shark DNA. The highest records of moisture content, crude protein and crude fat were achieved by red tilapia fed the lowest and highest levels of probiotic and the lowest level of amino yeast, respectively. These records were significantly (P≤0.05) higher than those of the other groups. The highest body moisture was achieved by non-injected fish fed the lowest level of probiotic, and this moisture differed (P≤0.05) significantly from that of DNA injected fish fed the lowest level of amino yeast and their control group, and that of non-injected fish fed the highest level of amino yeast and their control group. Moreover, the highest body protein contents were obtained by red tilapia injected with shark DNA and fed the highest level of probiotic and differ (P≤0.05) significantly from those of non-injected fish fed with mixed probiotic, and those of

**Table 3.** Effect of shark DNA injection and different additive levels on body composition of red tilapia (*Oreochromis* sp.).

| Treatment | Moisture | Crude protein | Crude fat |
|---|---|---|---|
| **At the start** | 73.87 | 13.56 | 5.28 |
| **At the end** | | | |
| Type of DNA (T) | | | |
| DNA | 72.69±0.64 $^{b}$ | 18.09± 0.72$^{a}$ | 6.30±0.40$^{a}$ |
| Non-DNA | 73.47±0.92 $^{a}$ | 17.24± 0.87$^{b}$ | 6.20±0.29$^{b}$ |
| **Type of Additive (A)** | | | |
| Control | 72.02±0.78$^{e}$ | 17.23±1.02$^{d}$ | 6.17±0.06$^{bc}$ |
| P1 | 74.02± 1.09$^{a}$ | 17.51±0.66$^{c}$ | 6.31±0.26$^{b}$ |
| P2 | 73.63±0.69$^{b}$ | 18.72±0.60$^{a}$ | 6.26±0.72$^{b}$ |
| A1 | 72.82±0.22$^{d}$ | 18.17±0.32$^{b}$ | 6.48±0.32$^{a}$ |
| A2 | 72.76±0.33$^{d}$ | 17.30±0.74$^{cd}$ | 6.20±0.19$^{bc}$ |
| P1+A1 | 73.21±0.27$^{c}$ | 17.09±0.97$^{d}$ | 6.10±0.30$^{c}$ |
| **Interactions T x A** | | | |
| DNA x C | 71.35±0.07$^{c}$ | 18.11±0.06$^{ab}$ | 6.17±0.08 |
| DNA x P1 | 73.08±0.11$^{abc}$ | 16.94±0.17$^{ab}$ | 6.09±0.03 |
| DNA x P2 | 73.03±0.04$^{abc}$ | 19.23±0.11$^{a}$ | 6.88±0.04 |
| DNA x A1 | 72.64±0.06$^{bc}$ | 18.43±0.11$^{ab}$ | 6.75±0.14 |
| DNA x A2 | 73.04±0.05$^{abc}$ | 17.93±0.20$^{ab}$ | 6.07±0.18 |
| DNA X P1+A1 | 72.98±0.03$^{abc}$ | 17.92±0.23$^{ab}$ | 5.84±0.08 |
| Non-DNA x C | 72.70±0.09$^{bc}$ | 16.34±0.08$^{b}$ | 6.17±0.06 |
| Non-DNA x P1 | 74.95±0.21$^{a}$ | 18.07±0.13$^{ab}$ | 6.53±0.04 |
| Non-DNA x P2 | 74.23±0.06$^{ab}$ | 18.02±0.10$^{ab}$ | 5.64±0.10 |
| Non-DNA x A1 | 73.01±0.08$^{abc}$ | 17.91±0.16$^{ab}$ | 6.21±0.06 |
| Non-DNA x A2 | 72.48±0.04$^{bc}$ | 16.66±0.04$^{ab}$ | 6.32±0.08 |
| Non-DNA X P1+A1 | 73.44±0.06$^{abc}$ | 16.26±0.06$^{b}$ | 6.35±0.07 |

Means having different superscripts within column in a main effect are significantly different (P≤0.05).
P1 and P2, 0.3 and 0.5% of probiotic, respectively; A1 and A2, 0.5 and 1.0 % of amino yeast, respectively.

amino yeast and their control (Table 3).

Data presented in Table 4 show also that, feed intake, feed conversion ratio (FCR) and protein efficiency ratio (PER) had surpassed the red tilapia injected with shark DNA significantly (P≤0.05). The highest feed intake was achieved by red tilapia fed the highest level of amino yeast, but did not differ (P≤0.05) significantly from those of fish fed the lowest and the highest levels of probiotic and their control. The highest FCR was recorded by the control group, but did not differ (P≤0.05) significantly from that of fish fed the highest level of amino yeast. Red tilapia fed the lowest level of amino yeast show significant (P≤0.05) superiority of PER and PR%, but did not differ (P≤0.05) significantly from those of fish fed the lowest and the highest levels of probiotic. Moreover, the highest record of ER % was obtained by the red tilapia fed the lowest level of amino yeast, but did not differ (P≤0.05) significantly from that of fish fed the highest level of probiotic.

## DISCUSSION

Red tilapia injected with shark DNA had significant (P≤0.05) superiority of growth performance, body composition and feed utilization compared with non-injected group. The improvement of growth performance, body composition and feed utilization in the present work may be explained by Hemieda et al. (2004); they reported the genetic investigation of Nile tilapia injected directly with shark DNA (into skeletal muscles). The concentrations of such DNA up to 40 µg/0.1 ml/fish probably activated gradually cell proliferation in modified muscle tissues. Also, the measurements of DNA content in the muscles of modified fish indicated that shark DNA may be acting as a mutagen and it had no carcinogenic effect. This is mostly responsible for the enhancement of the productive performance shown in the modified fish injected with foreign DNA. Moreover, Martinez et al. (2000) and Lu et al. (2002) found that anabolic stimulation and average

**Table 4.** Effect of shark DNA injection and different additive levels on feed utilization of red tilapia (*Oreochromis* sp.).

| Treatment | Feed intake (g) | FCR | PER | PR% | ER% |
|---|---|---|---|---|---|
| **Type of DNA (T)** | | | | | |
| DNA | 19.18±2.47$^{a}$ | 1.62±0.16$^{b}$ | 2.60±0.25$^{a}$ | 49.28±6.21 | 27.89±3.45 |
| Non-DNA | 17.63±1.76$^{b}$ | 1.78±0.21$^{a}$ | 2.37±0.27$^{b}$ | 48.04±5.87 | 26.99±3.92 |
| **Type of Additive (A)** | | | | | |
| Control | 18.62±1.67$^{ab}$ | 1.97±0.21$^{a}$ | 2.14±0.25$^{c}$ | 41.21±4.57$^{c}$ | 23.37±1.74$^{d}$ |
| P1 | 19.48±0.95$^{a}$ | 1.61±0.06$^{b}$ | 2.59±0.11$^{ab}$ | 49.49±3.72$^{ab}$ | 28.06±2.11$^{bc}$ |
| P2 | 19.27±3.77$^{a}$ | 1.60±0.08$^{b}$ | 2.61± 0.15$^{ab}$ | 53.91±1.19$^{a}$ | 29.46±1.23$^{ab}$ |
| A1 | 16.34±0.74$^{c}$ | 1.53±0.12$^{b}$ | 2.75±0.24$^{a}$ | 55.45±4.21$^{a}$ | 32.43±3.29$^{a}$ |
| A2 | 19.53±1.68$^{a}$ | 1.78±0.13$^{ab}$ | 2.35±0.21$^{bc}$ | 44.88±3.55$^{bc}$ | 25.11±2.00$^{cd}$ |
| P1+A1 | 17.20±1.01$^{bc}$ | 1.69±0.19$^{b}$ | 2.50±0.32$^{ab}$ | 47.04±2.98$^{bc}$ | 26.21± 2.76$^{bcd}$ |
| **Interactions T x A** | | | | | |
| DNA x C | 17.95 ± 0.29 | 1.88 ± 0.07 | 2.22±0.11 | 40.37±2.56 | 22.73±1.23 |
| DNA x P1 | 19.83 ± 0.86 | 1.56 ± 0.02 | 2.67±0.05 | 52.61±0.54 | 29.87±0.40 |
| DNA x P2 | 22.91 ± 1.38 | 1.54 ± 0.02 | 2.71±0.05 | 53.45±0.62 | 28.59±0.47 |
| DNA x A1 | 16.94 ± 0.61 | 1.47 ± 0.07 | 2.84±0.18 | 56.17±3.83 | 32.43±0.21 |
| DNA x A2 | 20.88 ± 1.31 | 1.73 ± 0.15 | 2.43±0.30 | 44.09±5.32 | 25.34±3.10 |
| DNA X P1+A1 | 16.59 ± 0.98 | 1.52 ± 0.07 | 2.76±0.16 | 49.00±3.15 | 28.40±1.73 |
| Non-DNA x C | 19.29 ± 2.08 | 2.01 ± 0.27 | 2.06±0.37 | 42.04±7.30 | 24.02±2.43 |
| Non-DNA x P1 | 19.13 ± 0.91 | 1.67 ± 0.00 | 2.51±0.08 | 46.38±1.53 | 26.26±0.51 |
| Non-DNA x P2 | 15.61 ± 0.03 | 1.67 ± 0.07 | 2.51±0.15 | 54.37±1.74 | 30.34±1.12 |
| Non-DNA x A1 | 15.75 ± 0.14 | 1.59 ± 0.14 | 2.65±0.33 | 54.73±6.05 | 32.43±5.69 |
| Non-DNA x A2 | 18.68 ± 0.51 | 1.83 ± 0.08 | 2.28±0.14 | 45.68±2.67 | 24.89±1.46 |
| Non-DNA X P1+A1 | 17.81 ± 0.56 | 1.87 ± 0.06 | 2.34±0.09 | 45.07±1.15 | 24.02±0.82 |

Means having different superscripts within column in a main effect are significantly different (P≤0.05).
P1 and P2, 0.3 and 0.5% of probiotic, respectively; A1 and A2, 0.5 and 1.0 % of amino yeast, respectively.
Feed conversion ratio (FCR) = dry feed intake / gain.
Protein efficiency ratio (PER) = gain / protein intake.
Protein retention (PR%) = protein increment / protein intake (100).
Energy retention (ER %) = energy increment / energy intake (100).

protein synthesis were higher in transgenic than that of non-transgenic fish. The results of the present study are consistent with these findings.

The results obtained by El-Zaeem (2004), El-Zaeem and Assem (2004), Hemeida et al. (2004) and Assem and El-Zaeem (2005) showed that the dose of 40 µg/0.1 ml/fish of shark DNA was more effective in stimulating most growth performance, body composition and immunity traits of *O. niloticus* and *T. zillii*. These traits were significantly higher (P≤0.05) than those of the other injected doses of DNA and their control. El-Zaeem (2012) produced grey mullet, *Mugil cephalus* with accelerated growth through direct injection of foreign DNA isolated from the liver of shark (*Squalus acanthias* L.) or African catfish (*Clarias gariepinus*) into muscles of fingerlings fish at the dose of 40 µg/fish. The results showed a significant (P≤0.05) improvement in most of the growth performance and body composition parameters of grey mullet fingerlings injected with shark DNA compared to both grey mullet injected with catfish DNA and the control fish. While the results of FCR and PER indicated that fish injected with shark DNA or catfish DNA had significant (P≤0.05) superiority compared to their control. El-Zaeem et al. (2012) stated that dietary protein can be spared down to 22% protein by direct injection of shark DNA into skeletal muscles of red tilapia. Thus, feed costs can be reduced by a further reduction in dietary protein. The results of the present work are consistent of these findings.

In addition, the different levels of probiotic and amino yeast were more effective in stimulating most of the productive performance traits compared to the control group and the mixed of probiotic and amino yeast. These results are consistent with the findings reported by El-Tawil et al. (2012) in *Mugil cephalus;* Amer and El-Tawil (2011) in red tilapia; Li and Gatlin (2004) in hybrid striped bass, *Morone chrysops* × *M. saxatilis*; Essa et al. (2010) in *O. niloticus* and Abdel-Tawwab et al. (2010) in *Sarotherodon galilaeus*. These stimulation may be due to improvement in intestinal microbial flora balance which in

turn will lead to better nutrient digestibility, higher adsorption quality and increased enzyme activities (Waché et al., 2006; Suzer et al., 2008; Sáenz de Rodriganez et al., 2009).

The result of the present study indicates a possible easy and rapid way for improving red tilapia (*Oreochromis* sp.) characteristics.

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