PARTIAL AND TOTAL REPLACEMENT OF FISH MEAL BY FISH MEAL ANALOG IN PRACTICAL DIETS OF BOTH NON SEX-REVERSED AND SEX-REVERSED NILE TILAPIA, *Oreochromis niloticus* (L.) FINGERLINGS.

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ABSTRACT

Two 12-week feeding trials were conducted to determine the amount of dietary fish meal analog that could replace fish meal in diets of non sex-reversed and sex-reversed Nile tilapia. Fish in both trials were fed five isonitrogenous and isocaloric diets that contained fish meal, fish meal analog or both as the dietary animal protein source. Fish meal (40%) was used as the only source of animal protein in the control diet. Percent replacement of fish meal by fish meal analog on the basis of crude protein were as follows: 0 % (control diet 1), 25 % (diet 2), 50 % (diet 3), 75 % (diet 4) and 100 % (diet 5). Diet 6 was used as a practical diet for Nile tilapia formulated in Egypt. Average body weight, weight gain and specific growth rate (SGR) of non sex-reversed tilapia fed test diets 2 and 3 were not significantly different ($P \geq 0.05$) from those of fish fed the control and diet 6. Feed conversion ratio (FCR), protein efficiency ratio (PER) and feed efficiency ratio (FER) of non sex-reversed fish fed diets 2, 3 and 4 were not significantly different ($P \geq 0.05$) from those in fish fed control and practical diets. The results of sex-reversed Nile tilapia trial exhibited the same trend. The results of whole-body composition of non-sex-reversed and sex-reversed fish indicated that replacement of fish meal by fish meal analog in the diets has no negative effects on protein and fat concentrations. These findings suggest that up to 50% of fish meal protein can be replaced by fish meal analog protein in non sex-reversed and sex-reversed Nile tilapia fingerlings.

INTRODUCTION

The protein component for fish diets is usually composed of a large proportion of fish meal which is also the most expensive component of the diet. Fish meal has well balanced amino acids profile, whereas, the majority of the protein sources presented are either deficient in some essential amino acid or suffer from an imbalance of amino acids (Tacon and Jackson 1985).

Aquaculture feeds represent a growing market for marine by-products, primarily fish meal. Approximately 700,000 mt of fish meal, representing 10% of world production was used in fish feeds worldwide in 1988. By the year 2000, the consumption of fish meal by the aquaculture industry is expected to double, while world production of fish meal is expected to remain constant (Barlow, 1989). Substitution of plant protein or animal protein sources for fish meal in fish feeds are two ways that a shortage of fish meal might be remediated. Although use of plant protein sources will result in an increase in solid waste output of fish farms, and this may be an unacceptable practice in some areas, this could be the most economical way to cut on feed costs in aquaculture.

Many studies has been done to evaluate new protein sources in fish diets as substitutes for fish meal. Among the protein sources studied are poultry by-product and feather meal. Dietary combination of poultry by-product and feather meal has been employed to replace the fish meal protein in practical diet for rainbow trout (Alexis *et al*., 1985; Steffens, 1994); coho salmon (Markert *et al*., 1977; Higgs *et al*., 1979); chinook salmon (Brannon *et al*., 1977; Roley *et al*., 1977; Westgate, 1979; Fowler, 1981, 1990, 1991) and Atlantic salmon (Bergstrom, 1973). Additionally, the data have been collected with other fresh water fish species, such as common carp (Steffens, 1988) and Nile tilapia (Gaber, 1996). Also fish meal analog has been employed to replace up the fish meal protein in starter diets for channel catfish larvae (El-Saidy, 1995).

The main objective of this study is to evaluate the use of fish meal analog as a partial or total replacement of fish meal in practical diets for a non sex-reversed (Trial I) and sex-reversed Nile tilapia fingerlings (Trial II). We focused on comparison between two batches of fish with sex ratio 1 : 1 (non sex-reversed) and thermally treated fish (called here sex-reversed) which should be dominated by males (Baroiller *et al*., 1995).

MATERIALS AND METHODS

<u>Fish used :</u>

Nile tilapia, *Oreochromis niloticus* was obtained from the River Nile (Egypt) as a broodstock and kept in fish research laboratory, Collage of Agriculture, Menofiya University, Egypt. After hatching the newly hatched fry were collected and divided into two groups and one of them was exposed to high temperatures of 32 - 34 C for 30 days (sex reversed). This procedure should increase the percentage of male up to 91 % (Baroiller *et al*., (1995). In the present study we followed the same procedure and sexing was made before starting the trial for about 350 fish which were treated with high temperature. Aproximately, 320 fish were males and only 30 fish were females (91.4 % males : 8.6 % females). The second group was kept at 22 - 24 C for 30 days and used as non-sex-reversed (Trial I). Two groups were reared separately in the same laboratory to an approximate weight of 5 g. Fish were then allocated to 36 experimental 60L glass aquaria (18 aquaria for each trial). Each aquarium in the two trials was stocked with 15 fish.

Five experimental diets and one practical diet (Table 1) were fed to fish in three aquaria per diet during 12 weeks. Fish meal and animal by-products (meat and bone meal, blood meal, feather meal and poultry by-product meal) which were used as ingredients of fish meal analog were purchased from commercial sources and diets processed at the Ohio State University Feed Meal, Wooster, Ohio, except diet 6 (practical diet) was processed in fish research laboratory, College of Agriculture, Menofiya

University, Egypt. This is a practical diet formulation for Nile tilapia (El-Saidy and Gaber, 1998). The diets were broken up and sieved into appropriate pellet sizes. All diets were sealed in plastic bags and placed in frozen storage. Portions were transferred to a refrigerator every week as needed for feeding.

The utilization of dietary protein for fish growth was assessed by comparing the mean final body weight, weight gain, percentage weight gain, specific growth rates (SGR) (% per day), feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER).

Growth response parameters were calculated as follows : SGR (% per day) = ({log W_t - log W_i}/ T) x 100, where W_t is the weight of fish at time t, W_i is the weight of fish at time 0, and T is the rearing period in days ; FCR = total dry feed fed (g) / total wet weight gain (g); FER = (total wet weight gain (g) / total dry feed fed (g)) x100 ; PER = wet weight gain (g) / amount of protein fed (g) (Dabrowski et al., 1989).

Experimental system and animals

The feeding trials were conducted in 36 glass aquaria. Each aquarium containing 60 liter of dechlorinated tap water. About one third of water volume in each aquarium was daily replaced by aerated fresh water after cleaning and removing the accumulated excreta. All aquaria were supplied with compressed air. A photoperiod of 12 h light, 12 h dark (08.00 to 20.00 h) was used. The illumination was supplied by fluorescent ceiling lights.

Water temperature and dissolved oxygen were measured every other day using a YSI oxygen meter (YSI Model 58 Yellow Springs, OH.) Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectrophotometer. Total alkalinity and chloride were monitored twice weekly using the titration method, pH was monitored twice weekly using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, OH). During the 12-week feeding trials, the water-quality parameters averaged (± SD): water temperature, 27.8 ± 0.8 C; dissolved oxygen, 6.7 ± 0.5 mg /L ; total ammonia, 0.20 ± 0.14 mg /L ; nitrite, 0.07 ± 0.05 mg / L ; total alkalinity 180 ± 46 mg / L ; chlorides, 573 ± 151 mg /L ; pH, 8.5 ± 0.16.

Two sets of 270 fish of non sex-reversed and sex-reversed Nile tilapia fingerlings average initial weight of 5.10 ± 0.07 g (Trail I) and 5.11 ± 0.06 g (Trail II) were collected as uniform as possible. Fifteen fish were randomly stocked into each aquarium with three replicates per treatment. After stocking, to minimize stress of handling, fish from each aquarium were weighed every two weeks. All fish were fed initially 4% of the body weight daily and gradually decreased to 3% daily. Tilapia were fed twice a day (0800 and 1600 h) 6 days per week. At the end of the feeding trials, three fish per aquarium were killed by decapitation and stored frozen in polyethylene bags, for whole-body composition analysis (protein, fat, moisture and ash analysis), according to AOAC methods (1990).

Statistical analysis

After the trials were terminated the data were analyzed as general factorial (two way ANOVA). The interaction between the two treatments (sex) and test diets was not significant (F calculated was 0.36). So, we analyzed the two trials separately using one way ANOVA. (Statistical analysis system, 1988). Duncan's multiple range test was used to compare differences among individual means for each trial. Treatments effect were considered significant at $P \leq 0.01$. All percentages and ratios were transformed to arcsin values prior to analysis (Zar, 1984).

RESULTS

Trial I:

The results of growth for non sex-reversed Nile tilapia are presented in Table 2 and Fig. 1. Growth parameters exhibited significant differences ($P \leq 0.01$) among the experimental diets. There was no mortality in all groups of fish fed the test diets during the experiment. Fish fed diet 1 containing 100% fish meal (control diet) had the highest values of final body weight, weight gain, and specific growth rate. The lowest values were recorded in fish fed diet 5 (containing 100% fish meal analog). There were no significant differences between fed fish diet 1 (control) and 2 and 3, in respect to SGR. Therefore, fish meal can be replaced by fish meal analog in Nile tilapia diets up to 50% without significant decrease in growth performance.

Feed utilization parameters of non sex-reversed Nile tilapia fed different experimental diets are presented in Table 3. Feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) exhibited significant differences ($P \leq 0.01$) among the experimental diets. The best values of FCR, FER and PER were recorded with groups of fish fed diet 1 (control). The lowest results were recorded with groups of fish fed diet 5 which contained 100% fish meal analog. There were no significant differences between diet 1 (100% fish meal) and diets 2 and 3. This experiment confirmed that fish meal can be replaced by fish meal analog in the diets of non sex-reversed Nile tilapia up to 50% without significant effects on the feed utilization.

No significant differences ($P > 0.05$) were observed in moisture, protein, fat and ash contents in non sex-reversed Nile tilapia fed the experimental diets (Table 4).

Trail II

When sexing was made again by the end of the experiment the ratio was changed to be 76.3% males : 24.7% females. The results of growth parameters for sex reversed Nile tilapia are presented in Table 5 and Fig. 2. Growth parameters exhibited significant differences ($P \leq 0.01$) among the experimental groups at the end of the experiment. There was no mortality for all groups of fish. Fish fed diet 1 containing 100% fish meal (control diet) had the highest values of final body weight, weight gain, and specific growth rate, but here no significant difference between fish fed diet 1 (control) and diet 2 which contained 25% fish meal analog. Therefore, fish meal can be replaced by fish meal analog in sex reversed Nile tilapia diets up to 25% without a decrease in growth performance.

Feed utilization parameters of sex-reversed Nile tilapia fed different experimental diets are presented in Table 6. Feed conversion ratio, feed efficiency ratio, protein efficiency ratio exhibited significant differences ($P \leq 0.01$) among experimental diets. The best food utilization was recorded in groups of fish fed diet 1 (control). There were no significant differences between fish fed diet 1 and fish offered 2, 3 and 4 (25 - 75 %fish meal analog). Therefore, fish meal can be replaced by fish meal analog in the diets of sex reversed Nile tilapia up to 75 % without significant effects on the feed utilization.

In general, groups of sex-reversed Nile tilapia (Trial II) fed the test diets had faster growth rate and better food utilization than groups of non-sex-reversed Nile tilapia (Trial I) fed the same diets. This could be due to the proportion of males which was higher in trial II (76.2 %) than in trial I (50 %).

The data on the whole-body composition of sex-reversed Nile tilapia fed the experimental diets for 12 weeks is presented in Table 7. Significant differences ($P \leq 0.05$) were observed in the moisture and ash contents of fish fed the experimental diets. The crude protein and crude fat levels did not differ ($P \leq 0.05$). The body ash level was significantly higher in fish fed diet 5 which contained 100% fish meal analog, in comparison with fish fed diets 1 and 2.

DISCUSSION

In the present study we used two batches of fish with sex ratio 1 : 1 (non sex-reversed) and thermally treated fish (called here sex-reversed). When sexing was made for sex-reversed fish (Trial II) by the end of the experiment the ratio was changed to be approximately three males : one female (76.3% males : 24.7% females). Groups of sex-reversed Nile tilapia fed test diets (Trial II) had faster growth rate and better food utilization than groups of non sex-reversed fish fed the same test diets (Trial 1) This may be related to the proportion of males which was higher in Trial II than Trial I. Similar findings were reported by Gaber and El-Saidy (1998) for *Tilapia zilli.*

The present study exhibited that fish meal analog which was composed of meat and bone meal, feather meal, blood meal and poultry by-product meal (25% each), can replace fish meal up to 50% in practical diets of Nile tilapia fingerlings. However, 100% fish meal analog (diet 5) had resulted insignificantly lower growth rate and feed utilization. Similar results have been reported by Lee and Bai (1997) for juvenile Nile tilapia. The authors reported that an animal by-product, hemoglobin powder, seemed to effectively replace up to 50% of fish meal in Nile tilapia diets. El-Saidy (1995) using channel catfish larvae found that diets containing 100% fish meal analog had produced significantly lower growth rate and feed utilization. Other authors reported that between 30% and 75% of dietary fish meal protein could be replaced by animal by-products. Tacon *et al*. (1983) reported that hexane extracted meat and bone meal or meat and bone meal plus blood meal (4 : 1), both supplemented with methionine, can replace up to 50% of fish meal protein in diets fed to Nile tilapia juveniles for six weeks. This is in agreement with the results of Viola and Zohar (1984), who reported that up to 50% of fish meal could be replaced by poultry by-products in the diets of tilapia hybrids. In addition, Otubusin (1987) found that blood meal levels exceeding 50% of fish meal protein in diets fed to Nile tilapia fingerlings reared in cages for 120 days resulted in a significant reduction in fish growth, while 10% level was the most efficient. The differences among these results may have been related to protein source, quality and processing of fish meal replacer, fish species and size, duration of experimental period and culture systems.

In the present study whole-body composition at the termination of the feeding trials showed that there was no significant difference ($P > 0.05$) in moisture, protein, fat and ash contents of non-sex-reversed Nile tilapia (Table 4). In contrast, sex-reversed Nile tilapia (Trial II) exhibited significant differences ($P < 0.05$) in moisture and ash. The differences in moisture and ash in trial II (Table 7) it may be related to higher proportion of males which grow faster than females.

In conclusion, the present study revealed that fish meal analog can replace up to 50% of fish meal in practical diets of Nile tilapia without any adverse effects on growth, feed utilization and whole-body composition of fish. In addition, these protein sources used in fish meal analog mixture are available at much lower prices than menhaden or herring fish meals.

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Table 1. Diet composition used in the present study (%).

	Diets					
	1	**2**	**3**	**4**	**5**	**6** *
Ingredients (%)						
FM menhaden	20.00	15.00	10.00	5.00	0.00	--
FM herring	20.00	15.00	10.00	5.00	0.00	--
FM analog	0.00	9.50	19.01	28.51	38.01	--
Yeast	9.00	9.00	9.00	9.00	9.00	--
Corn gluten meal	15.00	15.00	15.00	15.00	15.00	--
Extract soybean meal	13.00	13.00	13.00	13.00	13.00	--
Whey	7.00	7.00	7.00	7.00	7.00	--
Methionine	0.00	0.10	0.21	0.31	0.41	--
Lysine	0.00	0.12	0.25	0.37	0.49	--
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	--
Vitamin Mixture **	0.50	0.50	0.50	0.50	0.50	--
Mineral Mixture **	1.50	1.50	1.50	1.50	1.50	--
Alphacel	3.00	2.23	1.63	0.96	0.28	--
Vit. C-MP	0.05	0.05	0.05	0.05	0.05	--
Choline chloride	0.10	0.10	0.10	0.10	0.10	--
Cod oil	10.35	11.30	12.25	13.20	14.16	--
Total	100.00	100.00	100.00	100.00	100.00	--
Calculated values (%) based on nutrient tables or nitrogen analysis						
Crude protein	46.50	46.70	47.00	47.20	47.40	--
Methionine	1.20	1.20	1.20	1.20	1.20	--
Lysine	2.96	2.96	2.96	2.96	2.96	--
G. Energy kcal Kg^{-1}	4117	4117	4117	4117	4117	--

* Local diet (Source: El-Saidy and Gaber, 1998).

** Source: Dabrowski, K. (1990).

Table 2. Weight gain, Percentage weight gain and Specific growth rate (SGR % / day) of Nile tilapiafingerlings initial weight 5.10 ± 0.07g. Fish were treated with 22-24 C for one month after hatching (non sex-reversed) prior to feeding experimental diets. Values are means ± SEM of three replicate groups. Means not having the same superscript letter in the same column are significantly different (P ≤ 0.01).

Diets	Fish meal (%)	Fish meal analog (%)	Weight gain (g / fish)	Weight gain (%)	Specific growth rate (% / day)
1	100	0.0	21.61 ± 0.68 [a]	427.96 ± 16.8 [a]	1.98 ± 0.04 [a]
2	75	25	20.80 ± 0.03 [ab]	402.88 ± 2.9 [b]	1.92 ± 0.01 [a]
3	50	50	20.31 ± 0.47 [bc]	401.83 ± 8.5 [b]	1.92 ± 0.02 [a]
4	25	75	19.05 ± 0.39 [c]	370.86 ± 5.2 [c]	1.84 ± 0.01 [b]
5	0.0	100	14.84 ± 0.16 [d]	288.89 ± 0.9 [d]	1.62 ± 0.01 [c]
6*	-	-	20.14 ± 0.27 [bc]	399.31 ± 1.9 [b]	1.93 ± 0.02 [a]

***** Practical diet for Nile tilapia after El-Saidy and Gaber (1998).

Table 3. Feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) of non-sex-reversed Nile tilapia fingerlings. Values are means ± SEM of three replicate groups. Means not having the same superscript letter in the same column are significantly different (P ≤ 0.01).

Diets	Fish meal (%)	Fish meal analog (%)	Feed conversion ratio (FCR)	Feed efficiency ratio (FER)	Protein efficiency ratio (PER)
1	100	0.0	1.55 ± 0.02 [a]	0.643 ± 0.007 [a]	2.02 ± 0.02 [a]
2	75	25	1.62 ± 0.03 [a]	0.617 ± 0.009 [b]	1.93 ± 0.03 [b]
3	50	50	1.60 ± 0.01 [a]	0.623 ± 0.003 [ab]	1.95 ± 0.01 [ab]
4	25	75	1.72 ± 0.03 [b]	0.580 ± 0.010 [c]	1.82 ± 0.03 [c]
5	0.0	100	1.98 ± 0.05 [c]	0.507 ± 0.012 [d]	1.58 ± 0.04 [d]
6	-	-	1.59 ± 0.01 [a]	0.630 ± 0.001 [ab]	1.97 ± 0.01 [ab]

Table 4. Whole-body composition of non sex-reversed Nile tilapia fed the experimental diets for 12 weeks (% of dry matter basis). Values are means ± SEM of 3 replicate groups.

Diets[1]	Moisture	Protein	Fat	Ash
1	73.4 ± 0.3	58.3 ± 0.5	23.5 ± 0.8	14.7 ± 0.7
2	73.1 ± 0.4	57.9 ± 0.6	23.8 ± 0.3	14.1 ± 0.8
3	72.9 ± 0.7	57.6 ± 0.3	24.2 ± 0.6	14.2 ± 0.6
4	73.5 ± 0.4	57.4 ± 0.3	23.5 ± 0.6	14.6 ± 0.8
5	72.2 ± 0.5	57.9 ± 0.9	22.7 ± 0.6	14.8 ± 0.8
6	72.8 ± 0.4	57.8 ± 0.6	24.1 ± 0.7	13.8 ± 0.7

[1]See Table 1.

Table 5. Weight gain, Percentage weight gain and Specific growth rate (SGR % / day) of Nile tilapiafingerlings,treated with 32-34 C for one month after hatching (sex-reversed) fed experimental diets. Values are means ± SEM of 3 replicate groups. Means not having the same superscript letter in the same column are significantly different (P ≤ 0.01).

Diets	Fish meal (%)	Fish meal analog (%)	Weight gain (g/fish)	Weight gain (%)	Specific growth rate (% / day)
1	100	0.0	23.89 ± 0.16 [a]	471.6 ± 0.2 [a]	2.08 ± 0.003 [a]
2	75	25	23.15 ± 1.22 [ab]	447.9 ± 21.9 [ab]	2.02 ± 0.047 [ab]
3	50	50	21.76 ± 0.57 [b]	424.2 ± 11.1 [b]	1.97 ± 0.026 [b]
4	25	75	19.29 ± 0.28 [c]	381.7 ± 2.5 [c]	1.87 ± 0.006 [c]
5	0.0	100	16.06 ± 0.33 [d]	314.8 ± 2.9 [d]	1.70 ± 0.009 [d]
6	-	-	21.90 ± 0.50 [b]	426.8 ± 9.7 [b]	1.98 ± 0.023 [b]

Table 6. Feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) of sex-reversed Nile tilapia fingerlings of initial weight of 5.11 ± 0.06 g. Values are means ± SEM of three replicate groups. Means not having the same superscript letter in the same column are significantly different (P ≤ 0.01).

Diets	Fish meal (%)	Fish meal analog (%)	Feed conversion ratio (FCR)	Feed efficiency ratio (FER)	Protein efficiency ratio (PER)
1	100	0.0	1.47 ± 0.058 [a]	0.68 ± 0.030 [a]	2.13 ± 0.08 [a]
2	75	25	1.51 ± 0.055 [a]	0.66 ± 0.020 [a]	2.07 ± 0.07[a]
3	50	50	1.55 ± 0.037 [a]	0.65 ± 0.010 [a]	2.02 ± 0.05 [a]
4	25	75	1.58 ± 0.003 [a]	0.63 ± 0.003 [a]	1.98 ± 0.01 [a]
5	0.0	100	1.80 ± 0.024 [b]	0.55 ± 0.010 [b]	1.73 ± 0.02 [b]
6	-	-	1.54 ± 0.036[a]	0.65 ± 0.020 [a]	2.03 ± 0.04 [a]

Practical diet for Nile tilapia after El-Saidy and Gaber (1998).

Table 7. Whole-body composition of sex-reversed Nile tilapia fed the experimental diets for 12 weeks[1] (% of dry matter). Values are means ± SEM of 3 replicate groups.

Diets[2]	Moisture	Protein	Fat	Ash
1	69.9 ± 0.4[a]	60.4 ± 0.8	24.1 ± 0.6	14.8 ± 0.2 [a]
2	70.7 ± 0.5 [ab]	58.9 ± 0.9	23.9 ± 0.5	14.6 ± 0.5 [a]
3	69.8 ± 0.6 [a]	58.6 ± 09	24.5 ± 1.4	15.3 ± 0.5 [ab]
4	71.9 ± 0.4 [bc]	57.7 ± 1.1	24.3 ± 1.3	15.8 ± 0.4 [ab]
5	72.5 ± 0.5 [c]	57.3 ± 1.0	23.5 ± 1.3	16.1 ± 0.4 [b]
6	70.2 ± 0.7 [a]	58.1 ± 1.2	24.3 ± 0.9	15.1 ± 0.3 [ab]

[1]Means not having the same superscript letter in the same column are significantly diferent (P ≤ 0.01).
[2]See Table 1.