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Optimum dietary protein level in supplementary feed for *Oreochromis niloticus* fry reared in net enclosures

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Abstract

The present experiment was carried out to determine the Optimum dietary protein level (DPL) in supplementary diets for Nile tilapia, *Oreochromis niloticus* fry. Three diets containing 20, 25 and 30% CP were compared to a control group (natural food group) through studying their effects on growth performance, feed utilization and carcass composition. Four groups each of twenty five tilapia fry with an average initial weight of 0.31±0.10 g /fish were placed in twelve net enclosures. Fish were fed twice a day (9.00 a.m. and 14.00 p.m.) six days a week for 90 days. Feeding rates described as: for the 1st 30 days on 15% , for the 2nd 30 days on 10% and the last 30 days of the entire period on 5% of fresh body weight. The results showed significant differences at ($P \leq 0.05$) in the final individual fish fresh body weights, average daily gain (ADG g/fish), specific growth rates (SGR %), feed conversion ratios (FCR), protein efficiency ratios (PER), and energy utilization (EU%) among the tested groups. Fish fed on the diet containing 25% DPL exhibited better growth performance in comparison to those fed on 30, 20% DPL or natural food group. The highest growth performance of *O. niloticus* was obtained at 25% DPL and the poorest was obtained at 30% DPL or natural food group. The lowest PER and PPV% of *O. niloticus* fry were obtained at 30% DPL. EU% increased by increasing DPL up to 25%, but there were no significant differences between 25% and 30% DPL. Increasing the DPL from 20 to 25% resulted in a significant increase at ($P \leq 0.05$) in the dry matter (DM), crude protein (CP) and values of energy content (Eco) of the whole fish body, while ash content decreased. On the other hand, lipid content decreased significantly in all treatments at ($P \leq 0.05$).Therefore, the current results confirmed that the diet containing 25% DPL is considered optimal for better performances of Nile tilapia fry under the present experimental conditions.

Keywords: Nile tilapia, fry, dietary protein levels, growth performance, nutrient utilization, and carcass composition.

1. Introduction

The Egyptian aquaculture production of Tilapia fish represents about 60% of total fish production (GAFRD, 2007). Tilapia is an ideal candidate for warm-water aquaculture. They spawn easily in captivity, use a wide variety of natural food as well as formulated feeds, tolerate poor water quality, and grow rapidly at warm temperatures. These attributes, along with relatively low input costs, have made tilapia widely cultured freshwater fish in tropical and subtropical countries (Biswas *et al*., 2005; El-Saidy and Gaber 2005; Peña-Mendoza *et al*., 2005; Borgeson *et al*., 2006; Tsadik and Bar, 2007 and Tahoun , 2007).

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 the ingredients that could be derived from plant or animal resources and manufacture practices (Glencross *et al*., 2007) Apart from developing low-cost diets different feeding management strategies such as on- demand feeding regimes (Andrew *et al*., 2002; Velazquez *et al*., 2006; Nobel *et al*., 2007).

Tilapias are categorized as herbivorous fish. They have special adaptations to separate algae and other particulate matter from water for ingestion, (Pullin and Lowe-Mc-Connell, 1982). Adult Nile tilapia *O. niloticus* are omnivores (Philippart, 1982), and feed on detritus, blue green or green algae, diatoms, macrophytes and Bacteria (Bowen, 1982). Protein requirements for optimum growth of the fish seem to be affected by numerous factors such as temperature, salinity, fish age and size, etc. (Cowey, 1976). However, Nile tilapia (31 g) fed with graded dietary protein from 14% to 30% and raised under laboratory conditions grows up to 100 gm without significant difference in growth rate (El-Dahhar *et al*., 1999). Under semi-intensive conditions which used *Chlorella and Scenedesmus sp* in green water systems are used and feeding is supplemented with low protein diets (up to 25%), lower fry densities must be used under these conditions (Balarin and Haller, 1982).

Therefore, the objective of this study was to assess the optimum protein level in the diets compared with control (natural food) improved growth performance, feed, and protein and energy utilization of Nile tilapia, *Oreochromis niloticus* fry reared in net enclosures.

2. Materials and methods

2.1. Fish and culture facility

Nile tilapia, (*Oreochromis niloticus*) fry of 0.31±0.10 g/fish obtained from Berseek Fish Hatchery, El-Behera Governorate were used in the present study. The experiment began on 1st September (2007) and ended after 90 days at the El-Max research station , National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt.

Twelve net-enclosures measuring (100 X 100 X 100 cm) were placed in an earthen pond (0.50 feddan and 1.00 m depth), each stocked with twenty five fish. The net-enclosures were randomly allocated into four treatments (three net-enclosures / treatment).

2.2. Experimental Diets

The control treatment, fish did not receive artificial feeding; however, the other three treatments 1, 2 and 3 three tested diets were formulated to contain dietary protein level 20, 25 and 30% as shown in Table 1. Ingredients were finely ground in a house blender and used in preparation of the experimental diets. Few drops of sunflower oil were added in the same time of mixing warm water (45°C) which was slowly added until the diets began to clump. Diets were processed by a California pellet mill machine and dried for 48 hrs at 60 - 80°C in a drying oven. The experimental pellets were soft enough for the fish to take and retain. The processed diet particle size was 0.6 mm in length and 2 mm diameter. The experimental fish were fed the test diets for one week as adaptation period for test diets. After the adaptation period, fish in each net enclosure were reweighed, and their initial weights were recorded. Fish were fed twice daily (9.00 and 14.00 hr) six days a week for 90 days. Feeding rates are described as: 15%, for first 30 days, then reduced to 10 % and 5 % for second and third months respectively of fresh body weight.

Table 1. Composition, and Proximate analysis of the experimental diets contain different dietary protein levels.

Item	Diets.		
	protein levels 20%	protein levels 25%	protein levels 30%
Ingredient (g/100g):-			
Fish meal	20	25	35
Soybean meal	15	20	15
Wheat bran	25	25	15
Yellow corn	35	25	25
Corn oil	3	3	3
Vitamin & mineral mix[2].	2	2	2
Proximate analysis (% dry weigh) [1]			
Dry matter	94.51	95.72	96.23
Crude protein	19.5	24.9	29.4
Ether extract	7.52	6.87	6.52
Crude fiber	3.32	2.81	2.25
Ash	3.32	2.81	2.25
Nitrogen free extract	8.84	9.53	10.92
Gross energy (GE) Kcal/100 g diet[2]	432.91	436.17	439.18
P/E ratio (mg protein/kcal)[3]	47.12	58.00	70.13

[1] Premix supplied the following vitamins and minerals (mg or IU) / kg of diet, vit. A, 8000 IU; vit. D3, 4000 IU; vit. E,50 IU; vit K3, 19 IU; vit. B2 25 mg; vit. B3, 69 mg; Nicotinic acid, 125 mg;Thiamin,10 mg; Folic acid, 7 mg; Biotin,7mg; vit. B12,75mg; Cholin, 400 mg; vit. C, 200mg; Mn, 350 mg; Zinc, 325 mg.
[2] Gross energy values were calculated according to NRC, (1993) using the following Calorific Values: 5.65, 9.45 and 4.12 (GE Kcal/100 g diet) of protein, ether extract (lipids) and Carbohydrates, respectively.
[3] P/E ratio =Protein/energy (P/E) ratio (mg CP/ Kcal GE).

2.3.Water Analysis

Water quality parameters such as dissolved oxygen, temperature, pH, ammonia, nitrate, nitrite and salinity were monitored periodicaly (every 14 days) during the experimental period. Dissolved oxygen was measured using an oxygenmeter; temperature was measured using a simple thermometer; ammonia; nitrate and nitrite were measured using a DREL, 2000 Spectrophotometer; salinity was measured using salinometer and pH was measured using pH meter.

2.4. Body composition analysis

At the beginning of the experiments, about twenty fish were collected and immediately frozen and reserved for initial proximate body chemical analysis. At the termination of the study, all fish in each net-enclosures were netted, weighed, frozen and kept for final body composition analyses. Fish samples were pulverized, and homogenized with Ultra-Tunax. The homogenized samples were oven dried at 60 - 80°C for 48 hrs. Proximate analyses of whole body, protein, lipid, and ash were performed according to standard AOAC (2000) methods.

2.5. Growth performance parameters

Total weight gain, average daily gain, specific growth rate, feed conversion ratio protein and energy utilization were determined according to Recker (1975) and Castell and Tiews (1980).

1) Total weight gain (g/fish) = (W_T-W_I)

Where: W_T: Final weight means of fish in grams and W_I: Initial weight means of fish in grams

2) Average daily gain (ADG) (g/fish/day) = total gain / time period (days).

3) Specific growth rate (SGR) % / day) = 100 × (Ln W_T - Ln W_I) / T

Where: Ln: Natural log and T is the number of days in the feeding period.

2.6. Nutrient Utilization parameters

1) Feed conversion ratio (FCR) = total feed intake (g) / total gain (g).

2) Protein efficiency ratio (PER) = total gain (g) / protein intake (g)

3) Protein productive value (PPV %) = (P_T – P_I) ×100 / protein intake (g)

Where: P_T: Final body protein content in fish carcass and P_I: Initial body protein content.

4) Energy utilization (EU %) = (E_T–E_I) ×100 / Energy intake (kcal)

Where: E_T: final energy amount in fish carcass (kcal) and E_I: initial energy amount in fish carcass (kcal).

2.7. Nitrogen Free Extract

Nitrogen free extract in the experimental diets was calculated using the following equation:

$$NFE=100- (Moisture + CP + EE + CF)$$

Where: NFE= Nitrogen Free Extract; CP= Crude Protein; EE=Ether Extract and, CF= Crude Fiber

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2.8. Statistical Analyses

Statistical analyses were performed using ANOVA, F-test, and L.S.D. procedures available within the MSTAT- C software package **(ver. 1.2, 1998).**

3. Results

3.1. Water quality investigation:

The water quality parameters monitored were within tolerable limits for Nile tilapia *O. niloticus* , fry. Recorded values were within the following ranges:

(a) Dissolved oxygen from 4.5 to 8.0 mg/l.
(b) pH from 6.5 to 7.8.
(c) Total ammonia from 0.10 to 1.5 mg/l.
(d) Nitrate from 3.5 to 5 mg/l.
(e) Nitrite from 0.01 to 0.65 mg/l
(f) Salinity from 2.00 to 3.50 ppm.
(g) Temperature from 21 to 24°C

3.2. Experimental diets

The proximate chemical analysis (%) of the experimental diets. Diets are shown in Table 1. Diets were approximately isoenergtic and containing different crude protein levels (20, 25, and 30% protein), respectively.

3.3. Growth performance

The effects of dietary protein levels (20, 25, and 30% DPL) compared with control group (natural food) on final body weight, weight gain; average daily gain (ADG g/fish/day) and specific growth rate (SGR % /day) are summarized in Table 2. Final body weight and weight gain showed a significant at ($P< 0.05$) by increasing the DPL up to 25% followed by 30% and control group, respectively.

The optimum ADG of Nile tilapia fry (0.30 g/ fish) was obtained with the 25% and 30% dietary protein with insignificant difference at ($P> 0.05$), while the poorest ADG was significantly obtained from fish fed on natural food only (control group).

The highest SGR%/ day of Nile tilapia fry was obtained at the 25%DPL followed by those fed on 20% DPL, the poorest SGR were obtained at 30% DPL and control group, respectively.

Table 2. Growth performance and Nutrient utilization parameters of Nile tilapia (*O. niloticus*) fed on different dietary protein levels.

Item	Control (Natural food)	Diets			L.S.D ($P<0.05$)[1]
		protein levels 20%	protein levels 25%	protein levels 30%	
Initial live weight(g/fish)	0.30±0.10	0.30±0.10	0.29±0.11	0.33±0.10	N.S
Final live weight (g/fish)	6.33±0.04^{d}	17.78±0.08^{c}	18.53±0.06^{a}	18.2±0.06^{b}	0.098
Weight gain (g/fish)	6.03±0.03^{d}	17.48±0.10^{c}	18.24±0.07^{a}	17.87±0.03^{b}	0.107
Average daily gain (g/day/fish)	0.07±0.00^{c}	0.19±.0.00^{b}	0.20±0.00^{a}	0.20±0.00^{ab}	0.009
Specific growth rate (%/day)	3.39±0.06^{c}	4.54±0.10^{ab}	4.61±0.05^{a}	4.46±0.09b	0.126
Feed intake (FI)	-	26.36±0.08^{a}	22.96±0.59^{b}	21.03±0.05^{c}	0.691
Feed conversion ratio (FCR)	-	1.51±0.01^{a}	1.26±0.03^{b}	1.18±0.01^{c}	0.036
Protein efficiency ratio (PER)	-	3.25±0.01^{a}	3.14±0.07^{a}	2.76±0.01^{b}	0.036
Protein productive value (PPV %)	-	47.16±0.13^{a}	47.08±1.57^{a}	38.86±0.07^{b}	3.12

[1]The mean in the same column bearing different superscript are significantly different at ($P< 0.05$) (n=75).

Table 3. Body composition of Nile tilapia (*O. niloticus*) fed on different dietary protein levels.

Protein levels %	Dry Matter %	% On dry wt. basis			Energy Content (Kcal/100g)
		Crude Protein	Lipid content	Ash	
At the start					
	23.8	53.8	22.54	19.5	500.1
At the end					
Control	24.5±0.07[c]	54.95±0.55[d]	22.31±0.18[a]	22.41±0.24[a]	520.5 ±1.53[c]
20	25.25±0.23[b]	57.33±0.53[b]	21.85±0.21[a]	20.82±0.31[bc]	529.6 ±4.79[ab]
25	25.78±0.24[a]	58.6±0.42[a]	21.72±0.30[a]	20.28±0.12[c]	535.5 ±4.65[a]
30	24.86±0.43[bc]	56.57±0.14[c]	21.92±0.79[a]	21.51±1.35[ab]	526.0 ±6.67[bc]
L.S.D(P<0.05)[1]	0.443	0.72	0.72	1.15	7.79

[1]Themean in the same column bearing different superscript are significantly different at (P< 0.05)

3.4. Feed and nutrient utilization

Feed conversion ratio (FCR); protein efficiency ratio (PER); protein productive value (PPV) and energy utilization (UE%) of Nile tilapia fry were significantly affected by DPL. Results of feed intake and FCR are shown in Table 2. The best FCR for Nile tilapia fry was obtained from 25% and 30% DPL with significant difference at ($P \leq 0.05$) as their values were 1.26 and 1.18, respectively, while the poorest FCR was obtained from 20% protein diets 1.51.

The lowest values of PER and PPV were obtained with diets containing 30% protein with significant difference at ($P \leq 0.05$) treatments (2.76 and 38.86, respectively). Energy utilization (%) increased with increasing dietary protein level, but there were insignificant difference between feeding treatments 25% and 30% DPL.

3.5. Body composition

Results of whole body composition of fish fed diets containing 20, 25 and 30% DPL are summarized in Table 3. The dry matter (DM %) and crude protein (CP %) were affected by DPL. The highest DM and CP in whole fish body were obtained from 25% DPL. The ash content increased with increasing the DPL, and there were insignificant difference between treatments at ($P \leq 0.05$).

4. Discussion

Protein is always considered of primary importance in fish (Jauncey and ross, 1982), thus significant protein supply is needed for rapid growth (Lovell, 1989). Protein is the most expensive macro nutrient in fish diet (Pillay, 1990).

The present study revealed significant effect of dietary protein level on growth performance of Nile tilapia fry as SGR increased by increasing dietary protein level from 20% to 25% but there were no significant difference.Similar findings have been reported in different authors for different tilapia species. El-Sayed and Teshima 1992) estimated that the dietary protein requirements of several species of tilapia ranging from 20% to 56% .The highest growth rate obtained in the present study may be due to the contribution of natural food organisms meeting daily requirements of the reared fish. Nile tilapia fry are diversified feeders, feeding on green and blue green algae, zooplankton and benthic organisms which contain high amount of protein and are believed to provide additional protein to fish (Omar, 1994). Lovell (1975) observed that natural food plays a key role in the determination of dietary protein requirements of fish under pond conditions. Results of growth performance of the present study are in partial agreement with the findings of Shiau and Hung (1990) who found better growth of hybrid tilapia fed on a diet containing 21% crude protein .On the other hand El-Sayed and Teshima (1992) found the optimal growth of Nile tilapia with fry which fed on a diet containing 45% crude protein. Winfree(1992) reported that many fish require live food when they are newly hatched because their mouth parts are so small. So, 20% dietary protein can be used in the presence of natural food which is found in earthen pond used in the current experiment for fry feeding. These results are in agreement with [Clark *et al.*, (1990); Shiau and Hung *et al.*(1990); Khater and Dawah,(2008)]who found the feeding supplemented with low protein diets (25%) with green algae for growth performance is more economical for rearing of *O. niloticus* and *O. aureus* fry. However, these results are in disagreement with El-Sagheer, (2001) who reported that increasing dietary protein level from 25% to 32% increased growth performance and nutrient utilization of tilapia. On the other hand, Bahnasawy (2009) found that a diet containing 30% crude protein is considered optimal for growth of monosex Nile tilapia (2.5 g average initial weight). Ahmad *et al*. (2004) found that dietary protein level was 45% for fry and 35% for growing adult without natural food. Also Tacon (1987) found that dietary protein level varies from 42% for fry and 35% for growing adult and Al-Hafedh *et al*. (1999) found that the best growth of Nile tilapia was obtained at high dietary protein levels 40 - 45% rather than 25-35% protein in the absence of live algae.

Feed conversion ratio (FCR) decreased with increasing dietary protein levels. The best FCR was obtained from 30% protein diet, while the poorest FCR was obtained by 20% protein diet, and there were significant differences between FCR obtained by 20, 25, and 30% protein deit. These results agree with the results of previous studies on tilapia species by Shiau and Hung (1990); Khater and Dawah (2008). On the other hand, Bahnasawy, (2009) found that better FCR values were obtained by diet contains 30% or 35%.

Protein efficiency ratio (PER); protein productive value (PPV %) and energy utilization EU in the present study are significantly affected by dietary protein level. The decrease of PER and PPV with increasing dietary protein level, the current results confirmed those reported by different author for different tilapia species (Kheir, 1997 and Ahmed *et al*., 2004).

4.1. Carcass composition of fish

In the present study, DM%, CP% and energy content within each group increased with increasing the dietary protein levels up to 25% DLP. However the ash content decreased. These results are in agreement with those reported by (Clark *et al.*, (1990); Shiau and Hung (1990) and Khater and Dawah, (2008)) and disagreement with the results of Magouze, (1990); Omar, (1994); EL-Sayed and Teshima (1992; Jauncey (1982) and Attack *et al*., (1979).

5. CONCLUSION

In conclusion, this study indicates that a diet containing 25% dietary protein level during the rearing of Nile tilapia in earthen pond enclosures with natural food is more suitable and economic for Nile tilapia fry performances.

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