Egypt. J. Aquat. Biol. & Fish., Vol.5, No. 3: 195- 212 (2001) *ISSN 1110 - 6131*

EFFECT OF PROTEIN LEVEL AND STOCKING DENSITY ON GROWTH PERFORMANCE, SURVIVAL RATE, FEED UTILIZATION AND BODY COMPOSITION OF NILE TILAPIA FRY (*OREOCHROMIS NILOTICUS* L.)

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Key words: Protein level, stocking density, fish growth, Nile tilapia, feed utilization and body composition.

ABSTRACT

This study was carried out to evaluate the growth response of Nile tilapia; *O.niloticus* L. to dietary protein levels at two stocking densities. This study was bifactorial designed (3 protein levels × 2 stocking densities). Fish (1.8-2.5 g/fish) was randomly distributed into the aquaria at a rate of 15 or 30 fish/100 L. The temperature was adjusted at 27±1 °C. Fish of each density were fed either a diet containing 25%, 35% or 45% CP with a feeding rate of 4% of body weight twice daily for 5 days a week for 70 days.

The obtained results showed that the final body weight, weight gain, weight gain %, specific growth rate (SGR)were positively affected by protein level and inversely affected by stocking density, but not affected by their interaction. Condition factor was significantly affected only with dietary protein levels ($P<0.05$), while survival rate did not differ significantly by protein levels or stocking densities. The maximum growth was obtained with 45% CP at low density (10.1 g/fish), whereas the lowest growth was obtained with 25% CP at high density (6.4 g/fish). On the other hand, the reduced growth at high density could be recovered by increasing the protein level.

Feed intake increased significantly with increasing dietary protein level only ($P<0.01$). Feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) were significantly affected by protein level and stocking density ($P<0.01$). The best FCR was obtained with 45% protein diet at low density (1.72) and with 35% under low density (1.81) with insignificant

(1.72) and with 35% under low density (1.81) with insignificant difference (P>0.05). The highest values of PER and PPV were obtained with 25% CP at low (1.913 and 30.54%, respectively) and high density (1.720 and 26.64%, respectively) with significant difference (P<0.01).

Moisture was significantly affected by protein level only (P<0.05), while crude protein, total lipids and ash were significantly affected by protein level and stocking density. The highest protein content in fish body was obtained with 45% CP at low and high densities (58.0% and 57.9%, respectively; P>0.05). The highest content of body lipids was observed in fish fed 25% protein diet at low and high densities (32.8 and 31.0%, respectively; P>0.05). The highest content of carcass ash was noticed in fish fed 45% protein diet at low and high densities with significant difference (15.6% and 16.8%, respectively; P<0.05).

INTRODUCTION

Tilapias are of the most important fish species for aquaculture all over the world (El-Sayed, 1999) and represent the species of choice due to their high growth rate, significant tolerance to environmental stress, easy of reproduction, and perhaps their unquestionable market demand. Nile tilapia (*Oreochromis niloticus*), in particular is widely accepted to Egyptian consumers.

Intensification of tilapia culture is a good solution for increasing fish production, and to optimize fish intensification, both feed quality and stocking density should be considered. Addition of artificial feeds plays an important role especially under conditions of heavy stocking, when natural feed supply has declined or completely disappeared. The added feeds should be rich in protein, carbohydrate and fats, and should also contain vitamins, minerals and growth-promoting substances to be physiologically balanced (Huisman *et al.*, 1979). However, the fish malnutrition would reduce growth performance and may cause disease or even death (Lovell, 1989). So, it is essential to develop suitable feeds to be used either as a supplementary diet in ponds or as a complete diet in tanks.

Protein is the most critical and/or costly ingredient in tilapia feeds, where protein sources represent about 60% or more of the cost of fish feeds. So optimal utilization of dietary proteins is essential for

economical production (Andrews, 1977). The level of dietary protein producing maximum growth of tilapia depends upon the protein quality, energy content of the diet, the physiological state of the fish, age, reproductive state, and the environmental factors such as temperature, salinity...etc (Lovell, 1989).

In aquaculture, fish size and production determine the price of fish, which in turn depends on the growth. Subsequently, control of size and production are two important tasks to meet the market demands, and increasing the stocking density is a way of dealing with problem of land shortage and increasing fish intensification. Stocking density is an important factor to take into account when ranking families or progeny groups for growth performance. In many cultivated fish species, growth is inversely related to stocking density and this is mainly attributed to social interactions (Holm *et al.*, 1990; Haylor, 1991; Miao, 1992; Huang and Chiu, 1997; Canario *et al.*, 1998; Irwin *et al.*, 1999; Silva *et al.*, 2000). However, social interactions through competition for food and/or space can negatively affect fish growth. On the other hand, the price of fish is determined by the market demand of supply (size and production), that in turn depends on their growth. Papst *et al.* (1992) suggested that in intensive aquaculture the stocking density is an important factor that determines the economic viability of the production system.

A close relationship has been found between stocking density, growth of Africa catfish *Clarias gariepinus* (Burchell) fry (Haylor, 1991) and redtail shrimp *Penaenus penicillatus* (Alock) (Miao, 1992). Moreover, Wallace *et al.* (1988) reported that high density culture reduced size variation in the early stage of Arctic charr (*Salvelinus alpinus* (Linnaeus), but Papst *et al.*, (1992) recorded that density had no effect on the size (weight) variation in juvenile Arctic charr. The objective of this study was to evaluate the effects of protein levels at two stocking densities on growth performance, survival rate, feed utilization and body composition of Nile tilapia fry (*O. niloticus* L.).

MATERIAL AND METHODS

This study was carried out at the Central Laboratory for Aquaculture Research, Abbassa, Abou Hammad, Sharkia Governorate, Egypt. Nile tilapia (*O.niloticus* L.) were collected from

Abbassa Hatchery, General Authority for Fish Resources Development. Fish size was 1.8-2.5 g/fish; the batch of fish was kept in indoor trough tanks for two weeks as an acclimatization period. Fish were fed on a commercial diet containing 25% protein during the acclimatization period. Fifty fish were frozen at -20 ^{o}C for analysis of body chemical composition.

This study was bifactorial designed (3 protein levels × 2 stocking densities). Fish with mixed sex were graded and randomly distributed into the aquaria at two stocking densities (15 and 30 fish/aquarium, respectively). Each aquarium containing 100 L of well-aerated tap water. Each aquarium was supplied with compressed air from air pump via air stones. The temperature was adjusted at 27±1^{o}C by using thermostatically controlled heaters. Aquaria of each density were fed either a diet containing 25%, 35% or 45% CP with a feeding rate of 4% of life body weight twice daily for 5 days a week for 70 days. Three aquaria were assigned for each protein level treatment within each fish stocking density. Feed formulation and chemical composition of each diet are presented in Table (1). Fish faeces of each aquarium were removed daily by siphoning about 50% of water volume of each aquarium and replaced by well-aerated tap water. Dead fish were removed and recorded daily. Fish in each aquarium was biweekly weighed and subsequently the amount of the given feed was calculated.

At the end of experiment period (70 days), fish were removed, counted and weighed. Different growth parameters and parameters of feed utiliwere calculated as described by Ahmad (200). Five fishes from each aquarium were taken to carry out chemical analysis of the fish body. Chemical analysis of feed ingredients, experimental diets and fish carcasses were carried out according to the methods of AOAC (1990). Moisture was determined by drying oven at 85 ^{o}C until fixed weight. Crude protein was determined by Kjeldahl method (total nitrogen x 6.25). Total lipids (ether extract) were determined by Soxhlet extraction method using petroleum ether for 16 hrs. Ash was determined by ashing the samples in muffle furnace at 550 ^{o}C for 6 hrs. Crude fiber was estimated according to Goering and Van Soest (1970). Growth energy of diet and fish carcass were estimated according to NRC (1993).

Data of growth performance, survival rate, feed utilization and chemical composition of fish body were subjected to two-way

ANOVA according to Snedecor and Cochran (1982). Differences between means were deduced using Duncan's new multiple range tests (Duncan, 1955).

RESULTS

The different growth parameters (final body weight, weight gain, weight gain %, specific growth rate (SGR) of Nile tilapia (*O. niloticus*) fed with 25, 35 or 45% protein diets at low and high densities are shown in Table (2). The obtained results show that the different growth parameters were significantly affected by protein level and stocking density ($P<0.05$), but not their interaction. Condition factor was significantly affected only with dietary protein levels ($P<0.05$), while survival rate did not differ significantly at protein level or stocking density (Table 2).

It is also noticed that fish growth was positively affected by protein level and inversely affected by stocking density. The maximum growth was obtained with 45% CP at low density (10.1 g/fish), whereas the lowest growth was obtained with 25% CP at high density (6.4 g/fish). On the other hand, the reduced growth under high density could be recovered by increasing the protein level. The weight gain of Nile tilapia at high density fed with 45% protein diet and that at low density fed with 35% protein diet was approximately the same (7.0 and 6.8 g/fish, respectively). Similarly, Nile tilapia at high density fed 35% protein diet gave the same weight gain (5.5 g) to that obtained by low density with 25% protein diet (4.9 g).

Results of feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) of Nile tilapia (*O. niloticus*) fed different protein levels at both densities are shown in Table 3. Feed intake increased significantly with increasing dietary protein level ($P<0.01$), but did not differ significantly at both stocking densities. Meanwhile, FCR, PER and PPV were significantly affected by protein level and stocking density ($P<0.01$). The best FCR was obtained with 45% protein diet at low density (1.72) and with 35% under low density (1.81) with insignificant difference ($P>0.01$), but the poorest FCR was obtained with 25% protein diet at low and high density (2.25 and 2.46, respectively). PER and PPV decreased with increasing protein level and stocking densities. The highest

values of PER and PPV were obtained with 25% CP at low (1.913 and 30.54%, respectively) and high density (1.720 and 26.64%, respectively) with significant difference ($P<0.05$). The lowest values of PER and PPV were obtained with the diet containing 45% CP at low and high density (1.340 and 1.25 for PER and 20.88% and 19.82% for PPV, respectively) with insignificant difference ($P>0.05$).

Results of the chemical composition of whole body fish are shown in Table 4. Moisture was significantly affected by protein level only ($P<0.05$) and did not differ as affected by stocking density. Other chemical components (crude protein, total lipids and ash) were significantly affected by protein level and stocking density. The highest protein content in fish body was obtained with 45% CP at low and high density (58.0% and 57.9%, respectively; $P>0.05$), while the lowest one was obtained with 25% CP at low and high density (54.34% and 55.0%, respectively; $P>0.05$). Carcass total lipids content decreased with increasing dietary protein level. The highest content of body lipids was existed in fish fed 25% protein diet at low and high density (32.8 and 31.0%, respectively; $P>0.05$), while the lowest one was obtained with fish fed 45% protein diet at low and high density (26.3 and 25.2%, respectively; $P>0.05$). The highest content of carcass ash was noticed in fish fed 45% protein diet at low and high density with significant difference (15.6% and 16.8%, respectively; $P<0.05$). Meanwhile, the lowest ones were obtained with fish fed 25% protein diet at low and high density with insignificant difference (13.0% and 13.9%, respectively; $P>0.05$).

DISCUSSION

The amount of researches concerning different tilapia species has increased significantly in the last years, including many areas of interest for aquaculture because of the high importance of tilapias allover the world. The stocking density and the optimum protein level still attract the attention of researchers, because these factors aim to higher profit, but without significant losses in growth ratio and environmental quality. Furthermore, the growth of tilapia depends upon the stocking density, dietary protein quality, energy content of the diet, the physiological status of the fish, age, reproductive state, and the environmental factors such as temperature, salinity...etc (Lovell, 1989).

It is important to take fish density into account when ranking families or progeny groups for growth performance, where fish density is an important factor affecting growth and maturation of wild and laboratory fish, besides food supply and its quality, genetics and environmental conditions (Smith *et al.*, 1978). Also, fish density could affect the efficiency of food utilization, where larger number of fish stocked in a pond decreases the amount of feed available to each fish.(Chang, 1988).

In this study, there was significant reduction in growth ($P<0.05$) with increasing stocking density at all protein levels. This result is in agreement with Huang and Chiu (1997) who studied the effects of stocking density (0.1, 0.2, 0.4, 1.6 and 3.2 fry/L.) for fry Nile tilapia, and found that the fish size and production were significantly affected by stocking density. Also, Canario *et al.* (1998) studied the effect of stocking density (0.35, 1.3 and 3.2 kg/m^3) on the growth of gilthead sea-bream, *Sparus aurata*, and found that fish in the highest density group grew 25% slower than fish in the lowest density group. Irwin *et al.* (1999) studied the effect of stocking density (0.7, 1.1, 1.5 and 1.8 kg/m^2) on the growth of turbot, *Scophthalmus maximus* for 45 days, and found that stocking density inversely affects growth rate and mean weights. Silva *et al.* (2000) also studied the effect of stocking density (2, 3 and 4 kg/m^3) on the growth of tetra-hybrid red tilapia, and found that final body weight gain was significantly higher at density of 2 and 3 kg/m^3, while the biggest biomass and feed consumption were observed at density of 4 kg/m^3. Moreover, other fish species showed an inverse relationship between stocking density and growth parameters, which were considered as the density dependent category, such as the cases found for Chinook salmon *Oncorhynchus tshawytscha* (Walbaum) (Martin and Wertheimer, 1989), Nile tilapia *O. niloticus* (Siddiqui *et al.*, 1989; Rosa *et al.*, 1990), African catfish (Haylor, 1991) and Arcatic charr, *Salvelinus alpinus* (JØrgensen *et al.*, 1993).

In this study, the condition factor and survival rates were not affected significantly with stocking density, because the stocking density here was below the critical level, however, the fish were small sized and with no competition for space. Also, Huang and Chiu (1997) found that condition factor and survival rate were not

affected significantly by stocking density. Moreover, the high survival rate of Nile tilapia at high density indicates its amenability to the intensive culture practice.

In the present study, the optimum dietary protein level for Nile tilapia fry reared at low and high density (15 and 30 fish/100 L, respectively) wa 45% and 25% respectively. It is also noticed that, the reduced growth can be recovered by increasing dietary protein level. Fish require diets relatively high in protein content, because their poor utilization of carbohydrates as energy source, thus sufficient supply of dietary protein is needed for rapid growth (Lovell, 1989). This result is in agreement with other authors. Tacon (1987) found that dietary protein level was varying from 42% for fry to 35% for growing adult fish. Moreover, EL-Sayed and Teshima (1991) reported that the dietary protein requirements of several species of tilapia have been estimated to range from 20 to 56%. Also, Al-Hafedh (1999) found that the better growth rate of Nile tilapia was obtained at high dietary protein levels (40-45%) rather than 25 - 35 % protein. On the other hand, Abdelghany (2000) and Khattab, *et al.* (2000) found that, the optimum dietary protein level for Nile tilapia was 35% and 37%, respectively.

In this study, feed intake was significantly affected by protein level, but not by stocking density. JØrgensen *et al.* (1993) found that daily feed intake of Arctic charr (*Salvelinus alpinus*) at stocking densities of 100 and 200 fish/m^3 was not significantly different. In this study, FCR decreased significantly ($P<0.05$) under low stocking density than high stocking density and the best FCR for Nile tilapia was obtained with 45% protein diet under low density. This result is in agreement with those obtained by Jauncey (1982), Ofojekwu and Ejike (1984) and De Silva and Perera (1985) who reported that FCR decreased with increasing dietary protein level. In this study, PER and PPV were affected by dietary protein level and stocking density, where their higher values were obtained under low density than high density. Dabrowski (1979) and De-Silva *et al.* (1989) found that PER decreased with increasing dietary protein content.

Contrarily, McGeachin *et al.* (1987) reported that growth of *O. aureus* (21.2 g average wt.) fed 36% protein diet in marine cages, where stocking densities of up to 300/m^3 was not affected. Also, Siddiqui *et al.* (1989) reported no difference in growth or FCR of *O. niloticus* (40.3 g average wt.) reared in brackish water (3.5 - 3.9

ppt) tank for 164 days on a 34% protein diet at densities of 16, 32 and 42.6 fish/m^3.

On the other hand, Watanabe *et al.* (1990) showed that the growth and feed conversion of Florida red tilapia fed two protein levels (28 and 32%) did not differ at densities ranging from 100 to 300/m^3. Also, they found that final mean weight, daily weight gain, specific growth rate and survival rate were higher and FCR lower for fish fed the 28% protein diet than those fed 32% protein diet under all densities. Omar *et al.* (1997) found that growth performance of Nile tilapia fingerlings fed with 30 and 40% crude protein and stocking densities 10, 30 and 40 fish/ 105L was significantly reduced. At higher dietary protein level and stocking densities, feed and nutrient efficiency, it was also significantly reduced. These results clearly showed that fish growth was best at the lowest stocking density and low dietary protein level tested.

This study revealed that the reduced growth at high stocking density might be recovered by increasing the dietary protein level for Nile tilapia fry. The optimum dietary protein is 45% for intensive fish farming in Egypt, where it realized the optimum growth and feed utilization

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Table 1: Ingredients and chemical composition and the experimental diet (on dry matter basis).

Ingredients	Dietary protein levels		
	25%	35%	45%
Fish meal	15.6	20.3	31.0
Soybean meal	20.0	40.0	50.0
Wheat bran	5.0	5.0	5.0
Ground corn	52.63	28.42	9.44
Fish oil + Corn oil (1:1)	2.0	2.0	2.0
Vitamins & minerals premix [1]	1.5	1.5	1.5
Ascorbic acid	0.06	0.06	0.06
Starch	2.21	1.72	0.0
Carboxymethyl cellulose	1.0	1.0	1.0
Total	100	100	100
Chemical analysis (%)			
Dry matter	92.48 ± 0.7	92.69 ± 0.6	93.09 ± 0.6
Crude protein	25.32 ± 0.24	35.41 ± 0.33	45.56 ± 0.46
Crude fat	5.87 ± 0.15	5.67 ± 0.25	5.99 ± 0.20
Ash	5.51 ± 0.23	6.31 ± 0.36	7.31 ± 0.37
Fiber	6.68 ± 0.15	5.50 ± 0.12	5.76 ± 0.13
NFE [2]	56.62	47.11	35.38
GE (Kcal/100 g) [3]	439.14	446.85	458.92

[1] Vitamin & minerals premix: each 2.5 kg contain vitamin A 12 MIU; D_3 2MI U, E 10 g; K 2g; B_1 1g; B_2 4g; B_6 1.5g; B_{12} 10mg; Pantothenic acid 10g; Nicotinic acid 20g; Folic acid 1g; Biotin 50mg; Choline chloride 500 mg; copper 10g; iodine 1g; iron 30g; manganese 55 g; zinc 55 g and selenium 0.1g.

[2] NFE (nitrogen free extract) = 100 – (protein + lipid + ash + fiber)

[3] GE (gross energy): Calculated after NRC (1993) as 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively.

Table 2. Different growth parameters of Nile tilapia (*O. niloticus*) fed different levels of dietary protein at two stocking densities (with 2-way ANOVA).

Items	Treatments					
	25% CP		35% CP		45% CP	
	S1	S2	S1	S2	S1	S2
Final weight (g/fish)	7.0 cd ± 0.14	6.4 d ± 0.17	8.9 b ± 0.23	7.6 c ± 0.09	10.1 a ± 0.03	9.1 b ± 0.43
Weight gain (g/fish)	4.9 cd ± 0.16	4.3 d ± 0.17	6.8 b ± 0.23	5.5 c ± 0.09	8.0 a ± 0.07	7.0 b ± 0.43
Wt. gain %	233.3	204.8	323.8	261.9	381.0	333.3
S G R (%)	1.720 d ± 0.032	1.591 d ± 0.041	2.063 b ± 0.04	1.837 c ± 0.016	2.344 a ± 0.016	2.094 ab ± 0.069
K factor (%)	1.563 ab ± 0.008	1.617 a ± 0.023	1.497 bc ± 0.026	1.560 ab ± 0.006	1.497 bc ± 0.031	1.530 bc ± 0.005
Survival (%)	93.3 a ± 1.9	93.3 a ± 2.2	93.3 a ± 2.2	95.6 a ± 1.13	96.7 a ± 2.2	96.7 a ± 1.9

Initial weight was 2.1 g/fish.
S1= 15 fish/100 L; S2 = 30 fish/100 L.
The same letter in the same row is not significantly different at $P<0.05$.

ANOVA

Source	df	Mean square				
		Final wt.	Gain	SGR	K factor	Survival rate
St	1	4.11**	3.83**	0.097**	0.004	30.42
Pr	2	12.35**	11.94**	0.387**	0.0104*	17.31
Pr x St	2	0.22	0.22	0.005	0.0048	2.42
Error	12	0.1506	0.1522	0.0049	0.0013	16.667

St = stocking density; Pr = protein level.
* $P<0.05$; ** $P<0.01$.

Table 3. **Feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) of Nile tilapia (*O. niloticus*) fed different dietary protein levels at two stocking densities (with 2-way ANOVA).**

Items	Treatments					
	25% CP		35% CP		45% CP	
	S1	S2	S1	S2	S1	S2
Feed intake (g feed/fish)	10.8 b ± 0.22	10.7 b ± 0.33	12.7 ab ± 0.13	11.6 ab ± 0.15	14.9 a ± 1.45	13.4 a ± 0.35
FCR	2.25 b ± 0.02	2.46 a ± 0.01	1.81 de ± 0.06	2.07 c ± 0.02	1.72 e ± 0.05	1.89 d ± 0.06
PER	1.913 a ± 0.052	1.720 b ± 0.052	1.623 b ± 0.049	1.467 c ± 0.043	1.340 cd ± 0.040	1.250 d ± 0.035
PPV (%)	30.54 a ± 0.88	26.64 b ± 0.77	24.82 b ± 0.72	22.25 c ± 0.64	20.88 cd ± 0.60	19.82 d ± 0.57

S1= 15 fish/100 L; S2 = 30 fish/100 L.

The same letter in the same row is not significantly different at P<0.05.

ANOVA

Source	df	Mean square			
		Feed intake	FCR	PER	PPV
St	1	0.281	0.216**	0.097**	28.4**
Pr	2	0.998**	0.501**	0.408**	103.5**
Pr x St	2	0.969	0.003	0.004	3.01
Error	12	1.2128	0.0051	0.0064	1.4919

St = stocking density; Pr = protein level.

* P<0.05; ** P<0.01.

Table 4. Proximate chemical analysis (%; on dry matter basis) of Nile tilapia (*O.niloticus*) fed different protein levels at two stocking densities (with 2-way ANOVA).

Items %	Initial	Treatments					
		25% CP		35% CP		45% CP	
		S1	S2	S1	S2	S1	S2
Moisture	75.5 ± 0.35	71.8 c ± 0.23	72.9 bc ± 0.31	73.5 ab ± 0.88	74.5 a ± 0.49	73.8 ab ± 0.39	73.4 ab ± 0.38
Crude protein	55.9 ± 0.17	54.34 c ± 0.33	55.0 c ± 0.10	56.5 b ± 0.29	57.7 a ± 0.23	58.0 a ± 0.15	57.9 a ± 0.34
Ether extract	30.3 ± 0.17	32.8 a ± 0.07	31.0 ab ± 0.32	28.83 bc ± 0.56	27.6 cd ± 0.54	26.3 d ± 1.64	25.2 d ± 0.36
Ash	13.8 ± 0.29	13.0 d ± 0.54	13.9 cd ± 0.17	14.7 c ± 0.13	14.6 c ± 0.23	15.6 b ± 0.20	16.8 a ± 0.10

S1= 15 fish/100 L; S2 = 30 fish/100 L.
The same letter in the same row is not significantly different at P<0.05.

ANOVA

Source	df	Mean square			
		Moisture	Crude protein	Total lipids	Ash
St	1	1.614	1.572*	8.36*	1.29*
Pr	2	4.658*	17.11**	57.04**	10.09**
Pr x St	2	1.035	0.671	0.242	0.601
Error	12	0.7283	0.197	1.755	0.2198

St = stocking density; Pr = protein level.
* P<0.05; ** P<0.01.