The Effect of Dietary Soybean Meal and Phytase Levels on Growth Performance and Body Composition of Fingerlings Nile Tilapia *Oreochromis niloticus* (L.)

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ABSTRACT

An experiment with 2.1± 0.1 g Nile tilapia Oreochromis niloticus was conducted to evaluate the influence of partially or totally substituting of fish meal (FM) protein by soybean meal (SM) in practical diets supplemented with 3 microbial phytase levels (0, 1000, 1500 IU). Three diets were formulated to be isonitrogenous (ca. 35% crude protein) and isocaloric (ca. 461 Kcal./100g protein). The control diet, diet 1, and diet 2 contained FM and SM, as merely the sole sources of protein respectively. Diet 3 contained 20% protein from fish meal and 15 % of its total protein from Soya bean meal. Each diet was supplemented with 3 levels of phytase (0, 1000, 1500 IU/Kg diet). Three replicates groups of fish per treatment were hand-fed twice a day for 90 days in glass aquaria. After 90 days, fish fed FM diet and fish fed SM diet supplemented with 1500 IU/Kg phytase had significantly (p<0.05) higher weight gain, absolute growth rate, specific growth rate, protein efficiency ratio, feed efficiency and feed conversion ratio than the rest of experimental groups. Supplemental phytase tend to increase bone ash, body and bone phosphorous in fish fed SM diet and FM plus SM diet. No significant differences in body composition among treatments were observed.

The present study demonstrates that a diet containing 35% crude protein with 20% protein from FM and 15% crude protein from SM supplemented with 1500 IU phytase is adequate for good growth of fingerlings Nile tilapia.

Key words: *Oreochromis niloticus*, Soybean, Phytase, Growth performance.

1. INTRODUCTION

Feeding cost in fish production is considered the most expensive item particularly dietary crude protein which is the most expensive component in fish diets. The use of plant protein sources to partially or entirely replacement expensive fishmeal protein diets has been a goal of fish nutritionists for many years. Among all the plant protein sources tested, soybean meal has been the most widely used. Soybean protein is considered as one of the best amino acid profiles of all protein -rich plant feedstuffs for meeting essential amino acid requirements of fish (Lovell, 1988). Defatted soybean meal contains 45-48% crude protein whereas alcohol washed concentrate contains 70-85% crude protein (Storebakken et al., 1998). Properly and adequately heat treated full-fat soybean meal, reduces level of antinutritional factors and increases amino acid availability. Soy bean meal has been included in fish feeds with positive results (Smith, 1977; Viola and Arieli, 1983). However, most attempts to replace fishmeal by extracted soybean, have led to growth reduction and low feed conversion rate in grass carp and other fish (Cho et al., 1974; Dabrowski and Kozak,1979, Jackson et al.,1982, Sadiku and Jauncey, 1995 and Eid et al.,1995) However, in nutritional studies, soybean meal replaced 25%, 30% and 40%, of the fish meal protein showed decrease in weight gain of fish Dabrowski et al., (1989). Oliva-Teles et al., (1994) and Sanz et al. (1994). Fish feedstuffs, especially which of plant sources, have a relatively high content of phosphorus. However, up to 80% of this phosphorus is in the form of phytate which is unavailability for utilization by fish (Sebastian et al., 1998 and Ravindran, 1999). Thus, it is necessary to supplement either phytase or other additive to improve the utilization of phosphorus as well as other nutrients which bound with phytate molecules (Ravindran et al., 1995). However, Informations on the effect of phytase on protein and amino acids availabilities, digestibility and mineral utilization in fish diets are limited (Vielma and Lall, 1998; Storebakken et al., 1998, Vielma *et al.*, 2000, Cheng and Hardy,2002 and Ellestad et al., 2003). The purposes of the present study were to assess the potential for partially or totally substituting protein from soy bean meal for fishmeal protein and to determine the effects of adding microbial phytase in the diet on growth performance of Nile tilapia.

2. MATERIALS & METHODS

2.1. Fish and rearing procedures

Six hundred fingerlings of *O. niloticus* (L.) weighing ca. 2.1±0.1g were used. The fish were randomly divided into eight groups of 25 fish each in triplicate.

The experiment was conducted in glass aquaria (80 × 50 × 40 cm) each of 160-L capacity at Department of Animal and Fish Production, Suez Canal University. Each aquarium was filled with dechlorinated tap water and continuous aeration was provided in all aquaria with automatic air pump. The groups of fish were randomly distributed, each of which in one aquarium. Water temperature was maintained at 25±1°C throughout the experiment by automatic heaters. The water quality was monitored daily for temperature, pH, oxygen, ammonia, hardness and combined nitrate/nitrite levels. Aquaria were cleaned every day, as far as possible, and the water was changed once a fortnight. Water conditions remained almost identical in the system throughout the experiment and all parameters measured were within the tolerance limits known for tilapia. The light was kept on a 12-h light dark cycle (07.00 to 19.00h)

2.2. Experimental diets

Three experimental diets were formulated as follows:

1. Diet 1 (control) contained fish meal (FM) as a main source of protein.
2. Diet 2 contained Soybean meal (SM, hexan extracted) as a main source of protein.
3. Diet 3 contained (20% crude protein from FM plus 15% crude protein from SM.

The three diets were supplemented with three levels of microbial phytase (0, 1000 and 1500 IU/kg diet). All diets were isonitrogenous (ca. 35% crude protein) and isocaloric (ca. 463.6 kcal/100g diet) (Table 1).

Experimental diets were prepared by first mixing all the dry ingredient thoroughly with oil and then cold water was added until stiff dough resulted. This was then passed through a food mixer (Hobart A200 Hobart Ltd., London). The homogenous paste was then extruded under pressure through a 2mm die plate. The resulting strands were air dried for 12 h at approximately 30 °C. The resultant pellets were then crumbled by hand to obtain the required pellet size according to fish size prior to feeding. All diets were kept in refrigerator until immediate period to feeding.

Prior to initiation of the experiment, the fish underwent a 2-week conditioning period during which they readily adjusted to standardized environmental conditions. Experimental diets were fed at 3% of fish wet weight per day. The amount of diet was divided into two equal feedings portions per day. Fish were fed for six consecutive days. Each group of fish was weighed individually every 10 days, and the amount of diet was adjusted accordingly. Fish were fed the test diets for 90 days. Methylene blue (4 mg/L) was added to the water after weighing the fish to reduce bacterial infection caused by handling.

The experimental diets were analyzed for moisture, protein, ether extract, crude fiber and ash by standard AOAC methods (AOAC, 19

2.3 Statistical Analysis

Analysis of variance was carried out according to SAS Institute, Inc., (1985) and the means were compared by multiple range tests (Duncan, 1955) and were considered statistically different at ($P < 0.05$).

3. RESULTS AND DISCUSSION

Data for growth performance of Nile tilapia fed the experimental diets for 90 days are presented in Table (2). The group of fish fed diets containing (20% crude protein from fishmeal +15% crude protein from soybean meal) in which of 48% of fishmeal protein was replaced by protein from soybean meal had weight gains, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and feed efficiency (FE) close to those of fishmeal diets (diets1, 2 and 3). However, the differences was not significant ($p<0.05$). On the other hand the groups of fish fed diets 4.5 and 6 which contains soybean meal as the only source of protein had significantly lower body weight gain, absolute growth rate (AGR), PER and FE than the rest of experimental groups. These data indicate that growth of Nile tilapia improved when fed a diet containing 40% protein from fishmeal and 34% from soybean meal than those fed diets containing soybean meal only. This is in agreement with studies reported on carp *Cyprinus carpio* (Nandeesha et al., 1990)., rainbow trout, *O mykiss* (Reinitz, 1980); channel catfish, *I punctatus* (Moshen and Lovell, 1990) and tilapia, *O. niloticus* × *O. aureus* (Shiau et al, 1990). Processing of soybeans into various meals, concentrates and isolates has a major influence on their nutritional properties (Wolf and Cowan, 1971). Growth and feed utilization responses of tilapia when replacing FM by soybean meals (SBM) has been reduced direct proportion to the percentage of soybean meal in the diet. This may be due to suboptimal amino acid balance and inadequate available phosphorus in soybean meal, and presence of antinutritional factors (including trypsin inhibitors) in the soybean meal. However growth has tended to be reduced in group of fish fed diets with soybean meal replacing all the fish meal. Similar results were obtained by Jackson et al. (1982). The soybean meal used in the present study was hexan extracted commercial product. It is known that antinutritional factors in raw or in adequately heated soybean meal can adversely affect the growth of carp (Viola and Arieli, 1983) and channel catfish (Wilson and Poe, 1985) and tilapia (Eid et al., 1995).

In the present study, weight gain, AGR, FCR, PER and FE were increased by phytase supplementation from 0 to 1500 IU/kg. These results were in agreement with the results of (Vielma and Lall, 1998; Storebakken et al, 1998; and Lanari et al, 1998). Within each protein source weight gain, absolute growth rate, SGR, FCR, PER and FE were improved by increasing phytase levels from 0 to 1500 IU/ kg diet. Similar results were obtained by El-Deeb et al., (2000) and Attia *et al;* (2001). Feed conversion ratio (FCR) and protein efficiency ratio were significantly ($P<0.05$) different among treatments (Table 2). Feed conversions (FCR) were close to the results of Webster et al. (1992). The group of fish which fed the diet where 15% of fish meal was replaced by soybean meal (diet 7, 8 and 9) gained slightly less in weight than fish fed the diet in which all of the protein originated from fish meal (diets 1, 2 and 3). However, the difference in growth was not significantly ($P<0.05$) different. In relation to the former findings soybean meal has one of the best amino acid profiles of any of the plant protein feed stuffs and the profile meets the essential amino acid requirements for fish (Lovell, 1988) . Dabrowski et al. (1989) stated that amino acid availability, especially, Methionine, was reduced if soybean- meal protein was used in excess of 50% of the diet which is in agreement with the findings of present study. The effects of dietary treatments on body composition are shown in Table (3). Results indicated that no significant differences ($P<0.05$) in percentage body protein, moisture and fat among treatments. Supplemental phytase had no effect on body composition. Similar results were obtained by Vielma *et al.* (2000), Attia et al. (2001). Supplemental phytase tend to increase bone ash of soybean fed fish (diets 4, 5 and 6) and fish meal plus soybean meal diets (7, 8 and 9), thus indirectly indicating successful gastrointestinal hydrolysis of phytase in soybean meal diets (Table 4). Enhanced phytate phosphorous (P) availability by supplemental phytase has been reported in trout (Lanari et al. 1998; and Vielma et al., 2000).

Bone ash is a sensitive criterion for available P supply (Vielma and Lall, 1998), which was also shown in the present study. Soybean meal diets and replacing FM with soybean meals significantly ($P\leq 0.05$) increased bone ash concentration (Table 4). In group of fish fed soybean meal diets, bone ash were 45.4, 45.75 and 45.85 % in fish fed with and without phytase supplementation and the differences were not significant. These results confirm the findings of Vielma *et al.* (2000).

Bone phosphorus generally paralleled bone ash. The requirement of phosphorus for maximum growth and normal bone mineralization for *O. niloticus* was reported to be less than 0.9% in the diet (Watanabe et al., 1980).

The present study demonstrates that a diet containing 35% crude protein with 20% protein from fish meal and 15% protein from soybean meal supplemented with 1500 IU phytase is adequate for good growth of Nile tilapia.

Table 1. Composition and proximate chemical analysis of experimental diets.

Ingredients	Diet 1.	Diet 2.	Diet 3.
Fish meal (50% protein)[1]	70	-	40
Soybean meal (44% protein)[1]	-	80	34
Corn grain	20	5	13
Fish oil	4	6	5
Corn oil	3	6	5
Vitamin Mix.[2]	1	1	1
Mineral Mix.[3]	2	2	2
Proximate Analysis (%)			
Moisture	6.20	5.80	6.10
Crude Protein	35.10	35.50	35.10
Crude Fat	14.20	14.10	14.10
Ash	6.40	5.30	6.20
Crude Fiber	1.80	5.10	3.80
NFE[4]	42.50	40.00	40.80
GE (K cal/100g)[5]	504.90	496.30	497.20
Total (P)	2.90	0.51	1.33

1. Local ingredient obtained from the market.
2. Vitamin premix provide the following per Kg dry diet: tochopherol acetate, 699 I.U; menadione sodium bisulfate complex 83 mg; thiamin mononitrate, 33 mg; riboflavin, 73 mg; pyridoxine HCl, 25 mg; d-calcium pantothenate, 147 mg; niacin, 262mg; myo-insitol, 184 mg; folic acid, 17.6 mg; B12, 0.08 mg; d-biotin, 0.83 mg; ascorbic acid, 124 mg.
3. Mineral premix provided the following per Kg dry diet: zinc sulfate ($ZnSO4$), 257 mg; manganese sulfate ($MnSO4$), 288 mg; ferrous sulfate ($FeSO4$), 69 mg; copper sulfate ($CuSO4$), 5.4 mg; potassium iodide (Kio3), 1.1 mg.
4. Nitrogen Free Extract 100- (% crude protein + crude lipid + crude fiber % + crude ash).
5. Gross energy calculated using values of 5.7 Kcal/g protein; 9.5 Kcal/g lipid and 4.0 Kcal/g carbohydrate.

Table 2. Growth performance of Nile tilapia fed different experimental diets for 90 days.[1]

Diet (Treatment)	1	2	3	4	5	6	7	8	9
Protein source	Fish meal			Soybean meal			Fish meal + Soybean		
Phytase concentration (IU)	**0**	**1000**	**1500**	**0**	**1000**	**1500**	**0**	**1000**	**1500**
Initial body wt (g)	2.1± 0.10	2.1± 0.10	2.1± 0.20	2.1± 0.10	2.1± 0.10	2.1± 0.10	2.1± 0.10	2.1± 0.10	2.1+ 0.10
Final body wt (g)	48.5± 0.17^{d}	53.1± 0.10^{c}	55.6± 0.01^{a}	36.1± 0.02^{f}	40.2± 0.10^{e}	41.6± 0.12^{c}	47.9± 0.12^{d}	50.6± 0.10^{c}	54.2± 0.01^{b}
Weight gain (g)	46.4± 0.16^{c}	51.0± 0.13^{b}	53.5± 0.12^{a}	34.0± 0.11^{e}	38.1± 0.14^{d}	39.5± 0.12^{d}	45.8± 0.13^{c}	48.5± 0.10^{b}	52.1± 0.11^{a}
Absolute growth rate (g/kg)	0.48± 0.06^{c}	0.54± 0.05^{b}	0.59± 0.03^{a}	0.37±0. 04^{e}	0.42±0. 06^{d}	0.44± 0.05^{d}	0.50± 0.06^{c}	0.54± 0.04^{b}	0.58± 0.03^{a}
SGR[2]	3.49± 0.03^{c}	3.55± 0.03^{b}	3.64± 0.04^{a}	3.16± 0.02^{f}	3.28± 0.02^{e}	3.32± 0.03^{d}	3.47± 0.02^{c}	3.53± 0.02^{b}	3.61± 0.04^{a}
FCR[3]	2.1± 0.03^{c}	1.8± 0.04^{b}	1.5± 0.03^{a}	2.4± 0.02^{f}	2.2± 0.04^{e}	2.0± 0.04^{d}	2.2± 0.03^{c}	1.4± 0.02^{b}	1.6± 0.03^{a}
PER[4]	1.35± 0.06^{c}	1.58± 0.05^{b}	1.89± 0.04^{a}	1.17± 0.02^{f}	1.28± 0.02^{e}	1.39± 0.03^{d}	1.28± 0.02^{c}	1.50± 0.03^{b}	1.78± 0.04^{a}
FE[5]	0.48± 0.03^{c}	0.55± 0.04^{b}	0.66± 0.02^{a}	0.42± 0.03^{f}	0.45± 0.01^{e}	0.50± 0.02^{d}	0.45± 0.01^{c}	0.53± 0.02^{b}	0.63± 0.02^{a}
Feed	91.14± 0.20^{c}	88.56± 0.17^{c}	80.25± 0.05^{c}	81.6±0. 07^{c}	83.82± 0.03^{d}	79.0± 0.05^{c}	100.7± 0.02^{a}	92.15± 0.02^{b}	83.36± 0.03^{d}
Protein	31.99± 0.25^{b}	31.08± 0.31^{b}	28.16± 0.27^{d}	28.96± 0.19^{d}	29.75± 0.26^{c}	28.04± 0.20^{d}	35.36± 0.25^{a}	32.34± 0.25^{a}	29.25± 0.20^{c}
Mortality	5± 0.10^{c}	5± 0.20^{c}	5± 0.23^{c}	9± 0.12^{a}	9± 0.23^{a}	9± 0.22^{a}	7± 0.33^{b}	7± 0.21^{b}	7± 0.22^{b}

1. Values are means ± S.E of three replications. Values in the same row followed by the same letter are not significantly different ($P > 0.05$).
2. SGR= [{Ln W_2- Ln W_1}/ time (days)] × 100 where W_2 final body weight; W_1 initial body weight.
3. Feed Conversion Ratio= g dry food fed/g live weight gain
4. Protein Efficiency Ratio= Gain in weight (g)/ protein intake (g) × 100.
5. Feed Efficiency (g) live weight gain/ g dry food fed.

Table 3. Initial and final carcass composition of *Oreochromis niloticus* fed experimental diets for 90 days (% on wet weight basis).

Diet No.	Moisture	Crude protein	Crude fat	Ash
Initial	75.08	15.93	4.57	4.42
1	75.10	15.70	4.61	4.59
2	74.90	15.80	4.71	4.49
3	74.80	15.90	4.81	4.49
4	75.10	15.70	4.60	4.60
5	74.80	15.80	4.70	4.70
6	74.70	15.90	4.77	4.63
7	74.90	15.70	4.70	4.70
8	75.10	15.80	4.80	4.30
9	75.02	15.90	4.88	4.20

Table 4. Bone ash and whole body phosphorus % of Nile tilapia *Oreochromis niloticus* fed experimental diets for 90 days (1).

Diet	Bone Ash% (2)	P% (2) Whole fish	P% (2) Bone
1	43.20 ± 0.20^{d}	24.40 ± 0.12^{cd}	5.35 ± 0.04^{d}
2	44.60 ± 0.20^{cd}	25.90 ± 0.20^{cd}	6.55 ± 0.04^{cd}
3	44.80 ± 0.21^{cd}	25.80 ± 0.04^{cd}	6.88 ± 0.05^{cd}
4	45.40 ± 0.22^{c}	26.10 ± 0.04^{c}	7.40 ± 0.02^{c}
5	45.75 ± 0.02^{c}	26.30 ± 0.05^{c}	7.60 ± 0.05^{c}
6	45.85 ± 0.21^{c}	26.80 ± 0.04^{c}	7.80 ± 0.02^{c}
7	45.40 ± 0.04^{c}	26.10 ± 0.02^{c}	7.20 ± 0.05^{c}
8	46.80 ± 0.04^{b}	26.80 ± 0.04^{b}	8.30 ± 0.04^{b}
9	47.90 ± 0.05^{a}	27.80 ± 0.05^{a}	8.81 ± 0.04^{a}

1. Values are means ± S.E of three replications. Values in the same row followed by the same letter are not significantly different (P> 0.05).
2. Means with common superscript in each column were not significantly different at 5% level.

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الملخص العربى

تأثير مسحوق فول الصويا ومستويات مختلفة من الفايتيز على مظاهر النمو وتركيب الجسم فى إصبعيات البلطى النيلى

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أجريت تجربة لتقييم التأثير الجزئى أو الكلى لإحلال بروتين مسحوق السمك ببروتين مسحوق فول الصويا فى العلائق المضاف لها ٣ مستويات من الفايتيز (٠، ١٠٠٠، ١٥٠٠ وحدة دولية). تم تكوين ثلاث علائق متوازنة (٣٥% بروتين خام) و (٤٦١ ك سعر حرارى/جم بروتين). احتوت العلائق المقارنة، عليقة ١ ، عليقة ٢ على مسحوق السمك ومسحوق فول الصويا كمصدر وحيد للبروتين على التوالى و العليقة ٣ احتوت على ٢٠% من البروتين مسحوق سمك، ١٥% من البروتين فول صويا مضاف لكل منهم ثلاث مستويات من الفايتيز (٠ ، ١٠٠٠ ، ١٥٠٠ وحدة دولية/كجم عليقة). مثلت كل معاملة ثلاث مكررات واستمرت التجربة ٩٠ يوم فى أحواض زجاجية. لوحظ فى نهاية التجربة أن هناك تأثير ملحوظ على الأسماك المغذاة على علائق محتوية على ١٥٠٠ وحدة دولية/كجم فى كل من مسحوق السمك وفول الصويا من حيث زيادة الوزن و معدل النمو المطلق، كفاءة الغذاء ومعدل تحويل الغذاء عن باقى مجاميع التجربة، لم يلاحظ أى فروق فى تركيب الجسم بين المعاملات. أوضحت هذه الدراسة أن العلائق المحتوية على ٣٥% بروتين (٢٠% مسحوق سمك، ١٥% فول صويا) المضاف لها ١٥٠٠ وحدة دولية/كجم فايتيز كافية لنمو جيد لاصبعيات البلطى النيلى.