Partial and compleate replacement of fish meal with a mixture of different plant protein sources in juvenile mono sex male Nile tilapia, *Oreochromis niloticus (L.)* diets.

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ABSTRACT

A plant protein mixture (PPM) was tested to replace fish meal (FM) in diets for juvenile mono sex male Nile tilapia, *Oreochromis niloticus*. Fish averaging (± SD) 3.27 ± 0.1 g were divided into 15 groups. Three groups were fed each of five isonitrogenous (30.4%) and isocaloric (4.7 kcal g^{-1}) diets replacing 0, 25, 50, 75, and 100% of fish meal protein with similar percentages of PPM (PPM 0, PPM25, PPM50, PPM75, or PPM100, respectively). The PPM consisted of 25% penut meal, 25% akara meal, 25% canola meal and 25% corn glutein meal. After 10 weeks of feeding, fish fed diets PPM50 exhibited growth performance not differing significantly from fish fed control diet. Moisture, protein and ash contents of fish fed control (Diet 1) were higher significantly than those of fish fed other diets. While fat content of fish fed control (Diet 1) was lower significantly than those of fish fed other diets. The highest value of moisture protein and ash contents was obtained on fish fed 100% FM protein control (Diet 1) and the lowest was obtained on fish fed 100% plant protein mixture meal protein (diet 5). Incorporation of plant protein mixture in mono sex male Nile tilapia diets significantly decreased moisture, protein and fat content of whole body. The highest value of lipid content was recorded with fish fed 100% plant protein mixture meal (Diet 5) and the lowest was recorded with fish fed 100% FM protein control (Diet 1). Therefore, these findings suggest that up to 50% of fish meal protein can be replaced by plant protein mixture (PPM) protein in mono sex male Nile tilapia diets without any adverse effects on growth performances, feed utilization and body composition.

Key words: Plant protein mixture, fish meal, mono sex Nile tilapia and body composition..

INTRODUCTION

Various oilseed cakes and meals are produced in Egypt on a large scale as by-products of the edible oil industry. These include, penut meal, akara meal, canola meal and corn glutein meal. These oil cakes and meals are fairly rich in protein and are traditionally used as feeds for farm animals.

In recent years, intensification of tilapia fry production in Egypt has made it essential to develop complete and supplemental diets for use in hatcheries and nursery ponds. Traditionally, fish meal has been the main source of protein in diets for fish fry. However, the increasing cost of

fish meal has restricted its use as a protein source for fish diets. Therefore, it is a matter of urgency that alternative protein sources for tilapia diets be found.

The efficiency of various alternative protein sources as partial or complete dietary replacement for fish meal has been evaluated in fish diets, e.g. poultry by-product (Gaber 1996), sunflower meal (El-Saidy & Gaber 2002a), soybean meal (El-Saidy &Gaber 1997; 2002b), linseed meal (El-Saidy & Gaber 2001), and cottonseed meal (Middendorp & Huisman 1995; El-Saidy 1999 ; Mbahinzireki et al., 2001).

Penut meal, akara meal, canola meal and corn glutein meal commonly incorporated in practical fish feeds (El-Sayed 1999), have been studied individually as plant protein replacements for fish meal. These plant by-product meals have high protein levels and favorable essential amino acids profiles, but they are deficient in one or more essential amino acids (NRC 1993). Therefore, it is important to study the nutritional value of combinations of plant proteins in order to replace fish meal in commercial fish diets without compromising growth and feed efficiency. The purpose of this study was to evaluate growth, feed utilization, whole body composition and apparent nutrient digestibility of mono sex male Nile tilapia, *Oreochromis niloticus* fed diets containing graded substitutions of a plant protein mixture (PPM) for fish meal (FM) protein.

MATERIALS AND METHODS

Culture conditions

Mono sex male Nile tilapia, fingerlings were obtained from a local fish farm (Foka Hatchery, Qalyubia Governorate, Egypt). Fish were acclimated to laboratory conditions for four weeks in 1000 l fiberglass tanks. The feeding trial was performed at the Fish Research Laboratory, College of Agriculture, Minufiya University, Egypt. At the beginning of the experiment, 15 glass aquaria (80 l) were each stocked with 10 fish with an average weight of 3.27 ± 0.1 g. The aquaria were supplied with freshwater (free of chlorine) at a rate of 250 ml $min.^{-1}$ with supplemental aeration. The aquaria were illuminated by overhead fluorescent lighting set on a 12 h light : 12 h dark cycle.

Diets and feeding regime

Five experimental diets were formulated to be isonitrogenous and isocaloric in terms of crude protein (30.4%) and gross energy (4.7 kcal g^{-1}). Energy value was calculated using the gross energy values for the macronutrients (5.6 kcal/g protein, 9.5 kcal/g fat and 4.1 kcal/g carbohydrate; fiber was not included in calculation). A mixture of plant proteins used in this experiment was obtained from Minufiya University, Egypt and consisted of penult meal, akara meal, canola meal and corn glutein meal, 25% each. The proximate composition of the ingredients used in diets formulation is given in Table 1. For the experimental diets (Table 2), FM was replaced by PPM on the basis of crude protein as follows: control diet 1 = 100% FM : 0% PPM, PPM25 diet 2 = 75% FM : 25% PPM, PPM50 diet 3 = 50% FM: 50% PPM, PPM75 diet 4 = 25% FM: 75% PPM and PPM100 diet 5 = 100% PPM : 0% FM. The calculated essential amino acids concentrations in the experimental diets (Table 3) met or exceeded the recommendation of Santiago & Lovell (1988) for Nile tilapia. The experimental diets were pelleted, freezedried and stored at - 20 ^{o}C until used. Each diet was fed manually to apparent satiation twice daily for 10 weeks to triplicate groups of fish. Feed intake was recorded daily. Fish in each aquaria were weighed at the start and every two weeks of the experimental period. Feeding was stopped 24 h prior to weighing.

Table 1: Proximate composition of feedstuffs used in the study (% dry matter).

Ingredients	Crude protein	Crude fat	Crude ash	Dry matter	Crude fiber	N-free extract
Fish meal	60.0	10.7	19.0	92.0	0.9	9.4
Penut meal	48.0	6.0	5.1	90.2	6.6	34.3
Canola meal	30.0	3.8	7.2	90.0	12.0	47.0
Akara meal	34.5	18.8	3.9	95.9	20.6	22.2
Corn Glutein meal	60.0	2.2	1.6	90.0	2.0	34.2
Mixture plant protein	43.1	7.7	4.5	91.5	10.3	34.4

Table 2: Feed formulation and proximate composition of diets used in the present study.

	Diets				
	1 (Control) (0% PPM)	2 (25% PPM)	3 (50% PPM)	4 (75% PPM)	5 (100% PPM)
Ingredient (%)					
FM (60 % C. P.)	20.00	15.00	10.00	5.00	0.00
PPM (43.1 % C.P.)	0.00	6.96	13.91	20.87	27.82
Soybean meal (44% CP)	35.00	35.00	35.00	35.00	35.00
Yellow corn meal	20.00	19.04	18.09	16.13	16.18
Wheat bran	10.50	10.50	10.50	10.50	10.50
Corn starch	5.50	4.50	3.50	3.50	1.50
Fish oil	4.00	4.00	4.00	4.00	4.00
Molasses	2.00	2.00	2.00	2.00	2.00
Vit. & Min.Premix[1]	2.00	2.00	2.00	2.00	2.00
Vitamin C [2]	0.10	0.10	0.10	0.10	0.10
DiCa Phosphate	1.40	1.40	1.40	1.40	1.40
Total (%)	100.00	100.00	100.00	100.00	100.00
Proximate analysis (%)[3]					
Moisture	7.87	8.23	8.48	8.38	8.28
Crude protein	30.60	30.50	30.40	30.23	30.23
Crude fat	14.60	14.72	15.04	15.01	14.78
Ash	7.95	8.70	8.49	9.39	9.55
Crude fiber	6.22	7.23	7.33	7.43	7.46
NFE[4]	32.76	30.62	30.26	29.56	29.70
Gross Energy (kcal/g diet)[5]	4.70	4.70	4.70	4.70	4.70

[1] Premix supplied according to Xie, et al. (1997).
[2] Phospitan C (Mg-L-ascorbyl-2-phosphate); Showa Denko K. K., Tokyo, Japan.
[3] Values represent the mean of three sample replicates.
[4] NFE (Nitrogen free extract) = 100 - (% moisture + % protein + % fat + % fiber + % ash).
[5] Gross energy calculated according to Sanz, et al. (1994).

Table 3: Calculated amino acid composition of diets used in the study (g per 100 g diet).

	Diets					
	1	2	3	4	5	Required[1]
Indispensable amino acids (IAA) [2]						
Arginine	2.08	2.44	2.86	3.29	3.63	1.33
Histidine	0.84	0.96	0.98	1.06	1.13	0.54
Isoleucine	1.59	1.62	1.91	2.08	2.24	0.99
Leucine	2.73	2.68	2.61	2.54	2.48	1.09
Lysine	2.48	3.01	2.43	2.17	1.90	1.63
Methionine	1.05	1.36	1.28	1.20	1.11	1.02
Phenylalanine	1.42	1.45	1.48	1.51	1.53	1.82
Threonine	1.40	1.42	1.46	1.50	1.54	1.15
Tryptophane	0.37	0.41	0.47	0.53	0.58	0.32
Valine	1.81	1.83	1.90	1.96	2.01	1.09

[1]From Santiago and Lovell (1988).
[2]Data obtained from National Research Council (1993).

Growth study

At the beginning of the growth study, 15 fish were sampled and stored at -20 ^{o}C for analysis of whole body composition. At the end of the growth study (10 weeks), four fish per tank were withdrawn for analyses and frozen at - 20 ^{o}C. Growth performances was determined according to Cho & Kaushik (1985) as follows:

SGR (specific growth rate) = (Ln final weight - Ln initial weight/No of days).
FER (feed efficiency ratio) = wet weight gain (g)/dry feed intake (g).
FCR (feed conversion ratio)= dry feed intake (g)/wet weight gain (g).
PER (protein efficiency ratio) = weight gain (g)/protein intake (g).

Water quality

Water temperature were recorded daily in one tank using a mercury thermometer suspended at 30 cm water depth. Dissolved oxygen (DO) was measured using a YSI model 56 oxygen meter (Yellow Springs Instrument Company, Yellow Springs, Ohio, USA) and pH by a pH meter (pH pen Fisher Scientific, OH, USA). Ammonia and nitrite were measured at weekly intervals and alkalinity and salinity at monthly intervals in one tank per dietary treatment according to (Golterman,et al., 1978). Sampling was performed between 0700 and 0800 h.

Analytical methods

Analyses of samples were made as follows: dry matter after desiccation in an oven (105 ^{o}C for 24 h); ash (incineration at 550 ^{o}C for 12 h); crude protein (micro kjeldahl, N x 6.25); crude fat (ether extraction by Soxlhet method); and crude fiber according to AOAC (1995).

Statistical analysis

The data were analyzed by one-way analysis of variance (SAS, Institute Inc, 1993). Duncan's multiple range test was used to compare differences among individual means. Treatment effects were considered significant at $p \leq 0.05$. All percentage and ratio were transformed to arcsin values before analysis (Zar, 1984).

RESULTS AND DISCUSSION

In the present study water quality parameters were as followes: water temperature ranged from 27.4 to 28.2 ^{o}C, dissolved oxygen 4.2 to 4.5 mg l^{-1}, pH 7.4 to 7.8, alkalinity 165 to 175 mg l^{-1} and total ammonia 0.39 to 0.42 mg l^{-1}. There were no significant differences in water quality parameters among the treatments during the whole experimental period. Water quality parameters were found to be within the acceptable range for tilapia growth (Stickney, 1979).

The present study demonstrated the potential of four plant protein meals for inclusion in commercial Nile tilapia feeds. As well as being of immediate importance for feed production in Egypt. There is little information in the scientific literature concerning the use of plant protein mixture in Nile tilapia feeds, particularly feeds produced under commercial conditions. Since tilapia production of is in excess of 45% of annually fish production in Egypt (CAPMS 1994) there is considerable benefit related to the replacement of part of the fish meal currently used in feeds.

A significant amount of research has been conducted on the replacement of fish meal with soybean meal as protein source in feeds for Nile tilapia (El-Saidy & Gaber 1997 and 2002b). They reported that soybean meal supplemented with 1% methionine only or 1% methionine plus 0.5 % lysine can totally replace fish meal in Nile tilapia diets.

In contrast to soybean meal, there is less information available on the use of other oil seed by-products meals in feeds for Nile tilapia. Oilseed cakes and meals such as linseed meal (Hossain, et al. 1997; El-Saidy & Gaber 2001), sunflower meal (Sanz, et al., 1994; El-Saidy & Gaber 2002a); Cottonseed meal (El-Sayed 1990; El-Saidy 1999; Middendrop & Huisman 1995; Mbahinzireki, al., 2001) have been used in Nile tilapia feeds.

The results of average final body weight , specific growth rate (SGR), weight gain %, feed conversion ratio (FCR) and protein efficiency ratio (PER) are presented in Table 4. Fish fed diet 2 which contained 25 % plant protein mixture meal protein showed the best average final body weight, specific growth rate (SGR), weight gain %, feed conversion ratio (FCR), and protein efficiency ratio (PER). However, the same parameters for fish fed diet 3 did not differ significantly from those of fish fed diet 1 (control diet). Diet 5 which contained 100 % protein from plant protein mixture meal resulted in the lowest significantly average final body weight, specific growth rate (SGR), weight gain %, feed conversion ratio (FCR), protein efficiency ratio (PER). The unsuitability of substitution at this level was further reflected by the lack of response of the fish to the test diet at feeding times. The survival rate at the end of the feeding trail was high and recorded more than 98% for all groups of fish fed the experimental diets. In the present study fish fed diet 2 (25% PPM) had faster growth rate and better food utilization than fish fed diet 5 (100 % PPM). Fish fed diets 3 (50% PPM) did not differ significantly ($P > 0.05$) from those of fish fed control diet 1 (100 % fish meal protein). The present study exhibited that plant protein mixture meal protein can replace fishmeal protein up to 50% in practical diets of mono sex male Nile

tilapia. This is in agreement with the results of Jackson et al. (1982), who fed *S. mossambicus* (13.9 g) isocaloric, isonitrogenous diets with varying levels of plant protein from cottonseed meal for 9 weeks. The best feed conversion ratio (FCR) and specific growth rate (SGR) were obtained at 50 % cottonseed meal. However, the fish grew at a reasonable rate even at a 100 % cottonseed meal inclusion level. Similar results have been reported for *Tilapia zillii* (El-Sayed, 1987), Nile tilapia (El-Sayed, 1990), Carp Hasan et al.(1997), Nile tilapia El-Saidy and Gaber, (2001), and Carp El-Saidy et al. (2005) and Nile tilapia (El-Saidy and Amal 2008).

In the present study, however, mono sex male Nile tilapia which has an accelerated growth in much higher water temperatures have shown very significant growth depression with plant protein mixture meal protein over 50% of dietary protein (Diets 4 & 5). This growth depression was further elaborated by the lack of response of the fish to the test diets at feeding times. This is in agreement with the results of Hashim et al., (1994) who reported that winged bean seed meal can not be used as a sole protein source for *O. niloticus* fry. Fish fed winged bean seed meal based diets exhibited poor growth performance, feed conversion ratio (FCR) and SGR. El-Saidy and Gaber (2001), El-Saidy and Amal (2008) and El-Saidy et al (2005) reported the same results with Nile tilapia and common carp, respectively.

The good growth, excellent condition and high survival in mono sex male Nile tilapia fed diets containing plant protein mixture meal protein up to 50% in the present study demonstrated that plant protein mixture meal protein had no adverse effect on fish performance at this level (50%) of plant protein mixture meal protein. However, increasing levels of plant protein mixture meal protein to 75% and 100 % in the diets 4 & 5 exhibited the adverse effect.

Table 4.The effect of partial and total substitution of fish meal (FM) by plant protein meal mixture (PPM) in practical diets for Nile tilapia fingerlings on growth performaces and feed utilization after 10 weeks experiment. Values are means ± SD[1].

	Diets				
Parameters	1	2	3	4	5
Initial body weight (g)	3.24 ± 0.10a	3.26 ± 0.2 a	3.27 ± 0.2a	3.31 ± 0.1a	3.25 ± 0.1a
Final body weight (g)	17.99 ± 0.26 b	21.50 ± 2.1a	17.30 ± 1.1b	14.63 ± 0.31c	13.09 ± 0.23 c
Weight gain (g)	14.75 ± 0.28 b	18.27 ± 2.1a	14.06 ± 1.1b	11.39 ± 0.29 c	9.85 ± 0.21 c
Weight gain (%)	455.8 ± 12.2 b	564.7 ± 68.4 a	434.6 ± 36.7 b	352.0 ± 6.1 c	304.4 ± 4.0 c
SGR (% / day)	1.21 ± 0.02 b	1.39 ± 0.11a	1.17 ± 0.07 b	1.01 ± 0.01 c	0.89 ± 0.06 d
Feed intake (g / fish)	18.12 ± 0.02ab	18.46 ± 0.98 a	17.35 ± 0.54 b	15.62 ± 0.47 c	14.25 ± 0.36 d
FCR (FI / WG)	1.23 ± 0.02 b	1.02 ± 0.06 a	1.24 ± 0.05 b	1.37 ± 0.07c	1.45 ± 0.01 d
FER (WG / FI * 100)	0.81 ± 0.20 b	0.99 ± 0.06 a	0.81 ± 0.04 b	0.73 ± 0.01c	0.69 ± 0.01 c
PER	2.66 ± 0.05 b	3.24 ± 0.20 a	2.66 ± 0.12 b	2.41 ± 0.01c	2.29 ± 0.01 c

[1]Mean in the same row bearing different superscript letter differ significantly ($P \leq 0.05$ level).

In the present study, the proximate composition of the experimental fish at the start and the termination of the feeding trial are shown in Table 5. Moisture, protein and ash contents of fish fed control (Diet 1) were higher significantly than those of fish fed other diets. While fat content of fish fed control (Diet 1) was lower significantly than those of fish fed other diets. The

highest value of moisture protein and ash contents was obtained on fish fed 100% FM protein control (Diet 1) and the lowest was obtained on fish fed 100% plant protein mixture meal protein (diet 5). Incorporation of plant protein mixture in mono sex male Nile tilapia diets significantly decreased moisture, protein and fat content of whole body. The highest value of lipid content was recorded with fish fed 100% plant protein mixture meal (Diet 5) and the lowest was recorded with fish fed 100% FM protein control (Diet 1) The results of El-Saidy et al. (2005) and Soltan et al (2008) exhibited the same trend.

In conclusion, the present study revealed that plant protein mixture meal protein can replace up to 50% of fish meal protein in practical diets of mono sex male Nile tilapia without any adverse effects on growth performance, feed utilization and body composition analysis of fish. In addition, Plant protein mixture meal used in the present study is available at much lower prices (1.9 LE/kg) than fishmeal (7.75 LE/kg) in many tropical and sub-tropical regions where tilapia culture is well established. Further research should be conducted under the field conditions.

Table 5: Initial and final whole body composition of mono sex male Nile tilapia fed experimental diets. Values are mean ± SD[1] of triplicate analyses.

Parameters	Initial	Diets				
		1	2	3	4	5
Moisture	78.78 ± 0.30	74.60 ± 0.40a	73.02 ± 0.19b	72.98 ± 0.63 b	70.88 ± 0.33 c	69.89 ± 0.77d
Protein	12.41 ± 0.29	56.93 ± 2.29a	54.74 ± 0.09a b	54.16 ± 0.35 b	53.71 ± 0.31b	52.93 ± 0.75 b
Fat	2.06 ± 0.20	14.10 ± 0.44 a	20.52 ± 0.81b	21.57 ± 0.12 c	22.29 ± 0.53c d	23.12 ± 0.13 d
Ash	4.42 ± 0.10	17.87 ± 1.42 a	15.52 ± 0.43 b	15.36 ± 0.33 b	14.52 ± 0.65 b	12.52 ± 0.09 c

[1]Mean in the same row bearing different superscript letter differ significantly (P≤ 0.05 level).

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