THE EFFECT OF FEEDING VARIOUS DIETARY PROTEIN LEVELS DURING GROWING ON GROWTH PERFORMANCE OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* L

MOHSEN ABDEL-TAWWAB[1], MOHAMMAD H. AHMAD[2] AND MEDHAT E. A. SEDEN[2]

1. *Department of Fish Ecology*
2. *Department of Fish Nutrition*

Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt
Corresponding author E-mail: mohsentawwab@yahoo.com

Abstract

This study was conducted to evaluate the effect of feeding schedules with different dietary protein content on the growth performance, feed utilization, whole-body chemical composition of Nile tilapia, *Oreochromis niloticus* cultured in cages (1 m^3 each). Fish were randomly distributed to the five treatments at a rate of 50 fish per cage. Twenty cages were randomly allocated in 4-replicate experiment. All fish fed on a 45% crude protein (CP) diet for the first 4 weeks to meet fish requirement in this stage. In the first group fish fed on a 45% CP diet for the last 8 weeks (T1), the second group fed on 35% CP diet for the last 8 weeks (T2), the third group fed on 35% CP diet for the second 4 weeks followed by 25% CP diet for the last 4 weeks (T3), the fourth group fed on 25% CP diet for the second 4 weeks followed by 35% CP diet for the last 4 weeks (T4), and the fifth group fed on 25% CP diet for the last 8 weeks (T5). Feed was offered to satiation and provided manually twice a day in the morning and in the afternoon), 5 days a week. The optimum fish performance was obtained at T1 - T3. Feed intake was higher at T1 - T3, than that at T4 and T5 and the best FCR was obtained at T2 and T3. PER were lower at T1 and T2 that that of T3 - T5, whereas APU was low in T1 only. On the other hand, no significant changes in EU values among the different treatments. There was no major change in the carcass composition except in ash content, which was higher in T4 and T5 only. This study recommended that the dietary protein schedule is an important management in tilapia culture, and the protein level could be reduced from 45% to 35% then to 25% with the increase in fish size.

Keywords: Nile tilapia, *Oreochromis niloticus*, growth performance, feeding regime, protein management, feed utilization, proximate chemical analyses.

INTRODUCTION

Diet supplementation is an important aspect of in aquaculture management especially in intensive or in semi-intensive fish culture, and is promising for increasing fish production (Diana, 1997; Abdelghany and Ahmed, 2002; Thankur *et al.*, 2004; Liti *et al.*, 2005; Abdel-Tawwab *et al.*, 2007). In aquaculture, diet is often the single largest operating cost item and can represent over 50% of the operating costs in intensive aquaculture (El-Sayed, 1999; 2004). This cost depends on many factors such

as protein level, the source, and type of ingredients that could be derived from plant or animal resources, and manufacture practices (see Glencross *et al.*, 2007). Apart from developing low-cost diets, different feeding management strategies such as on-demand feeding regimes (Andrew *et al.*, 2002; Velázquez *et al.*, 2006; Noble *et al.*, 2007) and/or good husbandry and pond management (Abdelghany *et al.*, 2002; Peterson and Small, 2006; Abdel-Tawwab *et al.*, 2007; Bascinar *et al.*, 2007; Kim *et al.*, 2007) could improve fish growth. The optimum feeding regimes/schedules of cultured fish is an important aspect in achieving efficient production and also could lead to significant saving in diet cost. The feeding schedule concept was developed taking into account the changes in the protein requirement and digestibility of cultured fish (Thoman *et al.*, 2004; Wu *et al.*, 2004; Hossain *et al.*, 2006). Therefore, optimization of dietary protein levels to accommodate the changing requirements of fish due to age/size could significantly enhance the protein utilization, thereby reducing the cost of diet formulation and nutrient loading into the culture system.

The culture of Nile tilapia, *Oreochromis niloticus* has been expanded dramatically in recent years in Egypt and worldwide (El-Sayed, 2006), and it is important to consider various protein regime/schedules and how they may influence tilapia growth and feed utilization. However, the fixed supplementation of high-protein diet would be wasteful because it is not digested to the same extent day in and day out. So, there is a possibility of saving significant amount of protein without hampering the growth through adaptation of protein schedules. This has led to hypothesis that it might be economical to feed the fish with diets of various protein content, instead of providing a constant level of recommended protein level in the diet. Therefore, the present study aimed to evaluate the effect of feeding schedules with different dietary protein content on growth performance, feed utilization, and whole-body chemical composition of Nile tilapia in cages.

MATERIALS AND METHODS

The experimental design and fish culture technique

Fry of Nile tilapia, Oreochromis niloticus (L.) were obtained from the fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Fish (1.5 - 2.2 g) were acclimatized in an outdoor cage for one week whereas fish were fed on low protein diet (20% crude protein) for satiation. After which, fish were transferred to the experimental cages. Before the experiment, a 100-g weight of fish was frozen at - 20 °C for chemical analysis at initial. Twenty fish cages (1 x 1 x 1.25 m width x length x depth) was inserted in 5 cement ponds (3 x 15 x 1.5 m W x L x D), however, each 4 replicates were inserted in one cement pond to

represent one treatment. The ponds were filled with freshwater derived from El-Ismailia canal and each cage was fixed 0.25 m above water surface. Fish were randomly distributed at a rate of 50 fish per cage. All cages fed on a 45% CP diet for the first 4 weeks to meet fish requirement in this stage. In the first group fish fed on 45% CP diet for the last 8 weeks (T1), the second group fed on 35% CP diet for the last 8 weeks (T2), the third group fed on 35% CP diet for the second 4 weeks followed by 25% CP diet for the last 4 weeks (T3), the fourth group fed on 25% CP diet for the second 4 weeks followed by 35% CP diet for the last 4 weeks (T4), and the fifth group fed on 25% CP diet for the last 8 weeks (T5). Feeding started on 1 August 2007 and continued for 12 weeks. The compositions of the supplemental diets are shown in Table 1. Fish were fed to satiation and the diets were offered manually twice a day (in the morning and in the afternoon), 6 days a week. Every week, one-half of ponds water was replaced by freshwater and fish from each cage were sampled every 2 weeks, and group-weighed to the nearest 0.1 g. Sampled fishes were returned to their respective cages immediately.

Table 1. Ingredients and proximate chemical composition (on dry matter basis) of the experimental diets.

Ingredients	Dietary protein levels		
	25%	35%	45%
Fish meal	11.25	16.11	21.97
Soybean meal	31.12	47.16	65.40
Ground corn	46.41	28.86	4.73
Corn oil	1.46	1.94	2.61
Cod oil	1.76	0.82	0.25
Minerals premix[1]	1.00	1.00	1.00
Vitamins premix[2]	1.00	1.00	1.00
Starch	6.00	3.11	3.04
Total	100	100	100
Chemical analysis (%)			
Dry matter	92.59	92.68	92.74
Crude protein	25.12	35.3	45.2
Crude fat	7.11	7.31	7.21
Ash	5.46	5.8	6.2
Fiber	5.99	5.7	5.5
NFE[3]	56.32	45.89	35.89
GE (Kcal/100 g)[4]	440.6	457.13	471.02

[1] Mineral premix (per kg of premix): $CaHPO_4.2H_2O$, 727.2 g; $MgCO_4.7H_2O$, 127.5 g; KCl 50.0 g; NaCl, 60.0 g; $FeC_6H_5O_7.3H_2O$, 25.0 g; $ZnCO_3$, 5.5 g; $MnCl_2.4H_2O$, 2.5 g; $Cu(OAc)_2.H_2O$, 0.785 g; $CoCl_3.6H_2O$, 0.477 g; $CaIO_3.6H_2O$, 0.295 g; $CrCl_3.6H_2O$, 0.128 g; $AlCl_3.6H_2O$, 0.54 g; Na_2SeO_3, 0.03 g.

[2] Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; α-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

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

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

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Vitamins premix[2]	1.00	1.00	1.00
Starch	6.00	3.11	3.04
Total	100	100	100
Chemical analysis (%)			
Dry matter	92.59	92.68	92.74
Crude protein	25.12	35.3	45.2
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Ash	5.46	5.8	6.2
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Water quality analyses

Water samples for chemical analyses were collected at 2-weeks intervals from four sites from each cement pond between 09:00 and 09:30 am at 30 cm depth. Dissolved oxygen and water temperature were measured at 30 cm depth with a YSI model 58 Oxygen Meter (Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Water conductivity was measured with a YSI model 33 Conductivity Meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Unionized ammonia was measured by using HACH kits (Hach Co., Loveland, CO, USA). The pH was measured using a pH-meter (Fisher Scientific, Denver, CO, USA). Total alkalinity and total hardness were measured by titration as described by Boyd (1984). Chlorophyll 'a' was determined by filtering 100 mL of water sample through Millipore filter paper (0.45 μm) and extracting it in 90% acetone. Chlorophyll 'a' was then measured spectrophotometrically according to Boyd (1984).

In all treatments, dissolved oxygen concentrations ranged from 6.6 to 7.4 mg/L. The ambient water temperature range was 24.5. 26.8 C. The pH range was 7.8 - 8.1, and unionized ammonia concentration ranged from 0.11 to 0.19 mg/L. Total alkalinity and total hardness ranges were 250 - 285 mg/L as CaCO3, and 235 - 290 mg/L as CaCO3, resp ctively. Chlorophyll 'a' content ranged from 7.6 to 11.4 μg/L. All previous water parameters are within the acceptable ranges for fish growth (Boyd, 1984).

Growth performance

Growth performance was determined and feed utilization was calculated as follows:

Weight gain = W2 - W1; where W1 and W2 are the initial and final fish weight, respectively; Daily weight gain = weight gain / T; where T is the number of days in the feeding period;

Specific growth rate (SGR) = 100 (lnW2 - lnW1) / T;

Feed conversion ratio (FCR) = feed intake / weight gain;

Protein efficiency ratio (PER) = weight gain / protein intake;

Apparent protein utilization (APU %) = 100 x (protein gain / protein intake);

Energy utilization (EU %) = 100 (energy gain / energy intake).

Proximate chemical analyses

After growth trial, five fish were collected from each cage and the proximate chemical analyses of whole-fish body were done according to the standard methods of AOAC (1990) for moisture, protein, total lipids, and ash. Moisture content was estimated by drying the samples to constant weight at 85 C in a drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by

6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours and ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 hours.

Statistical analysis

The obtained data were subjected to one-way ANOVA and mean separations were determined by using Duncan's multiple range test. Differences were considered significant at $P \leq 0.05$. All statistical analyses were made using SPSS for windows version 10 (SPSS, Richmond, Virginia, USA) as described by Dytham (1999).

RESULTS

Growth performance of Nile tilapia was significantly affected by protein management (Table 2). Fish in all treatments gradually grew with time, and the highest individual weight was obtained in the 12th week (Fig 1). The optimum final weight, weight gain, and SGR of Nile tilapia were obtained in T1 - T3, whereas the poorest growth performance was obtained at T4 - T5 ($P < 0.05$). Survival rate among the different treatments was almost the same (100%) except that of T2 and T5 (98.3%; Table 2).

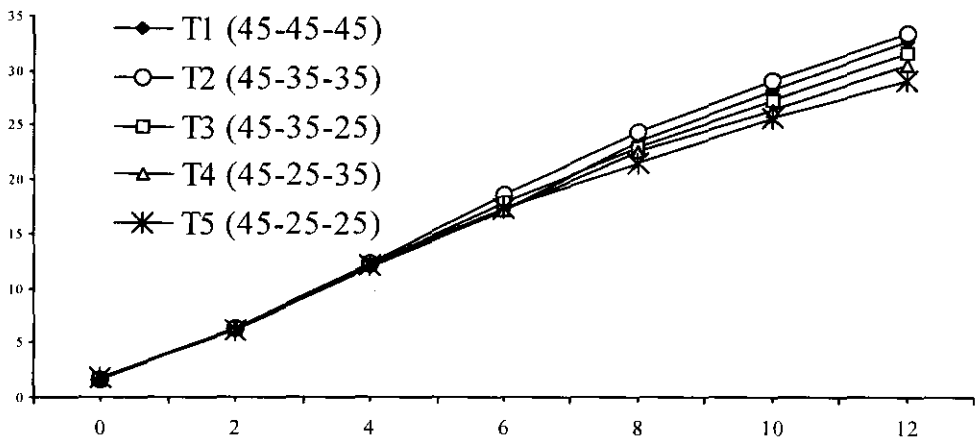

Figure 1. Changes in live body weight (g) of fry Nile tilapia fed on different protein schedules in cages for 12 weeks.

Feed utilization was approximately the same in T1 - T3, decreasing significantly in T4 and T5 ($P > 0.05$). FCR was significantly higher in T1 and T4 ($P<0.05$), while the best FCR was obtained at T2 and T3 (1.43 and 1.45, respectively; $P > 0.05$), while the poorest FCR was obtained at T5 (1.54; Table 2). As fish in all treatments grew, feed consumed and FCR increased by time and the highest (Figs 2 and 3).

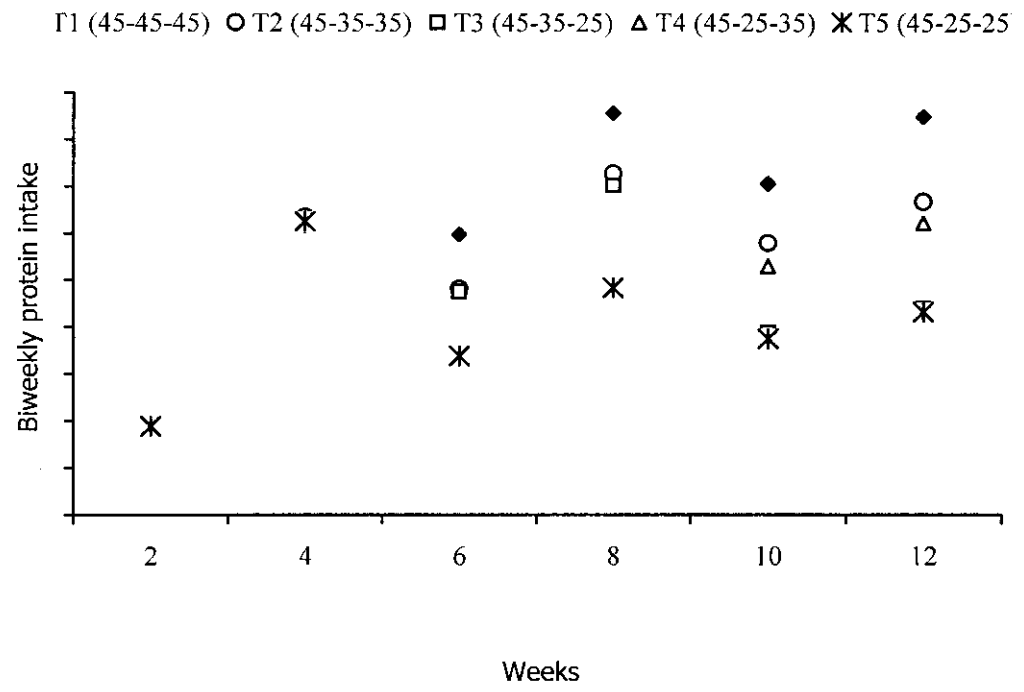

Figure 2. Changes in biweekly protein intake by fry Nile tilapia fed on different protein schedules in cages for 12 weeks.

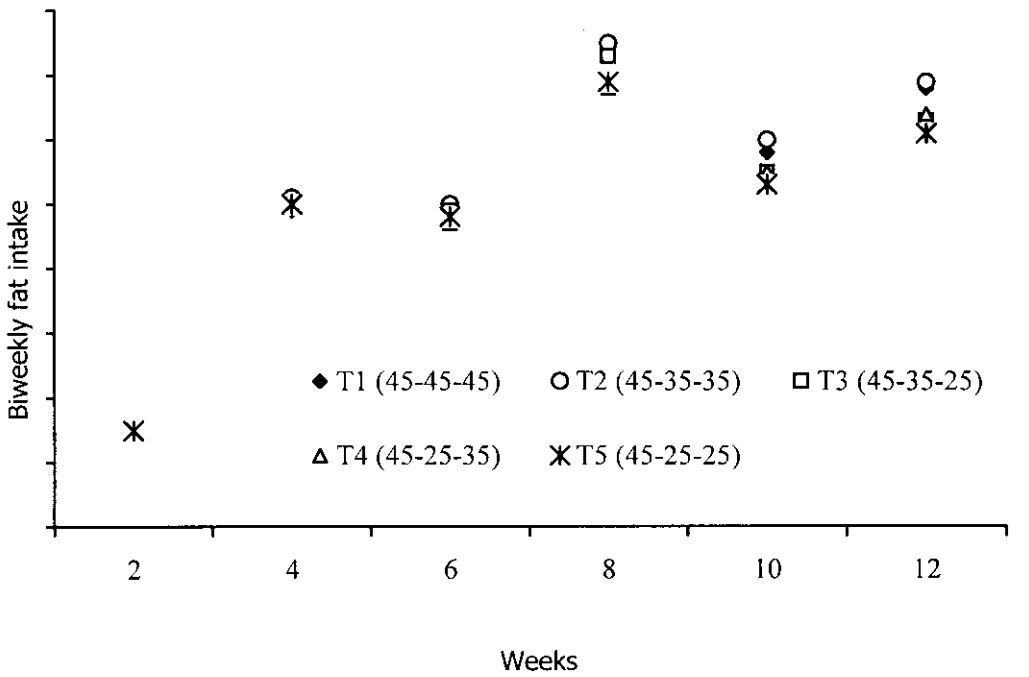

Figure 3. Changes in biweekly fat intake by fry Nile tilapia fed on different protein schedules in cages for 12 weeks.

TABLE 2. Growth performance, feed utilization, and production of Nile tilapia fed on diets with different protein schedules.

Item	45 – 45 – 45	45 – 35 – 35	45 – 35 – 25	45 – 25 – 35	45 – 25 – 25
	T1	T2	T3	T4	T5
Initial weight (g)	1.87±0.024 a	1.86±0.011 a	1.85±0.018 a	1.87±0.013 a	1.89±0.014 a
Final weight (g)	32.60±0.68 a	33.30±0.51 a	31.48±0.46 ab	30.58±0.47 b	29.10±0.45 b
Weight gain (g)	30.73±0.67 a	31.44±0.52 a	29.63±0.44 ab	28.71±0.49 b	27.21±0.46 b
Specific growth rate (%/day)	3.448±0.021 ab	3.541±0.027 a	3.475±0.014 ab	3.369±0.028 b	3.313±0.025 b
Survival rate (%)	100.0±0.0 a	98.3±1.7 a	100.0±0.0 a	100.0±0.0 a	98.3±1.7 a
Feed intake (g feed/fish)	45.5±0.52 ab	47.5±0.15 a	45.7±0.39 ab	44.9±0.28 b	44.8±0.22 b
Feed conversion ratio	1.48±0.039 ab	1.43±0.024 b	1.45±0.030 b	1.47±0.030 ab	1.54±0.023 a
Protein efficient ratio	1.61±0.082 c	1.91±0.110 bc	2.13±0.204 ab	2.03±0.110 ab	2.23±0.127 a
Apparent protein utilization (%)	25.94±0.754 c	30.47±1.758 b	33.67±2.016 a	31.50±1.819 ab	34.21±1.943 a
Energy utilization (%)	21.90±1.264 a	21.63±1.236 a	20.43±1.178 a	20.12±1.166 a	20.07±1.102 a

Means with same letter in same row are not significantly differed at $P < 0.05$.

Protein utilization was assessed through protein dependant parameters i.e. PER and APU. It is noticed that the lowest values of PER were obtained at T1 and T2 (1.61 and 1.91, respectively), whereas the highest PER values in T3 - T5 were approximately the same. Moreover, APU did not significantly affected by protein management in T2 - T5 (30.47 - 34.21%), meanwhile APU in T1 was the lowest (25.94%; $P < 0.05$). On the other hand, no significant changes in EU values among the different treatments ($P > 0.05$).

The contents of moisture, crude protein, and crude lipids in whole-fish body were not significantly affected by protein management ($P > 0.05$; Table 3). Ash content in whole-fish body was higher in treatments T4 and T5 (14.8 and 14.9%), while ash content in T1 - T3 did not vary ($P > 0.05$; Table 3).

Table 3. Carcass proximate chemical analyses (%; on dry matter basis) of Nile tilapia fed on diets with different protein schedules in cages.

Item	45 – 45 – 45	45 – 35 – 35	45 – 35 – 25	45 – 25 – 35	45 – 25 – 25
	T1	T2	T3	T4	T5
Moisture	75.3±0.76 a	75.4±0.42 a	75.8±0.52 a	75.9±0.38 a	75.7±0.69 a
Crude protein	64.7±1.08 a	64.4±1.55 a	65.0±1.13 a	64.1±1.28 a	62.8±1.48 a
Total lipids	20.2±1.05 a	20.9±1.16 a	20.1±1.13 a	20.3±0.85 a	21.4±0.73 a
Ash	13.2±0.25 b	13.7±0.42 b	13.6±0.28 b	14.8±0.57 a	14.9±0.26 a

Means with same letter in same row are not significantly different at $P < 0.05$.

DISCUSSION

As tilapia culture continues to expand worldwide, there is a growing pressure to minimize production cost associated with diet supplementation. Optimization of diets and feeding strategies are two mechanisms that could be utilized to help reach these goals. To facilitate the reduction in nutrient loading within culture systems, the concept of low-pollution diets has been adopted as a means to minimize waste output while maximizing the mass of fish produced (Cho *et al.*, 1994). In this regard, Hossain *et al.* (2006) demonstrated that the feeding with various protein schedules also help to reduce the nitrogen inputs substantially, as compared to continuous feeding of high-protein diets, thereby reducing the nitrogen loading into the culture systems.

Dietary protein is always considered to be of primary importance in fish feeding (Jauncey and Ross, 1982), thus sufficient supply of dietary protein is needed for rapid growth (Lovell, 1989). In the present study, results revealed that the optimum dietary protein management was obtained at T3. The high protein level in T1 did not significantly enhance the fish growth over other treatments. This result may be due to the fact that each fish size has a certain protein limit after which excess protein level could not be utilized efficiently (Siddiqui *et al.*, 1988; Wilson, 1989; Pillay, 1990; El-Sayed and Teshima, 1991; Ahmad *et al.*, 2004).

Hossain *et al.* (2006) demonstrated that there was no significant difference between the weight gain of sutchi catfish, *Pangasius hypophthalmus* (Sauvage) fed with a high-protein diet continuously (30% CP; H) and fish fed with one-day low protein (15% CP; L) and one day high-protein diets. The weight gain values in H and 1L/1H schedules were significantly higher than those obtained with other feeding schedules. Also, Nandeesha *et al.* (1993, 1995) and Srikanth *et al.* (1989) found similar results with Indian carp catla, *Catla catla* and common carp, *Cyprinus carpio*, respectively.

De Silva and Perera (1984) observed rhythmic variation in the protein digestibility of Nile tilapia, *O. niloticus* under laboratory conditions. They hypothesized that continuous, high-protein feeding may be wasteful as on a day when the digestibility efficiency of a fish is high, relatively lower protein intake would be sufficient to fulfill the requirement and vice versa. De Silva (1985) demonstrated that under laboratory conditions for Nile tilapia, by adopting mixed-feeding schedules, where a high-protein diet is alternated with a low-protein diet could obtain growth rates similar to those for fish maintained continuously on a high-protein diet. He pointed out that adoption of a mixed-feeding schedule would also result in substantial saving in diet cost.

Many authors worldwide studied the effect of dietary protein levels on the growth of Nile tilapia in different locations with conflicting results. The dietary protein requirements of several species of tilapia have been estimated to range between 20% and 56% (El-Sayed and Teshima, 1991). Hamza and Kenawy (1997) found that the diet of 40% protein proved to be more potent than other levels for Nile tilapia growth. Al-Hafedh (1999) and Al-Hafedh *et al.* (1999) found that better growth of Nile tilapia was obtained at high dietary protein levels (40-45%) compared to 25-35%. Ahmad *et al.* (2004) found that protein requirement for Nile tilapia is size dependant, however, fry need 45% CP, fingerlings need 35%, and fattening fish need 25% CP.

The continues feeding with a high protein diet (45% CP) in T1 neither enhanced feed intake nor FCR. However, feed intake was approximately the same in T1 - T3, and the best FCR was obtained at T2 and T3 (1.43 - 1.45, respectively). These FCR values are lower than that found by Al-Hafedh (1999) who found that FCR ranged from 1.6 to 2.5 for fry (0.51 g) and from 3.13 to 4.86 for fingerlings fish (45 g). This difference in FCR values may be because Al-Hafedh (1999) experiment was conducted in winter (18 - 25 °C). In this study, water temperature was 24.5 - 26.8 °C, which represents the optimum range of temperature for Nile tilapia growth (Boyd, 1984).

PER and APU are used as indicators of protein quantity and quality in the fish diet and fish body. So, these parameters are used to assess protein utilization and turnover, where they are related to dietary protein intake and its conversion into protein gain. In this study, the highest PER and APU values were obtained at T3 and T5. These results may be because the major part of weight gain is related to the deposition of protein, and the protein accretion is a balance between protein anabolism and catabolism. Jauncey (1982) reported that protein efficiency ratio and protein retention are known to be high at low level of protein inputs.

Hossain *et al.* (2006) demonstrated that FCR and PER values were lower in sutchi catfish when fed high protein diet (H) and other mixed-feeding schedules as compared to continuous feeding with low-protein diets (L). Similar trends were

reported for Indian major carps and common carp (Srikanth *et al.*, 1989; Nandeesha *et al.*, 1993, 1995). Furthermore, gastric emptying rate or solubility of the protein has been shown to affect the absorption and the utilization of dietary protein (Boirie *et al.* (1997; de la Higuera *et al.*, 1998; Epse *et al.*, 1999).

In the present study, PER ranged from 1.61-2.23 in an agreement with the findings of Al-Hafedh (1999), whereas PER in study of Khattab *et al.* (2000) ranged from 1.25 to1.98 for Nile tilapia collected from different locations in Egypt. Dabrowski (1979) reported different patterns of changes in PER in relation to dietary protein level and found that the relationship between dietary protein and PER differs from species to species. Jauncey (1982) and De Silva *et al.* (1989) also reported that FCR and PER decreased with increasing dietary protein level.

The contents of moisture, crude protein, and crude lipids in whole-fish body were not significantly affected by protein management except ash content, which was higher in fish body fed on T4 and T5. These results suggested that nutrient digestibility and deposition may not been affected by protein management. On the other hand, protein and lipids contents in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rate (Smith, 1981; Fauconneau, 1984; Soivio *et al.*, 1989; Abdel-Tawwab *et al.*, 2006).

Finally, it could be recommended that protein schedule is an important management in tilapia culture, and the protein level could be reduced from 45% to 35% then to 25% with the increase in fish size.

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