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USE OF SOYBEAN MEAL AND/ OR CORN GLUTEN MEAL AS PARTIAL SUBSTITUTES FOR FISH MEAL IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLING DIETS

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ABSTRACT

This experiment was conducted to evaluate both soybean meal (SBM) and corn gluten meal (CGM) as alternative dietary protein sources for fishmeal in Nile tilapia (*Oreocromis niloticus*) fingerlings. Fish with an average initial body weight of 4.35 ± 0.04 g were stocked into ten glass aquaria (100 X 30 X40 cm). Each tank contains 100 liters of freshwater and was stocked with ten fish. Five iso-nitrogenous diets (30%crude protein) containing different levels of SBM and CGM, (25 or 50%) were fed two times daily, 6 days a week for 70 days. Fish Growth rate, feed conversion ratio (FCR), protein efficiency ratio (PFR), protein productive value (PPV) and energy utilization (EU) were deteriorated with increasing both of SBM and CGM at 50% in the tested diets for tilapia (*Oreocromis niloticus*) fingerlings. Fish fed the diets supplemented with either CGM or SBM at 25% FM substitution level were not statistically different from those of fish fed the control diet (containing 40% fish meal (FM) as a sole protein source). Body compositions of various experimental fish were significantly affected ($P < 0.05$) by dietary treatments. Body water and body lipid were positively correlated ($P < 0.05$) with SBM and GM dietary inclusion level from 25% to 50 % in the tested diets, while both body protein and ash content showed negative relationship ($P < 0.05$). Survival rate of fish ranged from 90 to 100%, suggesting that up to 25% of fish meal protein can be replaced with either SBM or CGM in the diet of tilapia (*Oreocromis niloticus*) fingerlings, without affecting fish growth or nutrient composition.

INTRODUCTION

Aquaculture has expanded very rapidly and is now the fastest growing food-producing industry in the world. FAO (2004) estimated that by year 2030, over half of the fish consumed by the world's people would be produced by aquaculture. In Egypt tilapia production reached about 349,739 tones in the year 2003 (GAFRD, 2003), about 78.56 % from the total farmed fish production (445,181 tones). Assuming a mean feed conversion ratio of 1.8 for tilapia, total feed used should be nearly 629,530 tones per year. The primary objective for most fish farmers is to produce high quality fish at least cost. Feed typically account for 50-60 % of operating costs in efficient farms (Tacon, 1997 and Essa et al., 2004). The development of commercial aquafeeds has usually been based upon the use of fish meal (FM) as the main source of dietary protein. However, there is an urgent need for aquafeeds industry to reduce its total dependence upon FM because of its inadequate supply and escalating prices (Wassef et al., 2003 and El-Saidy & Gaber 2003).

According to Alvardo (1997) the pollution caused by aquafarms was high due to the use of animal protein sources such as fish meal and moist pellets, thereby good quality plant protein sources can reduce

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production costs, maintain profitability in fish farms so as to minimize aquatic environmental impacts. The dependency of tilapia upon fishmeal is not as great as that of carnivorous finfish and shrimp species, so it having an essentially herbivorous feeding habit (Morales et al., 1994).

Numerous studies have been conducted using processed soybean meal (SBM) as a fishmeal replacer within tilapia feeds (El-Sayed and Tacon, 1997; El-Ebiary & Essa, 2002; Wassef, 2005 and El-Sayed, 2006). Some of these studies have shown that between 67% to 100% of the dietary protein could be supplied in the form of SBM. Other studies recommended that SBM must not increase than 50% of fish diet (Shiau et al., 1987 and El-Saidy & Gaber 2003).

Soybean meal appeared to be better utilized by most fish species due to their nutritional quality, lower cost and availability, as compared to other plant protein sources (Alceste, 2000). Soy protein is considered the best plant protein source for meeting the essential amino acid requirements of tilapia and other fish species (Swick, 2001). It is highly digestible by fish and the digestion coefficients are comparable or higher than fish meal protein (Koprucu and Ozdemir, 2005). Corn gluten meal (CGM) has also been successfully used as dietary protein sources for tilapia. For example, the growth of *O. niloticus* fingerlings fed corn distillers grains with soluble gluten meal as partial protein sources was better than that of fish fed a 100% FM in control diet (Tudor et al., 1996). On a more practical level, corn gluten and gelatinized corn meal were evaluated as protein sources in tilapia feeds. Corn gluten was found to be a suitable protein source in a fish meal-free diet if the proper amino acid balance was maintained (Wu, et al. 1995). It is highly digestible by tilapia and the digestion coefficients are comparable to fish meal protein (Koprucu and Ozdemir, 2005). However, although plant protein sources may be a cheaper and more sustainable source of dietary protein for use by the aquaculture industry, their success or not will rest upon the shoulder and skills of the feed ingredient processor, the aquafeed formulator, the aquafeed manufacturer (Tacon, 1997).

The present study have been conducted to reduce current dependence of tilapia feeds upon fishmeal as animal protein source and investigate the optimum possibility of partial fishmeal by other plant proteins such as, soybean meal and/ or corn gluten meal.

MATERIALS AND METHODS

The present study was conducted in Fish Rearing Laboratory (NIOF), Alexandria. Ten glass aquaria with dimensions 100X40 X30 cm each were used to five feeding treatments (two-tanks/ treatment). Each tank-contained 105 liters of dechlorinated tap water (stored two nights before use) was stocked with ten Nile tilapia (*Oreocromis niloticus*) fingerlings weighing 4.35 ± 0.04 g per fish on average. The water in each aquarium was provided with sufficient aeration by means of an electric air pump. The experiment began in August 1st, 2005 and ended after ten weeks.

Experimental diets:

Five isocaloric iso-nitrogenous diets were formulated in which soybean meal protein (SMP) and corn gluten meal protein (CGMP) were partially replaced fishmeal protein (FMP). The fifth experimental diets were prepared as follows:

Diet 1: Control diet (100%fishmeal).

Diets 2 and 3: where 25and 50% of fishmeal protein were replaced by SBP respectively.

Diets 4 and 5: where25 and 50% of fishmeal protein were replaced by CGMP respectively.

The proportions of the other foodstuffs were varied in order to maintain crude protein, fat and fiber contents in the experimental diets. Ingredients and proximate composition of test diets are summarized in Table (1). The experimental diets were introduced to fish in a combined powder form. Fish were hand fed two times daily (9.00 and 14.00 hrs), 6 days a week, at a rate of 5% of fish body weight for 70 days. Proximate, amino acids composition and apparent digestibility of nutrients and energy in fish meal, corn gluten and soybean meal for Nile tilapia was shown in Table (2) according to Koprucu and Ozdemir (2005).

The amount of feed given to each aquarium was determined at the end of the study. Faeces and other remains were removed by siphoning every morning at 09.30 am, before the commencement of feeding. Throughout the experiment, each aquarium was partially (1/3 capacity of water) cleaned daily, and the water was completely changed two times a week. Every two weeks, fish were weighed and the experimental rations were adjusted accordingly. The set-up was placed under the natural day-length conditions, i.e., between 11 and 12 hours of light. Water temperature was recorded once daily at 10 am., 6 days a week. Weekly water analyses were carried out to determine pH values using pH meter. At the end of the experiment, fish in each aquarium were individually weighed, then were sacrificed and frozen at –20ºC for final body composition analyses. Initial body analyses were performed on a pooled sample of 30 fingerlings, which were weighed and frozen prior to the study. Proximate analyses of water, protein, lipid and ash in body fish carcass were performed according to standard AOAC (1995) methods. Body weight gain BWG (g/fish), specific growth rate SGR, (%/g/day), food conversion ratio FCR, protein efficiency ratio PER, protein productive value PPV, energy utilization EU and survival rate SR, (%) were computed according to Jauncey and Ross (1982). Statistical analyses of experimental data were conducted according to one-way analysis of variance ANOVA (Snedecor and Cochran, 1980), and means were compared using Duncan's multiple range test (1955) ($P > 0.05$).

Table 1. Composition and proximate analyses (% DM) of experimental diets.

Item	Diet No.				
	1(CTR)	2	3	4	5
Feed Ingredient (%):-					
Fish meal *	40	30	20	30	20
Soybean meal **	-	14	28	-	-
Corn gluten meal(CGM)***	-	-	-	10	20
Wheat bran	40	30	30	30	30
Yellow corn flour	15	21	17	25	25
Sun flower oil	3	3	3	3	3
Vitamin mix [1]	1	1	1	1	1
Mineral mix [2]	1	1	1	1	1
Proximate composition (% DM)					
Dry matter	92.3	90.89	90.63	90.95	90.75
Crude protein (CP)	31.62	31.13	30.74	31.42	31.38
Ether extract (EE	6.98	7.02	7.05	6.75	6.48
Crude fiber (CF)	4.67	4.59	5.21	4.34	4.68
Ash	11.62	10.31	8.47	9.53	7.64
Nitrogen free extract(NFE)	45.11	46.95	48.53	47.96	49.82
Gross energy kcal/100g [3]	434.08	439.41	444.13	442.74	447.77
Protein energy ratio (P/E mg P/Kcal GE	72.84	70.84	69.21	70.97	70.08

* Imported fish meal (62.6% P and 4.6 % L.).
** Hexane-extracted soybean meal (43.9% P and 4.8%)
*** Local CGM, 61.3% P and 2.2% L.)
[1]- Vitamin mixt / kg premix containing: 3300IU vitamin A, vitamin D, 410IU vitamin E, 2660mg vitamin $B^{1,}$ 133 mg vitamin$B^{2,}$ 580mg vitamin B^6, 410 mg vitamin B, ^{12} 50 mg biotin,9330mg colin chloride, 4000mg vitamin C, 2660 mg Inositol, 330mg para- amino benzoic acid, 9330mg niacin, 26.6mg pantothenic acid.
[2]- Mineral mix / kg: 325mg Manganese, 200mg Iron, 25mg Copper, 5mg Iodine and, 5 mg Cobalt.
[3]- Gross energy (GE kcal/100g diet) calculated according to NRC (1993) 5.65, 9.45, and 4.2 kcal/g diet of protein, fat and carbohydrates, respectively.

Table 2. Proximate, amino acids composition and apparent digestibility of nutrients and energy in fish meal. Corn gluten and soybean meal for Nile tilapia (Source: Koprucu and Ozdemir, 2005).

Components (in dry matter)	**International Feed Number of ingredients**			**Apparent digestibility of ingredients**		
	AM[1]	**CGM [2]**	**SBM [3]**	**AM[1]**	**CGM [2]**	**SBM [3]**
	5-01-985	5-28-242	5-04-604	5-01-985	5-28-242	5-04-604
Dry matter	92.1	91.4	90.8	91.6	93.2	90.9
Crude protein	71.1	66.2	53.7	90.5	89.0	87.4
Lipid	8.3	2.1	1.0	97.5	94.0	92.1
Fiber or chitin	1.1	1.8	3.9	90.1	96.1	95.2
Ash	14.2	2.5	6.2	38.1	74.9	71.6
Nitrogen-free extract	5.3	27.4	35.2	93.7	95.8	92.6
Calcium	4.1	0.2	0.3	17.1	20.3	20.9
Phosphorus	2.9	0.5	0.7	27.8	28.2	30.1
Gross energy	5.1	5.4	4.6	92.1	89.0	83.7
Amino acids						
Arginine	3.9	1.6	3.7	92.1	88.9	88.7
Histidine	1.7	1.4	1.3	92.4	90.0	88.9
Isoleucine	3.2	2.7	2.2	90.7	89.5	86.1
Leucine	5.1	10.1	3.7	90.8	87.7	86.5
Lysine	5.2	1.2	3.2	90.5	88.0	83.4
Methionine	2.1	1.7	0.7	94.0	94.1	84.0
Cystine	0.7	0.7	0.6	89.6	86.7	86.0
Phenylalanine	2.8	4.1	2.5	90.0	90.3	87.2
Tyrosine	2.3	3.4	1.8	90.5	86.0	85.7
Threonine	2.9	2.2	1.9	90.1	88.5	86.9
Valine	3.6	3.2	2.6	90.3	88.6	83.5
Alanine	5.3	5.4	3.0	93.2	94.0	86.0
Aspartic acid	6.8	5.0	6.1	91.4	89.4	88.0
Glutamic acid	10.2	12.6	10.0	92.8	90.2	92.1
Glycine	6.6	1.4	2.5	90.4	91.9	89.0
Proline	5.3	7.3	4.8	91.2	90.1	90.5
Serine	2.7	2.0	2.4	91.6	89.9	88.0
Average AAAD*				91.2	89.6	87.1

1 Anchovy meal 2 Corn gluten meal 3 Soybean meal * Average apparent amino acid digestibility.

RESULTS

Growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings fed the five test diets was summarized in Table (3). The results revealed a significant effect of substituting FM with SBM or CGM on fish growth indices. The 100% fishmeal –based diet (diet 1 CTR) produced the highest weight gain (WG, g/fish) and SGR (%/day). Both parameters significantly (P< 0.05) declined with increasing SBM or CGM dietary inclusion level. There were no significant differences in either growth parameters (WG & SGR) when fish fed 25% CGM or SBM (diets 4 and 2) respectively compared to fish fed the control diet of 100%FM (diet 1). Weight gain and SGR of fish fed diet 3 and 5 which contained 50% SBM and 50% CGM respectively were significantly lower than those of control diet. Survival rate gave the same trend as WG and SGR.

Feed and nutrient utilization indices of *O. niloticus* fed different levels of SBM and CGM are shown in Table (4). Feed intake (FI) was the highest among all when fish fed at 100% fishmeal diet (diet 1) but insignificantly differ from the other four diets. Diet 4 contained 75% FM and 25% CGM) gave the best FCR, PER, PPV and EU. There was no significant different for the same parameters (P< 0.05) when fish fed CTR diet (1) or diet 2, (75%FM +25% SBM). Fish fed either diet 3 or 5, which contained either 50% of SBM or 50% of CGM, gave the lowest values among all. Body composition was significantly affected (P< 0.05) by dietary treatments. As shown in Table (5), body water and lipids were positively correlated (P< 0.05) with elevation of SBM or CGM inclusion level from 25% to 50 % in test diets, while body protein and ash showed negative effect (P< 0.05) on the same level.

DISCUSSION

This study revealed an inverse effect of dietary SBM and CGM increased inclusion level on growth, feed utilization and survival rate of Nile tilapia fingerlings. It has further indicated that FM appeared to be a better protein source for tilapia than either of tested proteins. FDS (1994) has been recorded before that fishmeal is still generally the preferred protein source for use within compound aquafeeds for tilapia because of its high nutritional quality and biological value to that fish. In the present study, there were no significant difference in weight gain and FCR when fish fed 25% CGM or 25 % SBM (diet 4 or diet 2) respectively compared to fish fed 100%FM control diet (diet 1). Further increase of SBM or CGM in the tested diets up to 50% had resulted to a significant decrease of weight gain, FCR and survival rate of *O. niloticus.* These findings are in agreement with Shiau et al. (1987) demonstrated that fish meal can be partially replaced by soybean meal when the dietary protein level is below optimum for growth (24%). At the optimum level of dietary protein (32%) replacement of 30% catfish meal with soybean meal significantly depressed growth and feed efficiently. However, these were restored by addition of methionine to the level of the control diet. Tacon et al. (1983) reported that supplementation of 0.8% D, C-methionine to a diet in which 75% of brown fish meal was replaced by soybean meal improved the growth performance of *O. niloticus* to a level comparable to that of a fish meal diet. Viola and Arieli (1983) reported that soybean meal could be used to replace up to half of the fish meal in tilapia feeds having 25% crude protein content, without requiring any supplementation. Complete substitution of fish meal by soybean meal resulted in significant reduction of weight gain and feed efficiency which were not overcome by supplementation of oil, lysine, methionine and vitamins. Jackson et al. (1982) also observed a growth reduction of *Sarotherodon mossambicus* fed to a diet which 50% or more fish meal was replaced by soybean meal. They attributed this to the low level of sulfur amino acids and the presence of other factors such as trypsin inhibitor or haemaglutinins. The results also are in agreement with those previously obtained on mullet *Liza macrolepis* fingerlings when fed practical diets contained elevated levels of SBM as FM-replacer (El-Sayed 1994)). Mullet growth rate, feed conversion (FC), protein efficiency ratio (PER) and protein productive value (PPV) were deteriorated with increasing SBM level in the diets.

In the present work, Nile tilapia fingerlings fed the diet replacing 50% of fish meal protein with CGM (diet 5) showed considerably lower growth and feed utilization performance (Tables 3&4). Similarly, for replacement dietary groups substituting 25 % of fishmeal with CGM (diets4), all growth indices measured were comparable to those of the control diet containing fishmeal as a sole protein source (Table 3). Feed ingredient may appear from its chemical composition to be an excellent source of nutrients but will be of little actual value unless it can be digested and absorbed in the target species (Watanabe, et al.1996). The nutritive value of mixed rations depends on the nutrient composition of the individual feed components and the ability of fish to digest and absorb the nutrients (Riche, et al., 2001). Protein quality of dietary protein sources depends on the amino acid composition and their digestibility. Deficiency of an essential amino acid leads to poor utilization of the dietary protein and consequently reduces growth and decreases feed efficiency (Halver and Hardy, 2002).

Although SBM and CGM were highly digestible by tilapia and the digestion coefficients of protein are comparable to fish meal protein, and were not significant 87.4, 89.0 and 90.5, respectively there were deficient in amino acid (lysine and methionine) in SBM and CGM compared FM sea table 2 (Koprucu and Ozdemir, 2005). Also Wassef et al. (2003) cleared there were a shortage in amino acid (lysine and methionine) either in SBM or CGM compared to *O. niloticus* requirement.

There are several factors that may affect the high level for replacement among fish species, such as the specific amino acid requirement of fish, digestibility, and formulation of feeds.

Shimeno *et al*. (1993), on yellowtail, showed that the proper replacement level of fishmeal protein with CGM was 13-26%. On the other hand, Robaina *et al*. (1997) showed that replacing 20- 40% fish meal protein with CGM in the diet of gilthead seabream (*S. aurata*) had no adverse effects on the growth, feed efficiency, protein efficiency ratio, and digestibility. Similarly, a 40% substitution of fishmeal protein with CGM has been reported for rainbow trout diets (Morales *et al*. 1994). Part of the problem of using CGM at high level as a substitute for fishmeal in the diet of sea bass fry (*Dicentrarchus labrax*) is that the amino acid profile is insufficient to meet their requirements. Sea bass fed diet of 60% replacement FM level showed poor growth and feed utilization (El- Ebiary, et al., 2001). Takeuchi, et al. (1994) recorded that gelatinization of corn meal through extrusion of the feed provided some improvement in nutritional value, though tilapia hybrids were able to utilize raw corn meal nearly as effectively.

In conclusion, 25 % of dietary fish meal protein can be replaced by soybean meal or corn gluten meal protein without any adverse effect on growth, feed utilization and survival performance of *Oreochromis niloticus* fingerlings.

Table 3. Growth performance indices and survival rate of *O. niloticus* fingerlings fed diets contained different levels of soybean or corn gluten meal.

Diet	Initial Weight (g/fish)	Final Weight (g/fish)	Indices			Survival Rate (%)
			Weight Gain (g/fish)	ADG (mg/day/fish) [1]	SGR (% /day)[2]	
1 (CTR)	4.39	26.29 [a]	21.90 [a]	312.86 [a]	2.56 [a]	100 [a]
2	4.33	21.88[a]	17.55 [a]	250.71 [a]	2.31 [a]	100 [a]
3	4.37	18.29 [b]	13.92 [b]	198.86 [b]	2.05 [b]	80 [a]
4	4.35	24.72 [a]	20.37 [a]	291.00 [a]	2.48 [a]	100 [a]
5	4.31	19.10[b]	14.79 [b]	211.29 [b]	2.13 [b]	60 [b]
LSD(P<0.05)*	-	4.31	4.29	61.24	0.27	22.24

1 Control (100% FM)
2 (75% FM + 25% SB);
3 (50% FM + 50% SB);
4 (75% FM + 25% GM);
5 (50% FM + 50% GM).
[1] Average daily gain = body weight Gain / experimental period (d).
[2] Specific growth rate =(ln final weight – ln initial weight / time (d) X 100
Means with different letters within the same column differ significantly (P< 0.05).
* Least significant differences.

Table 4. Feed and nutrient utilization indices of *O. niloticus* fingerlings fed different levels of soybean or corn gluten meal, supplemented diets.

Diet	Feed utilization		Protein utilization		EU % [5]
	FI(g) [1]	FCR [2]	PER [3]	PPV % [4]	
1 Control	44.87 [a]	2.05 [b]	1.54 [a]	23.40 [a]	15.85[a]
2	35.16 [b]	2.00 [b]	1.62 [a]	24.21 [a]	16.09 [a]
3	35.76 [b]	2.57 [a]	1.27 [b]	18.80 [b]	12.43 [b]
4	39.50 [b]	1.94 [b]	1.64 [a]	24.69 [a]	16.36 [a]
5	36.51[b]	2.47 [a]	1.29 [b]	19.08 [b]	12.70 [b]
LSD (P< 0.05)	4.98	0.36	0.22	3.56	2.42

[1] Feed intake (g DM/fish).
[2] Food conversion ratio = dry fe ed (g) / live body gain (g)
[3] Protein efficiency ratio = live body gain (g)/ protein intake (g).
[4] Protein productive value = protein gain (g) / protein intake (g) X 100
[5] Energy utilization = Energy gain in fish (kcal) / energy intake (kcal) X 100
Means bearing different letters within the same column differ significantly (P< 0.05).

Table 5. Carcass composition (% DM) of *O. niloticus* fingerlings at start and end of feeding trial (70 d).

Diet	Water (%)	(% on DM)			Energy content (Kcal/100g)
		protein (%)	Lipids (%)	Ash (%)	
At start	77.18	58.92	17.67	23.42	499.88
At end 1	76.56 [b]	63.10 [a]	23.64 [b]	12.86 [b]	579.91 [a]
2	76.48 [b]	62.82 [a]	23.71 [b]	13.46 [a]	578.99 [a b]
3	76.76 [a]	62.44 [b]	24.21 [a]	13.34 [a]	581.57 [a]
4	76.52 [b]	62.86 [a]	23.62 [b]	13.54 [a]	578.37 [b]
5	76.82 [a]	62.48 [b]	24.06 [a]	13.44 [a]	580.38 [a]
LSD 0.05	0.19	0.51	0.33	0.34	1.88

[1] Energy contents (Kcal/ 100g) calculated according to NRC (1993) using the following calorific values; 5.65, 9.45 and 4.2 Kcal /g whole body of protein, fat and carbohydrate, respectively.

Means bearing different letters within the same column differ significantly (P< 0.05).

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