EVALUATION OF COTTON SEED MEAL AS PARTIAL AND COMPLETE REPLACEMENT OF FISH MEAL IN PRACTICAL DIETS OF NILE TILAPIA, *Oreochromis niloticus (L.)* FINGERLINGS

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(Received: July , 1999)

Key words : Cottonseed meal, Nile tilapia nutrition, feed utilization, apparent digestibility coefficient

ABSTRACT

The main objectives of this study was to determine the effect of L partial and total replacement of fish meal by cotton seed meal in practical diets of Nile tilapia fingerlings on growth performance, feed utilization, body composition and some biological measurements of Nile tilapia, Oreochromis niloticus (L.). Fish of an average weight 18.1 ± 0.2 g were stocked in 15 glass aquariums (80 l each) at a rate of 15 fish per aquarium. Fish meal (40 % of the diet) was used as the sole source of animal protein in the control diet. Percent replacement of fish meal by cottonseed meal on the basis of crude protein were as follows: 0 % (control diet A), 25 % (diet B), 50 % (diet C), 75 % (diet D) and 100 % (diet E). Diets were fed to fish at a rate of 4 %, then gradually reduced to 3 % of the total fish biomass daily, for a period of 14 weeks. The results of this study revealed that, the fish fed diet B had significantly ($P \le 0.01$) best average body weight, average body length, specific growth rate (SGR), weight gain %, feed conversion ratio (FCR), and protein efficiency ratio (PER) from those of fish fed control diet A and all other diets. The same parameters of fish fed diet C were not significantly different (P > 0.05) from those of fish fed the control diet A. Condition factor (K),

hepatosomatic index (HSI) and survival rate % of the fish fed experimental diets B and C were not significantly different (P > 0.05) from those of fish fed the control diet A. The best values of hematocrit % and hemoglobin % were recorded with groups of fish fed diet A (control) and diet B (25 % cottonseed meal) and then decreased significantly (P \leq 0.01) with increasing cottonseed meal level in diets C, D, and E. Body composition of crude protein and crude fat (wet and dry basis %) and apparent digestibility coefficient of crude protein and crude fat of the fish fed experimental diets B and C did not differ significantly (P > 0.05) from those of fish fed the control diet A. Therefor, these findings suggest that up to 50 % of fish meal protein can be replaced by cotton seed meal protein in fingerlings Nile tilapia diets.

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 plant 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 byproducts, 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.

The use of cotton seed meal as a supplemental fish feed has been examined in salmonids (Herman, 1970; Fowler, 1980), catfish (Dorsa et al., 1982; Robinson et al., 1984b) and tilapia (Jackson et al., 1982; Ofojekwu

and Ejike, 1984; Robinson et al., 1984a; El-Sayed 1987). The results of these studies are somewhat confusing. For example, Jackson et al.(1982) and El-Sayed (1987) found that *Sarotherodon mossambicus* and *Tilapia zillii*, respectively utilized cotton seed meal efficiently as protein source even at a 100 % inclusion level. On the contrary, Ofojekwu and Ejike (1984) reported that *Oriochromis niloticus* exhibited poor growth when fed on cotton seed meal-based diets.

One of the problems which limit the use of cotton seed meal as a fish feed is its gossypol content. Beside being toxic to some fishes, gossypol may render lysine unavailable (Jauncey and Ross, 1982). However, the response of fishes to gossypol is species specific. Rainbow trout fed on cotton seed meal-based diet containing 0.03 % gossypol exhibited poor growth and high mortality (Herman, 1970). Tilapia (Robinson et al., 1984a) and channel catfish (Dorsa et al., 1982; Robinson et al., 1984b) on the other hand, tolerated higher levels of free gossypol without adverse effects on their growth rates.

The present study was therefore carried out to evaluate the effects of partial and complete replacement of fish meal protein by cotton seed meal protein in practical diets on the growth, feed utilization efficiency, body composition and some biological measurements of Nile tilapia, *Oreochromis niloticus (L.)* fingerlings.

MATERIAL AND METHODS

Experimental diets

Five experimental diets were formulated. Diet A (control), with 40 % fish meal protein was formulated to be a high-quality commercial tilapia fish diet. The other four diets (diets B, C, D and E) contained 25, 50, 75 and 100 % cotton seed meal protein replacement of fish meal protein (Table 1). All diets were formulated to be isonitrogenous (44.2 % protein) and isocaloric (4173 kcal gross energy per kg of diet).

In preparing the diets, dry ingredients were first ground to a small particle size (approximately 250 μ m) in a Wiley mill. Ingredients were

thoroughly mixed and then thoroughly added water to obtain a 30 % moisture level. Diets were passed through a mincer with die into 0.4-mm diameter spaghetti - like strands and were dried under sun for 8 h. After drying the diets were broken up and sieved into appropriate pellet sizes. Percentage protein of the diets was determined by micro-kjeldahl, percentage fat was determined by ether extract method, and moisture was determined by drying (100 C) until constant weight (AOAC, 1990). Gross energy (GE) was estimated from the diet ingredient according to NRC (1993).

Experimental system and animals

The feeding trial was conducted in 15 glass aquaria each containing 80 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 for oxygen requirements. 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 Model 58 oxygen meter. 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 14-week feeding trial, the water-quality parameters averaged (\pm SD): water temperature, 27.6 \pm 0.9 C: dissolved oxygen, 6.5 \pm 0.5 mg /l : total ammonia, 0.20 \pm 0.14 mg /l : nitrite, 0.07 \pm 0.05 mg / l : total alkalinity, 181 \pm 45 mg /l : chlorides, 575 \pm 150 mg /l : pH, 8.5 \pm 0.2.

A set of 225 Nile tilapia (*Oreochromis niloticus*) fingerlings average weight 18.1 ± 0.2 g were collected from the stock of fish research laboratory in Shebin El-Kom, Faculty of Agriculture, Minufiya University

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and were used for the feeding trial. Fifteen fish were randomly stocked into each aquarium with three replications per treatment. After stocking, to minimize stress of handling, fish from each aquarium were weighed every 2 weeks and at the end of the feeding trial. Total length of each fish was measured at the end of the trial. All fish were fed initially 4% of the total body weight daily and gradually decreased to 3% daily. Tilapia were fed twice a day (0800 and 1600 h) 6 days per week for 14 weeks.

At the start and end of the feeding trial a number of fish were killed by decapitation (10 at stocking and three fish per aquarium at the end), fish flesh were obtained, homogenized in a blender, stored in polyethylene bags, and frozen for subsequent protein, fat, moisture and ash analysis, according to AOAC, (1990). Samples of blood were obtained for three individual fish per group from the caudal vasculature. Then, haemoglobin (Hb) and haematocrit were determined according to methods of Houston, (1990). The fish were anaesthetized with tricaine methanesulphonate (MS-222) and stored at -18 °C pending analysis. Three fish from each group were randomly selected and measured for determination of hepatosomatic index (HSI; liver weight x 100/body weight) and condition factor (CF; body weight x 100/body length³).

Growth performance and feed conversion were measured in terms of final individual fish weight (g), total length (mm), survival (%), specific growth rate (SGR, % day ⁻¹), feed conversion ratio (FCR), protein efficiency ratio (PER), and food intake(% body weight). Growth response parameters were calculated as follows : SGR (% day⁻¹) = ({In W_t - In 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): PER = wet weight gain(g) / amount of protein fed (g) : Food intake = total dry feed fed (g/fish) (Richardson, et al., 1985).

Apparent nutrient digestibility:

During the second month of the experiment, the feces were collected from each aquarium every morning before feeding . The feces were collected on filter paper for drying and subsequent chemical analysis. Apparent nutrient digestibility were calculated using the formula of Maynard and Loosli (1969).

Apparent nutrient digestibility (%) = 100 - {100 X $\frac{\% \text{ Cr2 O3 in feed}}{\% \text{ Cr2 O3 in feces}}$ X $\frac{\% \text{ Nutrient in feces}}{\% \text{ Nutrient in feed}}$ }

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the SAS ANOVA procedure (Statistical analysis system, 1988). Duncan's multiple range test was used to compare differences among individual means. Treatment effect were considered significant at $P \le 0.01$. All percentage and ratio were transformed to arcsin values prior to analysis (Zar, 1984).

RESULTS

The results of average body weight, body length, specific growth rate (SGR), weight gain %, feed conversion ratio (FCR), protein efficiency ratio (PER)), condition factor (K), hepatosomatic index (HSI) and survival rate are presented in Table 2 and Fig. 1.

Fish fed diet B which contained 25 % cottonseed meal protein had significantly ($P \le 0.01$) the highest average body weight, average body length, specific growth rate (SGR), weight gain %, feed conversion ratio (FCR), and protein efficiency ratio (PER) from those of fish fed diets A, C, D, and E ,which contained 100 % fish meal protein, 50 %, 75 % and 100 % cottonseed meal protein, respectively. While groups of fish fed diet C contained 50 % cottonseed meal protein were not significantly different (P > 0.05) from those of fish fed the control diet A. The lowest values of average body weight, body length, SGR, weight gain %, FCR, and PER were recorded with groups of fish fed diets D and E which contained 75 % and 100 % cottonseed meal, respectively.

Condition factor (K), hepatosomatic index (HSI) and survival rate % of the fish fed experimental diets B (25 % cotton seed meal) and diet C (50 % cotton seed meal) were not significantly different (P > 0.05) from those of fish fed the control diet A (100 % fish meal protein).

Hematocrit % and hemoglobin % which determined as a hematological indictors are illustrated in Table 3. The highest values of hematocrit % and hemoglobin % were recorded with groups of fish fed control diet A and diet B (25 % cotton seed meal protein). Then decreased significantly ($P \le 0.01$) with increasing cottonseed meal protein in diets C, D and E.

The results of body composition analysis on wet and dry matter basis % are shown in Table 4. Body moisture and ash contents were not significantly different (P > 0.05) among all experimental diets and control diet. protein and fat contents of fish fed 25 % cottonseed meal (diet B) and 50 % cotton seed meal protein (diet C) were not significantly different (P > 0.05) from those of fish fed the 100 % fish meal protein control (diet A).

The results of apparent nutrient digestibility coefficient of protein and fat for Nile tilapia fed diets containing 100 % fish meal protein control diet A and different dietary cottonseed meal protein replacement of fish meal are presented in Table 5. Fish fed on diet A, with 100 % protein contribution from fish meal showed the highest values of digestibility coefficients of protein and fat. However, the digestibility coefficients of protein differ significantly (P > 0.05) when the fish meal protein was replaced with 25 % and 50 % cottonseed meal protein diets B and C, respectively. Increasing levels of substitution of fish meal protein with cottonseed meal protein resulted in decreased digestibility coefficient of protein and fat.

From the above results it can be suggest that up to 50 % of fish meal protein can be replaced by cottonseed meal protein in fingerlings Nile tilapia diets.

DISCUSSION

In the present study fish fed diet B had faster growth rate and better food utilization than fish fed control diet A and all other experimental diets. Fish fed diet C (50 % cottonseed meal protein) did not differ significantly (P > 0.05) from those of fish fed control diet A (100 % fish meal protein). The present study exhibited that cottonseed meal can replace fish meal up to 50 % in practical diets of Nile tilapia fingerlings. 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 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) and Nile tilapia (El-Sayed, 1990).

In the present study, however, tilapia which has an accelerated growth in much higher water temperatures have shown very significant growth depression with cottonseed meal protein over 50 % of dietary protein (diets 4 & 5). This growth depression was further elaborated by decreased hematological indicators and most likely related to toxic effect of gossypol. This is in agreement with the results of Ofojekwu and Ejike (1984) who reported that cottonseed meal can not be used as a sole protein source for *O. niloticus*. Fish fed cottonseed meal based diets exhibited poor growth performance, feed conversion ratio (FCR), SGR and PER.

The ability of other fishes to utilize cottonseed meal as a protein source seems to be species specific. Fowler (1980) found that cottonseed meal was efficiently utilized as a replacement for fish meal in diets for Chinook salmon (1.5 g) and coho salmon (6.9 g) up to 34 and 22 %, respectively. Dorsa et al. (1982), on the other hand, reported that diets containing more than 17 % cottonseed meal depressed the growth rates of age-0 channel catfish.

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The use of cottonseed meal in fish feeds is limited by its gossypol content. Gossypol is toxic to a wide range of animals (Lovell, 1980). However, its effect on fishes is species dependent. Herman (1970) reported that 0.03 % free gossypol was toxic to rainbow trout. Dorsa et al. (1982) found that channel cat fish can tolerate up to 0.09 % free gossypol in their diets without any suppressive effect on their growth. Furthermore, Robinson et al. (1984a) found out up to 0.2 % free gossypol can be safely added to the diets of *T. aurea*, and reduced growth of the fish was due to cyclopropionic acids contained in glanded and glandless cotton seeds, not to the free gossypol content.

The good growth, excellent condition and high survival in Nile tilapia fed diets containing cottonseed meal protein up to 50 % in the present study demonstrated that gossypol had no adverse effect on fish performance at those levels of cottonseed meal (25 and 50 %). However, increasing levels of cottonseed meal protein to 75 and 100 % in the diets exhibited the adverse effect of high levels of gossypol in those diets. Free gossypol lowers protein quality of cottonseed meal by binding lysine during heating and the extrusion process and resulted in poor growth performance for fish fed those diets contained high levels of cottonseed meal. Steve (1990) reported that during processing, free gossypol is bound to cottonseed protein resulting in bound gossypol and unavailable amino acids. This binding reduces the protein quality, especially with regard to lysine availability. Lysine is believed to be the primary amino acid that is bound to free gossypol.

In the present study body composition analysis (wet and dry matter basis %) at the termination of the feeding trial showed that there was no significant difference (P > 0.05) in moisture and ash contents of Nile tilapia fed experimental diets (Table 4). In contrast, fish fed diet A, B and C contained 100 % fish meal protein, 25 % cottonseed meal protein and 50 % cottonseed meal protein, respectively, exhibited significant differences (P < 0.05) in protein and fat contents from those of fish fed diets 75 % and 100 % cottonseed meal protein. it may be related to higher growth of fish fed control diet A and diets B and C which grow faster than fish fed diets D and E (75 and 100 % cottonseed meal protein).

In the present study tilapia fed diet A, with 100 % fish meal protein showed the highest values of digestibility coefficients of protein and fat. However, the digestibility coefficients of protein and fat did not differ significantly (P > 0.05) when the fish meal protein was replaced with 25 % and 50 % cottonseed meal protein diets B and C, respectively. Increasing levels of substitution of fish meal protein with cottonseed meal protein resulted in decreased digestibility coefficient of protein and fat. This is in agreement with the results of Herman (1970) who reported a reduced digestibility of protein and fat for rainbow trout fed diets containing cottonseed meal with gossypol for a period of 4 months. This could well be due to the reduced digestibility of protein-gossypol complexes resulting in protein and /or amino acid deficiencies such as methionine which cause a disturbances in protein and fat metabolism.

In conclusion, the present study revealed that cottonseed meal protein can replace up to 50 % of fish meal protein in practical diets of Nile tilapia without any adverse effects on growth, feed utilization and body composition analysis of fish. In addition, cottonseed meal used in the present study is available at much lower prices than fish meal in many tropical and sub-tropical regions where tilapia culture is well established. Further research should be conducted in order to minimize negative impact of increased ration of cottonseed meal in Nile tilapia diets, gossypol has to be detoxified.

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Table 1: Composition and proximate analysis of experime	ntal diets for
Nile tilapia fingerlings (dry matter %).	

Ingredients	Diet A	Diet B	Diet C	Diet D	Diet E	
	(0% cot.)	(25% cot.)	(50% cot.)) (75% cot.)) (100% cot.)	
Menhaden fish meal	20.00	15.00	10.00	5.00	0.00	
Herring fish meal	20.00	15.00	10.00	5.00	0.00	
Cotton seed meal	0.00	14.70	29.72	44.11	58.80	
Krill hydrolysates	4.50	4.50	4.50	4.50	4.50	
Wheat middlings	28.00	21.20	14.40	7.50	0.60	
Corn gluten meal	11.60	12.60	13.60	14.60	15.60	
Yeast	6.00	6.00	6.00	6.00	6.00	
L-Methionine	0.00	0.10	0.20	0.30	0.40	
L-Lysine	0.00	0.20	0.40	0.60	0.80	
Vitamin mixture [*]	0.50	0.50	0.50	0.50	0.50	
Mineral mixture [*]	0.50	0.50	0.50	0.50	0.50	
Vitamin C	0.05	0.05	0.05	0.05	0.05	
Choline, chloride	0.10	0.10	0.10	0.10	0.10	
Cod oil	8.00	8.90	9.80	10.70	11.60	
Cellulose	0.25	0.15	0.03	0.04	0.05	
$Cr_2 O_3$	0.50	0.50	0.50	0.50	0.50	
Proximate analysis (%):						
Moisture	8.72	8.31	7.90	7.48	7.05	
Crude protein	44.40	44.37	44.27	44.01	44.00	
Crude fat	14.40	14.50	14.60	14.70	14.80	
Crude fiber	5.30	5.22	5.10	5.10	5.40	
Ash	10.49	10.05	10.36	8.36	7.74	
Nitrogen free extract	25.41	25.86	25.67	27.83	28.06	
Energy (Kcal/kg diet)) 4180.00	4177.00	4176.00	4170.00	4163.00	

* After Dabrowski, K. (1990).

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	Diets				
Items	A	В	С	D	E
Initial weight g/fish	18.1 ± 0.3^{a}	18.2 ± 0.1^{a}	18.1 ± 0.2^{a}	18.1 ± 0.1 ^a	18.2 ± 0.1 ^a
Final weight g/fish	55.6 ± 1.0^{b}	68.1 ± 1.7^{a}	56.9 ± 3.6^{b}	$46.0 \pm 0.5^{\circ}$	$45.9 \pm 1.2^{\circ}$
Final length cm /fish	$14.3 \pm 0.2^{\text{ b}}$	15.3 ± 0.1 ^a	14.8 ± 0.1^{ab}	12.9 ± 0.1 ^c	12.8 ± 0.3 ^c
Weight gain %	207.0 ± 3.6^{b}	273.9 ± 9.4^{a}	215.1 ± 21.6^{b}	154.2 ± 3.7 ^c	$154.0 \pm 8.0^{\circ}$
SGR % day -1	1.1 ± 0.01^{b}	1.3 ± 0.03^{a}	1.1 ± 0.06^{b} 0.9	$\pm 0.01^{\circ}$ 0.9	$9 \pm 0.03^{\circ}$
FCR	1.6 ± 0.01 ^b	1.3 ± 0.02 ^a	$1.6 \pm 0.11^{\text{ b}}$	2.0 ± 0.09^{c}	2.0 ± 0.08 ^c
PER	1.4 ± 0.01 ^b	1.7 ± 0.04^{a}	1.4 ± 0.09^{b}	$1.1 \pm 0.05^{\circ}$	1.1 ± 0.05 °
Feed consumed g/fish	60.8 ± 1.20^{ab}	64.9 ± 1.60^{a}	61.3 ± 1.80^{a}	56.9 ± 1.30^{t}	54.5 ± 0.30 bc
Condition factor (K)	2.1 ± 0.05^{a}	1.92 ± 0.01^{ab}	1.95 ± 0.03^{ab}		1.69 ± 0.03 ^c
H.S.I. %	1.8 ± 0.06^{a}	$1.9\pm0.08^{\rm \ a}$	2.1 ± 0.11^{ab}	2.6 ± 0.05 ^c	2.9 ± 0.06^{d}
Survival rate %	100 ^a	100 ^a	100 ^a	93.3 ^b	93.3 ^b

 Table 2: Growth performance, efficiency of feed utilization, hepatosomatic index and survival rate of Nile tilapia fed experimental diets.

Values are means \pm SE of three replications.

Means in the same row, having different superscript letters, are significantly different ($P \le 0.01$).

Table 3: Hematocrit (%) and hemoglobin (g/dl) in Nile tilapia fed fish meal protein (control diet) and different dietary levels of cottonseed meal protein replacement (diets B -E).

Cottonseed meal replacement (%)	Hematocrit (%)	Hemoglobin (g /dl)
0	35.9 ± 0.52^{a}	8.74 ± 0.22^{a}
25	31.1 ± 1.73^{a}	6.97 ± 0.25 ^a
50	16.8 ± 0.88 ^b	3.25 ± 0.25 ^b
75	$9.9 \pm 1.26^{\circ}$	1.56 ± 0.15 ^c
100	9.3 ± 1.17 ^c	1.72 ± 0.13 ^c
	replacement (%) 0 25 50 75	replacement (%)(%)0 35.9 ± 0.52^{a} 25 31.1 ± 1.73^{a} 50 16.8 ± 0.88^{b} 75 9.9 ± 1.26^{c}

Values are means \pm SE of three replications.

Means in the same column, having different superscript letters, are significantly different (P \leq 0.01).

Table 4: Effect of different dietary cottonseed meal protein level in Nile tilapia diets on chemical composition of fish flesh (wet and dry basis %). Values are means \pm SE.^{1,2}

Ash (%)
1.2 ± 0.16
1.4 ± 0.05
1.4 ± 0.09
1.5 ± 0.05
1.6 ± 0.02
0.4^{b} 5.6 ± 0.80
1.4^{a} 6.3 ± 0.30
0.5^{ab} 6.2 ± 0.30
0.9^{b} 6.7 ± 0.20
1.2° 7.1 ± 0.02
E E

 1 a,b,c,d means in the same column bearing different letter differ significantly at 0.05 level.

² Composition of fish slaughtered at the beginning of the experiment dry basis (moisture 79.6 %; crude protein 72.5 %; fat 9.6 % and ash 12.4 %), wet basis (crude protein 14.80 %; fat 1.96 and ash 2.53 %).

Table 5: Apparent nutrients digestibility coefficients for Nile tilapia fingerlings fed diets containing different dietary cotton seed meal protein replacement.

	Cottonseed meal replacement (%)	Digestibility coefficients		
Diets		Crude protein	Crude fat	
A (control)	0	87.8 ± 0.2^{a}	76.2 ± 0.3^{a}	
В	25	87.9 ± 0.2^{a}	$75.5\pm0.9^{\text{ ab}}$	
С	50	86.8 ± 0.4 ^{ab}	$73.4\pm1.3^{\text{ ab}}$	
D	75	85.7 ± 0.3 ^b	71.1 ± 0.3 ^b	
Ε	100	84.0 ± 0.6 ^c	63.3 ± 2.8 ^c	

Values represent the means of three samples.

a,b,c, means in the same column bearing different letter differ significantly at 0.01 level.

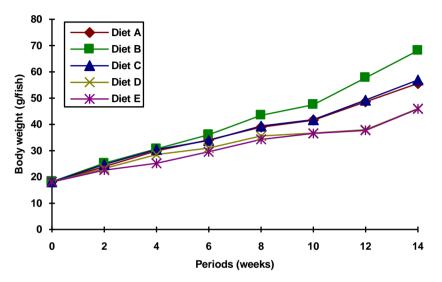


Figure. 1: Changes in average body weight of Nile tilapia fed different experimental diets for 14 weeks.

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