

Effect of Replacing Fish Meal by a Mixture of Different Plant Protein Sources in Nile Tilapia (*Oreochromis niloticus* L.) Diets

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Abstract: This study was designed to determine the maximum replacing levels of Fish Meal (FM) by a Plant Protein Mixture (PPM) in eight diets for Nile tilapia. The PPM consisted of cottonseed, sunflower, canola, sesame and linseed meals. FM in the basal diet was replaced by PPM in the diets at replacing levels of 15, 30, 45, 60, 75, 90 and 100%. A total number of 480 Nile tilapia were randomly distributed into eight treatments, each in three replicates. After 12 weeks of feeding, replacement of 15, 30 or 45% of FM by PPM did not significantly affected Feed Intake (FI), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and the Apparent Digestibilities Coefficient (ADC) of Dry Matter (DM), Crude Protein (CP) and Ether Extract (EE), while the highest replacing levels (60, 75, 90 or 100%) significantly ($P < 0.05$) reduced these parameters. Growth parameters were relatively parallel to those of FI, FCR and PER whereas, replacement up to 45% exhibited Body Weight (BW), Body Length (BL), Weight Gain (WG) and Specific Growth Rate (SGR) not differing significantly ($P < 0.001$) from the fish fed control diet. Compared to control, increasing PPM in the diets significantly reduced hemoglobin, hematocrite and the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The incorporation of PPM in diets did not significantly affect whole-body Dry Matter (DM) and Crude Protein (CP). From economic view, replacement of FM by PPM up to 45% in tilapia diets reduced feed costs/kg diet and feed costs/kg weight gain by 11.40 and 6.74%, respectively.

Key words: Replacement % fish meal % mixture plant protein % growth % feed utilization % Nile tilapia

INTRODUCTION

The intensification of fish production in Egypt has made it essential to develop complete and supplemental diets for use in aquaculture. Traditionally, fish meal is the preferred dietary protein source for many farmed fish species and is appreciated for its amino acid balance, vitamin content, palatability and un-identified growth factors [1]. However, increasing cost of fish meal has restricted its use as a protein source for fish diets. Therefore, plant proteins appear to be the most suitable alternatives for fish meal in fish diets.

Various oilseed meals are produced in Egypt on a large scale as by-products of the edible oil industry. These include cottonseed, sunflower, soybean and linseed meals. The efficiency of the various alternative protein sources as a partial or complete replacement for fish meal has been individually evaluated in fish diets, e.g.

sunflower meal [2, 3], soybean [4, 5], linseed meal [6, 7], canola [8] and cottonseed meal [9]. Individually, these plant by-product meals are fairly rich in protein and favorable essential amino acid profiles, but they are deficient in one or more essential amino acids and contained various quantities of anti-nutritional factors [10]. Some studies have also stressed that a mixture of plant protein source is more appropriate to obtain adequate amino acid profile compared to the incorporation of a single plant protein source [11, 12]. Recently, comparative studies conducted on rainbow trout, turbot, sea bass and sea bream recommend to complete substitute of fish meal by a mixture of plant protein. All diets were supplemented with L-amino acids to meet the amino acid requirements estimated for rainbow trout [10]. Results were disappointing and, compared to a control diet, growth retardation was observed even in rainbow trout. Beside the effects of known or unknown anti-

nutritional factors, a deficiency of one or more amino acid was suspected, suggesting that supplementation of diet according to amino acids needs available in [10] was not sufficient. [13] suggested that amino acid profile of fish meal reflects well the fish amino acid needs which could imply to supplement plant protein based diets at higher levels than required by [10].

The present, study was carried out to evaluate the nutritional value of combinations of plant proteins in order to replace fish meal in tilapia diets.

MATERIALS AND METHODS

Nile tilapia fingerlings were obtained from the World Fish Center at Abbassa, Sharkya Governorate, Egypt and acclimated to laboratory conditions in 1700-L fibreglass tanks. The feeding trial was performed at the Fish Nutrition Lab. (College of Agriculture, Benha University, Egypt).

Diets and feeding regime: Eight experimental diets were formulated (Table 1) to be isonitrogenous (30% CP) and isocaloric (2700 Kcal ME kg⁻¹). Cottonseed, sunflower, linseed, seasmе meals were obtained from local market, while canola meal was obtained from the Agricultural Research Center, Dokki, Egypt and these meals were mixed (20% for each) to obtain the PPM. Fish meal in the control diet was replaced (based on protein content) by PPM at 15, 30, 45, 60, 75, 90 and 100% levels. In preparing the diets, dry ingredients were first ground to a small particle size and mixed thoroughly with added water to obtain a 30% moisture level. Diets were passed through a mincer with diameter of 2 mm and were sun-dried for 3 days.

Culture conditions: At the beginning of the experiment, 24 glass aquaria (100×50×40 cm ml) were supplied with freshwater (180 L for each) at a rate of 1L min⁻¹ with supplemental aeration and each aquarium was stocked by 20 fish (2.61-2.71 g). Fish were fed the diets at a daily rate of 10% (during the 1st month), then reduced to 7% (2nd month) and 4% (3rd month) of total biomass. Fish were fed 6 day/week (twice daily at 9.00 am and 3.00 pm). The amount of feed was bi-weekly adjusted according to the changes in body weight throughout the experimental period (90 days).

Digestibility trial: A chromic oxide marker was included (0.5%) in all experimental diets. During the last three weeks of the experiment, fish provided the experimental

diets and feces were collected daily as described by [14]. Feeds and collected feces were dried to a constant weight. Proximate analysis of the diets and feces were conducted in 6 triplicates for Dry Matter (DM) Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF) and ash. Chromic oxide levels were determined in the diets and feces [15] and apparent digestibility coefficients for the nutrients were calculated according to [10] by the equation: $100 - 100 \left[\frac{(\% \text{ marker in diet} / \% \text{ marker in feces}) \times (\% \text{ nutrient in diet} / \% \text{ nutrient in feces}) \right]$.

Growth and feed utilization parameters: Growth performance and feed utilization parameters were determined according to [16] as follows:

$$\text{Specific growth rate (SGR)} = \frac{(\ln W_2 - \ln W_1)}{t} \times 100$$

Where:- Ln = the natural log, W1= initial fish weight; W2 = the final fish weight in “grams” and t = period in days.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake (g)}}{\text{wet weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{weight gain (g)}}{\text{protein intake (g)}}$$

Water quality: Parameters of water quality were determined according to the methods of [17]. Ammonia and nitrite were measured at weekly intervals, while water temperatures were recorded daily in each tank using a mercury thermometer suspended at 30-cm water depth. Also, dissolved oxygen was measured daily by oxygen meter and pH by pH meter.

Blood samples and liver function: Blood samples were obtained from fish at the end of experimental period. Five fish per tank were randomly chosen and anaesthetized by ethylene glycol mono-phenol ether. Blood samples were collected from the caudal vein using heparinized 27-gauge needles and tuberculin syringes. Hematocrite (Ht) was determined using the micro-Ht method described by [18]. Hemoglobin (Hb) was determined using the total Hb kit (Sigma Diagnostics, Sigma, St Louis, MO. USA) which is standardized procedure using the cyanomethemoglobin method. Liver was removed, homogenized and assigned for determination of Aspartate transaminase (AST) and Alanine transaminase (ALT) according to [19].

Chemical analysis: At termination of the experiment, three fish were randomly sampled from each tank and

subjected to the chemical analysis of whole fish body. Chemical analysis of fish, diets and feces were determined according to the methods of [20].

Statistical analysis: The statistical analysis of data was carried out by applying the computer program [21] by adopting the model: $Y_{ij} = \mu + \alpha_i + e_{ij}$ Where, Y_{ij} = the observation on the j^{th} fish eaten the i^{th} diet; μ = overall mean, α_i = the effect of i^{th} diet and e_{ij} = random error.

RESULTS

Water quality: During the whole experimental period, water temperature ranged from 23.15 to 30.16°C, dissolved oxygen from 4.55 to 6.23 mg/L, pH from 7.71 to 7.89 and total ammonia from 0.12 to 0.16 mg/L. There were no significant differences in water quality parameters among treatments during the whole experimental period indicating that, the experimental diets has not detrimental effects on the surrounding water quality of experimental fish.

Feed utilization: The highest feed intake (FI) and the best Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) were obtained for fish fed the control diet and these

parameters were did not significantly affected when 15, 30 or 45% of FM was replaced by PPM. The highest replacing levels (60, 75, 90 or 100%) significantly adversed FCR and significantly reduced FI and PER (Table 2).

Apparent nutrient digestibility: Compared to the control group, replacing up to 45% of FM by PPM did not significantly ($P < 0.05$) changed apparent digestibility coefficient (ADC) for DM, CP and EE, while the highest replacing levels significantly ($P < 0.05$) decreased ADC for DM, CP and EE. It is interesting to note that, the highest NFE digestibility coefficient was observed for the diets PPM0 and PPM15 and the lowest value observed for the diet PPM100 where FM was completely replaced by PPM (Table3).

Growth performance: As described in Table 4, replacing of FM by PPM protein up to 45% in tilapia diets had no significant effect on growth performance including BW, BL, WG and SGR, while the highest replacing levels significantly ($P < 0.01$) reduced these parameters. The worst growth parameters obtained for fish fed the diet PPM100. In contrast, no significant difference was observed among fish fed the diets PPM, PPM15, PPM30 and PPM45.

Table 1: Composition and proximate analysis of the experimental diets

Ingredients	Diets							
	D1	D2	D3	D4	D5	D6	D7	D8
Fish meal (72% CP)	16	13.6	11.2	8.8	6.4	4.0	1.6	0
Soybean meal (44% CP)	35	35.0	35.0	35.0	35.0	35.0	35.0	35
Plant protein mixture (33% CP)	0	5.1	10.2	15.3	20.4	25.5	30.6	35
Yellow corn	32	32.0	32.0	32.6	31.2	28.5	25.8	22
Wheat bran	10	7.3	4.6	1.3	0.0	0.0	0.0	0
Vegetable oil	4	4.0	4.0	4.0	4.0	4.0	4.0	5
Vit. & Min. Mixture ¹	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Cr2O3	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sum	100	100.0	100.0	100.0	100.0	100.0	100.0	100.0
<i>Proximate analysis (determined on dr. matter basis)</i>								
Dry matter (DM)	96.55	95.87	96.12	97.46	96.67	95.77	96.13	96.45
Crude protein (CP)	30.80	30.53	30.27	30.00	29.70	29.73	29.80	29.50
Ether extract (EE)	6.24	6.81	6.02	5.87	5.58	6.11	6.00	6.32
Crude fiber (CF)	9.16	9.24	9.13	9.35	9.52	9.50	9.54	9.73
Ash	8.25	8.46	8.55	8.56	8.44	8.88	8.90	8.99
NFE ²	45.55	44.96	46.03	46.22	46.76	45.78	45.76	45.46
ME ³ (Kcal/kg diet)	2712.00	2705.00	2714.00	2736.00	2715.00	2706.00	2717.00	2710.00
P/E ratio ⁴	113.57	112.87	111.53	109.65	109.39	109.87	109.68	108.86

¹Vitamin & mineral mixture/kg premix: Vitamin D₃, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g; B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg, Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg

²Nitrogen free extract (NFE) = 100 - (CP + EE + CF + Ash)

³Metabolizable energy was calculated from ingredients based on [10] values for tilapia

⁴Protein to energy ratio in mg protein/kcal ME

Table 2: Feed efficiency of Nile tilapia as affected by replacing fish meal by a mixture of plant protein source

Experimental diets	FI (g/fish)	FCR	PER
PPM0 (Control)	41.50a	1.50c	2.17a
PPM15	40.94a	1.53c	2.14a
PPM30	40.93a	1.54c	2.15a
PPM45	40.86a	1.58c	2.11a
PPM60	37.44b	1.72b	1.96b
PPM75	36.12b	1.80b	1.83b
PPM90	34.66b	1.90b	1.76b
PPM100	30.66c	2.17a	1.56c
<i>Pooled SE</i>	±0.45	±0.02	±0.03
<i>Probability</i>	0.0061	0.0089	0.0046

Means followed by the different letters in each column for each trait are significantly different (P<0.05)

Table 3: Apparent digestibility coefficients (ADC) for different nutrients in the experimental diets

Diets	DM	CP	EE	NFE
PPM0 (Control)	83.56a	81.25a	81.65a	75.25a
PPM15	82.34a	81.85a	81.44a	76.63a
PPM30	83.74a	82.69a	80.24a	71.24b
PPM45	82.67a	80.89a	78.89a	71.76b
PPM60	80.15b	77.57bc	75.65b	70.34b
PPM75	76.90c	74.84bc	75.24b	70.64b
PPM90	75.57c	73.17c	75.65b	71.68b
PPM100	76.71c	73.38c	74.22b	68.64c
<i>Pooled SE</i>	±1.88	±1.67	±2.33	±1.56
<i>Probability</i>	0.0511	0.0346	0.0308	0.0505

Means followed by the different letters in each column for each trait are significantly different (P<0.05)

Table 4: Growth performance of Nile tilapia as affected by replacing fish meal by a mixture of plant protein

Experimental diets	No.	BW (G)		BL (cm)		WG (g/fish)	SGR
		Initial	Final	Initial	Final		
PPM0 (Control)	60	2.61	30.34a	5.60	11.60a	27.73a	2.73a
PPM15	60	2.66	29.46a	5.57	11.44a	26.80a	2.67a
PPM30	60	2.68	29.30a	5.70	11.34a	26.62a	2.66a
PPM45	60	2.61	28.49a	5.60	11.33a	25.88a	2.66a
PPM60	60	2.67	24.43b	5.70	10.80b	21.76b	2.46b
PPM75	60	2.65	22.76bc	5.63	10.73b	20.11b	2.39b
PPM90	60	2.67	20.90c	5.63	10.64b	18.23c	2.29c
PPM100	60	2.71	16.84d	5.57	9.76c	14.13c	2.03c
<i>Pooled SE</i>		±0.21	±1.23	±0.46	±1.67	±0.68	±0.07
<i>Probability</i>		0.2881	0.0013	0.4325	0.0013	0.0056	0.0036

Means followed by the different letters in each column for each trait are significantly different (P<0.05)

Blood parameters and liver functions: Compared to control group, hemoglobin and hematocrite decreased proportionally with increasing incorporation level of PPM in the experimental diets (Table 5). All fish fed diets with PPM protein replacement had significantly (P<0.001) lower hematocrite and hemoglobin compared to control group and the opposite trend was observed for the levels of liver enzyme (ALT and AST) where the increased levels of PPM in the diet significantly increased the levels of ALT and AST.

Proximate analysis of fish whole-body: DM and CP of whole body showed some variation (but not significant) and increased with increasing level of PPM in diets (Table 6). The whole-body content of EE and ash significantly (P<0.05) increased with increasing the PPM content of tilapia diets.

Table 5: Blood parameters and liver functions of fish groups fed the experimental diets

Diets	Hemoglobin			
	(g dLG ^l)	Hematocrite (%)	ALT	AST
PPM0(control)	7.10a	26.90a	43.17d	50.00d
PPM15	5.37c	19.80b	43.33d	65.67c
PPM30	5.50bc	11.87c	47.67c	61.67c
PPM45	5.27c	21.00b	52.33b	60.00c
PPM60	4.23e	13.50c	56.67ab	77.33a
PPM75	5.63b	13.50c	58.00ab	76.33a
PPM90	4.77d	14.37c	56.77ab	70.00b
PPM100	5.43bc	21.37b	63.00a	75.33a
<i>Pooled SE</i>	±0.07	±0.76	±0.46	±0.67
<i>Probability</i>	0.0001	0.0001	0.0001	0.0001

Means followed by the different letters in each column for each trait are significantly different (P<0.05)

Table 6: Proximate analysis of fish whole-body (based on dry matter)

Experimental diets	DM	CP	EE	Ash
PPM0 (control)	25.18	68.58	13.12a	11.23b
PPM15	25.23	67.72	13.26a	11.93b
PPM30	25.95	67.20	14.74ab	12.02ab
PPM45	27.52	66.67	14.36ab	12.80ab
PPM60	25.91	66.12	15.22ab	12.59ab
PPM75	26.52	66.11	16.89b	12.25ab
PPM90	27.16	66.79	15.87b	15.81b
PPM100	27.76	67.78	16.39b	15.12a
<i>Pooled SE</i>	±1.88	±1.85	±0.86	±0.97
<i>Probability</i>	0.0761	0.0881	0.0431	0.0511

Means followed by the different letters in each column for each trait are significantly different (P<0.05)

Table 7: Feed costs (L.E) for producing one kg weight gain by fish fed the experimental diets

Diets	Costs (L.E)/ton	Relative to control (%)	Decrease in feed cost (%)	FCR	Feed costs * (L.E)/kg Weight gain	Relative to control (%)	Decrease in Feed costs* (L.E)/kg Weight gain
PPM0	3065.0	100.00	0.00	1.50	4.60	100.00	0.00
PPM15	2948.0	96.18	3.82	1.53	4.51	98.04	1.96
PPM30	2831.0	92.37	7.63	1.54	4.36	94.78	5.22
PPM45	2715.5	88.60	11.40	1.58	4.29	93.26	6.74
PPM60	2595.0	84.67	15.33	1.72	4.46	96.96	3.04
PPM75	2471.3	80.63	19.37	1.80	4.45	96.74	3.26
PPM90	2347.5	76.59	23.41	1.90	2.46	96.96	3.04
PPM100	2260.0	73.74	26.26	2.17	4.90	106.52	+6.52

*Feed costs/kg weight gain = FCR × costs of kg feed, Local market price (L.E./ton) for feed ingredients used for formulating the experimental diets when the experiment was started; fish meal 8000 LE/ton, yellow corn 1250; soybean meal 2500; plant protein mixture 2000; wheat bran 1000; com oil 4000 LE/ton and vit.& Min. Mixture 10 LE/kg

Economical evaluation: As described in Table 3 and 4, replacement of FM by PPM up to 45% in tilapia diets did not significantly affected all growth and feed utilization parameters and reduced feed costs/kg diet and feed costs/kg weight gain by 11.40 and 6.74%, respectively (Table 7). The highest replacing levels significantly reduced all growth and feed utilization parameters and also reduced feed costs/kg diet. Complete replacement of FM by PPM increased feed costs/kg weight gain by 6.52%.

DISCUSSION

Since fish are poikilothermic, their food requirement will be related to activity and hence to water temperature. Water temperature expressed as mean values ranged from 23.15 to 30.16°C, dissolved oxygen (4.55-6.23 mg LG^l); pH (7.71-7.89), total ammonia (0.12-0.16 mg LG^l). These values are in consistence with the means needed for tilapia growth [22]. These means indicated that the experimental diets has no adetrimental effect on the surrounding water where the experimental tilapia had been stocked. Therefore, all fish were in normal activity. The experimental diets were formulated to be almost iso-nitrogenous and iso-caloric. Accordingly, any differences in the performance of fish received such diets could be attributed to the quality and feeding value of the tested materials and levels used. Digestibility values are an important parameter to consider in the diet formulation and in determing the utilization of a feed. Feedstuffs which are poorly digested would be of limited value to an animal. The obtained results clearly showed that, the replacement of up to 45% FM by PPM allowed FI, FCR, PER and ADC for DM, CP and EE similar to those exhibited by the control groups (FM based diet) and the same trend was

also obtained for growth parameters (BW, BL, WG and SGR). The highest replacing levels (more than 45%) significantly reduced FI, FCR, PER and the ADC for the different nutrients (DM, CP and EE) and also negatively affected growth parameters (BW, BL, WG and SGR). These results suggests that the apparent protein digestibility for PPM is lower than that of FM for Nile tilapia. Possible reasons for the reduced feed utilization, digestibility and growth parameters recorded at the highest replacing levels of FM by PPM (more than 45%) may be related to the high-crude fiber, presence of identified or unidentified anti-nutritional factors and poor platability of PPM which reduce FI and adversed FCR and PER [23].

Mucilage in linseed (5-8%) could increase the delay of diet retention in stomach affecting FI through feedback on satiety signals. Also, mucilage has a large capacity to bind to water and increases intestinal viscosity thus, reduce nutrient digestibility [24]. Canola meal contains phenolic compounds (such as sinapine and tannine) that may reduce palatability [25] and reduce protein digestibility [26]. Canola meal also contain glucosinolates which act as anti-thyroid factors [27]. The high fiber content of canola meal may be reduces protein and energy digestibility [28].

Phytic acid (present in linseed meal) negatively affects the utilization of minerals which can be seen by its ability to bind up to 75% of all phosphorus [29]. It can chelate di-and tri-valent metals including calcium, magnesium, zinc and iron into compounds that are less easily absorbed in the intestine. Phytic acid inhibit activities of some digestive enzymes such as pepsin, trypsin and alpha-amylase [30]. Cyanogenic compounds in linseed meal act as a toxic agent for fish [31].

Cottonseed meal usually contains 0.4 to 1.7% gossypol. Free gossypol, when present in large quantity in the diet, has been shown to be toxic to mono-gastric animals including fish [32]. Consequently, the increased levels of PPM in the diets may reduce growth, feed intake, feed and protein utilization, digestibilities of the different nutrients and histological changes in the liver and kidney [33].

The finding that the incorporation of more than 45% of FM by PPM significantly decreased fish growth was in agreement with the poor growth reported by [34] who found that, replacement of FM by a PPM (lupin, corn gluten and wheat gluten meal) in the diets of juvenile turbot (*Psetta maxima*) up to 50% did not significantly affected growth rate, while the highest replacing levels (75 or 100%) significantly reduced growth rate. On the other hand, [35] substituted FM by a PPM (soybean, cottonseed, sunflower and linseed meals) in diets of Nile tilapia. They found that, the partial or complete replacement of FM by PPM exhibited growth performance not differing significantly from the fish fed the control diet. Also [36] with rainbow trout, *Oncorhynchus mykiss* found that FM could be entirely replaced by a mixture of plant proteins (cottonseed meal and soybean meal) and animal by-product proteins without adverse effect on growth rate and feed utilization. The different findings reflect the fact that the utilization of PPM differs considerably depending on the kind and quality of meals incorporated in the diets.

The hematological variables hemoglobin and hematocrite, taken as an indicator of the rate of hemoglobin synthesis to red cell formation and erythrocyte fragility [37]. The hemoglobin (7.10 g dL⁻¹) and hematocrite (26.90%) levels of control group in our study were within the normal levels [38]. Hemoglobin and hematocrite levels in fish fed diets containing 60% PPM protein were about half lower than control group. The lower hemoglobin and hematocrite levels in Nile tilapia fed PPM-containing diets is thought due to the binding of phytic acid and gossypol molecules and the other toxic factors in PPM to minerals (iron) and/or amine group of amino acids causing their low availabilities in the body and increased erythrocyte fragility.

ALT and AST enzymes are two of the thousands kinds of liver enzymes and a kind of transferase. They have the function of transferring amino group from alpha-amino acids to alpha-keto acids. Large amount of ALT and AST is released into blood mostly during liver cell damage. Thus, detection of serum level of ALT and AST tells monitored liver cell damage. Compared to control serum, level of transferases (ALT and AST)

significantly increased with the increased PPM in the experimental diet. Cellular damage indicators (ALT and AST) significantly ($P < 0.001$) increased as PPM increased in the diets indicating the abnormal liver function and this may be due to increasing the identified or un-identified anti-nutritional and toxic factors presented in PPM.

Concerning proximate whole-body composition, DM and CP contents of Nile tilapia not influenced by dietary protein source. Similarly [35] in Nile tilapia, [12] in turbot, [39] in rainbow trout [40] in carp and [41] in yellowtail did not find any effects of PPM on the whole-body protein content. In contrast to our results, they also found that, the whole-body fat and ash contents did not significantly varied when compared to the control. This was expected as fish in all treatments did not grow essentially at the same rate. [42, 43] reported that body fat content is closely related to weight gain and inversely related to body moisture content.

From the economic standpoint, replacement of FM with cheaper PPM in a practical diet for Nile tilapia can alleviate the problem of low FM availability and high cost. Feeding costs in fish production is about 50% of the total production costs [44]. All other costs in the present study are constant, therefore, the feeding costs required to produce one kg gain in weight could be used to compare the different experimental treatments. The calculated figures showed that, the cost of one ton feed mixture was reduced in all replacing levels of FM by PPM and the replacing level 45% reduced feeding costs by 11.4% and decreased feed costs/kg weight gain by 6.74%. In this respect, [35] found that, partial or complete replacement of FM by a mixture of plant protein sources significantly reduced incidence costs and improved profit indices compared to the basal diet. The present study indicted the potential of PPM for inclusion in commercial Nile tilapia feeds, as well as being of immediate importance for feed production in Egypt.

From the all aforementioned results, it could be detected that PPM could be utilized by tilapia safely and efficiently as alternative protein instead of 45% of FM without adverse effects on the performance of tilapia. This observation is supported by the ADC for DM, CP and EE values for diets containing mixtures of plant protein meals. In addition, these plant protein sources are locally available at much lower prices than imported FM. Further researches are required to determine the feasibility of improving the nutritional value of the available plant protein source and using PPM composed of different combinations of ingredients and to examine the effects of PPM use in diets on large sizes of fish under the field conditions.

REFERENCES

1. Tacon, A.G., 1993. Feed ingredients for warm water fish: fish meal and other feedstuffs. FAO. Circ., No. 856, FAO, Rome, pp: 64.
2. El-Saidy, D.M.S. and M.M.A. Gaber, 2002a. Evaluation of dehulled sunflower meal as a partial and complete replacement for fish meal in Nile tilapia, *Oreochromis niloticus* (L.), diets. In: Proceedings of the First Annual Scientific Conference on Animal and Fish Production (Fac. Agric., Al-Mansoura Univ., Egypt. September 24-25, 2002, pp: 193-205.
3. Ibrahim, M.S., 2007. Nutritional requirements for tilapia fry during nursing period. M. Sc. Thesis, Fac. Agric. Benha University, Egypt.
4. El-Saidy, D.M.S. and M.M.A. Gaber, 2002b. Complete replacement of fish meal by soybean meal with dietary l-lysine supplement for Nile tilapia *Oreochromis niloticus*, fingerlings. Journal of the World Aquaculture Society, 33: 297-306.
5. Soltan, M.A., M.K. Ibrahim, Fatma A. Hafez and A.F. Fath El-Bab, 2001. Effect of partial and total replacement of fish meal by soybean meal on growth and proximate analysis of Nile tilapia (*Oreochromis niloticus*). Egypt. J. Nutr. & Feeds, 4 (Special Issue): 799-812.
6. El-Saidy, D.M.S. and M.M.A. Gaber, 2001. Linseed meal: its successful use as a partial and complete replacement for fish meal in practical diets for Nile tilapia *Oreochromis niloticus*. In: Proceedings of the Second International Conference on Animal Production and Health in Semi-Arid Areas (Fac. Envir. Agric. Sci., Suez Canal Univ., El-Arish-North Sinai, Egypt. pp: 635-643.
7. Soltan, M.A., 2005a. Partial and total replacement of soybean meal by raw and heat treated linseed meal in tilapia diets. Egypt. J. Nutr. & Feeds (Special Issue), 8 (1): 1091-1109.
8. Soltan, M.A., 2005b. Potential of using raw and processed canola seed meal as an alternative fish meal protein source in diets for Nile tilapia (*Oreochromis niloticus*). Egypt. J. Nutr. & Feeds (Special Issue), 8 (1): 1111-1128.
9. Saudi, A., 2008. An experimental toxicobromatological evaluation of feeding cottonseed meal and alpha-tocopherol to tilapia fish. Ph.D. Thesis, Fac. Agric. Benha University, Egypt.
10. NRC, 1993. National research Council. Nutrient Requirements of Fish. National Academy Press, Washington, DC, pp: 114.
11. Watanabe, T., H. Aoki, V. Viyakarn, M. Maita, Y. Yamagata, S. Satoh and T. Takeuchi, 1995. Combined use of alternative protein sources as a partial replacement for fish meal in a newly developed soft-dry pellet for yellowtail,. Suisan Zoshoku, 43: 511-520.
12. Regost, C., J. Arzel and S.J. Kaushik, 1999. Partial or total replacement of fish meal by corn gluten meal in diet for turbot, *Psetta maxima*. Aquaculture 180: 90-117.
13. Mambrini, M. and S.J. Kaushik, 1995. Indispensable amino acid requirements of fish: Correspondence between quantitative data and amino acid profiles of tissue proteins. J. Appl. Ichthyol., 11: 240-247.
14. Hajen, W.E., D.A. Higgs, R.M. Beames and B.S. Dosanjh, 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus ishawytscha*) in sea water: I. Validation to technique. Aquaculture, 112: 321-332.
15. Fenton, T.W. and M. Fenton, 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci., 59: 631-634.
16. Cho, C.Y. and S.J. Kaushik, 1985. Effect of protein intake on metabolizable and net energy values of fish diets. In: Nutrition and Feeding in Fish (ed. by C.B. Cowey, A.M. Mackie & J.G. Bell), Academic Press, London, UK, pp: 95-117.
17. APHA, 1992. Standard methods for the examination of water and waste water. American Public Health Association. Washington, DC.
18. Brown, B.A., 1988. Routine hematology procedures. In: Hematology: Principle and Procedures. Brown B.A. (Ed.). Leo and Febiger, Philadelphia, PA. USA, pp: 7-122.
19. Reitman, S. and F. Frankel, 1957. A colorimetric method for determination of oxaloacetic transaminase and serum glutamic pyruvic transaminase. A. J. Clin. Path., 28: 56-60.
20. AOAC (Association of Official Analytical Chemists) 1990. Official Methods of Analysis. 15th Edn. AOAC, Arlington, VA, USA.
21. SAS 1996. SAS Procedure Guide "version 6.12 Ed". SAS Institute Inc., Cary, NC, USA.
22. Stickney, R.R., 1979. Principles of Warmwater Aquaculture. Wiley-Interscience, New York, USA.
23. Luo, L., M. Xue, X. Wu, X. Cai, H. Cao and Y. Liang, 2006. Partial or total replacement of fishmeal by solvent-extracted cottonseed meal in diets of juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition, 12: 418-424.

24. Fedeniuk, R.W. and C.G. Biliaderis, 1994. Composition and physicochemical properties of linseed (*Linum usitatissimum*) mucilage. J. Agric. Food Chem., 42: 240-247.
25. McCurdy, S.M. and B.E. March, 1992. Processing of canola meal for incorporation in trout and salmon diets. JAOCS, 69: 213-220.
26. Krogdahl, A., 1989. Alternative protein source from plants contain antinutrients affecting digestion in salmonids. In: Takeda, M. and T. Watanabe (Eds.). Proc. of the 3rd Intl. Symp. On Feeding and Nutrition in Fish, Laboratory of fish nutrition, Tokyo University of fisheries. Tokyo, Japan, pp: 253-261.
27. Teskeredzic, Z., D.A. Higgs, B.S. Dosanjh, J.R. McBride, R.W. Hardy, R.M. Beames, J.D. Jones, M. Simell, T. Vaara and R.B. Brides, 1995. Assessment of undephytinized and dephytinized rapeseed protein concentrate as source of dietary protein for juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 131: 261-277.
28. Higgs, D.A., U.H.M. Fagerlund, J.R. McBride, M.D. Plotnikoff, B.S. Dosanjh, J.R. Markert and J. Davidson, 1983. Protein quality of Alex canola meal for juvenile chinook salmon (*Oncorhynchus tshawytscha*) considering dietary protein and 3,5,3-triiodo-L-thyronine content. Aquaculture, 34:213-238.
29. NRC, 1998. National Research Council. Nutrient Requirements of Swine, 10th ed. National Academy of Science, Washington, DC, pp: 189.
30. Liener, I.E., 1994. Implications of antinutritional components in soybean foods. Crit. Rev. Food Sci. Nutr., 34: 31-67.
31. Poulton, J.E., 1989. Toxic compounds in plant foodstuffs. In: Food Proteins: J.A.O.C.S., 31: 381-401.
32. Barros, M.M., C. Lim and P.H. Klesius, 2002. Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth immune response and resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri* challenge. Aquaculture, 207: 263-279.
33. Kissil, G.W., I. Lupatsch, D.A. Higgs and R.W. Hardy, 1997. Preliminary evaluation of rapeseed protein concentrate as an alternative to fish meal in diets for gilthead seabream (*Sparus aurata*). Bamidgeh, 49: 135-143.
34. Fournier, V., C. Huelvan and E. Desbruyeres, 2004. Incorporation of a mixture of plant feedstuffs as substitute for fish meal in diets of juvenile turbot (*Psetta maxima*). Aquaculture, 236: 451-465.
35. El-Saidy, D.M.S. and M.M.A. Gaber, 2003. Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia *Oreochromis niloticus* (L.), diets. Aquaculture Research, 34: 1119-1127.
36. Lee, K.J., K. Dabrowski, J.H. Blom, S.C. Bai and P.C. Stromberg, 2002. A mixture of cottonseed meal, soybean meal and animal byproduct mixture as a fish meal substitute: Growth and tissue gossypol enantiomer in juvenile rainbow trout (*Oncorhynchus mykiss*). J. Anim. Physiol. Anim Nutr. (Berl.), 86 (7-8): 201-213.
37. Barraza, M.I., C.E. Coppock, K.N. Brooks, D.L. Wilks, R.G. Saunders and J.G. Latimer, 1991. Iron sulfate and feed pelleting to detoxify free gossypol in cottonseed diets for dairy cattle. J Dairy Sci., 74: 3457-3467.
38. Sun, L.T., G.R. Chen and F.F. Chang, 1995. Acute responses of blood parameters and comatose effects in salt-acclimated tilapias exposed to low temperature. Journal of Thermal Biology, 20: 299-306.
39. Moyano, F.J., G. Cardenete and M. De la Higuera, 1992. Nutritive value of diets containing high percentage of vegetable protein for trout, *Oncorhynchus mykiss*. Aquatic Living Resources 5: 23-29.
40. Pongmaneerat, J., T. Watanabe, T. Takeuchi and S. Satoh, 1993. Use of different protein meals as partial or total substitution for fish meal in carp diets. Bulletin of Japanese Society of Scientific Fisheries, 59: 1249-1257.
41. Shimeno, S., T. Masunoto, T. Tujita, L. Mima and S. Uenos, 1993. Alternative protein sources for fish meal in diets of young yellowtail. Nippon Suisan Gakkaishi, 59: 137-143.
42. Barros, M.M., C. Lim, J.J. Evans and P.H. Klesius, 2000. Effects of iron supplementation to cottonseed meal diets on growth performance of channel catfish, *Ictalurus punctatus*. J. Appl. Aquacult., 10: 65-86.
43. Yildirim, M., C. Lim, P. Wan and P.H. Klesius, 2003. Growth performance and immune response of channel catfish (*Ictalurus punctatus*) fed diets containing graded levels of gossypol-acetic acid. Aquaculture, 219: 751-768.
44. Collins, R.A. and M.N. Delmendo, 1979. Comparative economics of aquaculture in cages, raceways and enclosures. In: Advance in aquaculture, England, Fishing News Books, pp: 427.

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