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Effect of Dietary Protein Levels on Growth Performance and Body Composition of Monosex Nile Tilapia, *Oreochromis niloticus L.* Reared in Fertilized Tanks

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Abstract: Juvenile monosex Nile tilapia (*Oreochromis niloticus*) were fed four dietary protein levels (17%, 25%, 30% and 35%) to investigate their effects on growth performance, carcass composition and survival rate. The experiment was carried out in concrete tanks (2.9m x 1.4m x 1m), stocking each tank with 160 fish (50 fish/m^3). The diets were offered to the fish (2.5±0.1g average initial weight) at a level of 3% of body weight, six days a week for 180 days. In addition to the experimental diet, inorganic fertilizer was added to each tank at a rate of 6.8 mg/l of premix superphosphate and urea. The results showed that a significant effects of dietary protein on growth performance of the reared fish. Weight gain and specific growth rate increased significantly with increasing dietary protein levels between 17% and 30%. but, 35% crude protein showed insignificant increase in growth parameters. The protein efficiency ratio was inversely correlated with dietary protein levels. The protein content of the fish muscle increased with increasing dietary protein level while the lipid content decreased. The diet had no significant effect on survival rate of the fish. From the present results, diet containing 30% crude protein is considered optimal for growth of monosex Nile tilapia, under the present experimental conditions and it is recommended for feeding monosex tilapia juveniles.

Key words: Monosex, nile tilapia, protein requirements, growth performance and body composition

INTRODUCTION

Aquaculture is growing in Egypt and becoming an increasingly important source of fish available for human consumption. Tilapia is a highly popular aquaculture product because of its fast growth rate and ability to grow in extremely diverse and adverse conditions , so it is considered the most intensive cultivated freshwater fish in Egypt (Abdel-Hakim *et al.,* 2001 ; El-Sayed, 2002).
Nutrition and feeding play a central role in sustainable aquaculture and therefore, feed resources as well as costs continue to dominate aquaculture needs. Feed accounts for 40-60% of the production costs in aquaculture, with protein sources accounting for a significant proportion of this cost (Fotedar, 2004). Protein is main major dietary nutrient affecting performance of fish (Lovell, 1989). It provides the essential and non-essential amino acids which are necessary for muscle formation and enzymatic function and in part provides energy for maintenance (Yang *et al.,* 2002). It is important to minimize the amount of protein used for energy, because protein is usually the most expensive major constituents in a diet. Excess protein in fish diet may be wasteful and cause diets to be unnecessarily expensive (Ahmad, 2000). The energy needs of the fish can be met by less expensive lipid and carbohydrate sources. The protein requirement of Nile tilapia was estimated to be from 25% to 45% of diet (De Silva and Perera, 1985; Siddiqui *et al.,* 1988; El-Sayed and Teshima, 1992; Omar, 1994a,b; Kheir, 1997; Abdel-Hakim *et al.,* 2001; Swick, 2001). Monosex (males) Nile tilapia have been widely cultured in Egypt. Males are used for monosex culture because male tilapia grow faster than females. Females use considerable energy in egg production and do not eat when they are incubating eggs. Male monosex culture permits the use of longer culture periods, higher stocking rates and fingerlings of any age (Rakocy and McGinty, 1989).
Nile tilapia are commonly grown in semi-intensive culture using fertilization to increase primary production that is used by tilapia for food (Diana *et al.,* 1991) . Fertilizers can be used to reduce the quantity and expense of supplemental feeds. An increase in natural food has a much greater effect on tilapia production. Results of Brown *et al.* (2002) indicated that reducing feeding rates either by delaying the introduction of feeds or by feeding less than the amount required for satiation had no effect on growth or yield of fish reared in fertilized ponds, suggesting that this approach may be useful to farmers wishing to reduce costs without compromising sales.
The objective of the present study was therefore to assess the optimum protein levels required for optimum growth of monosex Nile tilapia (*Oreochromis niloticus*) reared in fertilized concrete tanks.

MATERIALS AND METHODS

Juveniles monosex Nile tilapia with an average weight of 2.5±0.1g were used in this study. The fish were obtained from private fish hatchery , transported in aerated plastic bags and acclimatized to laboratory conditions for about one week. Eight concrete tanks were used for this experiment. Each tank was 2.9m long,

Table 1: Formulation and chemical composition of the test diets on dry weight basis

	Dietary protein levels (%)			
Components	17	25	30	35
Diet constituent (g/100g diet)				
Soybean	10	30	52	72
Wheat bran	25	19	35	13
Ground yellow corn	51	35	9	11
Corn gluten meal	5	4	-	-
Molasses	6.5	6	-	-
Fish meal	1	4	-	-
Yeast	-	-	2	1
Starch	-	0.5	0.5	1.5
Vitamines and minerals	1.5	1.5	1.5	1.5
Total	100.0	100.0	100.0	100.0
Chemical analyses(% of dry matter)				
Crude protein	17.2	25.2	30.2	35.2
Crude fat	3.17	2.84	2.31	1.72
Ash	3.64	4.61	5.39	5.44
Fiber	4.87	5.33	7.54	6.83
NFE*	71.12	62.02	54.56	50.81
GE**(Kcal/100g)	419.23	423.80	416.38	423.60

*Nitrogen-free extract = 100 - (crude protein + crude fat + ash + fiber). **Gross energy, calculated after NRC(1993) as 5.64 , 4.11 and 9.44 Kcal /g for protein, NFE and lipid, respectively.

1.4m wide and 1m deep. The tanks were filled by about 10 cm thick soil base and the level of water was kept at 80 cm deep to maintain the water volume at 3.2m^3. The stocking density was 160 fish per tank (50 fish/m^3). The fish were divided into duplicated four groups to feed by four experimental diets (Table 1) containing 17%, 25%, 30%, 35% crude protein (CP). Each experimental diet was offered to the fish at a rate of 3% of body weight once a day for 6 days a week for 180 days. In addition to the experimental diets, inorganic fertilizer was added to each tank at a rate of 6.8 mg/l of superphosphate and urea premix (806.5g superphosphate and 270g urea) (Abdel-Baky *et al*., 2000). Every day, the water was renewed partially by clean free-chlorine tap water. To follow up the fish growth, fish samples (20-30 fish) from each tank was weighed monthly. At the end of the experiment, a sample of five fish from each tank were taken and frozen for subsequent proximate analyses of carcass composition. Crude protein was determined using Kjeldahl system, crude lipid was determined by ether extraction, moisture content was determined by drying at 105°C for 24 hours and ash was determined by a muffle furnace at 550°C for 4 hours (AOAC, 1995).

Growth performance of the experimental fish was calculated as described by Ahmad *et al*. (2004) as follows:

Weight Gain (WG) = final weight (g) - initial weight (g).

Average Daily Weight Gain (ADG) = weight gain (g) / time (days).

Food Conversion Ratio (FCR) = diet fed (g) / total wet weight gain(g).

Specific Growth Rate (SGR) = 100 (Ln final weight (g) - Ln initial weight (g)) / time (days).

Protein Efficiency Ratio (PER) = wet weight gain (g) / amount of protein given (g).

Table 2: Common phyto-and zooplankton (organism/l) groups in experimental tanks

	Number (organism/l)	
Plankton groups	Minimum	Maximum
Phytoplankton		
Chlorophycea	0.06x105	2.3x105
Bacillariophyceae	0.03x105	1.2x105
Cyanophyceae	0.01x105	3.8x105
Euglenophyceae	0.09x105	4.1x105
Zooplankton		
Protozoa	0.10x105	3.6x105
Rotifera	0.15x105	4.4x105
Copepoda	0.003x105	0.4x105
Cladocera	0.002x105	0.12x105
Ostracoda	0.001x105	0.06x105

Data were subjected to analysis of variance (ANOVA) to determine the effect of dietary protein levels on growth performance and body tissue composition of the fish . If ANOVA indicated significant treatment effects, the least significant difference (LSD) test was used to determine differences among individual treatment means (Snedecor and Cochran, 1989). Differences were considered significant at $P \leq 0.05$. Physico-chemical characteristics of water and plankton standing crops were recorded .

RESULTS

The water quality parameters monitored were with the tolerable limits for tilapia. Water temperature ranged from 18°C to 32°C, dissolved oxygen from 5.4 to 7.8 mgl^{-1} and pH from 6.8 to 7.9. The common phyto-and zooplankton groups found in experimental tanks are presented in Table 2. Weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio for juvenile tilapia after the feeding trial are presented in Table 3. Growth performance increased significantly ($P<0.05$) with increasing dietary protein levels from 17% to 30%, but there was no-significant increase with the diet of 35% protein diets. FCR decreased with increasing dietary protein levels and ranged from 2.03 - 2.42. PER ranged from 1.36 to 2.43 and decreased significantly ($P<0.05$) with increasing dietary protein levels to 30%, but 35% CP diet showed non-significant decrease. There was no significant differences in survival of tilapia fed the four diets. Overall, survival was high and ranged from 95.6% to 97.5%. Carcass composition data are presented in Table 4. The tissue protein content increased significantly ($P<0.05$) with increasing dietary protein levels. Conversely, lipid contents decreased significantly with increasing dietary protein levels up to 30% CP ($P<0.05$). The monthly growth of the reared fish was presented in Fig. 1.

DISCUSSION

The present study revealed a significant effects of dietary protein on growth performance of juvenile monosex Nile

Table 3: Growth parameters of tilapia fed practical diets with different protein levels

	Protein levels				ANOVA	
Parameters	17%	25%	30%	35%	F	Sig.
Initial weight (g/fish)	2.5±0.1	2.5±0.1	2.5±0.1	2.5±0.1	-	-
Final weight(g/fish)	35.2±4.8[a]	42.3±3.24[b]	49.07±1.76[c]	49.08±2.82[c]	12.526	0.002
Weight gain, g/fish	32.7±4.73[a]	39.8±3.2[b]	46.57±1.68[c]	47.3±2.79[c]	12.526	0.002
Average daily gain, g/fish/day	0.18±0.03[a]	0.22±0.02[b]	0.259±0.02[c]	0.262±0.02[c]	14.028	0.001
Specific growth rate (SGR)	1.47±0.08[a]	1.55±0.05[b]	1.65±0.02[c]	1.66±0.03[c]	10.559	0.004
Feed conversion ratio (FCR)	2.42±0.13[a]	2.12±0.17[b]	2.11±0.15[b]	2.03±0.12[b]	5.753	0.021
Protein efficiency ratio (PER)	2.43±0.09[a]	1.89±0.13[b]	1.65±0.1[c]	1.36±0.09[c]	39.219	0.000
Survival rate, %	95.7±2.5	95.6±1.9	97.5±1.5	96.3±2.7	1.597	0.265

Values with different superscripts within each row are significantly different at P< 0.05.

Table 4: Carcass analysis (dry weight basis) of tilapia fed practical diets with different protein levels

	Protein levels				ANOVA	
Parameters (%)	17%	25%	30%	35%	F	Sig.
Moisture	76.40±0.44[a]	76.73±0.38[ab]	77.17±0.25[bc]	77.8±0.36[c]	8.327	0.008
Crude protein	64.57±0.29[a]	65.13±0.15[b]	65.93±0.15c	66.53±0.15[d]	58.804	0.00
Crude lipid	20.83±0.15[a]	19.87±0.55[b]	18.67±0.35[c]	18.17±0.25[c]	33.740	0.00
Ash	14.87±0.21[a]	14.53±0.15[ab]	14.40±0.40[b]	14.13±0.11[b]	4.648	0.037

Values with different superscripts within each row are significantly different at P< 0.05.

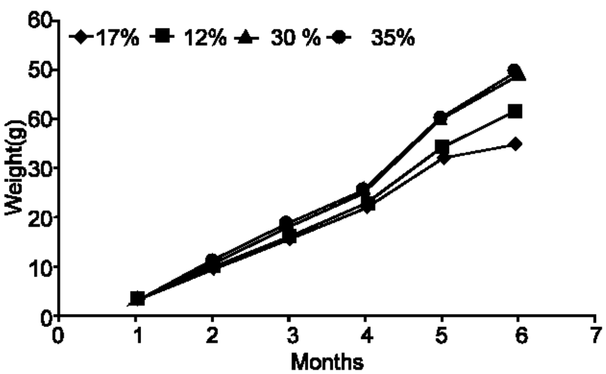

Fig. 1: Growth of the reared fish as affected by dietary protein levels.

tilapia (Table 3). Weight gain and specific growth rate increased significantly with increasing dietary protein levels from 17% to 30% with non- significant increase by the diet of 35% CP. Therefore, 30% CP diet is considered an economical and optimal for rearing tilapia using fertilization. Similar findings have been reported by different authors for different tilapia species (Wang *et al.*, 1985; Siddiqui *et al.*, 1988; Omar, 1994b; Abdel-Hakim *et al.*, 2001; Coyle *et al.*, 2004). The previous authors reported that the optimum dietary protein level for growth of Nile tilapia was 30% crude protein.

Many authors obtained conflicting results from their studies on tilapia nutrition. The dietary protein requirements of several species of tilapia have been estimated to range from 20% to 56% (El-Sayed and Teshima, 1992). Balarin and Halfer (1982) made a general conclusion that fry of tilapia <1g require diet with 35-50% protein, 1-5g fish require diet with 30-40 protein and 5-25g fish require diet with 25-35% protein. Wee and Tuan (1988) found that the minimum dietary protein requirements for non-spawning and spawning *O. niloticus* was 27.5% and 35% crude protein respectively. Similarly, De Silva *et al.* (1989) demonstrated that the most economical dietary protein requirement for young tilapia (1 to 5g) was 28 %. However, maximum growth was achieved at about 34%. Nile tilapia reared in cages exhibited their highest growth on diet containing 32% CP (Kheir, 1997) and 28-32% CP (Abdel-Hakim and Moustafa, 2000). In support to this, Wilkinson (2003) reported that the growth rate of juvenile tilapia increase as dietary protein content is raised until a plateau is reached at around 30-34%, further increase in dietary protein lead to decline in growth rate thereafter. Jauncey (1982) showed that the growth of juvenile *O. mossambicus* increased with dietary protein levels up to 38 - 40% respectively and thereafter decreased with increasing protein levels in diets. In addition, Santiago *et al.* (1982) reported that the optimum dietary protein level for *O. niloticus* fry was between 35 and 40%. The optimum dietary protein level for growth of Nile tilapia collected from different localities in Egypt was 27%, 37%, 32% (Khattab *et al.*, 2000).

The considerable variations in the results recorded previously for optimum dietary protein requirements for maximum growth might be due to the variations in fish size and age, stocking density, protein quality, hygiene and environmental conditions or other unknown factors which mask the standardization of the parameters (Ahmad *et al.*, 2004)

The high growth rate obtained In the present study may be due to the contribution of natural food organisms (Table 2) to daily requirements of the reared fish. Nile tilapia are diversified feeders, feeding on green and blue green algae, phytoplankton, diatoms, zooplankton and benthic organisms which contain high amount of protein and are believed to provide additional protein to fish (Omar, 1994b). Lovell (1975) observed that natural food plays a key role in the determination of dietary protein requirements of fish under pond conditions.

In the present study, FCR decreased wih increasing dietary protein levels and ranged from 2.03 - 2.42. The best FCR was obtained from 35% protein diet, while the poorest FCR was obtained by 17% protein diet, but there was no significant difference between FCR obtained by 25, 30, 35% protein diet. This results agree with the results of previous studies on tilapia species (Omar,1994b; Kheir,1997; Abdel-Hakim *et al.,* 2001; Siddiqui *et al.,* 1988). On the other hand, Wee and Tuan (1988) stated that better FCR values were obtained with increasing dietary protein levels up to 42.5% and deteriorated slightly by diet containing 50%. FCRs found by Al-Hafedh (1999) ranged from 1.6 to 2.5 for fry (0.51g) and from 3.13 to 4.86 for fingerlings fish (45g). Abdel-Tawaab (2004) recorded FCR of four Egyptian Nile tilapia strains as follow: 2.21, 1.8, 2.7 and 2.03 for Abbassa, Aswan, Manzalah and Mariut respectively.

PER, in the present study, is significantly affected by protein levels and manifests that protein utilization was obtained at low protein level (Table 3). The decrease of PER with increasing dietary protein level have also been reported by different authors for different tilapia species (Jauncey, 1982; Wee and Tuan, 1988; Shiau and Huang, 1989; Kheir, 1997; Ahmad *et al.,* 2004). This is mainly because more dietary protein is used as energy when high protein diets are fed to fish (Kim *et al.,* 1991). In support, Shimeno *et al.* (1981) found that increasing dietary carbohydrate and fat caused a reduction in the activities of amino acid-degrading enzymes in the hepatopancreas and resulted in a low nitrogen excretion rate and a high protein efficiency ratio. Dabrowski (1977) reported different patterns of changes in PER in relation to dietary protein level and found that the relationship between dietary protein and PER differ from species to species. In the present study, there was a significant increase in muscle protein and a decrease in lipid content with increasing dietary protein (Table 4). This relationship was also noted by Dabrowski (1977) on grass carp, Jauncey (1982) on *Sarotherodon mossambicus*, Fah and Leng (1986) on guppy, *Poecilia reticulata*, Shiau and Huang (1989) on hybrid tilapia (*O.niloticus* X *O.aureus*), Wee and Tuan (1988), Kheir (1997), Al-Hafedh (1999) on Nile tilapia (*O.aureus*). The increase in muscle protein and decrease in lipid content with increasing dietary protein levels may be attributed to the high carbohydrate and low protein content in the diet of low protein level (Table 1). The excess carbohydrate in the diet may be converted into body fat for storage (Fah and Leng, 1986).

In conclusion, this study indicate that a diet containing 30% crude protein appear to be more suitable and economical for Nile tilapia growth.

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