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REPLACING FISH MEAL PROTEIN IN NILE TILAPIA DIETS: UTILIZATION OF COTTON SEED MEAL AND ITS EFFECTS ON GROWTH PERFORMANCE, BODY COMPOSITION AND SOME BIOLOGICAL AND HEMATOLOGICAL PARAMETERS

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ABSTRACT: *The main objective of this study was to determine the effect of 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 and hematological measurements of Nile tilapia were determined. Fish of an average initial weight of 7.7 g and initial length of 7.5 cm were stocked in 15 glass aquaria (200 l each) at a rate of 20 fish per aquarium. Fish meal (46.68 % 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 5 % and then gradually reduced to 2 % of the total fish biomass daily, for a period of 16 weeks. The results of this study revealed that, fish fed diet B had significantly ($P \leq 0.01$) the best average body weight, gain in weight (g/fish), gain in weight %, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and feed consumed (g/fish) from those of fish fed control diet A and all other diets. The same parameters of fish fed diet C were not significantly different from those fed the control diet A. Condition factor (K), hepatosomatic index (HSI) Gonadosomatic index of female (GSI) and survival rate % of fish fed experimental diets B and C were not significantly different from those fed the control diet A. Incorporation of cottonseed meal in the diets did not affect the gonadosomatic index of male Nile tilapia. The best values of hematocrit % and hemoglobin % were obtained 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. Chemical composition of fish flesh crude protein and crude fat (wet and dry basis %) and apparent digestibility coefficient of crude protein and crude fat of fish fed experimental diets B and C did not differ significantly from those fish fed the control diet A. From the above results and the economic evaluation of the study it may be concluded that, up to 50 % of fish meal protein can be replaced by cotton seed meal protein in fingerlings Nile tilapia diets without decreasing the growth performance and feed utilization parameters and seems to be the lowest cost to maximize the net profit without depressing the growth.*

Key words: *Nile tilapia, growth performance, feed utilization, cotton seed meal, body composition .*

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 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.

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 *Oreochromis 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. Besides 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 and hematological measurements of Nile tilapia, *Oreochromis niloticus (L.)* fingerlings.

MATERIALS AND METHODS

Experimental diets:

Five experimental diets were formulated. Diet A (control), with 28 % fish meal protein was formulated to be a high-quality commercial tilapia fish diet. The other four 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 (30.4 % protein) and isocaloric (4.0 kcal gross energy per g of diet).

In preparing the diets, dry ingredients were first ground to a small particle size (approximately 250 µm) in a Wiley mill. Ingredients were thoroughly mixed and then thoroughly added water to obtain a 40 % moisture level. Diets were passed through a mincer with die into 0.4-mm diameter spaghetti - like strands and were dried under sun for 10 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, 1995). 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 200 liter of de-chlorinated 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 16-week feeding trial, the water-quality parameters averaged (± SD): water temperature, 27.5 ± 0.7 C: dissolved oxygen, 5.2- ± 0.5 mg /l: total ammonia, 0.20 ± 0.14 mg /l: nitrite, 0.05±0.03 mg / l: total alkalinity, 182±45 mg /l: chlorides, 550±120 mg / l: pH, 7.6± 0.16.

Table 1. Composition and proximate analysis of the five experimental diets fed to Nile tilapia (*Oreochromis niloticus*) fingerlings.

		Diets				
Ingredients	Crude Protein	A	B	C	D	E
		Control	CSM 25 %	CSM 50 %	CSM 75 %	CSM 100 %
Menhaden fish meal	(60%CP)	46.68	35.01	23.34	11.67	0.0
Cotton seed meal	(35%CP)	0.0	20.0	40.0	60.0	80.0
Corn meal	(8% CP)	17.73	17.73	17.73	17.73	0.0
Rice bran	(15%CP)	4.36	0.0	0.0	0.0	9.49
Fish oil		2.0	2.0	2.0	2.0	2.0
Soy bean oil		2.0	2.0	2.0	2.0	2.0
Vit & Min Premix[1]		2.0	2.0	2.0	2.0	2.0
Dicalcium phosphate		1.5	1.5	1.5	1.5	1.5
Molasses		1.5	1.5	1.5	1.5	1.5
L. lysine		0.0	0.5	0.5	0.5	0.5
L. Methionine		0.0	0.5	0.5	0.5	0.5
Corn starch maize		21.72	16.75	8.42	0.59	0.5
Vitamin C complex		0.01	0.01	0.01	0.01	0.01
Chromium oxide (Cr_2O_3)		0.5	0.5	0.5	0.5	0.5
Total (%)		100	100	100	100	100
Proximate composition (%)						
Dry matter		89.22	89.13	89.17	89.50	89.38
Crude Protein		30.08	30.42	30.42	30.42	30.42
Ether extract		5.46	5.72	5.79	5.87	5.07
Crude fiber		5.22	5.28	5.14	5.82	5.85
Nitrogen Free Extract [2]		42.23	41.68	41.21	41.02	41.53
Crude ash		6.21	6.03	6.61	6.37	6.51
GE (Kcal/g) [3]		3.99	3.99	3.98	3.98	3.93
Free gossypol(mg kg^{-1})		0.0	11.42	22.85	34.27	45.69

[1]Vitamins and minerals mixture (mg or IU if mentioned kg^{-1}diet): vitamin A, 8000 IU; vitamin D3, 4000 IU; vitamin E 50 IU; vitamin K3, 19IU;vitamin B2, 25mg; vitamin B3, 69mg; nicotinic acid, 125mg; thiamine, 10mg; folic acid, 7 mg; biotin, 7mg; vitamin B12,75mg; choline, 400mg and vitamin C, 200 mg. 300 mg I, 100mg Co, 100mg Si, 50000mg Zn, 70000mg Mn, 30000mg Fe, 4000 Cu, and CaCo3 even 1 KG.

[2]Nitrogen-free extract =100 - (moisture+ crude lipid+ crude protein+ crude fiber+ ash).

[3]GE (Gross energy) was calculated according to NRC (1993) by using factors of 5.65, 9.45 and 4.22 Kcal per gram of protein, lipid and carbohydrate, respectively.

A set of 300 Nile tilapia (*Oreochromis niloticus)* fingerlings average weight 7.7 ± 0.2 g were collected from a hatchery in kafer El Sheikh governorate and were used for the feeding trial. Twenty fish were randomly stocked into each aquarium with three replications per diet. 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 every 2 weeks and at the end trial. All fish were fed initially 5% of the total body weight daily and gradually decreased to 2% daily. Tilapia were fed twice a day (1000 and 1400 h) 6 days per week for 16 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, (1995). 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^{o} 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 length^3).

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}) = ({ln W_t - ln 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, 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 was calculated using the formula of Maynard and Loosli (1969).

Apparent nutrient digestibility (%) =

$$100 - \{100 \times \frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}} \times \frac{\%\ \text{Nutrient in feces}}{\%\ \text{Nutrient in feed}}\}$$

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. Diet effect was considered significant at $P \leq 0.01$. All percentages and ratios were transformed to arcsin values prior to analysis (Zar, 1984).

RESULTS

1. Growth performance:

The initial body weight (IBW) of all fish in all diets were 7.7g per fish of Nile tilapia (*Oreochromis niloticus*) and the final body weight of the five experimental diets are shown in Table (2). After sixteen weeks of feeding trial the results indicated that the final body weight of the diet A (Control) (CSM 0%), diet B (CSM 25%) and diet C (CSM 50%) were 38.0 ± 3.2, 38.5 ± 2.3 and 34.4 ± 2.4 g, respectively and there were no significant differences between them. However, diet D (CSM 75%) and diet E (CSM 100%) were 29.1 ± 2.3 and 20.6 ± 2.1, respectively. They were significantly different (*$P \leq 0.01$*) from the control diet and other experimental diets.

Table 2. Initial weight, final weight, weight gain, weight gain percentage and specific growth rate (SGR) of Nile tilapia (*Oreochromis niloticus*) fingerlings fed five experimental diets.

	Diets [1, 2]				
Items	A (100% FM)	B (25% CSM)	C (50% CSM)	D (75% CSM)	E (100% CSM)
Initial weight g/fish	7.7 ± 0.01	7.7 ± 0.01	7.7 ± 0.01	7.7 ± 0.01	7.7 ± 0.01
Final weight g/fish	38.0 ± 3.2 ^{a}	38.5 ± 2.3 ^{a}	34.4 ± 2.4 ^{ab}	29.1 ± 2.3 ^{b}	20.6 ± 2.1 ^{c}
Gain in weight g/fish	30.3 ± 3.2 ^{a}	30.8 ± 2.3 ^{a}	26.7 ± 2.4 ^{ab}	21.4 ± 2.3 ^{b}	12.9 ± 2.1 ^{c}
Weight gain %	393.3 ± 41.9 ^{a}	400.3 ± 29.9 ^{a}	347.2 ± 30.9 ^{ab}	277.7 ± 29.8 ^{b}	167.5 ± 26.9 ^{c}
SGR (% day $^{-1}$)	1.65 ± 0.09 ^{a}	1.67 ± 0.06 ^{a}	1.55 ± 0.07 ^{ab}	1.38 ± 0.08 ^{b}	1.01 ± 0.10 ^{c}

[1]Values are means ± SE of three replications.
[2]Means in the same row, having different superscript letters, are significantly different ($P \leq 0.01$).

The gain in weight from the initial body weight of the five experimental diets of Nile tilapia (*Oreochromis niloticus*) are shown in Table (2). The result from table show that the gain in weight of the diet A(Control), diet B (CSM 25%) and diet C (CSM 50%) were 30.3 ± 3.2 , 30.8 ± 2.3and 26.7 ± 2.4 g, respectively and there were no significantly differences (*P > 0.05*) among them. But the diet D (CSM 75%) and diet E (CSM 100%) were 21.4 ± 2.3 and 12.9 ± 2.1 g, respectively and there were significant differs (P ≤ 0.01) among them and the control diet. The percentage of weight gain in diet A(Control), diet B (CSM 25%) and diet C (CSM 50%) were 393.3 ± 41.9, 400.3 ± 29.9 and 347.2 ± 30.9, respectively of the initial body weight (7.7 g) and there were no significant difference (*P > 0.05*) among them, however diet D (CSM 75%) and diet E (CSM 100%) were 277.7 ± 29.8 and 167.5 ± 26.9, respectively of the initial body weight (7.7 g) which mean that were significant differs (P ≤ 0.01) between them and diet A (control).

The specific growth rate (% /day) of the five experimental diets are shown in Table (2) of Nile tilapia (*Oreochromis niloticus*). Results showed that the specific growth rate (% /day) of the diet A(control), diet B (CSM 25%) and diet C (CSM 50%) were 1.65 ± 0.09, 1.67 ± 0.06 and 1.55 ± 0.07, respectively and there were no significant differences (*P > 0.05*) among them. Diet D (CSM 75%) and diet E (CSM 100%) showed values of 1.38 ± 0.08 and 1.01 ± 0.10, respectively and there were significantly different (*P ≤ 0.01*) compared to the control diet.

2- Feed utilization:

Feed utilization, feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER) and feed consumed (g/fish) of Nile tilapia (*Oreochromis niloticus*) fingerlings fed five experimental diets are shown in Table (3). The best value of feed conversion ratio (FCR) of 2.13 ± 0.07 was recorded with groups of fish fed diet B and followed by diet A (control diet) which was 2.17 ± 0.17 then followed by diet C of 2.23 ± 0.15 and followed by diet D which had FCR of 2.47 ± 0.15. There were no significant differences (*P > 0.05*) among the four diets. The highest value of feed conversion ratio was found in diet E it was 3.80 ± 0.51 and it differ significantly (P ≤ 0.01) from diet A (control) and other diets.

Table 3. Feed utilization, feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER) and Feed consumed (g/fish) of Nile tilapia (*Oreochromis niloticus*) fingerlings fed five experimental diets.

	Diets [1,2]				
Items	A (100% FM)	B (25% CSM)	C (50% CSM)	D (75% CSM)	E (100% CSM)
FCR	2.17 ± 0.17 [a]	2.13 ± 0.07 [a]	2.23 ± 0.15 [a]	2.47 ± 0.15 [a]	3.80 ± 0.51 [b]
FER	0.47 ± 0.03 [a]	0.47 ± 0.02 [a]	0.45 ± 0.03 [a]	0.40 ± 0.02 [a]	0.27± 0.03 [b]
PER	1.54 ± 0.10 [a]	1.56 ± 0.06 [a]	1.47 ± 0.09 [a]	1.32 ± 0.08 [a]	0.89 ± 0.11 [b]
Feed consumed g/fish	64.5 ± 2.94 [a]	64.7 ± 2.4 [a]	59.4 ± 1.9 [ab]	52.9 ± 2.5b [bc]	47.02 ± 1.98 [c]

[1]Values are means ± SE of three replications.
[2]Means in the same row, having different superscript letters, are significantly different (P ≤ 0.01).

Feed efficiency ratio (FER), are shown in Table (3). The best value of feed efficiency ratio (FER), of the five experimental diets were diet A (control), B, C and D there were 0.47 ± 0.03, 0.47 ± 0.02, 0.45 ± 0.03 and 0.40 ± 0.02, respectively. There were no significant differences (*P > 0.05*) among the four diets. The lowest value of feed efficiency ratio was found in diet E it was 0.27± 0.03 and it differ significantly (*P ≤ 0.01*) from diet A (control) and other test diets.

Protein efficiency ratio (PER) of Nile tilapia (*Oreochromis niloticus*) fingerlings fed five experimental diets are shown in Table (3). The best value of protein efficiency ratio (PER) of the five experimental diets was diet B it was 1.56 ± 0.06 followed by diet A (control) it was 1.54 ± 0.10 then followed by diet C it was 1.47 ± 0.09 and followed by the last good PER was found in diet D it was 1.32 ± 0.08. There were no significant differences among the four diets. The lowest value of protein efficiency ratio was found in diet E (0.89 ± 0.11), significantly (*P ≤ 0.01*) different from diet A (control) and other diets.

Feed consumed (g/fish) of Nile tilapia (*Oreochromis niloticus*) fingerlings fed five experimental diets are shown in Table (3). The best value of feed consumed (g/fish) of the five experimental diets was diet A (control) it was 64.5 ± 2.94 followed by diet B the highest value it was 64.7 ± 2.4 then followed by diet C it was 59.4 ± 1.9. There were no significant differences (*P > 0.05*) among the three diets. Diet D and E which had feed consumed of 52.9 ± 2.5 and 47.02 ± 1.98, respectively, and there were no significant differences between them but they were significantly (*P ≤ 0.01*) different from diet A (control) and other diets.

3-Fish measurements:

Condition factor (K), Hepatosomatic index (H.S.I), Gonadosomatic index (G.S.I) and survival rate of Nile tilapia (*Oreochromis niloticus*) fingerlings fed five experimental diets are shown in Table (4). The best value of condition factor (K) of the five experimental diets was diet A (control) it was 2.10 ± 0.05 then followed by diet C and B there were 1.95 ± 0.03 and 1.92 ± 0.01, respectively and they were no significant differences between them and diet A (control) then followed by diet D (1.7 ± 0.06) then diet E (1.69 ± 0.03). They were significantly (*P ≤ 0.01*) different from the control diet and other diets.

The lowest value of hepatosomatic index (H.S.I) of 1.8 ± 0.06 was obtained with groups of fish fed diet A (control diet), then followed by diet B it was 1.9 ± 0.08 and there were no significant differences (*P > 0.05*) between them, then diet C it was 2.1 ± 0.1. There was no significant differences (*P > 0.05*) between it and control diet. The highest value of H.S.I was recorded with groups of fish fed diets D and E. They were 2.6 ± 0.05 and 2.9 ± 0.06, respectively. They were differ significantly (*P ≤ 0.01*) from diet A (control diet) and other diets.

Table 4. Condition factor (K), hepatosomatic index (H.S.I), gonadosomatic index (G.S.I)[1] and survival rate of Nile tilapia (*Oreochromis niloticus*) fingerlings fed five experimental diets.

	Diets [2,3]				
Items	A (100% FM)	B (25% CSM)	C (50% CSM)	D (75%CSM)	E (100% CSM)
Condition factor (K)	2.10 ± 0.05^{a}	1.92 ± 0.01^{ab}	1.95 ± 0.03^{ab}	1.7 ± 0.06^{c}	1.69 ± 0.03^{c}
H.S.I. %	1.8 ± 0.06^{a}	1.9 ± 0.08^{a}	2.1 ± 0.11^{ab}	2.6 ± 0.05^{c}	2.9 ± 0.06^{d}
G.S.I % (M)	2.39 ±1.40^{a}	1.13 ±0.74^{a}	1.52 ±0.75^{a}	1.89 ±0.96^{a}	2.27 ±0. 43^{a}
G.S.I % (F)	4.74 ±0.45^{a}	4.68 ±0. 26^{a}	4.20 ±1.53^{a}	3.29 ±1.12^{ab}	1.82 ±1.45
Survival rate %	100^{a}	100^{a}	100^{a}	93.3^{b}	93.3^{b}

[1]GSI (M) gonadosomatic index for male =100 x (gonad weight (g)/body weight (g)); GSI (F), gonadosomatic index for female=100 x (gonad weight (g)/body weight (g).
[2]Values are means ± SE of three replications.
[3]Means in the same row, having different superscript letters, are significantly different (*P ≤ 0.01*).

Gonadosomatic index (GSI) of males and females of Nile tilapia fed cottonseed meal based diet are presented in Table 4. It is evident from this table that cottonseed meal did not significantly affect the gonadosomatic index of male Nile tilapia fed cottonseed meal as a partial or complete replacement of fish meal. On the other hand cottonseed meal based diet significantly (*P ≤ 0.01*) affected the gonadosomatic index of female Nile tilapia. The highest value of gonadosomatic index of female of Nile tilapia was recorded with groups of fish fed the control diet A. There were no significant differences between it and diets B, C and D. The lowest value of gonadosomatic index was recorded with groups of Nile tilapia fed diet E which contained 100% cottonseed meal based diet and this diet was differ significantly from control and other diets.

The survival rate of Nile tilapia (*Oreochromis niloticus*) fingerlings fed five experimental diets was affected with cottonseed meal based diets. It was 100% for groups of fish fed diets A, B and C. There were no significant difference (*P > 0.05*) among them. While the survival rate % for groups of fish fed diets D and E was 93.3% .They were differ significantly (P ≤ 0.01) from the control diet and other diets.

4. Blood parameters:

Hematocrit (%) and hemoglobin (g/dl) in Nile tilapia (*Oreochromis niloticus*) fed cottonseed meal based diets are presented in Table (5). Hemoglobin (g/dl) data indicated that the best value of hemoglobin (g/dl) was 8.74 ± 0.22 recorded with groups of fish fed control diet A followed by 6.97 ± 0.25 for groups of fish fed 25% cottonseed meal diet B. There were no significant difference (*P > 0.05*) between the two diet. Hemoglobin for fish fed diet C was 3.25 ± 0.25 and it was significantly differ (*P ≤ 0.01*) from the control diet and diet D and E which come latest they were 1.56 ± 0.15 and 1.72 ± 0.13, respectively, and there were no significant difference (*P > 0.05*) between them but they are significantly differ (*P ≤ 0.01*) from the control diet.

Hematocrit (%) data are presented in Table (5). The Hematocrit (%) best value of 35.9 ± 0.52 was recorded with fish fed diet A control followed by 31.1 ± 1.73 for fish fed diet B which had 25% cottonseed meal There were no significant difference (*P > 0.05*) between the two diet, then followed by diet C it was 16.8 ± 0.88 and it was significantly differ (*P ≤ 0.01*) from the control diet and diet D and E come latest they were 9.9 ± 1.26 and 9.3 ± 1.17, respectively. There were no significant difference (*P > 0.05*) between diets D and E, but they are differ significantly (*P ≤ 0.01*) from the control diet.

Table 5. Hematocrit (%) and hemoglobin (g/dl) of Nile tilapia (*Oreochromis niloticus*) fed cottonseed meal based diets as a partial and complete replacement of fish meal.

Diets [1,2]	Cottonseed meal replacement (%)	Hematocrit (%)	Hemoglobin (g /dl)
A (control)	0	35.9 ± 0.52 [a]	8.74 ± 0.22 [a]
B	25	31.1 ± 1.73 [a]	6.97 ± 0.25 [a]
C	50	16.8 ± 0.88 [b]	3.25 ± 0.25 [b]
D	75	9.9 ± 1.26 [c]	1.56 ± 0.15 [c]
E	100	9.3 ± 1.17 [c]	1.72 ± 0.13 [c]

[1]Values are means ± SE of three replications.
[2]Means in the same column, having different superscript letters, are significantly different ($P \leq 0.01$).

5. Chemical composition of fish flesh (wet and dry basis %):

Chemical composition of fish flesh (wet basis %) at the end of the experiment are presented in Table (6). It is evident from this table that, the highest percent of crude protein was recorded with groups of fish fed diet B. It was 17.6 ± 0.28 then diet A (control) it was 17.1 ± 0.10 and then followed by diet C it was 17.4 ± 0.19 and there were no significant difference ($P > 0.05$) among the three diet. Crude protein content of the fish fed diets D and E were 16.8 ± 0.01 and 16.2 ± 0.25, respectively. They were differ significantly ($P \leq 0.05$) from the control diet and other diets. The highest value of crude fat was recorded with fish fed diet B. It was 3.1 ± 0.4 then diet C it was 2.9 ± 0.1. They were no significant differences ($P > 0.05$) between them. Diet A (control) and diet D exhibited the same value of 2.5 ± 0.1 and 2.5 ± 0.2, respectively, then followed by diet E it was 2.2 ± 0.3 and it was significantly differ ($P \leq 0.05$) from diet A (control) and diet B. The moisture and ash content (wet bases %) did not influenced by cottonseed meal based diets. The values of ash content for fish fed diets A, B, C, D and E were 1.2± 0.16, 1.4 ± 0.05, 1.4 ± 0.09, 1.5 ± 0.05 and 1.6 ± 0.02, respectively. The moisture content for fish fed diets A, B, C, D and E were 78.3 ± 0.2, 77.7 ± 0.3, 77.9 ± 0.3, 77.7 ± 0.1 and 77.6 ± 0.2, respectively.

Chemical composition of fish flesh (dry basis %) at the end of the experiment are presented in Table (6). It is evident from this table that, the highest value of crude protein content was recorded with fish fed diet B. It was 79.7 ± 0.2 then diet A (control) it was 78.8 ± 0.4 and then followed by diet C it was 78.1 ± 0.2. Diet B had significantly ($P \leq 0.05$) the highest crude protein content compared with diet A (control) and other diets. The lowest value of crude protein was recorded with fish fed diets D and E. It was 75.3 ± 0.2 and 72.8 ± 0.2, respectively, they were differ significantly ($P \leq 0.05$) from the control and other diets. The highest value of crude fat was recorded with fish fed diet B. It was 13.9 ± 1.4 then fish fed diet C it was 13.3 ± 0.5. They were no significant difference ($P > 0.05$) between them. Crude fat content of fish fed diet A (control) was 11.4 ± 0.4 and diet D was 11.1 ± 0.9. They were no significant difference ($P > 0.05$) between them. The lowest value of fat content (10.0 ± 1.2) was recorded with fish fed diet E and it differ significantly ($P \leq 0.05$) from control and other diets. The ash content of groups of fish fed diets A, B, C, D and E were 5.6 ± 0.8, 6.3 ± 0.3, 6.2 ± 0.3, 6.7 ± 0.2 and 7.1 ± 0.02, respectively. The moisture content of fish fed diets A, B, C, D and E were 78.3 ± 0.2, 77.7 ± 0.3, 77.9 ± 0.3, 77.7 ± 0.1 and 77.6 ± 0.2, respectively. Incorporation of cottonseed meal in Nile tilapia diets as partial and complete replacement of fish meal did not significantly affect the moisture and ash contents of fish flesh at the end of the experiment.

Table 6. 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 ± SE.[1, 2]

Diets	Cottonseed meal Replacement (%)	Protein (%)	Fat (%)	Ash (%)	Moisture (%)
			Wet basis (%)		
A (control)	0	17.1 ± 0.10^{ab}	2.5 ± 0.1^{b}	1.2 ± 0.16	78.3 ± 0.2
B	25	17.6 ± 0.28^{a}	3.1 ± 0.4^{a}	1.4 ± 0.05	77.7 ± 0.3
C	50	17.4 ± 0.19^{ab}	2.9 ± 0.1^{ab}	1.4 ± 0.09	77.9 ± 0.3
D	75	16.8 ± 0.01^{b}	2.5 ± 0.2^{b}	1.5 ± 0.05	77.7 ± 0.1
E	100	16.2 ± 0.25^{c}	2.2 ± 0.3^{c}	1.6 ± 0.02	77.6 ± 0.2
			Dry basis (%)		
A (control)	0	78.8 ± 0.4^{b}	11.4 ± 0.4^{b}	5.6 ± 0.8	78.3 ± 0.2
B	25	79.7 ± 0.2^{a}	13.9 ± 1.4^{a}	6.3 ± 0.3	77.7 ± 0.3
C	50	78.1 ± 0.2^{b}	13.3 ± 0.5^{ab}	6.2 ± 0.3	77.9 ± 0.3
D	75	75.3 ± 0.2^{c}	11.1 ± 0.9^{b}	6.7 ± 0.2	77.7 ± 0.1
E	100	72.8 ± 0.2^{d}	10.0 ± 1.2^{c}	7.1 ± 0.02	77.6 ± 0.2

[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 %).

6. Apparent digestibility coefficients:

Apparent digestibility coefficients for Nile tilapia fingerlings fed five experimental diets containing different dietary cottonseed meal protein replacement; data are presented in Table (7). The highest value of 87.9 ± 0.2 for apparent digestibility coefficient of crude protein was recorded with diet B. followed by 87.8 ± 0.2 for diet A (control). There were no significant differences ($P > 0.05$) between them. The apparent digestibility coefficient of crude protein for diet C was 86.8 ± 0.4 and there were no significant difference ($P > 0.05$) between it and control diet A. Diets D and E had the lowest values of apparent digestibility coefficient of crude protein it was 85.7 ± 0.3 and 84.0 ± 0.6, respectively. They were differ significantly ($P \leq 0.01$) from diet A (control).

The highest value of 76.2 ± 0.3 for apparent digestibility coefficient of crude fat was recorded with groups of fish fed diet A (control). The values of apparent digestibility coefficient for diets B, c and D were 75.5 ± 0.9, 73.4 ± 1.3 and 71.1 ± 0.3, respectively. There were no significant differences between them and control diet A. The lowest value of 63.3 ± 2.8 for apparent digestibility coefficient of crude fat was recorded with groups of fish fed diet E and it differ significantly from the control and other diets.

Table 7. Apparent nutrients digestibility coefficients for Nile tilapia fingerlings fed diets containing different dietary cotton seed meal protein replacement.

		Apparent Digestibility Coefficients	
Diets [1,2]	Cottonseed meal replacement (%)	Crude protein	Crude fat
A (control)	0	87.8 ± 0.2 [a]	76.2 ± 0.3 [a]
B	25	87.9 ± 0.2 [a]	75.5 ± 0.9 [ab]
C	50	86.8 ± 0.4 [ab]	73.4 ± 1.3 [ab]
D	75	85.7 ± 0.3 [b]	71.1 ± 0.3 [b]
E	100	84.0 ± 0.6 [c]	63.3 ± 2.8 [c]

[1]Values represent the means of three samples.
[2]a,b,c, means in the same column bearing different letter differ significantly at ($P \leq 0.01$) level.

DISCUSSION

This study demonstrates that up to 50% CSM could be used to replace fish meal as a protein source in the diet of tilapia without affecting growth performance of fish. Beyond that level growth was depressed and the fish population experienced mortality when fed 100% CSM diet. Wu *et al.* (1996) indicated that a 40% protein diet for tilapia achieved the best feed conversion ratio and is suitable to examine alternative protein sources. The SGR and FCR of fish in the present experiment were directly affected by dietary protein source in fish fed with CSM-based diets. Ofojekwu & Ejike (1984) reported that CSM could not be used as a sole protein source for *O. niloticus* because they exhibited poor growth, food conversion and specific growth rate.

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 Nile tilapia (El-Saidy 1999; Mbahinzireki *et al.* 2001 and El-Saidy and Gaber 2002).

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 D and E). 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) and El-Saidy (1999) who reported that cottonseed meal cannot 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 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.

The results in the present study indicated that cottonseed meal can be replaced fish meal up to 50% of the diet without significant affects on feed utilization parameters. This is in agreement with that of Mbahinzireki (1999) who indicated that cottonseed meal can only partially replace FM as a source of protein in compound feed at a limited amount of no more than 50% for tilapia raised in recirculation systems. We are in agreement with Mbahinzireki *et al.* (2001) and El-Saidy and Gaber (2002) who reported that up to 50% CSM could be used to replace fish meal as a protein source in the diet of tilapia without affecting the feed utilization parameters and the overall growth performance of fish beyond that level growth was depressed and the fish population experienced mortality when fed 100% CSM diet. Lim and Webster (2006) indicated that CSM is an important protein source for terrestrial animals. However, its utilization in fish feeds is limited, mainly because of the presence of gossypol and low available lysine. (Barros *et al.*, 2002) reported that free gossypol, when present in large quantities in the diet, has been shown to be toxic to monogastric animals, including fish. Mbahinzireki *et al.* (2001) reported that using CSM plant protein sources as alternatives to fishmeal may be limited by the presence of a number of anti-nutritional factors. These include various protease inhibitors, phytic acid, tannins, a high fibre content and natural toxicants such as gossypol, erucic acid, glucosinolates and iso thio-cyanates (Davies *et al.* 1990). In fact, besides gossypol, cottonseed also contains cyclopropenoid fatty acids, namely, malvalic and sterculic acids, which have been shown to have detrimental effects on salmonid fish (Hendericks *et al.* 1980). The amount of dietary CSM that can be used as a protein source for tilapia seems to depend mainly on its free gossypol and available lysine content (El-Saidy and Gaber, 2004). El-Sayed (1990) mentioned that the effect of poor appetite the use of CSM in fish feed is limited because of gossypol toxicity. Besides being toxic to some fishes, gossypol may render lysine, an essential amino acid, unavailable (Jauncey and Ross 1982). However, this action was diminished as the diets with CSM were supplemented with the recommended amount of synthetic lysine. Free gossypol is reported to be a membrane-active agent with cytocidal properties and the ability to inhibit membrane-bound enzymes, causing haemolytic anaemia at high concentrations (Makinde *et al.* 1997). Li *et al.* (2008) concluded that CSM derived from GM (genetically modified) cotton crops, Roundup Ready Flex, Bollgard Roundup Ready, Bollgard II Roundup Ready, and Bollgard II Roundup Ready Flex at a level of 20% in the diet did not adversely affect feed consumption, growth, FCR, survival, behaviour, and body composition of channel catfish, *Ictalurus punctatus*. These CSM are nutritionally equivalent to CSM derived from conventional cotton varieties when fed to juvenile channel catfish. Yue and Zhou (2008) reported that CSM could not be used as a sole protein source for *O. niloticus* because they exhibited poor growth, food conversion and specific growth rate. However, other studies El-Saidy and Gaber (2004) reported that Nile tilapia can grew well on diets containing 100% CSM protein as a replacement for fish meal with supplemental iron (1:1 iron to free gossypol ratio).

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. 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). This is in agreement with that of El-Saidy (1999). Lee *et al.* (2006) indicated that fillet of rainbow trout produced by fish fed CSM-containing diets can be safely consumed by humans.

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 El-Saidy (1999) who reported a reduced digestibility of protein and fat for Nile tilapia fed diets containing cottonseed meal with gossypol for a period of 14 weeks. 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 disturbance in protein and fat metabolism. Same results were reported by Herman (1970) for rainbow trout. The present study showed that ADC values of nutrients in CSM were comparable with those in other oilseed meals. El-Saidy and Gaber (2002) reported that ADCs of crude protein in soybean meal were 78.7 - 88.9% for Nile tilapia. Cheng and Hardy (2002) found the ADCs of crude protein in CSM were 81.6 - 87.9% for rainbow trout. These values are in agreement of our results where digestibility of crude protein in CSM-based diets ranged from 84% to 87.9%. The results are also in agreement with Mbahinzireki *et al.* (2001) who reported that ADCs of crude protein decreased as dietary gossypol level increased in tilapia (*Oreochromis sp.*) feeds. It is well known that the rate of digestion and nutrients assimilation in fish may be influenced by various physiological and a biotic factors, including fish size, ration level and temperature (Windell *et al.*1978; NRC 1993). The lower digestibility observed for the diet E can be attributed to free gossypol presented in CSM. Results of these studies indicate that total replacement of FM with CSM reduced the nutritional value of the diets.

The present study indicated that HSI of Nile tilapia fingerlings are affected positively by increasing levels of CSM in the diets D and E (75 % CSM and 100 % CSM) respectively. Our study reported a decrease in hematocrit and hemoglobin values with an increase in dietary gossypol and that's in agreement with (Blom *et al.*, 2001; Dabrowski *et al.*, 2001; Rinchard *et al.*, 2003). Herman (1970) and Mbahinzireki *et al.* (2001) who reported that reduced hematocrit and hemoglobin values were one of the most common indicators of gossypol toxicity in fish.

The present study indicated that gonadosomatic index (GSI) of males Nile tilapia were not influenced by CSM diets. Our results are in agreement with those of Dabrowski *et al.*, (2000) and Rinchard *et al.*, (2003). They reported that gonadosomatic index (GSI) of male rainbow trout not negatively affected by increasing levels of cottonseed meal in their diets. Results of the present study indicate that GSI of females Nile tilapia significantly influenced with CSM levels and the lowest values were recorded with groups of fish fed diet E (100 % CSM). The same results were reported by Dabrowski *et al.,* (2000) and Blom *et al.,* (2001) in their studies on rainbow trout fish. Lee *et al.* (2006) based on the 35-month long feeding study, indicated that the high dietary supplementation of CSM (up to 58.8/100 g) or its complete substitution for FM did not have detrimental effects on growth of rainbow trout brood stock. However, reproductive performance of males and females might be impaired by high dietary CSM inclusions over 29.4 g CSM/100 g.

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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استبدال بروتين مسحوق السمك فى علائق أسماك البلطى النيلى: الاستفادة من كسب بذرة القطن وتأثيراته على النمو والأداء ومكونات الجسم وبعض الصفات البيولوجية والهيماتولوجية

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قسم إنتاج الدواجن – كليه الزراعة بشبين الكوم– جامعة المنوفية– مصر

الملخص العربى

أجريت هذه الدراسة لمدة ١٦ أسبوع فى الاحواض الزجاجية على أسماك البلطى النيلى لدراسة الاستبدال الجزئى أو الكلى لبروتين مسحوق السمك ببروتين مسحوق كسب بذرة القطن فى علائق إصبعيات أسماك البلطى النيلى وتأثيراته على النمو والأداء والاستفادة الغذائية ومكونات الجسم وبعض الصفات البيولوجية والهيماتولوجية. وعلى ذلك تم عمل خمسة علائق تجريبية. العليقة الاولى تحتوى على مسحوق السمك بمعدل ٤٦.٦٨% كمصدر وحيد للبروتين الحيوانى وذلك لاستخدامها كعليقة للمقارنة (كنترول). تم استبدال بروتين مسحوق السمك بنسبة ٠ % ,

٢٥ %, ٥٠ %, ٧٥ %, ١٠٠ % ببروتين مسحوق كسب بذرة القطن للعلائق من ١الى ٥ على التوالى و كانت العلائق كلها متماثلة فى الطاقة والبروتين . أجريت التجربة فى ١٥ حوض زجاجى سعة كل منها ٢٠٠ لتر مياة وقسمت الأسماك إلى ١٥ مجموعة متساوية كل مجموعة ٢٠ سمكة متوسط وزن ابتدائى ٧.٧ جم / سمكة ومتوسط طول ابتدائى ٧.٥ سم/ سمكة ووزعت عشوائيا على العلائق (٣ مجموعات/ عليقة) غذيت الأسماك بهذه العلائق بمعدل ٥ % من وزن الجسم الحى يوميا وخفضت تدريجيا إلى ٢ % من وزن الجسم الحى فى اليوم لمدة ١٦ أسبوع. أظهرت النتائج زيادة معنوية عند مستوى ٠.٠١ فى متوسط وزن الجسم النهائى والزيادة فى وزن الجسم (جم/سمكة) والزيادة النسبية فى وزن الجسم و معدل النمو النسبى اليومى و معامل التحول الغذائى وكفاءة استخدام البروتين والغذاء المستهلك (جم/سمكة) فى مجموعة الأسماك التى غذيت على العليقة المحتوية على ٢٥ % بروتين كسب بذرة القطن بالمقارنة بالكنترول وباقى العلائق كذلك وجد أن العليقة المحتوية على ٥٠ % بروتين كسب بذرة القطن لاتختلف معنويا عن العليقة الكنترول. وجد أن معامل الحالة والوزن النسبى للكبد ودليل المناسل للإناث ومعدل الحيوية للأسماك التى غذيت على العلائق المحتوية على ٢٥ % و ٥٠ % بروتين كسب بذرة القطن لاتختلف معنويا عن الأسماك التى غذيت على العليقة الكنترول. وجد أن اضافة كسب بذرة القطن إلى العلائق عند كل المستويات لم يؤثر معنويا على دليل المناسل لذكور أسماك البلطى النيلى. وجد أيضا أن الهيماتوكريت والهيموجلوبين كانت أعلى معنويا فى الأسماك التى غذيت على العليقة الكنترول والعليقة ٢٥ % بروتين كسب بذرة القطن بالمقارنة بباقى العلائق. ووجد أيضا أن مكونات الجسم من البروتين والدهن الخام وكذلك معاملات هضم كل من البروتين والدهن الخام للأسماك التى غذيت على العليقتين ٢٥ % و ٥٠ % بروتين كسب بذرة القطن كانت لاتختلف معنويا عن الأسماك التى غذيت على العليقة الكنترول ١٠٠% بروتين مسحوق السمك. ومن النتائج السابقة والتقييم الاقتصادى للتجربة يتضح أنه من الممكن استبدال بروتين مسحوق السمك جزئيا حتى ٥٠% باستخدام بروتين كسب بذرة القطن فى علائق إصبعيات أسماك البلطى النيلى دون أى تأثيرات معنوية على النمو والأداء والاستفادة الغذائية ومكونات الجسم ومعامل الهضم الظاهرى للعلائق كذلك تبدو أنها أفضل عليقة من الناحية الاقتصادية لتعظيم الربح وتقليل تكاليف التربية بدون نقص فى معدلات نمو الأسماك.