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GROWTH PERFORMANCE AND ELECTROPHORETIC PATTERN OF F_1 AND F_2 INJECTED OREOCHROMIS NILOTICUS WITH CHICKEN DNA

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ABSTRACT

In the present investigation we tried transfer chicken DNA to *Oreochromis niloticus* and evaluate its effect on the growth performance and the electrophoretic pattern of sarcoplasmic protein. To achieve this purpose, mature males and females received two concentrations of fractionated chicken DNA (10µ and 20µ) injected in intraperitoneal cavity on two times through one week.

The first generation of injected parents (F_1IP) were fed with practical diets containing different protein levels (20%, 25%, 30%, 35% and 40%) for 178 days to evaluate the feeding level which produce the best growth rate. The growth performance and electrophoretic pattern of the F_1IP and F_2IP and chicken were studied.

It's clear that:

1- the growth performance increased in both F_1IP and F_2IP compared with control

2-the electrophoretic patterns of sarcoplasmic proteins of the studied groups showed presence from (15 to 17) fractions in control fish; from (15 to 19) fractions in both F_1IP at different protein levels in diet; (17) fractions in both F_2IP and (20) fractions in chicken.

Fractions (7, 17 and 29) appeared in chicken and in two generations of injected parents and this refer to gene transfer occurred.

INTRODUCTION

Transgenesis is a method which is in current use in fish production purposes. A number of fish species are under focus for gene transfer experiments and can be divided into two main groups: animals used in aquaculture (Hew *et al.,* 1995; Chen and Lu, 1998) and model fish used in basic research (Chen and Lu, 1998). Among the major food fish species are carp, *Cyprinus spp., Tilapia, Oreochromis spp.,* Salmon, *Salmo spp., Oncorhynchus spp.* and channel catfish, *Ictalurus punctatus.* On the other hand zebra fish, *Danio rerio, medaka, Oryzias latipes* and gold fish, *Carassius aurata* are used in basic research.

Many investigators have been engaged in the development of this technology (Lathe *et al.,* 1987; Powers *et al.,* 1998; Sheela *et al.,* 1999; Ali, 2000 and others). It has been used to improve some biological characters in cultured fish, such as growth promotion, salinity tolerance and diseases resistance. In Egyptian farms it was found to be desirable to introduce such techniques which seem to be promising and would attract the attention of various fish culturists. According to the available literature, this technique is rarely applied in Egypt. In the present investigation we tried to use transgenesis in order to

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improve growth rate of the fish and use the electrophoretic analysis to monitor it on the electrophoretic patterns of protein in F1 and F2 of the transgenic *O. niloticus.*

MATERIAL AND METHODS

Mature samples of Nile *tilapia* (family: Cichlidae) used in the present study were obtained from a fish farm near Alexandria (Barzik fish farm). Fish were kept under observation in large glass aquaria for 10 days to be acclimatized to laboratory conditions before being used in the experiments.

High molecular weight DNA was extracted and purefied from proteins from the pituitary glands of Broilers, at market ages, according to the method of Sambrook et al. (1989) with some modifications according to Abdel-Fattah (1995).

The DNA concentration was estimated from the optical Density (O.D) reading of UV-spectrophotometer at wave-length of 260nm. [1.0 O.D.= 50µg DNA/ml of the solution, according to Charles (1970)].

The brood stock (at spawning condition) were put in three aquaria each contain three females and two males. The fish in the first aquarium were injected with 10µ,fractionated chicken DNA, the fish in the second aquarium were injected with 20µ fractionated chicken DNA and the fish in the third aquarium were injected with saline citrate (0.1 x) as a control. Injection were in the intraperitoneal cavity in the gonads.

The fry of each treatment from the first experiment were divided into 5 aquaria (1, 2, 3, 4, 5) and were fed protein diet with different protein concentrations (20, 25, 30, 35, 40% cp) respectively, each having the same gross energy level (350 kcal/100gm diet), for 178 days.

Selected large males and females from the first generation were chosen from a F_1IP and F_2IP quaria which were fed on 30% cp (the diet that gave the best growth according to last experiment) to produce the second generation. The fry of the second generation were fed for 178 days with 30% protein diet. The growth performance were estimated for the F_1IP and F_2IP and control.

Bulked samples of skeletal muscle represent some individuals of each experiment in the F_1 and F_2 were dissected and extracted in appropriate volume of distilled water and used for SDS electrophoresis analysis.

RESULTS

Table 1 and 2 indicate that the best concentration of the utilized protein in the fish feed was 30% crude protein since it gave the maximum weight, maximum weight gain and maximum daily gain. Also, 30% crude protein in the diet leads to the best FCR in both F_1IP and non-injected (control) fish, also it was noticed that the SGR value at 30% protein level was greater than the other crude protein concentrations in both F_1IP and control fish.

Table (3) shows the performance of second generation of injected and non-injected parents of *Oreochromis niloticus* fed on 30% crude protein for 178 days. It is clear that, final weight increased significantly (P<0.001) at both F_2IP with 10µ and 20µ as compared with the non- injected one. Therefor the weight gain at F_2IP of *Oreochromis niloticus* injected with 10µ and 20µ were 1.7 and 1.5 times greater than the non-injected fish. The average daily gain of fish increased significantly (P<0.002) in fish of F_2IP with 10µ and 20µ than that in non-injected one.

Muscular sarcoplasmic protein patterns of first generation of *Oreochromis niloticus* at different protein levels in diet were compared with the patterns of *Oreochromis niloticus* after transferred the two concentrations (10µ and 20µ) DNA of broiler chicken; also were compared with the patterns of second generation at 30% crude protein in the diet that gave the best growth performance and the pattern of sacroplasmic protein of chicken.

The intention of the present work is to examine the general genetic polymorphism and structure of variability among F_1IP of *Oreochromis niloticus* and control at different levels of protein in diets but not to test specific hypothses about forces influencing maintaining variability in *Oreochromis niloticus*. Bulked samples of sarcoplasmic protein were chosen for this study.

Tables 4 and 5 show the relative mobility of sarcoplasmic proteins for the first and second generation of *Oreochromis niloticus* of injected parents and non-injected (control) parents and chicken. It is clear that the electrophoretic patterns of the studied groups showed the presence (15 to 17) fractions in control fish, (15 to 19) fractions in both F_1 of injected parents at different protein levels in diet and 20 fractions in chicken, while 17 fractions in F_2 of both injected parents with 10µ and 20µ.

Each fraction was studied at two levels, the first was the relative mobility which shows the relative genetic distance in which the fraction migrates from the point of application to its position on the electrophertogram. The second was the relative concentration of each fraction which indicates the concentration of protein in the fraction.

From tables (4 and 5) it is clear that fractions (1, 13, 19 and 30) are conserved fractions which appeared in all fish and chicken.

Fractions (4, 6, 9, 10, 12, 16 and 26) appeared in F_1 of the injected and the non-injected parents of *Oreochromis niloticus*. This indicates that these fractions are conserved and specific for *O. niloticus*. Fractions (3, 5, 8, 21, 23 and 31) appeared in chicken only which indicate that they are constant and specific for chicken. Fractions (2, 11, 14, 15, 18, 20, 24, 25, 27 and 28) showed different degree of appearance in some injected and non-injected fish. Such fractions are polymorphic fractions.

Fractions (7, 17 and 29) are specific for chicken. However, they appeared in F_1 of the injected parents and not in the control. This may be due to gene transfer. However it must be verified.

Fraction (7) appeared in fish of injected parents with 10µ chicken DNA at 20%, 30% and 40% crude protein in diet. However, fraction (17) appeared in fish injected with 10µ, at 20%, 25% and 35% crude protein in diet. It also appeared in fish of injected parents with 20µ at 20% protein in diet.

Fraction (29) which appeared in FI of parents injected with 10µ chicken DNA at 40% crude protein in diet. Table (8) shows the comparison of relative concentration percentage of sarcoplasmic protein for the second generation of injected parents of *Oreochromis niloticus* with 10µ and 20µ chicken DNA, and fed with 30% crude protein in diet and the non-injected one.

It is clear from Table (6, 7) that at 20%, 25%, 30%, 35% and 40% crude protein in the diet there was an increase in the relative area of some fractions and decrease in others in both injected parents compared with the control.

Also total protein content nearly increased in injected parents compared with the control at all concentrations of crude protein in diet.

Table (1): Performance of F_1 (first generation) of injected with 10µDNA and non-injected parents of *Oreochromis niloticus* fed different dietary protein levels for 178 days.

Performance indices	Injected					non-injected				
	CP%					CP%				
	20%	25%	30%	35%	40%	20%	25%	30%	35%	40%
IW (g/fish)	1.23 ± 0.03	1.20 ± 0.081	1.20 ± 0.052	1.22 ± 0.71	1.24 ± 0.103	1.14 ± 0.105	1.15 ± 0.08	1.17 ± 0.10	1.16 ± 0.09	1.16 ± 0.081
FW (g/fish)	65.50 ± 4.12^{*a}	57.01 ± 3.85^{*b}	77.11 ± 4.11^{*c}	75.91 ± 6.23^{*c}	66.26 ± 4.82^{*a}	46.06 ± 3.25^{a}	39.60 ± 4.52^{b}	44.12 ± 7.12^{a}	38.21 ± 3.12^{b}	49.67 ± 4.03^{a}
WG (g/fish)	64.27 ± 6.23^{*a}	55.81 ± 4.12^{*b}	75.91 ± 8.25^{*c}	74.69 ± 4.62^{*c}	65.26 ± 4.62^{*a}	44.92 ± 2.87^{a}	38.45 ± 3.12^{b}	42.95 ± 4.52^{a}	37.05 ± 2.13^{b}	48.51 ± 4.12^{a}
ADG (g/fish/d)	0.361 ± 0.036*	0.314 ± 0.041*	0.426 ± 0.081*	0.420 ± 0.103*	0.367 ± 0.084*	0.252 ± 0.0132	0.216 ± 0.012	0.241 ± 0.102	0.208 ± 0.085	0.273 ± 0.062
SGR% $(d)^{-1}$	0.970 ± 0.103	0.942 ± 0.095	1.016 ± 0.045*	1.008 ± 0.035*	0.972 ± 0.0012	0.902 ± 0.041	0.863 ± 0.103	0.886 ± 0.058	0.853 ± 0.037	0.917 ± 0.1003
FI (g)	74.84 ± 1.03^{*a}	74.42 ± 4.03^{*a}	79.65 ± 3.62^{*b}	87.71 ± 4.103^{*c}	78.08 ± 5.12^{*b}	71.50 ± 4.62^{a}	58.43 ± 5.22^{b}	56.64 ± 6.32^{b}	57.72 ± 4.17^{b}	73.68 ± 8.25^{a}
FCR	1.164 ± 0.062*	1.333 ± 0.102*	1.049 ± 0.095*	1.174 ± 0.062*	1.196 ± 0.074*	1.592 ± 0.078	1.520 ± 0.068	1.319 ± 0.1032	1.558 ± 0.036	1.519 ± 0.014
Survival rate (%)	90	85	95	95	90	75	88	90	84	90

I.w = Initial weight (g)
Fw= Final weight
WG = weight gain
ADG = Average daily gain (g/fish/d)
FI= Feed intake

* significant at $P<0.05$
FCR= Food conversion ratio
SGR (% days)$^{-1}$ = Specific growth rate
CP= precent of crude protein in diet

Means with the same letter in the same row and treatment are not significant at $P>0.05$.

Table (2): Performance of F_1 (first generation) of injected with 20μDNA and non-injected parents of *Oreochromis niloticus* fed different dietary protein levels for 178 days

Performance indices	injected					non-injected				
	CP%					CP%				
	20%	25%	30%	35%	40%	20%	25%	30%	35%	40%
IW (g/fish)	1.19 ± 0.085	1.18 ± 0.103	1.20 ± 0.074	1.17 ± 0.103	1.19 ± 0.036	1.14 ± 0.105	1.15 ± 0.08	1.17 ± 0.10	1.16 ± 0.09	1.16 ± 0.081
FW (g/fish)	49.81 ± 3.12^{a}	48.16 ± 4.52^{*a}	62.01 ± 6.52^{*b}	51.14 ± 4.8^{*c}	46.06 ± 9.56^{a}	46.06 ± 3.25^{a}	39.60 ± 4.52^{b}	44.12 ± 7.12^{a}	38.21 ± 3.12^{b}	49.67 ± 4.03^{a}
WG (g/fish)	48.62 ± 3.21^{*a}	46.98 ± 4.12^{*a}	60.81 ± 6.12^{*b}	49.97 ± 4.103^{*a}	44.87 ± 2.13^{*c}	44.92 ± 2.87^{a}	38.45 ± 3.12^{b}	42.95 ± 4.52^{a}	37.05 ± 2.13^{b}	48.51 ± 4.12^{a}
ADG (g/fish/d)	0.273 ± 0.062	0.264 ± 0.018	0.342 ± 0.031	0.281 ± 0.038	0.252 ± 0.041	0.252 ± 0.0132	0.216 ± 0.012	0.241 ± 0.102	0.208 ± 0.085	0.273 ± 0.062
SGR% $(d)^{-1}$	0.911 ± 0.03	0.905 ± 0.015	0.959 ± 0.085	0.922 ± 0.012	0.892 ± 0.082	0.902 ± 0.041	0.863 ± 0.103	0.886 ± 0.058	0.853 ± 0.037	0.917 ± 0.1003
FI (g)	90.34 ± 4.02^{*a}	96.61 ± 5.21^{*a}	90.51 ± 3.98^{*a}	77.83 ± 4.62^{*b}	75.79 ± 8.12^{b}	71.50 ± 4.62^{a}	58.43 ± 5.22^{b}	56.64 ± 6.32^{b}	57.72 ± 4.17^{b}	73.68 ± 8.25^{a}
FCR	1.858 ± 0.085^{*a}	2.056 ± 0.101^{*a}	1.488 ± 0.085^{b}	1.558 ± 0.069^{*b}	1.892 ± 0.081^{*a}	1.592 ± 0.078	1.520 ± 0.068	1.319 ± 0.1032	1.558 ± 0.036	1.519 ± 0.014
Survival rate (%)	84	85	95	90	92	75	88	90	84	90

I.w = Initial weight (g)
Fw= Final weight
WG = weight gain
ADG = Average daily gain (g/fish/d)
FI= Feed intake

* significant at $P<0.05$
FCR= Food conversion ratio
SGR (% days)$^{-1}$ = Specific growth rate
CP= precent of crude protein in diet

Means with the same letter in the same row and treatment are not significant at $P > 0.05$.

Table (3): Performance of F_2 (second generation) of injected and non-injected parents of *Oreochromis niloticus* fed on 30% protein level for 178 days

Performance indices	Injected		non-injected
	10 µ	20 µ	
Initial weight (g/fish)	0.122 ± 0.062	0.081 ± 0.001*	0.163 ± 0.0036
Final weight (g/fish)	84.00 ± 2.03*	73.85 ± 1.03*	50.63 ± 1.82
Weight gain (g/fish)	83.88 ± 1.62*	73.77 ± 1.52*	50.467 ± 1.84
Average daily gain (g/fish/d)	0.471 ± 0.021*	0.414 ± 0.035*	0.284 ± 0.041
SGR (%days)$^{-1}$	1.594 ± 0.024	1.663 ± 0.0142	1.400 ± 0.008
Feed intake (g)	98.08 ± 1.42*	91.60 ± 1.52*	87.13 ± 2.03
FCR	1.169 ± 0.038*	1.242 ± 0.041*	1.726 ± 0.021
Survival rate (%)	96	95	89

* significant at $P<0.05$
FCR= Food conversion ratio
SGR (%/day)= Specific growth rate

Table (4): Relative mobility of sarcoplasmic proteins of F_1 (First generation) of *Oreochromis niloticus* for injected and non-injected parents

No. of fraction	CP% / R.M.	Injected										non-injected					Chicken
		10 µ					20 µ										
		20%	25%	30%	35%	40%	20%	25%	30%	35%	40%	20%	25%	30%	35%	40%	
1	0.99	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.94	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1
3	0.87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
4	0.84	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
5	0.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
6	0.80	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
7	0.79	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1
8	0.78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
9	0.75	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
10	0.73	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
11	0.71	1	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1
12	0.69	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
13	0.65	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	0.59	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
15	0.56	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0
16	0.54	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
17	0.49	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1
18	0.47	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
19	0.46	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	0.43	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1
21	0.39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
22	0.32	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
23	0.30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
24	0.29	1	1	1	1	0	1	0	1	1	0	1	0	1	1	0	1
25	0.28	1	1	0	1	0	1	0	0	1	0	1	0	0	1	0	1
26	0.26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
27	0.25	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0
28	0.19	1	1	1	1	0	1	0	1	1	0	1	1	1	1	0	1
29	0.17	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1
30	0.15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Total No. of band		19	18	17	19	18	18	16	16	17	15	17	15	16	17	15	20

R.M.= Relative mobility CP%= concentration of crude protein in diet

Table (5): Relative mobility of sarcoplasmic proteins of F_2 (second generation) of *Oreochromis niloticus* for injected and non-injected parents

No. of fractions	R.M	Injected		non-injected	Chicken
		10µ	20µ		
1	0.99	1	1	1	1
2	0.94	1	1	1	1
3	0.87	0	0	0	1
4	0.84	1	1	1	0
5	0.83	0	0	0	1
6	0.80	1	1	1	0
7	0.79	0	0	0	1
8	0.78	0	0	0	1
9	0.75	1	1	1	0
10	0.73	1	1	1	0
11	0.71	0	0	0	1
12	0.69	1	1	1	0
13	0.64	1	1	1	1
14	0.59	0	0	0	1
15	0.56	1	1	1	0
16	0.54	1	1	1	0
17	0.49	1	1	0	1
18	0.47	1	1	1	0
19	0.46	1	1	1	1
20	0.43	1	1	1	1
21	0.39	0	0	0	1
22	0.32	1	1	1	0
23	0.30	0	0	0	1
24	0.29	0	0	0	1
25	0.28	0	0	0	1
26	0.26	1	1	1	0
27	0.25	0	0	0	0
28	0.19	0	0	0	1
29	0.17	0	0	0	1
30	0.15	1	1	1	1
31	0.03	0	0	0	1
Total No. of fractions		17	17	16	20

R.M.: Relative mobility

Table (6): Relative concentration of sarcoplasmic proteins of F_1 (First generation) of *Oreochromis niloticus* for injected with 10μDNA and non-injected parents

No. of fraction	Injected					non-injected					Chicken
	CP%					CP%					
	20%	25%	30%	35%	40%	20%	25%	30%	35%	40%	
1	1.48	1.19	1.55	0.68	2.98	1.50	0.84	1.17	1.19	1.35	0.59
2	-	-	-	-	0.16	-	-	-	-	0.35	0.48
3	-	-	-	-	-	-	-	-	-	-	0.67
4	3.06	2.29	1.69	2.03	1.60	1.58	1.65	1.73	1.95	1.00	-
5	-	-	-	-	-	-	-	-	-	-	0.44
6	2.06	2.48	1.40	2.18	0.76	1.60	1.39	1.69	1.51	1.02	-
7	1.08	-	0.97	-	0.22	-	-	-	-	-	0.29
8	-	-	-	-	-	-	-	-	-	-	1.12
9	2.74	2.72	2.25	4.29	2.58	2.50	2.48	2.11	1.96	1.39	-
10	3.60	4.29	2.91	3.21	3.94	3.67	2.51	3.86	4.21	3.35	-
11	1.25	1.19	0.78	6.75	-	0.95	0.92	0.93	0.93	-	0.23
12	1.49	1.34	1.05	1.37	0.31	2.34	2.00	0.86	1.20	1.52	-
13	1.07	2.19	2.00	1.74	0.76	1.76	1.45	1.31	1.18	0.93	0.75
14	-	-	-	-	0.71	-	-	-	-	-	2.06
15	1.77	1.75	1.64	1.45	1.40	1.66	-	1.26	1.70	1.24	-
16	2.45	2.86	2.65	2.92	1.96	2.89	1.15	2.49	2.55	2.12	-
17	0.96	0.68	-	0.70	-	-	-	-	-	-	2.37
18	0.99	0.90	1.52	0.79	-	1.93	1.20	0.89	0.72	1.41	-
19	2.60	2.81	1.90	2.09	0.66	1.70	3.11	1.97	1.72	1.03	1.29
20	-	-	-	-	-	-	-	-	-	0.65	2.26
21	-	-	-	-	-	-	-	-	-	-	0.97
22	-	-	-	-	1.23	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	2.00
24	0.90	0.87	0.95	1.03	-	1.18	-	1.05	0.88	-	1.11
25	1.57	1.68	-	0.81	-	0.81	-	-	1.23	-	0.33
26	2.92	3.03	1.67	2.67	0.66	2.05	2.82	1.85	2.34	1.27	-
27	-	-	-	0.86	0.97	-	1.48	-	-	-	-
28	1.20	1.29	1.05	1.39	-	0.56	1.64	0.91	0.82	-	0.37
29	-	-	-	-	0.51	-	-	-	-	-	0.63
30	1.04	0.90	1.19	1.01	0.60	1.42	0.93	1.49	1.14	0.54	1.37
31	-	-	-	-	-	-	-	-	-	-	1.12
Total protein	34.23	34.46	27.17	31.97	22.01	30.1	25.57	25.57	27.23	19.17	20.45

CP%= concentration of crude protein in diet

Table (7): Relative concentration of sarcoplasmic proteins of F_1 (First generation) of *Oreochromis niloticus* for injected with 20µDNA and non-injected parents

No. of fraction	Injected					non-injected					Chicken
	CP%					CP%					
	20%	25%	30%	35%	40%	20%	25%	30%	35%	40%	
1	1.92	1.18	1.47	0.70	2.57	1.50	0.84	1.17	1.19	1.35	0.59
2	-	-	-	-	0.17	-	-	-	-	0.35	0.48
3	-	-	-	-	-	-	-	-	-	-	0.67
4	2.40	1.39	1.56	1.39	1.37	1.58	1.65	1.73	1.95	1.00	-
5	-	-	-	-	-	-	-	-	-	-	0.44
6	1.89	1.48	1.54	1.01	1.37	1.60	1.39	1.69	1.51	1.02	-
7	-	-	-	-	-	-	-	-	-	-	0.29
8	-	-	-	-	-	-	-	-	-	-	1.12
9	3.45	2.69	2.05	3.37	2.37	2.50	2.48	2.11	1.96	1.39	-
10	4.00	2.30	2.13	1.97	1.37	3.67	2.51	3.86	4.21	3.35	-
11	0.97	1.07	0.81	0.43	-	0.95	0.92	0.93	0.93	-	0.23
12	2.55	0.91	1.19	1.62	1.94	2.34	2.00	0.86	1.20	1.52	-
13	2.00	0.81	1.48	1.43	1.41	1.76	1.45	1.31	1.18	0.93	0.75
14	-	-	-	-	-	-	-	-	-	-	2.06
15	1.89	1.31	1.38	1.47	1.53	1.66	-	1.26	1.70	1.24	-
16	3.20	1.25	1.82	1.62	2.41	2.89	1.15	2.49	2.55	2.12	-
17	1.80	-	-	-	-	-	-	-	-	-	2.37
18	1.40	1.50	2.35	0.63	1.00	1.93	1.20	0.89	0.72	1.41	-
19	1.11	3.09	1.04	1.22	0.96	1.70	3.11	1.97	1.72	1.03	1.29
20	-	-	-	-	0.44	-	-	-	-	0.65	2.26
21	-	-	-	-	-	-	-	-	-	-	0.97
22	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	2.00
24	1.13	-	1.40	1.28	-	1.18	-	1.05	0.88	-	1.11
25	1.09	-	-	0.90	-	0.81	-	-	1.23	-	0.33
26	2.40	2.17	1.32	1.93	1.99	2.05	2.82	1.85	2.34	1.27	-
27	-	1.32	-	-	-	-	1.48	-	-	-	-
28	0.85	-	1.06	0.92	-	0.56	1.64	0.91	0.82	-	0.37
29	-	1.70	-	-	-	-	-	-	-	-	0.63
30	0.83	1.16	1.20	1.46	0.34	1.42	0.93	1.49	1.14	0.54	1.37
31	-	-	-	-	-	-	-	-	-	-	1.12
Total protein	34.88	25.33	23.80	23.35	24.24	30.1	25.57	25.57	27.23	19.17	20.45

CP%= concentration of crude protein in diet

Table (8): Relative concentration of sarcoplasmic proteins of F_2 (second generation) of *Oreochromis niloticus* for injected and non-injected parents

No. of fractions	Injected		non-injected	Chicken
	10µ	20µ		
1	0.89	0.65	0.46	0.59
2	0.40	0.41	0.29	0.48
3	-	-	-	0.67
4	1.41	0.68	0.87	-
5	-	-	-	0.44
6	1.25	0.96	0.84	-
7	-	-	-	0.29
8	-	-	-	1.12
9	1.81	1.78	1.75	-
10	3.15	0.38	0.34	-
11	-	-	-	0.23
12	1.88	1.91	1.70	-
13	1.55	1.22	0.68	0.75
14	-	-	-	2.06
15	1.37	0.74	1.52	-
16	2.30	0.82	2.35	-
17	0.39	0.73	-	2.37
18	0.66	0.86	0.97	-
19	1.56	0.75	0.95	1.29
20	0.73	0.49	0.58	2.26
21	-	-	-	0.97
22	0.54	0.77	0.78	-
23	-	-	-	2.00
24	-	-	-	1.11
25	-	-	-	0.33
26	1.91	0.78	1.06	-
27	-	-	-	-
28	-	-	-	0.37
29	-	-	-	0.63
30	0.52	1.01	1.12	1.37
31	-	-	-	1.12
Total protein	22.32	14.94	16.26	20.45

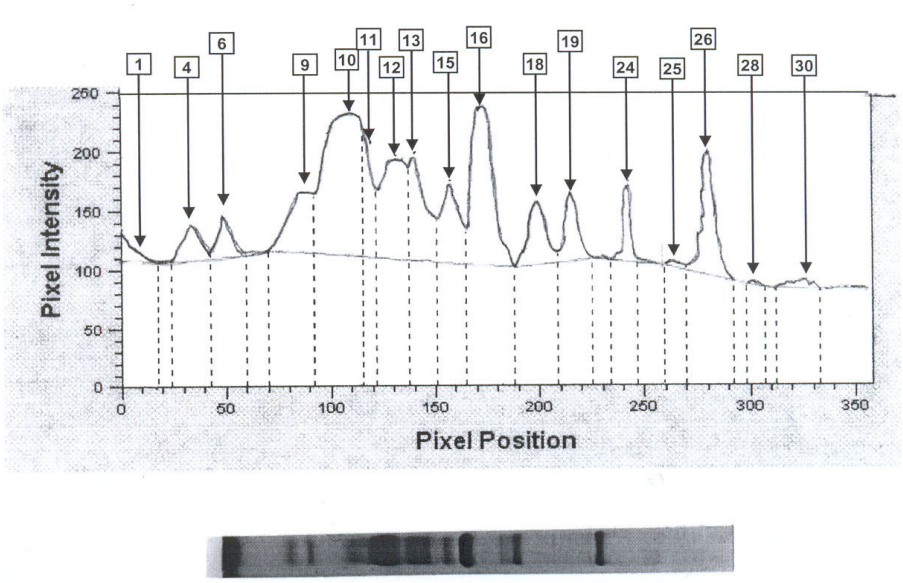

Figure (1): Scans of SDS electrophoretic pattern of sarcoplasmic protein of first generation for control (non-injected parents) *Oreochromis niloticus* at 20% crude protein in diet.

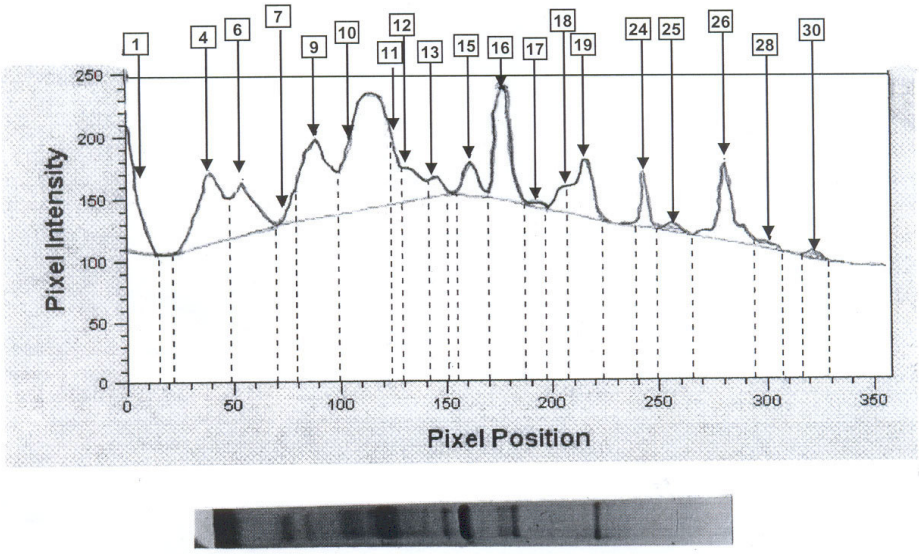

Figure (2): Scans of SDS electrophoretic pattern of sarcoplasmic protein of first generation for *Oreochromis niloticus* injected parents with 10µ DNA at 20% crude protein in diet.

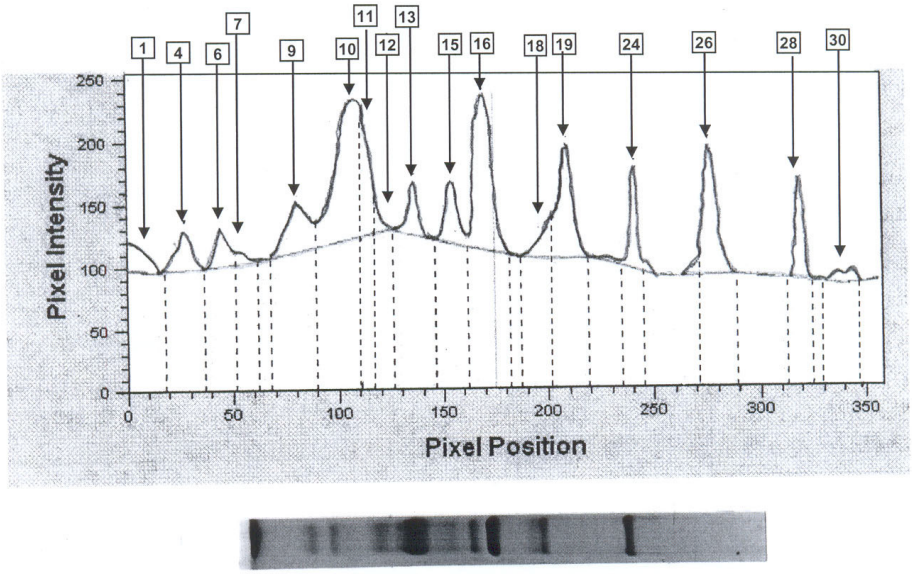

Figure (3): Scans of SDS electrophoretic pattern of sarcoplasmic protein of first generation for *Oreochromis niloticus* injected parents with 10µ DNA at 30% crude protein in diet.

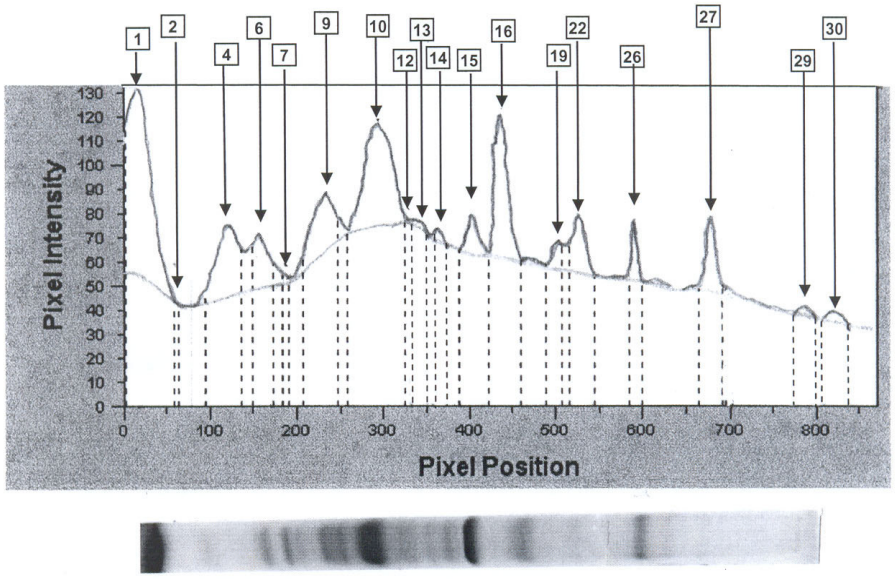

Figure (4): Scans of SDS electrophoretic pattern of sarcoplasmic protein of first generation for *Oreochromis niloticus* injected parents with 10µ DNA at 40% crude protein in diet.

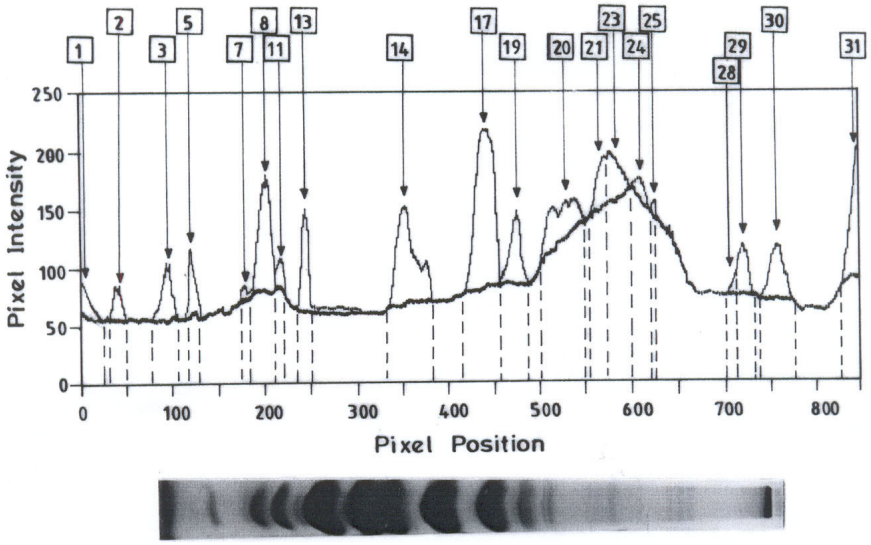

Figure (5): Scans of SDS electrophoretic pattern of sarcoplasmic protein of chicken.

DISCUSSION

The present results indicate that the best concentration of the utilized protein in fish feed was 30% crude protein. Mazid et al. (1979) noticed that the diet containing about 30% crude protein, appeared to be more efficiently utilized by the *Tilapia zillii* in terms of protein deposition than diets with higher levels of crude protein.

The electrophoretic analysis showed that the number of fractions in sarcoplasmic protein of F_1IP *Oreochromis niloticus* injected with chicken DNA ranged from (15 to 19) fractions, while the control ranged from (15 to 17) fractions in the first generation and ranged from (16 to 17) in the second generation. This means that the protein became more active in the F_1IP after injection with fractionated DNA of chicken. Also, we showed different degrees of appearance of some fractions (polymorphic) in some F_1IP and control. Appearance of the polymorphic protein state may be due to the effect of crude protein in diet and low conservation of these fractions. The appearance of fractions 7, 17 and 29 in both F_1IP only, may be due to transmitted DNA. The electrophoretic patterns of muscle myogen and serum protein were used to verify gene transfer (Tsai and Tsing, 1992; Kuo and Tsai, 1993; Tsai *et al.,* 1995; Hsih *et al.,* 1997; Chen *et al.,* 1998 and Ahmed, 1999).

A genetic analysis of alliloforms with identical mobility in the electric field, but differing in the intensity of bands staining makes it possible to detect a variation in the regulating genes (Kirpichinkov, 1981).

At 30% crude protein in diet, the relative area percentage of fractions number (1, 12, 13, 15, 18 and 28) increased in both F_1IP than control one but there were a decrease in fractions number (6, 26 and 30) in the first

generation. In the second generation there was an increase in fractions number (1, 2, 6, 12 and 13).

The introduced gene may be linear or circularized, include the cloning vector DNA or not (Maclean, 1987). The linear form of fragmented chicken DNA allows them to pass easily through the micropores of cell membrane, then nucleus membrane of germ cell of female and male *O. niloticus*. Bending (1981) reported that the linear DNA molecules rapidly ligate and concatemerize after injection into unfertilized eggs. Chourrout *et al.* (1986) pointed out that transformation was higher with linearized than with circular plasmids (75% versus 40%).

Using total DNA (fragmented) enables all genes to enter the cell. Organisms from the zygote cell to the multi-cellular, multi-tissue, mature. The gene will enter randomly in some cells. The only functional genes are those which are functional in the tissue from which the DNA was isolated and it will be only functional in the cells of the host which are similar to cells of the tissue from which the donor DNA was isolated. This means that genes of DNA isolated from pituitary glands will be functional only if they enter pituitary gland cells of the host (Ali, 2000). The specificity of donor genes is developed by their introns (Ali, 2000). Moreover, it has been proven that the insertion of DNA causes repair and correction of crossing-over, which causes reciprocal recombination (Abd El–Aal, 1992; Soliman, 1992 and Sharaf El-Din, 1992).

Expression of the chicken DNA in the modified *Oreochromis niloticus,* may have occurred through preventing recipient cells DNA degrading enzymes (DNAses) from destroying such foreign molecules. It could be attributed to the DNA methylation process in chicken which seems to be similar or having high homology with that of *O. niloticus***,** thus donor methylation can withstands the same protection in recipient cells. It has been reported that the differential methylation of the cytosine in many animals can protect against DNAse activities and that DNA methylation processes can provide means of explaining the stability of the committed state, while allowing for its modification in suitable circumstances (Latchman, 1990 and Moav, 2000).

The increase in growth rate (Food efficiency) and relative concentration of sarcoplasmic protein in F_1IP and F_2IP with 10µ compared with both F_1IP and F_2IP with 20µ may the fact that the former concentration is more suitable. Kang *et al.* (1999) stated that the integration rate depends on the concentration of DNA, DNA form, buffer composition, age of the ovum and the structure of chromosome. The higher amount of DNA led to much lower survival of the embryo (Chourrout *et al.,* 1986) while lower amount of DNA produce a higher number of surviving animals, but tend to give less transgenic. Hsih *et al.* (1997) reported that, rGH did not enhance fish growth at all concentrations tested.

Tsai *et al.* (1995) suggested that higher dosage rGH treatments (1 and 2 µg/g) had less effect on growth enhancement than the lower one 0.1µg/g but higher than the untreated one. The adverse effects caused by higher dosage treatments were also observed by Agellon *et al.* (1988).

In the present research there are indications that refer to possibility of gene transfer.

1. Increase the growth for the injected fish than control one.
2. Appearance of new fractions such as 7, 17 and 29.
3. Increase the relative area protein bands for the injected fish.

It may be concluded that the methods used in the present paper, using naked fragmented DNA of pituitary gland intraperitoneal, could offer certain advantages such as simplicity holding a great potential for introduction of foreign genes like growth hormone into large patches of fertilized egg.

It is not worthy that such indications persuted through the generation. However the

present results need to be verified through PCR methods.

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REFERENCES

Abd-El-Aal, S. Kh. (1992): The effect of nucleotide sequences of repetition dicotolydon on microbial genetic repair. Ph.D. Thesis, Fac. Of Agric., Al-Azhar Univ., Egypt.

Abd-El-Fattah, M.O. (1995): Micro and macro DNA Lesions induced by some % drugs. Ph.D. Thesis, Genetics Dept., Fac. Of Agric., Alex. Univ., Egypt.

Agellon, LB.; C. J. Emery, J.M. Jones; S.L. Davis, A.O. Dingle and T.T. Chen (1988): Promotion of rapid growth of rainbow trout (*salmo grirdneri*) by a recombinant fish growth hormone. Can. J. Fish. Aquat. Sci., 45:146-151.

Ahmed, O.M.A. (1999): Genetic engineering on poultry breeding effect of introducing pituitary glands DNA as foreign genes on local chickens performance. Ph.D. faculty of Agriculture, Alexandria University.

Ali, A.M.M. (2000): The role of genomic DNA and introns in gene transfer. Int. J. Biotechnology, Vol. 2, No. 5, In press.

Bending, M.M. (1981): Persistence and express ion of histone genes injected into xenopus eggs in early development. Nature, Vol. 292: 65-67.

Charles, P. (1970): Isolation of Deoxyribonucleic acid: Uptake of Informative Molecules by Living cells. Ledouix, L. Ed. North Holland Publishing company, Amsterdam, London.

Chen, J.Y.; H.L. Tsai, C.Y. Chang, J.I. Wang, S.C. Shen and J.L. Wu, (1998): Isolation and characterization of tilapia (*oreochromis mossambicus*) insulin-like growth factors gene and proximal promoter region: DNA cell Biol. 17, 359-376.

Chen, T.T. and J.K. Lu, (1998): Transgenic fish technology: Basic principles and its application in basic and applied research. In: De la fuente J. and castro F.O. eds. Gene transfer in aquatic organisms. RG Landes company and Germany: Springer-verlag, Austin, Texas, USA. PP. 45-73.

Chourrout, D.; R. Guyomard, and L.M. Houdebine (1986): High efficiency gene transfer in rainbow trout (*salmo gairdneri Rich*) by microinfection into egg cytoplasm. Aquaculture, 51: 143-150.

Hew, C.L.; G.L. Fletcher, and P.L. Davies, (1995): Transgenic salmon: tailoring the genome for food production. Journal of Fish Biology 47:1-19.

Hsih, M.H.; J.C. Kuo, and H.J. Tasi, (1997): Optimization of the solubilization and renaturation of fish growth hormone produced by Escherichia coli. Appl. Microbiol. Biotechnol, 48: 66-72.

Jiang, Y.(1993): Transgenic fish- gene transfer to increase disease and cold resistance. Aquaculture, 111:31-40.

Kang, J.H.; G. Yoshizaki, O. Homma, C.A. Strussmann, and F. Takashima, (1999): Effect of an osmotic differential on the efficiency of gene transfer by electroporation of fish spermatozoa. Aquaculture, 173:297-307.

Kirpichnikov, V.S. (1981): Genetic bases of fish selection. Springer-verlag, Berlin, Heidelberg, New York, pp. 19, 33, 34, 158-160 and 171-181.

Kuo, J.L. and H.J. Tsai, (1993): Electrophoretic analysis of recombinant fish growth hormone secreted from *saccharomyces cerevisiae* by using α-factor-leader sequence journal of the fisheries society of Taiwan, Vol. 20, No. 1: 51-58.

Latchman, D.S. (1990): Eukaruotic transription factors. Biochem. J., 270 (2): 281-289.

Lathe, R.; Clark, A.J.; Archibald, A.L.; Bishop, L.O.; Simons, P.; Wllmut, I. (1987): Novel product from livestock. In: Exploiting new technologies in animal breeding. Ed. By Smith, C.; King, J. W. B.; Mckay, J.C, Oxford Univ. Press

Mazid, A.M.; Tanaka, Y.; Katayama, T.; Asadur, M.; Rahman, K.; Simpson, L. and Chichister, C.O. (1979): Growth response of (*Tilapia zillii*) fingerlings fed isocaloric diets with variable protein levels. Aquaculture, 18:115-122.

Maclean, N.; D. Penman, and Z. Zhu, (1987): Introduction of novel genes into fish. Bio/technology, 5:257-261.

Moav, B. (2000): Methylation and transgene expression in transgenic common carp. Proceeding of Genetics in Aquaculture. Townsville 673. Astralia.

Powers, D.A.; M. Gomez-Chiarri, T.T. Chen, and R. Dunham, (1998): Genetic Engineering of Finfish and shellfish. In: De la fuente J. and Castro F.O. eds. Gene transfer in aquatic organisms. RG Landes company and Germany, Springer-verlag, Austin, Texas, USA. PP. 17-34.

Sambrook, J.; E.F. Fritsch, and T. Maniatis, (1989): Molecular cloning: A laboratory manual. 2Ed. Cold spring Harber Laboratory, Cold spring Harber, New York.

Sharaf El-Din, Sh.H.M. (1992): The effect of inserting a foreign gene on reciprocal and non-rediprocal recombinants in *saccharomyces cerevisiae*. Ph.D. Thesis, Fac. of Agric. Al-Azhar Univ., Egypt.

Sheela, S.G.; T.J. Pandian, and S. Mathavan, (1999): Electroporatic transfer, stable integration, expression and transmission of pZpssyp GH and pZpssrp GH in Indian cat fish. Heteropneustes fossils (Bloch). Aquaculture Research. 30 (4): 233-248.

Soliman, E.A.M. (1992): The effects of nucleotide sequences repetition of a monocotolydon plant on microbial genetic functions. Ph. D. Thesis, Fac. of Agric. Al-Azhar Univ., Egypt.

Tsai, H.J., and C.F. Tseng, (1992): Epression of rainbow trout homone cDNA in E. coli from vector utlilizing tac promoter. J. Fish Soc. Taiwan 19: 45-53.

Tsai, H.J.; F.S. Tseng, and I.C. Liao, (1995): Electroporation of sperm to introduce foreign DNA: into the genome of loach (*Misgurnus anguillicaudatus*). Can. J. Fish. Aquat. Sci. 52,776-787.