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EFFECT OF SUBSTITUTING BLACK SEED CAKE (*NIGELLA SATIVA* L.) FOR SOYBEAN MEAL IN DIETS OF NILE TILAPIA (*OREOCHROMIS NILOTICUS* L.) ON GROWTH PERFORMANCE AND NUTRIENTS UTILIZATION

Yassir A. Khattab
Fish Nutrition Department, Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Egypt.

Key words: Black seed cake (*Nigella sativa* L.), Nile tilapia, soybeen meal, growth performance, nutrients utilization.

ABSTRACT

Five experimental diets were formulated to contain various percentages of black seed cake (BSC) as a partial replacement for soybean meal (SBM). The substitution levels were 0 (control), 10, 30, 50, and 70% of crude protein of BSC that replaced the same percentages of crude protein from soybean meal. All diets were isonitrogenous (35% crude protein) and isocaloric (477.49 Kcal/100 g diet) and protein energy ratio ranged from 72.90 to 74.34 mg protein/Kcal gross energy. Nile tilapia (*Oreochromis niloticus*) fingerlings were randomly distributed at stocking density of 20 fish per aquarium. The average initial weight of fish ranged from 16.0 to 17.5 g/fish. Each three aquaria (represented three replicates) of one treatment were fed on one of the experimental diets. Diets were given to fish at a rate of 3% of live body weight for 90 day. The daily feed was offered twice daily.

The obtained results revealed that the highest final weight, weight gain and SGR were recorded in fish group reared on diet 10% BSC 35.48, 16.09 (g/fish) and 0.81 (%/day) respectively, while the lowest values were recorded in fish group fed control 33.21, 16.09 (g/fish) and 0.47 (%/day) respectively. No significant differences in SGR, FCR, feed intake, survival rate and hepatosomatic index were observed among different treatments.

Results of DM, CP and ash in fish body composition did not show any significant differences under all treatments. Differences were significant in crude fat ($P<0.05$) among fish groups fed 70% BSC and either fed control or 50% BSC.

Concerning nutrients digestibility, results showed that crude protein and gross energy values under BSC diets were lower than

those in control. The values of apparent protein digestibility, apparent carbohydrate digestibility and apparent gross energy digestibility were higher in fish groups fed BSC diets than that of the control group.

INTRODUCTION

Soybean meal is used as a protein source for feeding of many fish species (Lovell, 1988). Most commercial fish diets currently contain 29.6-60% as average calculated of soybean meal (Mohsen and Lovell, 1990; Webester *et al.*, 1992). The nutritive value of soybean meal as a substitute for fishmeal in formulated diets has been investigated for many fish species (Viola *et al.*, 1982; Shiau *et al.*, 1988; Nengas *et al.*, 1996).

Recently, Soybean has become expensive because of the growing worldwide demand for human and animal feeding and increasing the cost of processing techniques for elimination of inhibition factors (Anti-trypsin). So, it might be feasible to use a new source of plant protein instead of Soybean meal.

Different sources of plant protein such as sun flower, rapeseed and cotton seed promoted reasonable growth even at high dietary inclusion levels of tilapia diets (Jackson *et al.*, 1982). Colzapro (rapeseed and peas) can be utilized in rainbow trout diets at levels as high as 20 % without negative effects on growth (Gomes *et al.*, 1993). Black seed is promising protein source used in fish feeding. However, black seed is cultivated in the Mediterranean region and Asia (Hutchinson, 1959). Nowadays, Egyptian farmers cultivate black seed as a medical crop and for oil production as well, where it is rich in oil (Abdel-Aal and Attia, 1993 a) and the defatted black seed Cake (BSC) has a high level of protein (Abdel-Aal and Attia, 1993 b).

Large price differences between Soybean meal and black seed cake (*Nigella sativa* L.) assures that feed costs will be reduced if soybean meal is substituted by black seed cake (BSC) in fish feed formulations. BSC protein can be used in formulating some balanced diets according to the complementary relationship between this protein and other plant proteins. When it was also substituted for 25 % of wheat protein in Albino rat diet, it remarkably raised the growth of rats by about 48 % and 21 % over the group receiving the unsupplemented wheat protein after two and four weeks (Sharobeem, 1996). Also, BSC was used as a protein source in poultry feeding (Khalifah, 1995; Zewil, 1996).

There is defecient data concerning the inclusion of BSC in fish diets. Therefore, the aim of this study was to use variable percentages of black seed cake (BSC) protein (0 – 70 %) as a partial replacement for soybean meal protein in diets for Nile tilapia (*O. niloticus*) and to investigate its effect on growth performance, survival rate, apparent digestibility of nutrients and reducing feed cost.

MATERIALS AND METHODS

Experimental diets:

Five experimental diets were formulated to contain various percentages of black seed cake (BSC) as a partial replacement for soybean meal (SBM). The chemical analysis of BSC and SBM are shown in Table (1). The experimental diets were formulated to contain (0) control, 10, 30, 50, and 70% of crude protein of BSC that was replaced the same percentages of crude protein from soybean meal. All diets were isonitrogenous (35% crude protein) and isocaloric (Table 2).

In preparing the diets, dry ingredients were ground to a small particle size in a Thomes-Wiley Laboratory Mill Model 4, USA. Ingredients were thoroughly mixed and water was added to make 40 % of the mixture (Shimeno *et al.*, 1985). Diets were passed with die through a 0.8 mm diameter "Spaghetti Machine, La Parmigiana, Model D45 LE, Italy" and dried at room temperature. After drying, the diets were transferred to plastic bags and stored in a freezer at – 20 °C until immediately prior to feeding. Also, a part of each diet was mixed separately with 0.5% chromic oxide and stored till measuring the apparent nutrient digestibility trail.

Experimental system and fish:

The feeding trial was conducted in fifteen glass aquaria. Each aquarium (75×40×50 cm) has a total volume of 100 L. The experimental aquaria were supplied with well aerated freshwater, using compressed air via air-stone. Fresh tap water was stored in fiberglass tanks for 24 h under aeration in order to dechlorenate the water. Each aquarium was cleaned daily and water exchange rate including fish faeces and remaining food were approximately 25% of total volume per day. Then each aquarium was refilled to a fixed volume again, using stored and well aerated fresh water. Water temperature was adjusted (26 – 27 ^{o}C) by a thermostat column heater for each aquarium.

Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from Fish Genetic Department, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia. Experimental fish were acclimated under wet laboratory conditions for two weeks. Fish were randomly distributed at stocking density of 20 fish per aquarium. The average fish initial weight ranged from 16.0 to 17.5 g/fish. Each three aquaria (representing three replicates) were fed on one of the experimental diets. Diets were given to fish at a rate of 3% of live body weight. The daily feed was offered two times at 9.00 a.m. and 13.00 p.m. Weight of fish was recorded biweekly and the amounts of given feed were readjusted according to increase in body weight.

After 6 hours of feeding, the faeces deposited by experimental fish was removed every 20 minutes, using a fitted siphon of plastic tube. The faeces was collected on fine mesh net, filtered on Whatman paper No. 1 for 1/2 hour to get rid of water, then dried and stored in a refrigerator for analysis.

Growth parameters:

At the end of 90 days after starting the experiment, fish in each aquarium were weighed and counted. Different growth parameters were calculated according to Khattab (1996).

Analytical methods:

Chemical analysis of feed ingredients, experimental diets, faeces and fish body were conducted according to (AOAC, 1984). Moisture content was determined by oven drying. Crude protein was determined indirectly from analysis of total nitrogen (crude protein = N×6.25) by the Kjeldahl method (AOAC, 1984). Crude lipid was determined by Soxhlet apparatus. Ash was determined by weighing samples in a porcelain crucible placed in a muffle furnace at 550 ^{o}C for 4 h. Crude fiber was estimated according to Goering and Van Soest (1970). To measure the apparent nutrient digestibility, chromic oxide was determined in the faeces and diets using the methods of Furukawa and Tsukahara (1966).

Statistical analysis:

The data were analyzed by analysis of variance using SAS program (1989). Duncan's multiple-range test (Duncan, 1955) was used to test the significance of mean difference among treatments.

RESULTS

1- Growth performance

The proximate analyses of the tested black seed cake (BSC) were 92.39, 83.10, 28.31, 11.33, 5.84, 37.62 and 9.29% for DM, OM, CP, EE, CF, NFE and ash calculated on dry matter basis, respectively as shown in Table (1).

The proximate chemical composition of formulated diets fed to Nile tilapia (*O. niloticus*) during experimental period are shown in Table 3. All the diets were isonitrogenous (35% crude protein), isocaloric (477.49 Kcal/100 g diet) and protein energy ratio had ranged from 72.90 Kcal to 74.34 Kcal /mg protein.

Available essential amino acids content of soybean meal (SBM) and black seed cake (BSC) and percentage of dietary requirements for Nile tilapia illustrated in Table (4). Most essential amino acids percentages of BSC protein content were higher than those of SBM protein except lysine and tryptophane (9.55, 2.88, 6.72, 7.40,4.07 and 5.20 Vs. 7.25, 2.18, 6.35, 7.08, 3.25 and 4.09 for arginine, Histidine, leucine, phenyalanine + tyrosine, threonine and valine respectively. Almost essential amino acids of BSC content were sufficient to cover the requirements for Nile tilapia except lysine, methionine + cystine and tryptophan.

The data concerned with the growth performance, feed utilization efficiency, survival rate, hepatosomatic index and the relative price to control ration are presented in Table (5). The average final weight (g/fish) increased considerably from the initial value in all the dietary treatments. However, the highest records in average final weight, weight gain and SGR were recorded in fish group reared on diet 10% BSC 35.48, 16.09 (g/fish) and 0.81 (%/day) respectively, while the lowest values was recorded with fish group fed control 33.21, 16.09 (g/fish) and 0.47 (%/day), respectively. No significant differences in SGR, FCR, feed intake, survival rate and hepatosomatic index were observed among different treatments.

Relative cost to control ration was decreased gradually by increasing replacement rate of BSC for SBM.

It can be noticed from Table (6) that results of DM, CP and ash in body composition did not show any significant differences among treatments. Differences are significant in crude fat ($P < 0.05$) between fish group fed 70% BSC and either groups fed on control and 50% BSC diets.

2- Nutrients digestibility

Table (7) illustrates the chemical composition of faeces dropping from fish fed experimental diets. Crude protein and gross energy values under BSC diets were lower than that in control.

Table (8) shows the apparent digestibility records of different nutrients for experimental diets fed to Nile tilapia. The values of apparent protein digestibility (APD), apparent carbohydrate digestibility (ACHOD) and apparent gross energy digestibility (AGED) were higher in fish groups fed BSC diets than that of control group. Subsequently, the differences in these parameters were significant ($P < 0.05$).

DISCUSSION

The proximate analysis of the tested black seed cake (BSC) were 92.39, 83.10, 28.31, 11.33, 5.84, 37.62 and 9.29 for DM, CP, EE, CF, NFE and ash, respectively as shown in Table (1). The present results are similar to those reported by Hashim and El-Kiey (1962), Nasr *et al.* (1996), Mohamed (1997) and Soliman *et al.* (2000).

The obtained results indicate that final weight, weight gain and SGR increased with increasing the inclusion level of BSC. These results may be due to the abundance of essential amino acids that satisfy fish requirements and/or the antimicrobial effect and the enhanced cellular immune response of black seed (Amal, 1997). Sharobeem (1996) clarified that 25% replacement of wheat protein in albino rats diet with *Nigella* cake remarkably raised their growth rate by about 43 and 21% over the group receiving unsupplemented diet after two and four weeks, respectively. Also, Nasr *et al.* (1996) found that weight gain and daily gain were higher significantly ($P<0.05$) between rabbit groups fed 10% BSC and groups fed 5% BSC and control (308, 223 and 196 g/head; 22, 16 and 14 g/day, respectively) at 10-12 weeks. Also, Amal (1997) reported that the antimicrobial effect of the ether extract of *Nigella sativa* L. improved the growth, health, body weight and weight gain of chickens by inhibiting the microorganisms which interfere with the nutrient supply of the chicken.

In the present study, there are no significant differences in FCR and PER values, which are similar to that obtained by Mohamed (1997) who replaced BSC for fishmeal in Nile tilapia diets. The mean value of FCR of 2.68 was recorded in the present study which is similar to the value of 2.65 reported by Nwanna and Daramola (2000) who used shrimp head waste as source of protein for *O. niloticus*.

In the present study, the apparent digestibility of protein (ADP) was improved by increasing replacement of BSC (85.65 increased to 92.25%). Nearly, similar results were obtained by Shiau and Huang (1989) in hybrid tilapia (81.5 to 87.86%) and Wee and Shu (1989) in Nile tilapia (74.37 to 89.65%). The apparent digestibility of carbohydrate (ADCHO) in the tested diets ranged from 27.84 to 47.61%, a range which broadly supports the finding of Degani *et al.* (1997) that carp can utilize from 22 to 55% of carbohydrate, and Chow *et al.* (1980) was showed that carp can utilize up to 48% of dietary starch. Also, Nasr *et al.* (1996) reported that the apparent digestibility of NFE was significantly ($P < 0.05$) higher for rabbits fed diets containing 5 or 10% BSC than that of control. The apparent digestibility of gross energy gradually increased by increasing BSC in diets which may be due to special characters of *Nigella sativa* oil.

DM, CP, and ash of body composition for Nile tilapia fed experimental diets were not affected by BSC replacement, which was supported by Soliman *et al.* (2000). There was significant difference ($P<0.05$) in body fat composition, AL-Gaby (1992) recorded that the oil of Egyptian *Nigella sativa* has linoleic and oleic acids (about 60 and 21%, respectively). These acids may have an important role in cell membrane lipid formation.

It could be concluded from the present study that BSC is promising protein source in diets of Nile tilapia (*O. niloticus*) and its inclusion could reach up to 70% replacement for soybean meal.

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Table 1. Chemical analysis of black seed cake (BSC) and soybean meal (SBM).

Test Ingredients	Items	DM	OM	CP	EE	CF	NFE	Ash
BSC	As feed	92.39	83.10	28.31	11.33	5.84	37.62	9.29
	DM	100.00	89.94	30.64	12.26	6.32	40.72	10.06
SBM	As feed	92.30	85.98	44.00	2.16	5.75	34.07	6.32
	DM	100.00	93.15	47.67	2.34	6.23	36.91	6.85

DM→ dry matter, OM→ organic matter; CP→ crude protein; EE→ ether extract; CF→ crude fiber; NFE→ nitrogen free extract.

Table 2. Experimental diets containing different levels of black seed cake (BSC).

Ingredients	Control	BSC diets replaced CP %			
	(0)	10	30	50	70
Herring fish meal (72 % CP)	19.36	19.36	19.36	19.36	19.36
Soybean meal (44 % CP)	47.86	43.07	33.50	23.93	14.36
Black seed cake (28.3 % CP)	0.00	7.46	22.33	37.21	52.08
Starch	22.39	20.64	17.13	13.64	10.14
Cellulose	1.09	0.93	0.63	0.31	0.00
Fish and corn oil (1:1)	5.24	4.48	2.99	1.49	0.00
Pfizer Broiler Premix*	1.50	1.50	1.50	1.50	1.50
Carboxymethyl cellulose	2.00	2.00	2.00	2.00	2.00
Ascorbic acid	0.06	0.06	0.06	0.06	0.06
Cr_2O_3	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100

*Each 2.5 kg of Pfizer Broiler premix contains: Vit. A, 12m.i.u.; Vit D2, 2 m.i.u.; Vit E, 10 gm; Vit K, 2 gm; Vit B1, 1gm; Vit B2, 4gm; Vit B6, 1.5 gm;Vit B12, 10 mg; Pantothenic acid, 10 gm; Nicotinic acid, 20gm; Folic acid, 1000mg; Biotin, 50 mg; Choline chloride 500 gm; Cooper, 10 gm; Iodine, 1gm; Iron, 30gm; Manganese, 55gm; Zinc, 55gm; Selenium, 0.1gm.

Table 3. Proximate chemical composition (% on dry matter basis) of the experimental diets.

Constituents	Control	BSC diets replaced CP %			
	(0)	10	30	50	70
Dry matter	92.24	93.21	92.96	92.78	91.95
Crude protein	35.61	35.12	35.22	34.80	35.11
Crude fat	8.38	7.40	8.96	8.17	8.48
Crude fiber	4.92	5.08	4.74	5.51	5.16
Ash	7.58	7.65	7.91	7.41	7.70
NFE[1]	43.51	44.75	43.17	44.11	43.55
Cr_2O_3	0.372	0.384	0.359	0.396	0.367
GE (Kcal/100g diet)[2]	478.99	472.73	480.13	477.34	478.27
P/E ratio	74.34	74.29	73.36	72.90	73.41

(1) NFE (nitrogen free extract) = 100 – (protein + lipid + ash + fiber)
(2) GE (gross energy): Calculated after NRC (1993) as 5.64, 9.44 and 4.11 Kcal/g for protein lipid and NFE, respectively.
(3) P:E ratio = mg protein/ Kcal gross energy.

Table 4. Available essential amino acid content of soybean meal and black seed cake and, percentage of dietary requirement for Nile tilapia (*O. niloticus*)

Essential amino acids	E.A.A, percentage of the protein		% requirement for Nile tilapia•
	Soybean meal (SBM)*	Black seed cake (BSC)**	
Arginine	7.25	9.55	4.20
Histidine	2.18	2.88	1.70
Isoleucine	4.01	4.12	3.10
Leucine	6.35	6.72	3.40
Lysine	5.82	3.83	5.10
Methionine + Cystine	2.25	2.54	3.20
Phenylalanine + Tyrosine	7.08	7.40	5.70
Threonine	3.25	4.07	3.60
Tryptophan	1.18	0.67	1.00
Valine	4.09	5.20	2.80

•From: Santiago (1985)., * From: Lovell (1989)., **From: Sharobeem (1996).

Table 5. Growth, feed utilization efficiency, feed intake and survival rate in Nile tilapia (*O. niloticus*) fed experimental diets during 90 days.

Items	Control (0)	BSC diets replaced CP % 10	30	50	70
Initial av. wt. (g/fish)	17.12^a ± 0.05	17.15^a ± 0.10	17.10^a ± 0.10	17.06^a ± 0.10	17.14^a ± 0.04
Final av. wt. (g/fish)	33.21^b ± 0.5	35.48^a ± 0.42	34.82^{ab} ± 0.48	34.81^{ab} ± 0.63	34.58^{ab} ± 0.80
Av. weight gain (g/fish)	16.09^b ± 0.45	18.33^a ± 0.43	17.37^{ab} ± 0.39	17.75^{ab} ± 0.62	17.44^{ab} ± 0.84
SGR (%)[1]	0.74^a ± 0.01	0.81^a ± 0.01	0.79^a ± 0.01	0.79^a ± 0.04	0.78^a ± 0.03
FCR[2]	2.96^a ± 0.05	2.57^a ± 0.41	2.61^a ± 0.03	2.63^a ± 0.12	2.67^a ± 0.14
PER[3]	1.06^a ± 0.02	1.11^a ± 0.02	1.09^a ± 0.01	1.01^a ± 0.04	1.07^a ± 0.54
Dry feed intake (g/fish)	47.65^a ± 0.58	47.16^a ± 0.37	46.27^a ± 0.43	46.68^a ± 1.06	46.57^a ± 0.16
Survival rate (%)	97.78^a ± 3.85	97.78^a ± 3.85	97.78^a ± 3.85	95.55^a ± 3.85	100.0^a ± 00.0
HSI[4]	1.66^a ± 0.10	1.95^a ± 0.04	1.51^a ± 0.03	1.93^a ± 0.05	1.54^a ± 0.08
Relative price to control ration	100.00	91.26	81.75	61.51	57.14

Means with the same superscript letters in the same row were not significantly different (P< 0.05).
Data are mean values ± SE (n = 3).
(1) Specific growth rate (%/day) = $\ln W_t - \ln W_o / T * 100$, W_o = initial wt, W_t = final wt, and T = time
(2) Feed conversion ratio = Total dry feed consumed (g) / Total wet weight gain (g).
(3) Protein efficiency ratio = wet weight gain (g)/ amount of protein fed (g).
(4) Hepatosomatic index = Liver weight/ fish body weight.

Table 6. Proximate carcass composition of Nile tilapia (*O. niloticus* L.) fed experimental diets for 90 days (% on dry matter basis).

Items	Control (0)	BSC Diets replaced CP % 10	30	50	70
Dry matter	27.22^a ± 0.83	26.08^a ± 0.21	26.66^a ± 0.25	27.24^a ± 0.38	27.80^a ± 0.01
Crude protein	52.56^a ± 0.40	52.65^a ± 0.52	53.24^a ± 1.20	53.77^a ± 0.91	52.55^a ± 0.69
Crude fat	24.75^b ± 0.40	25.67^{ab} ± 0.26	25.40^{ab} ± 0.16	25.08^b ± 0.03	26.58^a ± 0.76
Ash	22.41^a ± 0.41	21.38^a ± 0.07	21.14^a ± 0.86	21.15^a ± 0.35	20.87^a ± 0.56

Means with the same superscript letters in the same row were not significantly different (P< 0.05).

Table 7. Composition of faeces (% on dry matter basis).

Items	Control	BSC Diets replaced CP %			
	(0)	10	30	50	70
Crude protein	12.32^{a} ± 0.35	11.17^{a} ± 0.08	11.55^{a} ± 0.08	8.70^{b} ± 0.07	8.68^{b} ± 0.14
Ether extract	4.17^{ab} ± 0.24	4.08^{ab} ± 0.13	4.60^{a} ± 0.46	3.66^{b} ± 0.47	4.50^{a} ± 0.30
Carbohydrate	76.38^{ab} ± 1.61	75.24^{ab} ± 2.10	76.74^{a} ± 1.74	75.40^{ab} ± 1.80	73.72^{b} ± 1.70
Ash	7.13^{c} ± 0.94	9.51^{b} ± 0.28	7.11^{c} ± 0.66	12.24^{a} ± 1.22	13.10^{a} ± 1.02
Gross energy	422.77^{a} ± 1.12	410.75^{b} ± 0.89	421.96^{a} ± 1.10	393.51^{c} ± 0.65	394.42^{c} ± 0.76
Cr_2O_3	0.908 ± 0.01	0.992 ± 0.06	0.986 ± 0.10	0.977 ± 0.10	1.203 ± 0.12

Means with the same superscript letters in the same row were not significantly different ($P < 0.05$).

Table 8. Apparent digestibility (%) for experimental diets fed to Nile tilapia (*O. niloticus*) during the experimental period.

Items	Control	BSC diets replaced CP %			
	(0)	10	30	50	70
APD[1]	85.65^{b} ± 0.93	87.52^{b} ± 1.50	88.12^{ab} ± 1.34	89.75^{ab} ± 1.39	92.25^{a} ± 1.58
AFD[2]	79.50^{a} ± 1.85	78.55^{a} ± 1.86	81.64^{a} ± 1.23	81.55^{a} ± 1.57	83.57^{a} ± 1.37
ACHOD[3]	27.84^{c} ± 0.99	34.48^{b} ± 0.66	35.92^{b} ± 0.58	29.89^{c} ± 0.76	47.61^{a} ± 1.01
AGED[4]	63.92^{c} ± 0.98	66.07^{bc} ± 0.71	68.32^{b} ± 1.14	66.38^{bc} ± 0.69	74.58^{a} ± 0.72

Means with the same superscript letters in the same row were not significantly different ($P < 0.05$).
Data are mean values ± SE (n = 3).
(1) Apparent protein digestibility (%) = 100 - (100 × % Cr_2O_3 in feed/ % Cr_2O_3 in faeces × % protein in faeces / % protein in feed.
(2) Apparent fat digestibility.; (3) Apparent carbohydrate digestibility.; (4) Apparent gross energy digestibility.