

Effect of dietary replacement of fish meal by mixture of different plant protein sources on growth performance and some blood parameters of Nile tilapia, *Oreochromis niloticus*

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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 a 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, results revealed that replacement of 15, 30 or 45% of FM by PPM did not significantly affect 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 higher 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 on control diet. Compared to control diet, increasing PPM in the diets significantly reduced hemoglobin, hematocrit and increased the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The incorporation of PPM in diets did not significantly affect the whole-body dry matter (DM) and crude protein (CP).

From economic point of 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.

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 valued for its amino acid balance, vitamin content, palatability and unidentified growth factors (Tacon, 1993). However, the increasing cost of fish meal

has restricted its use as a protein source for fish diets. Therefore, plant proteins appear to be the most appropriate alternative 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 various alternative protein sources as partial or complete replacement for fish meal has been individually evaluated in fish diets, e.g. sunflower meal (El-Saidy & Gaber, 2002a ; Ibrahim, 2007), soybean (El-Saidy & Gaber, 2002b ; Soltan *et al.*, 2001), linseed meal (El-Saidy & Gaber, 2001 and Soltan, 2005a) canola (Soltan, 2005b) and cottonseed meal (Saudi, 2008). Individually, these plant by-product meals are fairly rich in protein and favourable essential amino acid profiles, but they are deficient in one or more essential amino acids and contained various quantities of anti-nutritional factors (NRC, 1993).

Some studies have also stressed that a mixture of plant protein sources is more appropriate to obtain adequate amino acid profile compared to the incorporation of a single plant protein source (Watanabe *et al.*, 1995; Regost *et al.*, 1999). Recently, comparative studies conducted in rainbow trout, turbot, sea bass and sea bream attempted to completely substitute fish meal by a mixture of plant proteins. All diets were supplemented with L-amino acids to meet the amino acid needs estimated for rainbow trout (NRC, 1993). 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 NRC (1993) was not sufficient. Mambrini & Kaushik (1995) 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 NRC (1993).

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 (Faculty 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, sesame 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

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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 to produce pellets and were sun-dried for 3 days.

Culture conditions: At the beginning of the experiment, 24 glass aquaria (100 × 50 × 40 cm) 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 the experimental diets 6 days/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).

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	35	35	35	35	35	35
Plant protein mixture (33% CP)	0	5.1	10.2	15.3	20.4	25.5	30.6	35
Yellow corn	32	32	32	32.6	31.2	28.5	25.8	22
Wheat bran	10	7.3	4.6	1.3	0	0	0	0
Vegetable oil	4	4	4	4	4	4	4	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	100	100	100	100	100	100
Proximate analysis (determined on dry matter basis)								
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	2705	2714	2736	2715	2706	2717	2710
P/E ratio ⁴	113.57	112.87	111.53	109.65	109.39	109.87	109.68	108.86

1-Vitamin & 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.

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

3- Metabolizable energy was calculated from ingredients based on NRC (1993) values for tilapia.

4- Protein to energy ratio in mg protein/kcal ME.

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 Hajen *et al.* (1993). 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 (Fenton & Fenton, 1979) and

apparent digestibility coefficients for the nutrients were calculated according to NRC (1993) by the equation:

$100 - 100 [(\% \text{ marker in diet} / \% \text{ marker in feces}) \times (\% \text{ nutrient in diet} / \% \text{ nutrient in feces})]$.

Growth and feed utilization parameters: Growth performance and feed utilization parameters were determined according to Cho & Kaushik (1985) as follows:

Specific growth rate (SGR) = $[(\text{Ln}W_2 - \text{Ln}W_1)/t] \times 100$ Where:- Ln = the natural log, W1 = initial fish weight; W2 = the final fish weight in “grams” and t = period in days.

Feed conversion ratio (FCR) = feed intake (g)/wet weight gain (g),

Protein efficiency ratio (PER) = weight gain (g)/protein intake (g),

Water quality: Parameters of water quality were determined according to the methods of APHA (1992). 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 taken and anaesthetized by ethylene glycol mono-phenol ether. Blood samples were collected from the caudal vein using heparinized 27-gauge needles and tuberculin syringes. Hematocrit (Ht) was determined using the micro-Ht method described by Brown (1988). Hemoglobin (Hb) was determined using the total Hb kit (Sigma Diagnostics, Sigma, St Louis, MO. USA) which is a standardized procedure using the cyanomethemoglobin method. Liver was removed, homogenized and assigned for determination of Aspartate transaminase (AST) and Alanine transaminase (ALT) according to Reitman and Frankel, (1957).

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 AOAC (1990).

Statistical analysis: The statistical analysis of data was carried out by applying the computer program, SAS (1996) 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 3.55 to 7.23 mg L⁻¹, pH from 7.71 to 7.89 and total ammonia from 0.12 to 0.16 mg L⁻¹. There were no significant (P<0.05) differences in water quality parameters among treatments during the whole experimental period, indicating that the experimental diets had no harmful effects on the surrounding water quality of experimental fish.

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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 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).

Table (2): Feed efficiency of Nile tilapia as affected by replacing fish meal by a mixture of plant protein sources.

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

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

Apparent nutrient digestibility: Compared to control group, replacing up to 45% of FM by PPM did not significantly (P<0.05) change 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 (Table 3).

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

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

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

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 parameters including BW, BL, WG and SGR, while the highest replacing levels significantly ($P<0.01$) reduced these parameters. The worst growth performance parameters were 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 (4): Growth performance of Nile tilapia as affected by replacing fish meal by a mixture of plant proteins

Experimental diets	No.	BW (G)		BL (cm)		WG (g/fish)	SGR
		Initial	Final	Initial	Final		
PPM0 (Control)	60	2.61	30.34 a	5.60	11.60 a	27.73 a	2.73 a
PPM15	60	2.66	29.46 a	5.57	11.44 a	26.80 a	2.67 a
PPM30	60	2.68	29.30 a	5.70	11.34 a	26.62 a	2.66 a
PPM45	60	2.61	28.49 a	5.60	11.33 a	25.88 a	2.66 a
PPM60	60	2.67	24.43 b	5.70	10.80 b	21.76 b	2.46 b
PPM75	60	2.65	22.76 bc	5.63	10.73 b	20.11 b	2.39 b
PPM90	60	2.67	20.90 c	5.63	10.64 b	18.23 c	2.29 c
PPM100	60	2.71	16.84 d	5.57	9.76 c	14.13 c	2.03 c
$\pm SE$		± 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 row for each trait are significantly different ($P<0.05$).

Blood parameters and liver function: Compared to the control group, hemoglobin and hematocrit values 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 hematocrit and hemoglobin values compared to the 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 function of fish groups fed the experimental diets.

Diets	Hemoglobin	(g dL ⁻¹)	Hematocrite	(%)	ALT	AST
PPM0(control)	7.10 a		26.90 a		43.17 d	50.00 d
PPM15	5.37 c		19.80 b		43.33 d	65.67 c
PPM30	5.50 bc		11.87 c		47.67 c	61.67 c
PPM45	5.27 c		21.00 b		52.33 b	60.00 c
PPM60	4.23 e		13.50 c		56.67 ab	77.33 a
PPM75	5.63 b		13.50 c		58.00 ab	76.33 a
PPM90	4.77 d		14.37 c		56.77 ab	70.00 b
PPM100	5.43 bc		21.37 b		63.00 a	75.33 a
$\pm SE$	± 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 row for each trait are significantly different ($P<0.05$).

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Economical evaluation: As described in Tables 3 and 4, replacement of FM by PPM up to 45% in tilapia diets did not significantly affect 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%.

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

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

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

Table (7): Feed costs (L.E) for producing one kg weight gain as affected by 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	1.50	4.60	100	0
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; corn oil 4000 LE/ton and vit.& Min. Mixture 10 LE/kg.

DISCUSSION

Since fish are poikiothermic, 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 L⁻¹); pH (7.71 - 7.89), total ammonia (0.12 - 0.16 mg L⁻¹). These values are in consistence with the means needed for tilapia growth (Stickney, 1979). These means

indicated that the experimental diets have no harmful, 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 difference 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 important parameters to consider in the diet formulation and in determining 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). This result 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 could be the highest replacing levels of FM by PPM (more than 45%). The high-crude fiber and poor palatability of PPM might reduce FI and adversed FCR and PER (Luo *et al.*, 2006). Other possible explanation for the low feed utilization, digestibility and growth performance at increasing levels of FM substitution by PPM may be resulting from the presence of identified or unidentified anti-nutritional factors in PPM which increased in the diet with increasing the substitution of FM by PPM that reduced feed utilization, digestibility coefficients for the different nutrients and fish growth.

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 reducing nutrient digestibility (Fedeniuk & Biliaderis, 1994).

Canola meal contains phenolic compounds (such as sinapin and tannin) that may reduce palatability (McCurdy & March, 1992) and reduce protein digestibility (Krogdahl, 1989). Canola meal also contains glucosinolates which act as anti-thyroid factors (Teskeredzic *et al.*, 1995). The high fiber content of canola meal may reduce protein and energy digestibility (Higgs *et al.*, 1983).

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 (NRC, 1998). 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 also has the ability to nonselectively bind to protein and inhibit activities of a number of digestive enzymes such as pepsin, trypsin and alpha-amylase (Liener, 1994). Cyanogenic compounds in linseed meal are a toxic for fish (Poulton, 1989).

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 (Barros *et al.*, 2002). 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 (Kissil *et al.*, 1997).

The finding that the incorporation of more than 45% of FM by PPM significantly decreased fish growth in agreement with the poor growth reported by Fournier *et al.* (2004) 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 affect growth rate, while the highest replacing levels (75 or 100%) significantly reduced growth rate. On the other hand, El-Saidy & Gaber (2003) substituted FM by a PPM (soybean, cotton seed, sunflower and linseed meal) 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. Moreover, Lee *et al.* (2002) with rainbow trout, *Oncorhynchus mykiss* found that FM could be entirely replaced by a mixture of plant proteins (cottonseed meal, soybean meal) and animal by-product proteins without adverse effect on growth rate and feed utilization. Such 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 of hemoglobin and hematocrite, taken as an indicator of the rate of hemoglobin synthesis to red cell formation and erythrocyte fragility (Barraza *et al.*, 1991). The hemoglobin (7.10 g dL⁻¹) and hematocrit (26.90%) levels of control group in our study were within the normal levels (Sun *et al.*, 1995). Hemoglobin and hematocrit levels in fish fed diets containing 60% PPM protein were about half lower than the control group. The lower hemoglobin and hematocrit levels in Nile tilapia fed PPM-containing diets is thought to be due to 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 thousand 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 amounts of ALT and AST released into blood mostly due to liver cell damage. Thus, detection of serum level of ALT and AST tells the existence of liver cell damage. Compared to control serum level of transferase enzymes (ALT and AST) significantly increased with each increase of 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. The increasing levels of toxic factors as the level of PPM increased in the diets showed the degree of liver cells damage.

Concerning proximate whole-body composition, DM and CP contents of Nile tilapia were not influenced by dietary protein source. Similarly, El-Saidy & Gaber (2003) in Nile tilapia, Regost *et al.* (1999) in turbot, Moyano *et al.* (1992) in rainbow trout, Pongmaneerat *et al.* (1993) in carp and Shimeno *et al.* (1993) 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 had not significantly varied when compared to the control. This is expected as fish in all treatments did not grow essentially at the same rate. Barros *et al.* (2000) and Yildirim *et al.* (2003) 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 (Collins & Delmendo, 1979). 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, El-Saidy & Gaber (2003) 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 research is 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.

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