

THE EFFECT OF PARTIAL REPLACEMENT OF SOYBEAN MEAL BY BLACK SEED MEAL IN PRACTICAL DIET ON GROWTH PERFORMANCE, FEED UTILIZATION AND WHOLE-BODY COMPOSITION OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* (L). FINGERLINGS

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ABSTRACT

Eight experimental diets were formulated to contain various percentages of black seed meal (BSM) as a partial replacement for soybean meal (SBM). The substitution levels were 0 (control), 10, 20, 30, 40, 50, 60, and 70% of crude protein of BSM that replaced the same percentages of crude protein from soybean meal. All diets were isonitrogenous (35% crude protein) and isocaloric (477.49 Kcal/100g diet). Diets were fed to triplicate groups of Nile tilapia, *Oreochromis niloticus* (L.) (17.1 ± 1.0 g) at a daily rate of 3% of live body weight given twice daily for 90 days. Results revealed that fish growth indicated as final weight, weight gain and SGR insignificantly decreased with increasing substitution level up to 30% BSM (P>0.05), while the lowest significant growth was recorded in fish group fed 40-70% BSM (P>0.05). No significant difference in survival rate was observed due to the increment in BSM level in the tested diets. Feed intake and PER significantly decreased, while FCR increased due to the increment in BSM level in the tested diets. No significant changes were observed in dry matter, crude protein, total lipids and ash in fish body composition at all tested diets.

Key words: Nile tilapia, growth performance, black seed meal, soybean meal, feed utilization, body composition.

INTRODUCTION

Feeding represents over 60% of the operation costs of aquaculture, and protein itself represents about 50% of feed costs in intensive culture. Therefore, the major challenge facing fish nutritionists in developing countries is the development of commercial, cost effective fish feeds using locally available, cheap and unconventional resources (El-Sayed, 2004). Soybean meal is the most

commonly used plant protein in fish feeds because it is palatable and is available at a cost much below fish meal (Lovell, 1988). It has been used as a protein source in diets for feeding of various fish species (Mohsen and Lovell, 1990; Shimeno *et al.*, 1992; Refstie *et al.*, 1998; Soliman *et al.*, 2000; Khattab 2002). However it has recently, become relatively expensive and scarce due to the competing demands with poultry and livestock feeding (El-Sayed, 1999).

Thus, considerable emphasis has been centered on the use of unconventional plant protein sources, particularly those not directly usable for human consumption (Richter *et al.*, 2003; Adebayo *et al.*, 2004; Fagbenro, 2004; Fiogbé *et al.*, 2004; Kaushik *et al.*, 2004; Glencross *et al.*, 2006; Zhou *et al.*, 2006). Formation of cheaper diets consisting of locally available ingredients could have a considerable economic advantage through cost reduction.

Black seed (*Nigella sativa* L.) plant is a member of *ranunculaceae* family and it is an annual herb that is widely grown in different parts of the world. It has been used for different (Rifat-uz-Zaman *et al.*, 2004). Egyptian farmers cultivate black seed for oil production as it is rich in oil (Abdel-Aal and Attia, 1993a) containing 35.6 - 41.6% fixed oil (Al Gaby, 1998), however, the defatted BSM contains a high level of protein (Abdel-Aal and Attia, 1993b) indicating the possibility to be used in formulating some animal diets. The present study was carried out to evaluate the potential of partial replacement of different levels of BSM as a protein source for SBM protein in practical diets for Nile tilapia, *Oreochromis niloticus* L. fingerlings.

MATERIALS AND METHODS

Diet preparation

Eight experimental diets were formulated to contain various percentages of black seed meal (BSM) as a partial replacement for soybean meal (SBM). The chemical analysis of BSM and SBM is shown in Table 1. The diets were formulated to replacement 0 (as control diet), 10, 20, 30, 40, 50, 60 and 70% of protein diet

with black seed meal. All diets are isonitrogenous (35% crude protein) and isocaloric (477.49 Kcal/100 g diet; Table 1).

The ingredients of each diet were separately blended with additional 100 ml of water to make a paste of each diet. The pastes were separately passed through a grinder, and pelleted in a modified paste extruder to form the tested diets. The diets were approximately similar in nutrient contents but containing different levels of BSM. The diets were stored in plastic bags in a refrigerator (- 2 °C).

Fish culture and feeding regime

Nile tilapia, *O. niloticus* L. fingerlings were obtained from the nursery ponds, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia. All fish were kept indoors and placed in a fiberglass tank for 2 weeks as an acclimation period to the laboratory conditions. Fifty fish were frozen at -20 °C for chemical analysis. Fish were randomly distributed at stock density of 20 fish per 100-L aquarium. The initial weight of fish averaged 16.5±1.0 g. Each aquarium was supplied with compressed air via air-stones using aquarium air pumps. Settled fish wastes were siphoned daily with a half of aquarium water, which was replaced by aerated water from the storage tank. Water temperature was about 27±1 °C. Each diet was given to fish at a daily rate of 3% of live body weight and given twice daily at 9.00 and 13.00 hours. Each diet was fed to triplicate aquaria of Nile tilapia for a period of 90 days. Fish in each aquarium were weighed biweekly and the amounts of given feed were readjusted according to the increase in body weight. The dead fish were daily recorded and removed.

Chemical analysis of diets and fish

The diets and fish were analyzed according to the standard methods of AOAC (1990) for moisture, protein, fat and ash. Gross energy was calculated according to NRC (1993).

Analysis of the Water Quality

Water samples were collected biweekly from each aquarium. Water temperature and dissolved oxygen were measured on site with a YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). The pH

was measured using a pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA). Unionized ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, Co., USA).

Growth parameters

Growth performance was determined and feed utilization was calculated as following:

$$\text{Weight gain} = W_2 - W_1 ;$$

Where W_1 and W_2 are the initial and final fish weight, respectively,

$$\text{Specific growth rate (SGR)} = 100 (\ln W_2 - \ln W_1) / T ;$$

Where W_1 and W_2 are the initial and final fish weight, respectively, and T is the number of days of the feeding period.

$$\text{Feed conversion ratio (FCR)} = \text{Feed intake} / \text{Weight gain}$$

$$\text{Protein efficiency ratio (PER)} = \text{Weight gain} / \text{Protein intake}$$

Economic evaluation:

The cost of feed to raise a unit of biomass of fish was calculated based on local retail sale market price of all the dietary ingredients at the time of the study. These prices (in LE/kg) were as follows: herring fish meal, 8; soybean meal, 2.5; Black seed meal, 1; starch, 2.0; fish oil, 7.0; corn oil, 5.0; Pfizer broiler premix, 7.0; carboxymethyle cellulose 6; cellulose 3.

Statistical analysis

The obtained data of fish growth, feed utilization, survival rate and proximate chemical composition were subjected to one-way ANOVA. Differences between means were tested at the 5% probability level using Duncan test. All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, USA) as described by Dytham (1999).

RESULTS

Water temperature value range was 27 – 29 C, dissolved oxygen concentrations range was 5.6 – 6.6 mg/L, pH range was 7.8 – 8.1, and unionized

ammonia concentration range was 0.09-0.17 mg/L. All the previous water quality parameters are within the acceptable range for tilapia growth Boyd, (1984). All the diets were isonitrogenous (35% crude protein), isocaloric (477.49 Kcal/100 g diet) and protein energy ratio ranged from 72.90 to 74.34 mg protein/ Kcal gross energy (Table 1). Available essential amino acids content of SBM and BSM, and percentage of dietary requirements for Nile tilapia are illustrated in Table 2. Most essential amino acids percentages of BSM protein content were higher than those of SBM content (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, phenylalanine + tyrosine, threonine and valine, respectively. Almost essential amino acids of BSM content are sufficient to meet with the requirements for Nile tilapia except lysine, methionine, cystine and tryptophan (Table 2).

Growth performance indicated by final weight, weight gain and SGR gradually decreased with the increase of BSM level in the tested diets (Table 3). The survival rate was slightly changed due to the inclusion of BSM in fish diets. Moreover, feed intake was significantly decreased with the increase of BSM level ($P < 0.05$; Table 4) except diets containing up to 20% BSM, while the optimum FCR value was obtained at 30% BSM level. On the other hand, PER were significantly decreased by the inclusion of BSM level in fish diet over 40% ($P < 0.05$; Table 4). The contents of dry matter, crude protein, total lipids and ash did not show any significant differences under all treatments ($P > 0.05$; Table 5). Crude protein content of the final carcass analysis ranged between 52.5% and 53.7%. Ash and lipid content of final carcass analysis ranged from 21.1 to 21.6 and 25.1% to 25.8%, respectively.

DISCUSSION

There were no significant variations ($P > 0.05$) in fish growth when fish fed on diets containing 0-30% BSM. Growth depression was obtained when fish fed on diets in which SBM was replaced by BSM at 40–70%. These results are in agreement with Abdel-Hakim *et al.*, (2008) who reported that, sesame seed cake

or sunflower meal could replace 30 % of soybean protein to reduce feed costs without any adverse effects on growth performance and feed utilization of Nile tilapia. Moreover, Abou Zeed *et al.*, (2008) found a possibility of partial replacement of soybean meal by sunflower meal up to 75 % without adverse effect on final body weight of Nile tilapia and similar trend was also observed for weight gain, specific growth rate. Adebayo *et al.* (2004) studied the replacement value of *Cassia fistula* seed meal (CFM) for soybean meal (SBM) in practical diets of *O. niloticus* fingerlings for 70 days and found that only fish fed on diet containing 17.0% substitution level of CFM were similar ($P > 0.05$) to fish fed on the control diet. Glencross *et al.* (2006) studied the palatability of some prototype lupin protein concentrates (PC) when fed to rainbow trout, *Oncorhynchus mykiss* and demonstrated that each PC was highly palatable at inclusion levels up to 40% of the diet. Zhou *et al.* (2006) studied the replacement of blue-green algae meal (BGAM) for soybean meal (SBM) in practical diets of gibel carp (*Carassius auratus gibelio*) for 12 weeks in a flow-through system. They found that fish growth was reduced when fish fed on diets containing more than 44.69% BGAM. Azaza *et al.* (2007) evaluated the use of green algae, (*Ulva rigida*) ulva meal (UM) as a replacement for soybean meal in a practical diet for Nile tilapia, where, Soybean meal was replaced by 0%, 10%, 20% and 30% of UM in an open circulation system for 75 days. They found that UM can substitute up to 20% of SBM in tilapia diet without any depressive effect on fish growth and feed utilization efficiency.

Deficiencies of one or more amino acids are known to limit protein synthesis, growth or both (Cowey, 1992; Cole and Van Lunen, 1994). Therefore, for protein synthesis, all amino acid building blocks must be present. The reduction in fish growth at 40-70% BSM replacement level may be attributed to deficiencies in essential amino acids as lysine, methionine, cystine and tryptophan as compared to Nile tilapia requirement (Table 2). Tryptophan deficiency in fish results in scoliosis, bending of the back bone in a vertical plane (Jauncey and Ross, 1982). In the present study no backbone abnormalities were observed and normal shape

was obtained for fish fed BSM-containing diets. These results could be attributed to sufficient amounts of tryptophan being provided by BSM.

Soliman *et al.* (2000) reported that BSM may contain toxic substances that depress fish growth. Takruri and Dameh (1998) reported that the seeds of *Nigella sativa* contain toxic glucosidal saponines. Tennekoon *et al.* (1991) reported that when aqueous extracts of black seed were administered orally to rats at a dose of 10 ml/kg body weight, resulted in a significant increase in serum gamma glutamyl transferase and ALT activities. The first enzyme is a membrane-bound enzyme and its release depends on the physiological effects. These two enzymes are generally regarded as ones of the most sensitive index of hepatic damage (Szezeklik *et al.*, 1961; Wilkinson, 1976).

On the other hand, previous studies reported that the oilseed meal contain anti-nutritional factors especially haemagglutinin and phytin (Balogun and Fetuga 1989). When a diet containing haemagglutinin and phytin are being digested, a portion of them remains bound on certain protein molecules of the diet making these inaccessible to digestive enzymes and, therefore, reducing protein digestibility and interfering with their bioavailability (Enujiugha and Agbede 2000). It is likely from this study that these anti-nutrients might have impaired the digestibility or absorption of some essential amino acid components of the diets containing BSM causing growth depression in Nile tilapia fingerlings at high levels of dietary inclusion.

Growth performance and nutrient utilization of Nile tilapia fingerlings fed at varying inclusion levels of BSM showed that BSM could be a substitute for SBM up to 30% in practical diets without affecting growth. BSM at a high substitution level in practical fish diet does not appear encouraging and of less practical and economic value judging from the poor FCR obtained from the experiment. There was a significant difference ($P < 0.05$) in feed intake, FCR and PER, as a result of the effects of the different treatments. Dry matter, crude protein and ash of body composition of Nile tilapia fed BSM-containing diets have not been affected. These results are similar to that obtained by Soliman *et al.* (2000).

Economic evaluation showed that diets containing higher levels of BSM were cheaper than diets containing higher levels of SBM (Table 3). As BSM inclusion in the diets increased up to 70 % level of replacement for SBM – protein, the cost of diet to produce 1 kg weight gain of Nile tilapia was gradually reduced, thereby increasing profitability of producers.

The present study conclusion reveals the inadequacy of total replacement of SBM by BSM in practical diets for Nile tilapia fingerlings. It could be concluded from the present study that black seed meal is promising protein source in diets for feeding Nile tilapia fingerlings and it may be recommended as alternative to soybean meal as a dietary protein source, however, its inclusion might replace up to 30% of soybean meal.

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Table 1. Ingredients and proximate chemical analysis of the experimental diets containing soybean meal (SBM) and black seed meal (BSM).

Ingredients	Levels of black seed meal (%)									
	SBM	BSM	0	10	20	30	40	50	60	70
Herring fishmeal (72% CP)			19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4
Soybean meal (44% CP)			47.9	43.1	38.3	33.5	28.7	23.9	19.2	14.4
Black seed cake (28.3% CP)			0.0	7.5	14.9	22.3	29.7	37.2	44.6	52.1
Fish and corn oil (1:1)			5.2	4.5	3.7	3.0	2.3	1.5	0.7	0.0
Pfizer broiler premix ¹			2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Starch			22.4	20.6	18.9	17.1	15.35	13.6	11.8	10.0
Carboxymethyl cellulose			2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cellulose			1.0	0.8	0.7	0.6	0.45	0.3	0.2	0.0
Ascorbic acid			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total			100	100	100	100	100	100	100	100
Proximate chemical composition (% on DM basis)										
Dry matter	92.3	92.4	92.2	93.2	92.8	93.0	93.1	92.8	92.6	92.0
Crude protein	44.0	28.3	35.6	35.1	35.1	35.2	35.1	34.8	35.0	35.1
Total lipids	2.2	11.3	8.4	8.4	8.3	8.2	8.3	8.5	8.6	8.9
Crude fiber	5.8	5.8	4.7	4.9	5.0	5.1	5.1	5.2	5.3	5.5
Ash	6.3	9.3	7.6	7.7	7.8	7.9	7.6	7.4	7.5	7.7
NFE ²	41.70	45.30	43.7	43.9	43.8	43.6	43.9	44.1	43.6	42.8
GE ³ (Kcal/100 g diet)			460.13	458.13	456.78	455.57	457.19	458.20	458.22	458.34
Protein/Energy ratio ⁴			77.37	76.62	76.84	77.27	76.77	75.95	76.38	76.58

¹ Pfizer broiler premix: each 2.5 kg contain vitamin A 12 MIU; D₃ 2MI U, E 10 g; K 2g; B₁ 1g; B₂ 4g; B₆ 1.5g; B₁₂ 10mg; Pantothenic acid 10g; Nicotinic acid 20g; Folic acid 1g; Biotin 50mg; Choline chloride 500 mg; copper 10g; iodine 1g; iron 30g; manganese 55 g; zinc 55 g and selenium 0.1g., ² NFE (nitrogen free, extract) = 100 - (protein % + lipids % + ash % + fiber %). ³ GE (gross energy) was calculated according to NRC (1993). ⁴ P/E ratio = mg protein / Kcal gross energy.

Table 2. Available essential amino acids content (% of protein) of soybean meal (SBM) and black seed meal (BSM), and the essential amino acids (EAA) requirement of Nile tilapia

Essential amino acids	SBM ¹	Defatted BSM ²	EAA requirement of Nile tilapia ³
Arginine	7.1	8.5	4.2
Histidine	2.4	2.9	1.7
Isoleucine	5.1	3.9	3.1
Leucine	7.4	6.7	3.4
Lysine	6.1	3.8	5.1
Methionine + Cystine	2.2	2.5	3.2
Phenylalanine + Tyrosine	6.2	6.7	5.7
Threonine	3.2	4.2	3.6
Tryptophane	1.3	0.8	1.0
Valine	4.6	5.2	2.8

1 after Lovell (1988), 2 after Sharobeem (1996), and 3 EAA requirement of Nile tilapia according to Santiago (1985).

Table 3. Growth performance of Nile tilapia fed diets containing different levels of BSM.

Items	Levels of black seed meal (%)							
	0	10	20	30	40	50	60	70
Final weight (g/fish)	36.6 a ± 1.81	35.7 a ± 0.77	35.2 ab ± 1.21	34.1 abc ± 0.45	32.7 bcd ± 0.44	32.0 cd ± 0.52	31.9 cd ± 0.75	30.7 d ± 0.23
Weight gain (g/fish)	19.5 a ± 1.76	18.6 a ± 0.73	18.1 ab ± 1.18	17.0 abc ± 0.44	15.6 bcd ± 0.42	14.9 cd ± 0.55	14.8 cd ± 0.70	13.6 d ± 0.21
SGR (%/day)	0.846 a ± 0.056	0.818 a ± 0.023	0.802 ab ± 0.039	0.767 abc ± 0.015	0.720 bcd ± 0.015	0.696 cd ± 0.021	0.683 cd ± 0.024	0.650 d ± 0.007
Survival rate (%)	100.0 a ± 0.00	95.9 a ± 1.68	95.9 a ± 1.68	97.9 a ± 1.69	95.9 a ± 1.68	95.6 a ± 3.05	97.9 a ± 1.69	95.9 a ± 1.68
Cost of diets (L.E.) per kg weight gain	3.32	3.23	3.13	3.04	2.95	2.85	2.76	2.67

The same letter in the same row is not significantly different ($P < 0.05$).

Table 4. Feed utilization by Nile tilapia fed diets containing different levels of BSM.

Items	Levels of black seed meal (%)							
	0	10	20	30	40	50	60	70
Fed intake (g feed/fish)	39.8 a ± 0.24	39.9 a ± 0.12	39.3 a ± 0.15	38.3 b ± 0.23	37.6 c ± 0.22	37.0 cd ± 0.27	36.3 de ± 0.39	36.0 e ± 0.12
FCR	2.04 c ± 0.19	2.15 bc ± 0.08	2.17 bc ± 0.14	2.25 bc ± 0.05	2.41 ab ± 0.05	2.48 ab ± 0.08	2.45 ab ± 0.10	2.65 a ± 0.03
PER	1.49 a ± 0.117	1.43 ab ± 0.048	1.41 ab ± 0.081	1.36 abc ± 0.026	1.27 abc ± 0.027	1.25 bc ± 0.035	1.26 bc ± 0.045	1.17 c ± 0.013

The same letter in the same row is not significantly different ($P < 0.05$).

Table 5. Proximate carcass composition of Nile tilapia fed diets containing different levels of BSM.

Items	Levels of black seed meal (%)							
	0	10	20	30	40	50	60	70
Dry matter	26.2 a ± 0.38	26.1 a ± 0.21	26.2 a ± 0.21	26.6 a ± 0.25	26.8 a ± 0.31	27.2 a ± 0.38	26.8 a ± 0.31	26.8 a ± 0.31
Crude protein	52.6 a ± 0.40	52.7 a ± 0.52	52.9 a ± 0.96	53.2 a ± 1.20	52.8 a ± 0.76	53.7 a ± 0.91	53.3 a ± 0.88	52.5 a ± 0.69
Total lipids	25.8 a ± 0.4	25.7 a ± 0.26	25.5 a ± 0.36	25.4 a ± 0.16	25.4 a ± 0.34	25.1 a ± 0.63	25.5 a ± 1.12	25.6 a ± 0.8
Ash	21.4 a ± 0.41	21.4 a ± 0.07	21.3 a ± 0.46	21.1 a ± 0.86	21.4 a ± 0.29	21.2 a ± 0.35	21.2 a ± 0.63	21.6 a ± 0.56

The same letter in the same row is not significantly different ($P < 0.05$).

تأثير الإحلال الجزئي لمسحوق فول الصويا بمسحوق حبة البركة في العليقة على أداء النمو والاستفادة من الغذاء وتركيب الجسم لأصبيغيات البلطي النيلي

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بالمعمل المركزي لبحوث الثروة السمكية بالعباسة- أبو حماد- شرقية

في هذه الدراسة تم تكوين ثمانية علائق متساوية فيما بينها في نسبة البروتين (٣٥,٠ %) ونسبة الطاقة ٤٧٧,٤٩ كيلو كالورى لكل ١٠٠ جم من العليقة ولكنها مختلفة في نسب إحلال بروتين مسحوق مخلفات حبة البركة محل بروتين مسحوق فول الصويا - وكانت نسب الإحلال هي صفر و ١٠، ٢٠، ٣٠، ٤٠، ٥٠، ٦٠ و ٧٠% على أساس وحدة البروتين لتغذية إصبيغيات البلطي النيلي (١٧,١ جم) - وكان معدل التغذية ٣ % من وزن الجسم والتغذية ٦ أيام في الأسبوع - والتغذية مرتين يوميا لمدة ٩٠ يوم . أوضحت النتائج أن إحلال بروتين مسحوق حبة البركة محل بروتين مسحوق فول الصويا (على أساس وحدة البروتين) لم يؤدي إلى نقص النمو والزيادة في الوزن والنمو النوعي حتى مستوى إحلال ٣٠ % . بينما حدث نقص معنوي في النمو والغذاء المأكول وزيادة في معدل التحويل عند زيادة نسبة الإحلال من ٤٠ - ٧٠ % من مسحوق فول الصويا . كانت نسبة الأسماك الحية في نهاية التجربة عالية في المعاملات المختلفة وكانت متماثلة إحصائيا كما لم يؤثر الإحلال الجزئي على التركيب الكيماوي لجسم الأسماك . نستنتج من هذه الدراسة أنه يمكن إحلال بروتين مسحوق حبة البركة محل بروتين مسحوق فول الصويا بنسبة ٣٠% في علائق البلطي النيلي على اعتبار أنه مصدر رخيص للبروتين . إن التقييم الاقتصادي للعلائق أظهر أن إحلال مسحوق حبة البركة محل مسحوق فول الصويا بنسبة ٧٠% بالعليقة كان الأفضل بين المعاملات من حيث السعر (الأرخص) ومن حيث تكلفة وحدة الزيادة في الوزن المكتسب بالأسماك.