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Full Length Research Paper

Effect of direct injection of shark DNA into skeletal muscles on the productive performance characteristics of red tilapia (*Oreochromis* sp.) fed at different dietary regimes

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The present work aims to study the effect of direct injection of shark (*Squalus acanthias* L.) DNA into skeletal muscles of red tilapia (*Oreochromis* sp.) fingerlings fed at different dietary regimes (three protein levels; 18, 22 and 26% each with two metabolizable energy levels; 244 and 260 kcal/100 g diet) on the productive performance. The results showed that growth performance, body composition and feed utilization of red tilapia injected with shark DNA had significant superiority (P≤0.05) compared with non-injected fish. Red tilapia fed 22% protein diet had significant improving (P≤0.05) most of the productive performance traits. Moreover, final body weight, weight gain, percent body weight increases, feed intake and protein retention percentage of red tilapia fed on diet containing 244 kcal/100 g, were significantly higher (P≤0.05) than those of fish fed on diet containing 260 kcal/100 g. These data suggests that dietary protein can be spared down to 22% protein by direct injection of shark DNA into skeletal muscles of fish. Thus, feed costs can be reduced by a further reduction in dietary protein. Therefore, the result of the present work indicates a possible easy and rapid way for improving fish characteristics.

Key words: Red tilapia, *Oreochromis* sp., deoxyribonucleic acid (DNA) transfer, productive performance, dietary regimes.

INTRODUCTION

Needs to increase fish production is considered one of the most important ways to raise animal protein production to provide humans with an essential source of animal protein. In recent years, aquaculture has been the fastest growing primary production industry worldwide, amounting to 39.4 million tons in 1998 (Tacon and Forster, 2000). Tilapia culture is and will continue to be important particularly for the lesser-developed countries in tropics (FAO, 2001). Red tilapias (a collective name for the large number of red, orange, gold and pink phenotypes) have become objects of interest for culturist and researchers throughout the world (Wohlfarth and Hulata, 1983). The cost of most prepared feeds depends on protein content in the diet and the expensive protein fraction should therefore be optimally utilized for growth rather than for maintenance of the fish (Lovell, 1989). Thus, reducing the amount of protein in tilapia feed is one of the most important interests of aquaculture investigators. Several studies have shown that providing adequate energy with dietary lipids can minimize the use of more costly protein as an energy source (Shiau and Huang, 1990; El-Tawil, 1998). However, excess energy

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may produce fatty fish, reduce feed consumption and inhibit proper utilization of other feed stuffs (Maynard et al., 1979; El-Dahhar and Lovell, 1995).

A major goal in introducing new materials into the fish genome is to establish new improved commercial strains for use in aquaculture (Martinez et al., 1999; Hinits and Moav, 1999; Maclean and Laight, 2000; El-Zaeem, 2001; Melamed et al., 2002; Sarmasik, 2003; Tsai, 2003). Genetically-modified fish offer new potential for increased production of cultured organisms. This technology allows the introduction of new traits, or improvement of old ones, in a way that is impossible to be achieved with conventional breeding methods (Alestrom, 1996). A foreign gene can be transferred into fish *in vivo* by introducing DNA either into embryos or directly into somatic tissues of adults (Sudha et al., 2001; Dunham et al., 2002; El-Zaeem, 2004a, b; El-Zaeem and Assem, 2004; Assem and El-Zaeem, 2005). A commonly used method to introduce foreign DNA into embryos includes microinjection, electroporation, sperm-mediated gene transfer, gonad-mediated gene transfer (Gong and Hew, 1995; Iyengar et al., 1996; Maclean, 1998; El-Zaeem, 2001, 2011; Lu et al., 2002; El-Zaeem et al., 2011). Gene transfer and expression following intramuscular direct injection of foreign DNA into skeletal muscles of fish has been achieved by several studies and indicates a possible easy and rapid way for improving fish characteristics (Hansen et al., 1991; Rahman and Maclean, 1992; Anderson et al., 1996; Tan and Chan, 1997; Xu et al., 1999; El-Zaeem, 2004a, 2012; El-Zaeem and Assem, 2004; Hemeida et al., 2004; Assem and El-Zaeem, 2005; El-Zaeem et al., 2012).

Furthermore, the expression of muscular injection of DNA is evident in several non-muscle tissues of fish, such as skin epithelia, pigment cells, blood vessel cells and neuron-like cells (Sudha et al., 2001). Therefore, the objective of this work was to study the effect of direct injection of shark (*Squalus acanthias* L.) DNA into skeletal muscles of red tilapia (*Oreochromis* sp.) fingerlings fed on different dietary protein and energy levels on growth performance, body composition and feed utilization.

MATERIALS AND METHODS

Fish origin

The red tilapia, *Oreochromis* sp. fingerlings used in this study was a hybrid, descended of an original cross of female *Oreochromis mossambicus* × male *Oreochromis niloticus* and obtained from Marine Fish Hatchery of GAFRD, Alexandria, Egypt. Red tilapia fry at one month post-hatching 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.

Preparation of genomic DNA

High molecular weight DNA was extracted according to Brem et al. (1988) method. Isolation of DNA was accomplished by reducing liver sample from shark (*S. acanthias* L.) to small pieces, which were then transferred to a microfuge tube and incubated overnight until the sample was digested in a buffer containing 50 mM Tris, 100 mM EDTA (pH 8.0), 100 mM NaCl, 0.1% 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 0.1 × SSC buffer (saline sodium citrate).

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 three weeks. Then, fingerlings with an initial live weight (7.86 ± 0.19 g) were divided randomly to 12 groups and three replicates for each group. Each replicate was held separately in a half of glass aquaria (total area, 100 × 34 × 50 cm), which was divided by plastic sieved connected with glass frame. The glass aquaria were supplied with fresh water at a rate of 0.5 L/min with supplemental aeration and stocked at 1.0 fish/10 L. Fish were fed twice daily with different pelleted dietary regime, to satiation, six days a week. Fish were weighed biweekly for 60 days.

Injection of foreign DNA in vivo

The DNA concentration of 40 µg/0.1 ml/fish (El-Zaeem, 2004a; El-Zaeem and Assem, 2004; Hemeida et al., 2004; Assem and El-Zaeem, 2005) were prepared using 0.1× 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 regimes were used in this study containing both animal and plant proteins sources (Table 1). Soybean meal to fish meal in a fixed ratio (2:1) were added at graded levels to achieve the three different protein levels (18, 22 and 26% crude protein) each with two metabolizable energy levels 244 and 260 kcal/100 g diet (air–dry basis) based on feedstuff values reported by NRC (1993). The high energy level in the diets containing 260 kcal/100 g, was obtained by adding 3.0% corn oil instead of yellow corn. 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. Boiling water was then blended into the mixture at the ratio of 50% for pelleting. The diets were pelleted using meat grinder with a 1.5 mm diameter.

Quantitative traits studied

The following parameters were measured; body weight (g), weight gain (g), 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

Table 1. Formulation and proximate analysis of the different tested diets used in the present study.

Ingredient	A (18%)	B (22%)	C (26%)	D (18%)	E (22%)	F (26%)
Wheat flour	39.0	33.0	27.0	39.0	33.0	27.0
Wheat bran	24.0	21.0	17.5	24.0	21.0	17.5
Soybean meal	11.0	19.0	26.0	11.0	19.0	26.0
Yellow corn	18.2	15.7	14.2	15.2	12.7	11.2
Corn oil	-	-	-	3.0	3.0	3.0
Fish meal	5.5	9.0	13.0	5.5	9.0	13.0
Bone meal	2.0	2.0	2.0	2.0	2.0	2.0
Vit and min mix*	0.3	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100	100
Proximate analysis %						
Protein	17.89	21.95	25.93	17.65	21.76	25.81
Moisture	9.53	10.15	10.22	10.15	10.09	9.41
Fat	14.83	15.75	15.88	14.95	15.92	15.99
Fiber	7.95	7.93	8.21	7.88	7.84	8.12
Nitrogen free extract (NFE)	38.77	34.11	29.59	38.37	34.32	30.55
Ash	11.03	10.11	10.17	11.00	10.07	10.12
Metabolizable energy (ME)	244.07	242.96	243.78	258.62	257.51	258.33

*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 B_1, 0.4 g; Vitamin B_2, 1.6 g; Vitamin B_6, 0.6 g; Vitamin B_7, 20.0 mg; Vitamin B_{12}, 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 and Selenium, 0.04 g.

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):

$$Y_{ijkl} = \mu + T_i + P_j + E_k + (TP)_{ij} + (TE)_{ik} + (PE)_{jk} + (TPE)_{ijk} + e_{ijkl}$$

Where: Y_{ijkl}: Observation of the ijklth parameter measured; μ: overall mean; T_i: effect of jth DNA; P_j: effect of ith protein; E_k: effect of Kth energy; $(TP)_{ij}$: interaction DNA by protein; $(TE)_{ik}$: interaction DNA by energy; $(PE)_{jk}$: interaction protein by energy; $(TPE)_{ijk}$: interaction among DNA, protein and energy; e_{ijkl}: random error. Significant differences (P≤0.05) among means were tested by the method of Duncan (1955).

RESULTS

Data of Table 2 show that the final body weight (FBW), weight gain (WG), specific growth rate (SGR%/day) and percent body weight increases (% BWI) of red tilapia (*Oreochromis* sp.) injected with shark DNA were significantly (P≤0.05) higher than those of non-injected fish. Moreover, the highest records of FBW, WG and % BWI were achieved by the red tilapia fed 244 kcal/100 g diet. These records were significantly (P≤0.05) higher than those of the other group fed 260 kcal/100 g diet. Furthermore, the highest FBW, WG and SGR %/day were obtained from red tilapia fed 22% protein diet, but did not differ significantly (P≤0.05) from those of fish fed 26% protein diet. While, red tilapia fed 22% protein diet had significant higher (P≤0.05) % BWI, compared with the fish fed 18 and 26% protein diets. In addition, mortality rates were 0.0% for all injected red tilapia and their control. The results of body composition of red tilapia according to treatments of the experiment are presented in Table 3. By the end of experiment, crude protein and crude fat of red tilapia injected with shark DNA were significantly (P≤0.05) higher than those of non-injected group. While moisture content showed no significant differences (P≤0.05) between red tilapia injected with shark DNA and non-injected group. Moreover, the highest protein content was recorded by the fish fed 26% protein diet, but did not differ significantly (P≤0.05) from that of fish fed 22% protein diet.

The highest crude fat was achieved by red tilapia fed 22% protein diet and significantly differed (P≤0.05) from those of the fish fed 18 and 26% protein diets. In addition, red tilapia fed 260 kcal/100 g, diet had significant lower (P≤0.05) moisture content compared with the fish fed 244 kcal/100 g diet. While, crude protein and fat of fish fed 260 kcal/100 g diet were significantly higher (P≤0.05) compared with the fish fed 244 kcal/100 g diet (Table 3). Data of Table 3 show also that, feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention percent (PR%) and energy retention percent (ER%) had surpassed the red tilapia injected with shark DNA significantly (P≤0.05). In addition, the highest

Table 2. Effect of foreign DNA injection and different dietary regimes on growth performance[1] of red tilapia (*Oreochromis* sp.).

Treatment	IBW (g)	FBW (g)	WG (g)	SGR %/day	% BWI
Type of DNA (T)					
DNA	7.89	19.27^{a}	11.44^{a}	1.74^{a}	146.67^{a}
Non-DNA	7.82	16.37^{b}	8.55^{b}	1.45^{b}	107.48^{b}
Protein (P)					
18%	7.81	15.70^{b}	7.86^{b}	1.38^{b}	101.79^{c}
22%	7.82	19.18^{a}	11.35^{a}	1.71^{a}	145.29^{a}
26%	7.95	18.58^{a}	10.78^{a}	1.68^{a}	134.15^{b}
Energy (E)					
244 kcal/ 100 g	7.89	18.27^{a}	10.38^{a}	1.60	131.78^{a}
260 kcal/ 100g	7.83	17.37^{b}	9.61^{b}	1.58	122.38^{b}
Interactions					
T×P	NS	NS	NS	*	NS
T×E	NS	NS	NS	NS	NS
P×E	NS	***	***	***	***
T×P×E	NS	***	***	***	***

(1) Mortality rates were 0.0% for all injected fish and their control. Means having different superscripts within column in a main effect are significantly different (P≤0.05). * P≤0.05, *** P≤0.001, NS: not significant. Initial and final body weight (IBW and FBW) = body weight at the start and end of experiment. Weight gain (WG) = final weight - initial weight. Specific growth rate (SGR%/day) = (Ln final weight - Ln initial weight) 100/number of days. Percent body weight increases (% BWI) = (final weight - initial weight) 100/initial weight.

Table 3. Effect of foreign DNA injection and different dietary regimes on body composition and feed utilization of red tilapia (*Oreochromis* sp.).

Treatment	Moisture	Crude protein	Crude fat	Feed intake (g)	FCR	PER	PR%	ER%
		At the start						
	73.27	10.68	7.68					
		At the end						
Type of DNA (T)								
DNA	73.02	14.62^{a}	8.38^{a}	19.77^{a}	1.83^{b}	2.64^{a}	45.97^{a}	25.54^{a}
Non-DNA	73.57	11.69^{b}	7.52^{b}	18.13^{b}	2.24^{a}	2.12^{b}	26.65^{b}	15.98^{b}
Protein (P)								
18%	73.36	12.72^{b}	7.14^{c}	17.78^{c}	2.46^{a}	2.42^{a}	36.54^{b}	17.31^{c}
22%	73.06	13.30^{a}	8.66^{a}	19.89^{a}	1.79^{b}	2.58^{a}	39.52^{a}	23.56^{a}
26%	73.47	13.44^{a}	8.05^{b}	19.18^{b}	1.85^{b}	2.13^{b}	32.87^{c}	21.41^{b}
Energy (E)								
244 kcal/100 g	73.60^{a}	12.44^{b}	7.09^{b}	19.28^{a}	2.00	2.40	38.87^{a}	18.39^{b}
260 kcal/100 g	72.99^{b}	13.87^{a}	8.81^{a}	18.62^{b}	2.06	2.35	33.74^{b}	23.13^{a}
Interactions								
T×P	*	***	***	NS	***	**	***	***
T×E	NS	***	***	*	NS	NS	**	***
P×E	NS	*	***	***	*	**	***	*
T×P×E	NS	***	**	**	***	***	*	***

Means having different superscripts within column in a main effect are significantly different (P≤0.05). * P≤0.05, ** P≤0.01, *** P≤0.001, NS: not significant, 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.

feed intake, PR and ER% were recorded by the red tilapia fed 22% protein diet, showing significant improvement (P≤0.05) compared with the other fish fed 18 and 26% protein diets. Also, the best FCR was significantly increased (P≤0.05) by the red tilapia fed 22% protein diet, showing higher mean, compared with the other fish fed 18% protein diet, but did not differ significantly (P≤0.05) from that of fish fed 26% protein diet. The results of PER revealed that red tilapia fed 22% protein diet had higher mean compared with fish fed 26% protein diet, but did not differ significantly (P≤0.05) from that of fish fed 18% protein diet. Moreover, the highest feed intake and PR% were achieved by the fish fed 244 kcal/100 g diet, which were significantly (P≤0.05) higher than those of the fish fed 260 kcal/100 g diet. While, red tilapia fed 260 kcal/100 g diet had significant higher (P≤0.05) ER% compared with the other fish fed on lower energy.

Insignificant differences (P≤0.05) were detected between two levels of energy with respect to FCR and PER.

DISCUSSION

The results obtained by 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 of growth performance, body composition and immunity traits of *O. niloticus* and *Tilapia zillii*. These traits were significantly higher (P≤0.05) than those of the other injected doses of DNA and their control. Also, El-Zaeem (2004a) reported that the optimal dose of foreign DNA isolated from the liver of common carp, *Cyprinus carpio* and African catfish, *Clarias gariepinus* and injected into *O. niloticus* and *T. zillii*, was 40 µg/0.1 ml/fish. These injected fish had significant (P≤0.05) improvement of growth performance, body composition and feed utilization compared with the other injected fish and their control. Similar results were recorded in the present study where the red tilapia injected with 40 µg/0.1 ml/fish of shark DNA had significantly (P≤0.05) improved of growth performance, body composition and feed utilization compared with non-injected fish. Moreover, the results of the present work are consistent with the findings of Brem (1989), Mandour (1996), El-Fiky and Mehana (1998), Martinez et al. (2000), El-Zaeem (2001, 2004a, b, 2011, 2012), El-Maremie (2007), Abd El-Hamied (2009), Elwan (2009) and El-Zaeem et al. (2011, 2012). They reported that, the transfer of foreign DNA has been shown to improve growth performance, body composition, feed utilization and other quantitative traits.

Chatakondi et al. (1995) and Dunham et al. (2002) reported that, the moisture and lipid contents were lower while the protein content was higher in the first and second generations of transgenic common carp muscle compared with their control genotype. Similar observations were reported in the present work concerning the moisture and protein contents but the lipid content was higher in the red tilapia injected with shark DNA compared with non-injected fish. The differences between these results may be due to a higher content of lipid found in the viscera of red tilapia injected with shark DNA, since the components of whole body-proximate composition were performed in this study, while the other study was concerned with the components of muscle-proximate composition. Also, Martinez et al. (2000) and Lu et al. (2002) found that anabolic stimulation and average protein synthesis were higher in transgenic than that of non-transgenic fish. The results of the present study are consistent with these findings. The improvement of most traits of growth performance, body composition and feed utilization in the present work may be explained according to Hemieda et al. (2004); they reported that, genetically investigation of Nile tilapia injected directly with shark DNA into skeletal muscles was carried out. 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.

Watanabe et al. (1990) reported that the production efficiency of Florida red tilapia with an initial body weight (8.78 g), held in sea cages is higher on 28% than on a 32% protein diet. Furthermore, Abdel-Tawwab et al. (2010) found that the optimum growth of tilapia fry was obtained at 45% protein, while fingerlings and advanced juvenile showed optimum growth performance with 35% protein diet. Higher improvement was obtained in the present study and the result suggests that dietary protein can be spared down to 22% protein by direct injection of shark DNA into skeletal muscles of fish. Thus, feed costs can be lowered by a further reduction in dietary protein. Therefore, the result of the present work indicates a possible easy and rapid way for improving fish characteristics.

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