

Total Replacement of Fish Meal By Soy Bean Meal, With Various Percentages of Supplemental L-Methionine, in Diets For Nile Tilapia (*Oreochromis niloticus*) Fry

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ABSTRACT

A 12-week feeding trial was conducted in aquaria with (0.9 ± 0.01 g) Nile tilapia, *Oreochromis niloticus* fry, to examine the effect of totally replacing fish meal with high (33%) percentage of soy bean meal (SBM) in prepared diet. Five isonitrogenous (31.5% crude protein) and isocaloric (2.84 kcal /g digestible energy of diet) diets were formulated. Diet 1 was similar to a high- quality commercial tilapia diet, containing 10 % fish meal and 24 % SBM. Diets 2-5 contained 0 % of fish meal and 33 % SBM with various amounts (0.5 %, 1 %, 1.5 % and 2 %) of L-methionine added. After 12 weeks, the results revealed that, there is a significant difference in the final individual weight, weight gain %, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and food intake among fish groups ($P \leq 0.01$). The best final individual weight, weight gain %, specific growth rate, feed conversion ratio, protein efficiency ratio (PER), and food intake were recorded with diet 3, which contained 33 % SBM and 1 % methionine and there was no significant difference between it and diet 1 (control). The survival rate did not differ among the fish groups and the morphological examination did not reveal any morphological differences in the fish fed diet 1 (control) and diets 2-5 which contained 33 % SBM with various percentages of supplemental L-methionine. Fish flesh compositions were not significantly different ($P > 0.05$) among treatments and averaged 75.87 %, 16.46 % and 9.69 % for percentage moisture, fat and ash, respectively, while for percentage protein was significantly different ($P \leq 0.01$) and the best result was achieved in case of fish fed diet 3 and diet 1(control). These suggest that a diet with 33 %(SBM) supplemented with 1% L-methionine can totally replace fish meal in a diet for Nile tilapia (*Oreochromis niloticus*) fry, without adverse effect on fish performance or body composition, when the dietary protein level is 31.5 % and fish fed to satiation.

INTRODUCTION

Fish meal is one of the most expensive ingredients in prepared fish diets. Fish nutritionists have tried to use less expensive plant protein sources to partially or totally replace fish meal. Soy bean meal (SBM) is considered to be one of the most nutritious of all plant protein foodstuffs (Lovell, 1988). However, growth has often been reduced in fish fed diets with SBM replacing all the fish meal (Cowey, et al., 1974; Lovell, et al., 1974; Murai, et al., 1986; Shiau, et al., 1987; Reigh and Ellis, 1992). One possible reason may be the activity of protease (Trypsin) inhibitors in crude or inadequately heated SBM

(Dabrowski and Kozak, 1979 ; Wilson and Poe 1985). However, this may not be of practical importance since commercially available SBM usually has little trypsin inhibitor activity if adequately processed (Webster, et al., 1992a). A second possible reason may be suboptimal amino acid balance of SBM (Dabrowski, et al., 1989). It has been reported that addition of supplemental methionine improved growth in common carp, *Cyprinus carpio* L., fed diets with soy flour (Murai, et al., 1982; 1986). A third possible explanation may be that the energy content of SBM is lower than that of fish meal in diets for fish (Viola, et al., 1983; Hilton and Slinger, 1986). Lastly, SBM may have reduced digestibility's of minerals, especially phosphorus, compared with fish meal (Liebowitz, 1981).

The purpose of the present study is to evaluate commercially available soy bean meal as total replacement for fish meal in diets and to know the effects of supplementation with various percentages of L-methionine on Nile tilapia fry.

MATERIALS AND METHODS

Experimental diets

Five experimental diets were formulated. Diet 1, with 24 % hexane-extracted SBM and 10 % menhaden fish meal was formulated to be a high-quality commercial tilapia fish diet. The other four diets (diets 2-5) contained 0 % fish meal, 33 % SBM and various percentages (0.5, 1, 1.5 and 2 %) of supplemental crystalline L-methionine (Table 1). Amino acid composition of the diets were calculated from tabular values provided for diet ingredients (NRC, 1993). All diets were formulated to be isonitrogenous (31.5 % protein) and isocaloric (2.84 kcal digestible energy per g of diet).

In preparing the diets, dry ingredients were first ground to a small particle size (approximately 250 μ m) in a Wiley mill. Ingredients were thoroughly mixed and then thoroughly added water to obtain a 30 % moisture level. Diets were passed through a mincer with die into 0.4-mm diameter spaghetti - like strands and were dried under sun for 8 h. After drying the diets were broken up and sieved into appropriate pellet sizes. Percentage protein of the diets was determined by micro-kjeldahl, percentage fat was determined by ether extract method, and moisture was determined by drying (100 C) until constant weight (AOAC, 1990). Digestible energy (DE) was estimated from the diet ingredient as established for *Oreochromis nilotica* (Santiago et al., 1982). Diets were also analysed for amino acid composition (Table 2 and Fig.1). Samples of diets were prepared for analyses by method 982.30, d and e (AOAC, 1990) and measured by using amino acid analyzer (Chromaspek-Rank Higer).

Experimental system and animals

The feeding trial was conducted in 15 glass aquaria each containing 80 liter of dechlorinated tap water. About one third of water volume in each aquarium was daily replaced by aerated fresh water after cleaning and removing the accumulated excreta. All aquaria were supplied with compressed air for oxygen requirements. Continuous illumination was supplied by fluorescent ceiling lights.

Water temperature and dissolved oxygen were measured every other day using a YSI Model 58 oxygen meter. Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectrophotometer. Total alkalinity and chloride were monitored twice weekly using the titration method, pH was monitored twice weekly using an

Table 1: Composition of a diet similar to a high-quality commercial tilapia diet (with fish meal) and experimental diets (without fish meal) fed to *Oreochromis nilotica* fry. All diets without fish meal contained various percentages of supplemental L-methionine.

	Diet				
	1	2	3	4	5
<u>Ingredient (%)</u>					
Menhaden fish meal	10.0	0.0	0.0	0.0	0.0
Meat meal	20.0	20.0	20.0	20.0	20.0
Soy bean meal	24.0	33.5	33.0	32.5	32.0
Wheat bran	20.0	20.0	20.0	20.0	20.0
Corn meal	20.0	20.0	20.0	20.0	20.0
Vegetable oil	5.0	5.0	5.0	5.0	5.0
Premix ¹	1.0	1.0	1.0	1.0	1.0
L-methionine	0.0	0.5	1.0	1.5	2.0
<u>Proximate analysis</u>					
Moisture	12.6	11.7	10.5	12.0	11.6
Crude protein	31.6	31.3	31.2	31.5	31.7
Crude fat	10.7	11.1	10.5	9.8	9.7
Ash	6.0	5.2	5.7	5.5	6.1
Crude fiber	5.0	5.0	5.0	4.8	4.7
CHO	34.1	35.7	37.1	36.9	36.2
DE (kcal /g) ²	2.8	2.9	2.9	2.8	2.8
P:E ratio ³	113	108	108	113	113

¹ Premix supplied the following vitamins and minerals(mg or IU)/ kg of diet, vit. A, 8000 I.U.; vit. D₃, 4000 I.U.; vit. E 50 I.U.; vit. K₃, 19 I.U.; vit. B₂, 25 mg; vit. B₃, 69 mg; Nicotinic acid, 125 mg; Thiamin, 10 mg; Folic acid, 7 mg; Biotin, 7 mg; vit. B₁₂, 75 mg; Cholin, 400 mg; vit. C, 200 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 Lovell, 1989).

²Digestible energy calculated based on 3.5 kcal /g for protein, 8.1 kcal /g for ether extract and 2.5 kcal /g for CHO (Santiago, et al., 1982).

³P:E = protein to energy ratio (as mg protein /kcal of DE).

electronic pH meter (pH pen; Fisher Scientific, Cincinnati, OH). During the 12-week feeding trial, the water - quality parameters averaged (\pm SD): water temperature, 27.4 ± 0.8 C: dissolved oxygen, 6.7 ± 0.5 mg /l : total ammonia, 0.20 ± 0.14 mg /l : nitrite, 0.07 ± 0.05 mg / l : total alkalinity, 180 ± 46 mg /l : chlorides, 573 ± 151 mg / l : pH, 8.5 ± 0.16 .

A set of 450 Nile tilapia (*Oreochromis niloticus*) fry average weight 0.9 ± 0.01 g were collected from the stock of fish research laboratory in Shebin El-Kom, Faculty of

Agriculture, Menofiya University and were used for the feeding trial. Thirty fish were randomly stocked into each aquarium with three replications per treatment. After stocking, to minimize stress of handling, fish from each aquarium were weighed every 2 weeks and at the end of the feeding trial. Total length of each fish was measured at the end of the trial. All fish were fed the quantity of food they could consume in 40 min. twice daily (0800 and 1600 h) for 12 weeks. At the start and end of the feeding trial a number of fish were killed by decapitation (15 at stocking and six fish per aquarium at the end), homogenized in a blender, stored in polyethylene bags, and frozen for subsequent protein, fat, moisture and ash analysis, according to AOAC, (1990).

Growth performance and feed conversion were measured in terms of final individual fish weight (g), total length (mm), survival (%), specific growth rate (SGR, % day⁻¹), feed conversion ratio (FCR), protein efficiency ratio (PER), and food intake(% body weight). Growth response parameters were calculated as follows : SGR (% day⁻¹) = $(\{\log W_t - \log W_i\} / T) \times 100$, where W_t is the weight of fish at time t, W_i is the weight of fish at time 0, and T is the rearing period in days : FCR = total dry feed fed (g) / total wet weight gain (g): PER = wet weight gain(g) / amount of protein fed (g) : Food intake = $(DFI \times 100) / (\{ W_{t+1} + W_t\} / 2)$, where DFI is mean daily dry food intake per fish (t, t+1) and W_t , W_{t+1} are the averaged wet weights at the start (t) and end (t+1) of the experimental period (Richardson, et al., 1985).

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the SAS ANOVA procedure (Statistical analysis system, 1988). Duncan's multiple range test was used to compare differences among individual means. Treatment effect were considered significant at $P \leq 0.01$. All percentage and ratio were transformed to arcsin values prior to analysis (Zar, 1984).

RESULTS AND DISCUSSION

Fish fed diet 3 and diet 1 were significantly ($P \leq 0.01$) longer (69.81 and 67.80 mm) than fish fed diet 2,4 and 5 (60.92, 61.89 and 61.54 mm, respectively) (Table 3). There was a significant difference ($P \leq 0.01$) in final individual weight, weight gain, weight gain %, specific growth rate, feed conversion ratio, protein efficiency ratio, and total food intake among treatments. The highest values of final individual weight, weight gain, weight gain %, specific growth rate, feed conversion ratio, protein efficiency ratio and total food intake were recorded with diet 3, which contained 33 % SBM and 1% L-methionine and there was no significant difference between diet 3 and diet 1 (control), but fish fed diets 2, 4, and 5 had significantly the lowest values (Table 3 and Fig., 2)). In contrast, the survival rate % (100 % for all diets) did not differ among the fish groups and the morphological examination did not reveal any morphological differences in the fish fed all experimental diets and high-quality control diet (Table 3). These data indicate that growth of Nile tilapia fry may not be adversely affected by feeding a diet containing a high percentage of soy bean meal (SBM) 33 % supplemented with 1 % methionine and contained 0 % fish meal (diet 3). This is in contrast to some studies on common carp (Nandeesh, et al., 1989),

Table 2 : Amino acid composition of experimental diets fed to Nile tilapia fry. (Amino acids expressed as percentage of diet)¹.

Amino acid	Required ²	Diet				
		1	2	3	4	5
Essential amino acid						
Arginine		1.33	2.02	1.91	1.90	1.89
1.87						
Histidine	0.54	0.91	0.86	0.86	0.85	0.85
Isoleucine	0.99	1.31	1.23	1.22	1.21	1.20
Leucine	1.09	2.10	1.90	1.88	1.87	1.86
Lysine	1.63	1.79	1.55	1.54	1.53	1.51
Methionine ³	1.02	0.75	0.88	1.18	1.58	1.88
Phenylalanine ⁴	1.82	1.30	1.25	1.24	1.24	1.22
Threonine	1.15	1.15	1.06	1.05	1.04	1.03
Tryptophan	0.32	0.32	0.30	0.30	0.30	0.29
Valine	1.09	1.52	1.39	1.38	1.37	1.36
Non-essential amino acid						
Alanine		1.70	1.52	1.52	1.33	1.30
Aspartic acid		3.42	3.80	3.81	3.83	3.87
Cystine		0.40	0.39	0.39	0.39	0.38
Glycine		2.44	2.21	2.20	2.19	2.18
Glutamic acid		4.50	5.04	5.27	5.47	5.67
Proline		1.81	1.79	1.76	1.69	1.63
Serine		1.34	1.33	1.31	1.30	1.29
Tyrosine		0.81	0.73	0.72	0.72	0.71

¹Values are means of two replications.

²From Santiago (1985).

³Approximately 60 % of cystine can substitute for methionine (Lovell, 1989).

⁴Approximately 50 % of Tyrosine can substitute for phenylalanine (Lovell, 1989).

Table 3 : Length, Weight, Gain in weight, Percentage weight gain, Survival, Specific growth rate (SGR), Feed conversion ratio (FCR), Protein efficiency ratio (PER), Food intake and Feed efficiency ratio (FER) of Nile tilapia fry (average initial weight 0.9 ± 0.01 g) fed a diet containing fish meal (diet 1) and four diets without fish meal and containing various percentages of supplemental L-methionine as shown in table 1. Values are means \pm SE of three replications. Means in the same row, having different superscript letters, are significantly different ($P \leq 0.01$).

	Diet				
	1	2	3	4	5
Total length (mm)	67.80 \pm 1.21 ^a	60.92 \pm 4.12 ^b	69.81 \pm 4.63 ^a	61.89 \pm 6.53 ^b	61.54 \pm 5.65 ^b
Final indiv. wt.(g)	6.37 \pm 0.24 ^a	4.70 \pm 0.04 ^b	6.61 \pm 0.06 ^a	4.88 \pm 0.12 ^b	5.04 \pm 0.12 ^b
Gain in weight (g)	5.37 \pm 0.24 ^a	3.71 \pm 0.04 ^b	5.62 \pm 0.06 ^a	3.88 \pm 0.12 ^b	4.04 \pm 0.12 ^b
Survival (%)	100.0 \pm 0.00 ^a	100.0 \pm 0.00 ^a	100.0 \pm 0.00 ^a	100.0 \pm 0.00 ^a	100.0 \pm 0.00 ^a
Weight gain (%)	537.3 \pm 24.3 ^a	374.7 \pm 4.40 ^b	563.5 \pm 4.50 ^a	388.3 \pm 12.3 ^b	404.0 \pm 12.0 ^b
SGR (% day ⁻¹)	0.96 \pm 0.02 ^a	0.81 \pm 0.01 ^b	0.98 \pm 0.01 ^a	0.82 \pm 0.01 ^b	0.84 \pm 0.01 ^b
FCR	2.98 \pm 0.21 ^{ab}	3.63 \pm 0.04 ^c	2.85 \pm 0.03 ^a	3.49 \pm 0.11 ^c	3.32 \pm 0.07 ^{bc}
PER	0.99 \pm 0.07 ^{bc}	1.19 \pm 0.01 ^a	0.93 \pm 0.01 ^c	1.15 \pm 0.03 ^a	1.09 \pm 0.02 ^{ab}
Total feed intake	15.9 \pm 0.45 ^a	13.45 \pm 0.12 ^b	15.92 \pm 0.37 ^a	13.54 \pm 0.02 ^b	13.40 \pm 0.15 ^b

FER 0.34 ± 0.03^{ab} 0.28 ± 0.03^c 0.35 ± 0.01^a 0.29 ± 0.01^c 0.30 ± 0.01^{bc}

Tilapia, *Oreochromis niloticus* x *O. aureus* (Shiau, et al., 1990), channel cat fish (Mohsen and Lovell, 1990), and blue catfish (Webster, et al., 1992a).

Results of the present study are in agreement with other studies (Davis and Stickney 1978 and Webster, et al., 1992b). Nile tilapia fed diets containing 0 % fish meal and 60 % SBM had similar growth rate when compared with fish fed commercial diets containing fish meal (Shiau, et al., 1990). No differences in weight gains were reported in blue tilapia, *Oreochromis aureus*, fed diets containing 0 % fish meal and 74 % SBM (36 % protein) compared with fish fed a commercial (36 % protein) diet containing fish meal (Davis and Stickney 1978). When protein level was below 30 %, fish fed diets containing fish meal, had higher weight gains compared with fish fed diets without fish meal. Since the essential amino acid levels in the 36 % protein diets exceeded the requirements of the fishes, it may be that the amino acid profiles of the lower-protein diets were not adequate when SBM totally replaced fish meal. In the present study percentage dietary protein was 31.5 %.

Although final body weights and weight gains were not significantly different between diet 1 and diet 3, fish fed diet 1 (containing 10 % fish meal and 24 % SBM) showed numerically smaller values compared with fish fed diet 3. This is unexplained since diet 1 was similar to diet 3 known to provide for good growth in Nile tilapia (NRC, 1993). All essential amino acids requirement for Nile tilapia were met or exceeded by diet 1 except methionine (Table 2). Diet 1 contained 0.75 % methionine (methionine plus 60 % of cystine), covered the requirement of Nile tilapia from methionine (1.02 %) (Santiago, 1985) also diet 3 covered the requirements of Nile tilapia from methionine and exceeded (1.18 %). This discrepancy does not appear large, but may be a factor in the somewhat reduced growth of fish fed diet 1. Fish meal quality may have been less than optimal.

Feed conversion ratio (FCR), PER, and feed intake were significantly different ($P \leq 0.01$) among treatments (Table 3). Feed intake and PER values were similar to values reported in Webster et al., 1992a). Feed conversion values in the present study are somewhat higher than reported in literature; however, they are consistent with FCR values found in other studies (Andrews and Stickney, 1972 and Webster, et al., 1992a,b). The FCR values tend to be higher due to the use of small (80 l) aquaria and the relatively slow feeding habits of Nile tilapia (Shiau, et al., 1990). Further, the colour of the diets (light brown) sometimes made them difficult to see in aquarium bottoms and may have resulted in overfeeding. However, feed supply must not be limiting in nutrition experiments and overfeeding is more desirable than underfeeding (Tacon and Cowey, 1985).

Fish flesh proximate composition at the end of the feeding trial resulted in no significant differences ($P > 0.05$) in percentage moisture, fat and ash and their averages were 75.78 %, 16.46 % and 9.69 % respectively. While in percentage protein, diet 3 significantly ($P \leq 0.01$) had higher protein percentage than the other experimental diets and control diet (Table 4). All diets were formulated to be similar in percentage protein and energy content.

Replacement of fish meal with SBM has had variable success. In those studies in which growth is reduced, several hypotheses have been suggested to explain the results; 1. suboptimal amino acid balance (NRC, 1993); 2. inadequate levels of phosphorus in SBM (NRC, 1993); 3. presence of antinutritional factors (including trypsin inhibitors) (Liener, 1980); and 4. inadequate levels of energy in SBM (Webster et al., 1995).

SBM has one of the best amino acid profiles of any plant protein foodstuff and the composition meet the requirements of essential amino acid for Nile tilapia (Lovell, 1988).

Amino acid analysis indicated that all diets met the amino acid requirements of Nile tilapia (Santiago, 1985). However, the biological value of amino acid from SBM may be less than indicated. Dabrowski et al., (1989) stated that methionine availability may be reduced when SBM comprises large percentage (> 50 %) of the diet. However, practical diets for Nile tilapia have percentage of SBM higher than 50 % without adverse effects on growth as reported by (Lovell 1988; Shiau et al., 1990 and Robinsone and Robinett, 1994).

Addition of supplemental methionine in fish diets has had variable success. Tacon et al., (1984) stated that addition of 0.2 % L-methionine to a diet deficient in methionine for rainbow trout, *Oncorhynchus mykiss*, did not improve fish growth when compared with commercial diet. However, Shiau et al., (1987) reported that addition of supplemental methionine improved growth of tilapia. Murai et al., (1986) reported that nutritional value of soy flour was improved by addition of 0.4 % crystalline L-methionine. This is in contrast to Andrews and Page (1974), who reported no improvement in weight gains when supplemental methionine was added to channel catfish diets. These conflicting data may be due to the higher level of sulphur amino acid in the basal diet fed by Andrews and Page (1974). It has been stated that crystalline methionine is rapidly absorbed in fish and degraded into methionine sulphoxide (Thebault, 1985; Murai et al., 1986).

Fish have high requirement for phosphorus; however, SBM is deficient in available phosphorus. Although SBM contains approximately 0.7 % phosphorus, only about one-third to one-half is biologically available to fish (Lovell, 1988). Diets used in the present study contained > 0.9 % available phosphorus, while the requirement for Nile tilapia is 0.9 % (NRC, 1993). It has been reported that SBM could mostly replace fish meal in diet for Nile tilapia if supplemental phosphorus was added (Liebowitz, 1981). Reinitz (1980) stated that weight gain in rainbow trout was positively correlated with the percentage of dietary phosphorus.

It is known that antinutritional factors in raw or inadequately heated SBM can adversely affect fish growth (Smith, et al., 1980; Robinson, et al., 1981 and Viola et al., 1983). Webster et al., (1992a) reported that use of heated SBM in diets did not increase growth of blue catfish, probably because of the already low level of trypsin inhibitor in commercial SBM used. However, Wee and Shu (1989) reported higher growth in Nile tilapia, *O. niloticus* (L) fed diets containing boiled SBM compared with fish fed diets containing fish meal or raw SBM. This was due to inactivation of the high trypsin inhibitor activity in SBM used and increasing digestibility of diets containing boiled SBM compared with raw SBM.

Some fish, such as red drum, *Sciaenops occlatus*, find SBM unpalatable and will not consume diets that have 0 % fish meal (Reigh and Ellis, 1992). Mohsen and Lovell (1990) reported that addition of animal by product to a SBM-based diet improved palatability. However, fish adapted to a SBM diet may have similar consumption rates to those of fish fed diets containing animal protein sources. In the present study, Nile tilapia fry appeared to consume diets containing 33 % SBM and 0 % fish meal with other animal protein sources and did not find them unpalatable.

The variable success of researchers in using SBM as a total replacement for fish meal, indicates the wide variation possible in the nutritive value of SBM for various fish

species and the influence of diet formulation on growth. The data from the present study indicate that a diet in which SBM totally replaces fish meal, with supplemental L-methionine at a level of 1 % may be fed to juvenile Nile tilapia (*O. niloticus* L.) fry without adverse effects on growth and body composition when the fish are fed to satiation, and the diet contains 31.5 % protein and vegetable oil. Further evaluation of soy bean meal -based diet on growth of Nile tilapia in production trial of longer duration should be conducted.

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