EFFECT OF DIETARY PROTEIN SOURCE AND FEEDING FREQUENCY ON GROWTH PERFORMANCE, PRODUCTION TRAITS AND BODY COMPOSITION OF NILE TILAPIA, *Oreochromis niloticus* (L.) CULTURED IN CONCRETE TANKS.

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SUMMARY

A 28-week feeding trial was conducted in concrete tanks with Nile tilapia, ***Oreochromis niloticus* (*L.*)** with an average initial weight and length of 50.87 ± 6.03 g and 14.4 ± 0.45 cm, respectively, to examine the effect of two protein sources and two feeding frequencies on growth performance, production traits and body composition. Twelve 4 m^3 concrete tanks (2 x 2 x 1.25 m, long, width, and height) each was stocked with 100 fish and fed diets containing either fish meal protein or soybean meal protein at two feeding frequencies of two times daily or four times daily (2 x 2 x 3 factorial experiment). The results revealed that there was no significant effect of dietary protein source on growth rate, whereas there was significant increase in growth rate with increasing feeding frequency ($P \leq 0.05$). The same trend was also observed for mean body weight (g), production rate (kg/m^3), specific growth rate (SGR % day $^{-1}$). The best final mean body weight (g), specific growth rate (SGR % day $^{-1}$), and production rate (kg / m^3) were recorded in groups of fish fed with feeding frequency four times daily. Whole fish moisture, protein, fat and ash contents were significantly influenced ($P \leq 0.05$) by protein source and feeding frequencies, however protein was not influenced with feeding frequency. Gross energy (kcal/100g) was significantly ($P \leq 0.05$) influenced by feeding frequencyies, but not by dietary protein sources. Economic evaluation indicated that soybean meal (Diet B) at four times feeding daily was the most cost-effective and affords a feed strategy for farmers in Egypt. We conclude that a soybean diet fed at four times daily is recommended for adult Nile tilapia reared in concrete tanks.
Keywords: *Nile tilapia, protein sources, feeding frequency, intensive culture, growth.*

INTRODUCTION

Tilapia is often cultured in freshwater ponds without supplemental feeding. Recent intensification of culture practices necessitates the use of prepared feed. Research has been conducted to determine the influence of protein sources and feeding frequency on tilapia growth in intensive culture. Some studies have been directed towards identifying low-cost, readily available, raw materials as protein sources for tilapia diets ***(Jackson, et al. 1982; Ofojekwa and Ejike, 1984; Gaber, 1996; El-Saidy and Gaber, 2002; El-Saidy and Gaber, 2003).***

The influences of feeding frequency on growth rates have been examined in several species, including channel catfish (***Ictalurus punctatus***), estuary grouper (***Epinephelus tauvina***) (***Chua and Tang 1978***), (***Oreochromis niloticus* and *Mugil cephalus***) (***Essa, 1996***), yellowtail flounder (***Limanda ferruginea***) (***Dwyer, et al. 2002***). For example, groups of channel catfish fed twice daily grew faster and used food more efficiently than fish fed 24 times a day (***Andrews and Page, 1975***), whereas for Nile tilapia in cages , high feeding frequency resulted in high growth rate, (***Essa, 1996***). For husbandry purposes, it is important to understand daily feeding pattern for different fish species, so that feeding time and amount can be set appropriately. However, there are relatively, few studies for daily feeding activity in cultured fish, (***Goddard, 1995***). Since fish may adapte physiologically and behaviorally to artificial feeding patterns entrained by food availability (***Talbot, 1994***), it is expected that fish fed at different feeding frequencies will show differences in appetite at the different feeding times.

In contrast, limited informations are available concerning the effects of dietary protein sources and feeding frequency on Nile tilapia reared in concrete tanks. Therefore, the purpose of the present study was to determine the effect of protein sources and feeding frequency on growth performance, production traits, body composition and the economic feasibility of Nile tilapia reared in concrete tanks using a water recirculation system.

MATERIALS AND METHODS

Experimental diets

Two isocaloric diets (17.0 kJ gross energy g^{-1} diet) were formulated to contain either fish meal protein (Diet A) or soybean meal protein (Diet B) (Table 1). Diet B contained 45% hexane- extracted soybean meal (SBM) and was formulated according to ***El-Saidy & Gaber (2002).***

All ingredients were first ground to a small particle size (approximately 250 μm) in a Wiley mill. Dry ingredients were thoroughly mixed prior to adding water to 40 % moisture. Diets were passed through a mincer with die into 3-mm diameter spaghetti-like strands, sun dried and stored in airtight containers. Proximate composition of the experimental diets was determined according to ***A.O.A.C. methods (1995***), while crude fiber in fish diets was determined according to methods of ***Berdon and Juko (1961***). Total carbohydrate content (NFE) of diets was calculated by difference. (100 **-** (moisture + crude protein + crude fat + crude ash + crude fiber)). Gross energy (GE) was calculated using the gross energy values for the macronutrients (23.4 kJ g^{-1} protein, 39.8 kJ g^{-1} fat and 17.2 kJ g^{-1} carbohydrate, fiber was not included in calculation) according to ***Lovell, 1989***.

Table 1. Composition and proximate composition of different dietary protein sources for production of Nile tilapia *O. niloticus* reared in concrete tanks.

	Diets	
Ingredients (%)	A (Fish meal protein)	B (Soybean meal protein)
Fish meal (66% C.P.)	30.0	0.0
Soybean meal (44 % C.P.)	0.0	45.0
Wheat bran (14 % C.P.)	23.0	20.0
Yellow corn meal	37.5	24.0
Soybean oil	5.0	5.0
Mineral and vitamin premix [1]	1.0	1.0
L-Methionine	0.0	1.0
L-Lysine	0.0	0.5
Dicalcium phosphate	1.4	1.4
Vitamin C	0.1	0.1
Molasses (as bender)	2.0	2.0
Total (%)	100	100
Proximate composition (%)[2]		
Moisture	10.7	11.0
Crude protein	26.0	26.0
Crude fat	9.8	9.7
Crude fiber	6.2	7.0
Ash	7.3	7.7
NFE [3]	40.0	38.6
Gross Energy (kJ/g diet)	17.0	17.0

[1] Premix supplied the following vitamins and minerals(mg or IU)/ kg of diet, vit. A, 8000 I.U.; vit. D_3, 4000 I.U.; vit. E 50 I.U.; vit. K_3, 19 I.U.; vit. B_2, 25 mg; vit. B_3, 69 mg; vit. B_6, 20 mg; Nicotinic acid, 125 mg; Thiamin, 10 mg; Folic acid, 7 mg; Biotin, 7 mg; Pantothenate, 15 mg; vit. B_{12}, 75 mg; Choline, 900 mg; vit. C, 500 mg; Manganese, 350 mg; Zinc, 325 mg; Iron, 30 mg; Iodine, 0.4 mg; Cobalt 2 mg; Copper, 7 mg; Selenium, 0.7 mg and 0.7 mg B.H.T. according to ***Xie, Cui, Yang & Liu (1997).***

[2] Values represent the mean of three sample replicates.

[3] Nitrogen free extract (NFE) = {100 **-** (moisture + crude protein + crude fat + ash + crude fiber)}

Experimental system and animals

The experiment was carried out at the outdoor installations of the Fish Research Laboratory, Faculty of Agriculture, Minufiya University, Egypt, from June 2nd until December 10th 2003. The experimental system consisted of 12 experimental concrete tanks. Each tank was 2 m long, 2 m wide and 1.25 m high. Water level in the concrete tanks was kept at 1 m depth to maintain the water volume of 4 m^3. The concrete tanks were supplied with freshwater at a rate of 4 l min^{-1} with supplemental aeration. The walls and bottoms of the tanks were brushed weekly to minimize algal growth. All tanks were drained and cleaned every 4 weeks during sampling.

Water temperature and dissolved oxygen were measured every other day using YSI model 58 oxygen meter (Yellow Springs Instrument Company, Yellow Springs, OH, USA). Total ammonia and nitrite were measured once weekly using a DREL 2000 spectrophotometer (Hach Co., Loveland, CO). Total alkalinity and chloride were monitored once a week using the titration method, and pH was monitored twice weekly using an electronic pH meter (pH pen, Fisher Scientific, Cincinnati, OH). During the 28-week feeding trial, the average water quality parameters (mean ± SD) were: water temperature, 27.5 ± 0.7 ^oC; dissolved oxygen, 5.2 ± 0.5 mg l^{-1}; total ammonia 0.2 ± 0.1 mg l^{-1}; nitrite, 0.05 ± 0.03 mg l^{-1}; total alkalinity, 182 ± 45 mg l^{-1}; chlorides, 550 ± 120 mg l^{-1}; pH, 7.6 ± 0.16.

A group of 1200 fingerlings Nile tilapia ***Oreochromis niloticus*** with an average initial weight and length of 50.87 ± 6.4 g and 14.4 ± 0.5 cm, respectively, were obtained from the stock of fish at the fish research laboratory in Shebin El-Kom, Faculty of Agriculture, Minufiya University. One hundred fish were randomly stocked into each concrete tank at a density of 25 fish/m^3. Fish from each tank were weighed individualy every 4 weeks and at the end of the trial. Total length of each fish was measured at the beginning and the end of the trial. Fish in three replicate tanks were fed the fish meal protein (Diet A) or soybean meal protein (Diet B) at feeding frequencies of two or four times daily. The amounts of feeding allowance was adjusted for each tank every 4 weeks. Tilapia was fed at a feeding rate of 2 % body weight daily in the first 20 weeks then reduced to 1.5 % feeding rate until the end of the experiment (28 weeks), 6 days per week for 28 weeks. At the end of the feeding trial, a sample of six fish from each tank were killed by decapitation, stored in polyethylene bags and frozen for subsequent protein, fat, moisture and ash analysis of whole fish according to ***A.O.A.C. (1995***). Gross energy (GE) of whole fish (kcal/100 g) was determined by Ballistic bomb calorimeter, Gallenkamp, England.

Growth response, production and feed utilization parameters were calculated as follows: SGR (% day $^{-1}$) = 100(Ln final weight - Ln initial weight)/ days; Total prodution = final biomass (kg/m^3); Net production = final biomass - initial biomass (kg/m^3); Gain in weight (g/fish)= mean final body weight - mean initial body weight; Gain in total length = mean final body total length -mean initial total length (cm/fish) ; Condition factor (K) = 100(Wt/L^3), where Wt is fish body weight (g), L is total length (cm); Feed conversion ratio (FCR) = total dry feed fed (g)/total wet weight gain (g); Feed intake (g/fish) was recorded daily and calculated at the end of the experiment. Net income was determined by the difference between the sale price of the fish after harvest and the costs of fingerlings and food according to ***Hengsawat,et al. (*1997).**

Statistical analysis

Data were analyzed by two-way analysis of variance using the SAS General Linear Models procedure (***Statistical Analysis Systems, 1993***). Significance between dietary protein sources, between feeding frequencies, and their interaction were determined using Duncan's multiple range test ***(Duncan's 1955***). Treatment effects were considered significant at (P ≤ 0.05). All percentage and ratio data were transformed to arcsin values prior to analysis ***(Zar, 1984***).

RESULTS

Mean gains in weight and length, specific growth rate (SGR % day^{-1}) and production rate (kg/m^3) are presented in Table (2). There were no significant differences in fish weight due to protein sources. However, there were significant differences due to the effect of feeding frequency, and the interactions between feeding frequency and protein sources. Diet (A) containing fish meal protein provided the highest weight of 194.3 g / fish while there is no significant (P ≥ 0.05) difference from fish fed on diet (B) containing soybean meal protein (Table 2). The fish fed four times daily feeding frequency had heaviest body weight of 194.5 g/fish . Statistical evaluation of fish weight revealed that increasing the feeding frequency significantly (P ≤ 0.05) increased the mean fish weight. Mean body length (cm), body gain (g), production rate (kg/m^3) and specific growth rate (SGR % day $^{-1}$) showed the same trend as mean fish weight (Table 2).

Results of net production (kg/m^3) and total production (kg/m^3) of the present experiment provided a picture for the feeding frequency and growth rate. Feeding frequency and interaction between protein source and feeding frequency showed significant (P ≤ 0.05) effect on net production and total production, while there was no effect of protein source. Fish fed at four times feeding daily showed a higher production rate than those fed two times daily (Table 2).

The effects of dietary protein source and feeding frequency on feed conversion ratio (FCR), total food intake (g/fish), protein efficiency ratio (PER) and condition factor (K) are shown in Table 3. In general, protein source, feeding frequency and interaction had no significant ($P \geq 0.05$) effects on feed conversion ratio, protein efficiency ratio and condition factor. The average feed consumption increased with increasing feeding frequencies and this was accompanied by an increase in weight gain. Total feed fed (g/fish) was not influenced by protein source, but significantly ($P \leq 0.05$) affected by feeding frequency and interaction (Table 3).

The effects of dietary protein source and feeding frequency on whole body proximate composition and energy content of Nile tilapia are presented in Table (4). Moisture content was low (71.7%) for fish fed fish meal protein (Diet A) and high (73.1%) for fish fed the soybean meal protein (Diet B). Moisture content was significantly ($P \leq 0.05$) influenced by dietary protein source, feeding frequency and interaction. Protein contents was influenced ($P \leq 0.05$) by dietary protein source but was not significantly ($P \geq 0.05$) influenced by feeding frequency. Dietary protein source and feeding frequency had significant ($P \leq 0.05$) effect on the fat and ash contents of whole body. Gross energy was not influenced ($P \geq 0.05$) by dietary protein source and interaction but was significantly ($P \leq 0.05$) affected by feeding frequency.

The economic calculation from the study is presented in Table (5). The feed cost and the total cost (L E) increased with fish fed fish meal protein (Diet A) and feeding four times daily. From the economic information, it can be concluded that the highest net profit (Lever Egyptian) was achieved with fish fed soybean meal protein (Diet B) and feeding rate of four times daily.

DISCUSSION

In the present study, there are some factors leading to intensive fish culture in recirculation systems. Increasing land costs and decreasing freshwater supplies are, the main reasons for intensification of fish farming in Egypt, though additional advantages include savings in manpower and easier stock management. Increased fish yields in conventional, static ponds or reservoirs was accomplished by a combination of management procedures, the most important factor among them being the use of supplementary feed, polyculture, and auxiliary aeration during the night (***Sarig 1989***). Higher yields were obtained in specially designed smaller units, 50-1000 m^3 (***Zohar, et al. 1985; Van Rijn, et al.1986***), which differ from conventional ponds in design. These are made of concrete or are plastic-lined, and their configuration allows periodical removal of organic matter from the bottom. Most of these units are operated in a semi-closed mode, allowing optimal use of water and hence, minimal water discharge. Due to their reduced environmental impact, their development is supported by national and regional authorities. Pollution control is, therefore, another factor underlying the development of intensive systems. Finally, culture of fingerlings (mainly tilapia) during off-season in heated, indoor systems is rapidly expanding, and so, heat conservation can be counted as an additional factor promoting the use of intensive recirculation systems.

Several investigators have determined the effect of dietary protein sources for tilapia and the results are not consistent. Among the plant protein sources, soybean meal has been shown to posses an acceptable amino acid profile for growth of many fish species (***Tacon, et al. 1983; Murai, et al.1986; El-Saidy and Gaber, 1997***) and may be used as the major protein sources in many fish diets (***Lovell, 1989 and El-Saidy and Gaber, 2002 and 2003***). In the present study, the growth performance showed no significant differences between tilapia groups fed fish meal protein and soybean protein diets, but showed significant increase with increasing feeding frequency four times daily. These results may attributed to fish at low feeding frequency might undergo metabolic changes as depressing protein assimilation and accumulation of phospholipids in the muscle. According to ***Kayano, et al. (1993***), young fish fed several times a day could effectively assimilate dietary protein to muscle. In previous studies, it was found that, four feeding daily represent the optimum frequencies for fish (***Holm, et al. 1990***; ***Kayano, et al. 1990 and Kayano, et al. 1993***).

Feeding frequencies had significant effect on growth, feed consumption, net production, total production and specific growth rate (SGR) in Nile tilapia. By the end of the experiment, fish fed at higher feeding frequency had gained significantly more weight and more length than fish fed the lower feeding frequency. Fish fed at higher frequency consumed larger quantities of food than those fed less often, but individual meal size was smaller. This is consistent with studies conducted on other fish species. (***Ishiwata, 1969***), where fish fed fewer meals per day tend to eat more per meal. Fish accomplished this by increasing stomach volume and became hyperphagic (***Grayton and Beamish, 1977; Jobling, 1982 and 1983; Ruohonon and Grove, 1998***). However, although fish fed at higher frequency consumed larger quantities of food, when the interval between meals is short, the food passes through the digestive tract more quickly, resulting in less effective digestion (***Liu and Liao, 1999***). Determining the optimal feeding frequency is important.

There was a strong trend for total production and net production (kg/m^3) to increase with increasing feeding frequency. These results agree with those of ***Essa, et al. (1989) and Kayano, et al.(1993***) that production in fish culture depends on daily feed consumption rate and feeding frequency. Production estimates, are based on biomass estimates adjusted for mortality and corrected for growth rate (***Chapman, 1968***). It is the basis for estimating economic yield for both fish culture operation and for natural fish population. Net production and harvest value were not dependent on protein source but dependent on feeding frequency. In addition, final harvest and production values were directly related to feeding frequency and there are some feeding frequencies at which growth rate is reduced and when it occurs, production will be reduced. The critical level in our experiment was a feeding frequency four times daily for maximum growth in adult Nile tilapia

From the above results and the economic evaluation, we can conclude that, a diet containing soybean meal protein at four times daily feeding frequency is recommended for adult Nile tilapia. These fish showed no significant responses to different dietary protein sources, but had significant increase with increasing feeding frequency. Thus a soybean protein diet plus the methionine and lysine to correct the essential amino acids ratio with four times feeding frequency would be cost effective and maintaining adequate growth and production of adult Nile tilapia in concrete tanks under the experimental condition.

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Table 5. Economic information for Nile tilapia reared in concrete tanks for 28 weeks fed two protein sources at two feeding frequency. Values are mean ± SE.

Items	Diet A (Fish meal protein) F.F.[1] (Two times daily)	Diet A (Fish meal protein) F.F. (four times daily)	Diet B (Soybean meal protein) F.F. (Two times daily)	Diet B (Soybean meal protein) F.F. (four times daily)
No. fish stocked/tank	100	100	100	100
No. fish harvested	100	100	100	100
Harvest (kg /m^3)	4.86 ± 0.18	4.78 ± 0.27	4.77 ± 0.27	4.86 ± 0.19
Harvest kg per tank (4m^3)	19.44 ± 0.70	19.11 ± 1.10	19.09 ± 1.10	19.45 ± 0.75
Food used (kg/tank)	37.4	37.6	37.7	38.3
Fingerling cost (LE) [2]	40.00	40.00	40.00	40.00
Food cost [3]	104.72	105.28	65.98	67.03
Total cost (LE.)	144.72	145.28	105.98	107.03
Value of harvest (9.5 LE. kg^{-1})	184.68	181.55	181.36	184.78
Net profit (LE.)	39.96	36.27	75.38	77.75

[1] FF = feeding frequency.
[2] LE. = Lever Egyptian.
[3] Food cost equal 2.80 and 1.75 LE for diet A (Fish meal protein.) and diet B (Soybean meal protein) , respectively, according to praises of year 2005.