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Evaluation of substituting black seed meal (*Nigella sativa* L.) as protein source in Nile tilapia diets

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The study seeks to evaluate the suitability of Black seed meal (*Nigella sativa* L.) (BSM) as a protein source in replacing increasingly expensive soybean meal (SBM) in Nile tilapia diets on growth performance, feed utilization body composition and economic evaluation of Nile tilapia, *Oreochromis niloticus*. Five isonitrogenous and isocaloric diets were formulated to contain different levels of black seed meal (BSM) as an alternative to soybean meal in Nile tilapia diets at different levels (T1, control, 0%, T2, 25%, T3, 50%, T4, 75% and T5, 100% for 60 days. Therefore, the results were conducted that, replacement of SBM by TBM up to 50% did not effect on growth performances (weight gain (WG), feed intake (FI)) and feed utilization (specific growth rate (SGR), relative growth rate (RGR) and protein productive value (PPV). Results: No differences were observed among the control and any other treatment in survival rate. Carcass crude protein did not affected by inclusion black seed meal levels in all experimental treatments. In contrast, ash content showed significant increasing with increase black seed meal levels in diets (P<005). From economic view, it was observed that replacing 50% of SBM by BSM in Nile tilapia diets reduces feed costs by 5.88% while replacing 75% and 100% of SBM by BSM led to increase feed costs by 17.38% and 16.27%, respectively. Inclusion BSM levels more than 50% of SBM in Nile tilapia diets led to decrease growth performance and feed utilization and get negative reflection on economic efficiency of diets.

Keywords: Nile tilapia (*Oreochromis niloticus*), black seed meal, soybean meal, growth performance, feed utilization and economical efficiency.

INTRODUCTION

During last few decades, aquaculture production had doubled (FAO, 2014 and Ogello et al., 2014). Nile tilapia is considered as one of the most important fish in aquaculture and widely practiced in many different regions with an annual growth rate more than 12% (FAO, 2014). Rabidly increasing in aquaculture production led to creating nutritional crisis and also increases the feeding costs which represent 60% in intensive aquaculture systems (Collins et al., 2013). Soybean meal is commonly used as a plant protein source in fish diets (Tonsy et al., 2006). However, soybean meal contains high level of protein (about 45-48%, CP) whereas, alcohol–washed, protein concentrate contains could be rich to 70-85% crude protein (Storebakken and Refstie, 2000). Increase using soybean in fish, animal and human nutrition led to necessary demand to identify new other plant protein sources (Abdel Hakim et al., 2008 and Olsen and Hansen, 2012). Search for cheaper new alternative of soybean such is urgent. Black seed, *Nigella sativa*, frequently referred as important medical crops in many different regions and is primarily consumed as medical oil and after oil extract could be used as a rich protein meal in diets (Abdel-Aal and Attia, 1993, El-Nattat and El-

Kady, 2007 and Hassan et al., 2009). Black seeds (*Nigella sativa*) are cultivated in the Mediterranean region and other place in Asia (Hutchinson, 1959). Few studies have appeared in the literature concerning the inclusion of black seed meal in fish diets (Hassan et al., 2009). Therefore, the present study was undertaken to evaluate the nutritive value and economical evaluation of using black seed meal as a protein source in Nile tilapia (*Oreochromis niloticus*) diets.

MATERIALS AND METHODS

Experimental system and fish:

Experimental fish were obtained from hatchery of the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharquia Governorate. Two hundred and twenty five fingerlings were randomly distributed to five treatments each treatment had triplicate groups in Complete Close Recirculation System in Animal Production Department, National Research Center, Egypt. Each aquarium was stocked with fifteen fish (average weight 6g) to evaluate the effect of partial and complete replacement of soybean meal by black seed meal during the experimental period (60 days).

Preparation of black seed meal (BSM) and feed ingredients analysis:

Black seed meal (BSM) was obtained from a local factory in Alexandria city where the oil was extracted from the black seeds crop. BSM was ground using a retsch mill and it passed through a 1.0 mm sieve resulting in producing which was stored in well closed plastic buckets until use.

Diets and feeding regime:

Five diets were formulated to contain different levels of black seed meal (BSM) (0%, 25%, 50%, 75% and 100%) in T1 (control), T2, T3, T4 and T5, respectively. A fixed feeding regime of 3% of body weight per day (dry food/whole fish) was employed and fish were fed two times daily in equal portions. Fish were fed for six consecutive days, weighed on the seventh day and feeding rates for the following week adjusted accordingly (NRC, 1993).

First diet (T1, 0%) was saved as a control diet containing protein from soybean meal. Soybean meal protein was replaced at different levels, 25%, 50%, 75% and 100%, by black seed meal (BSM) in T2, T3, T4 and T5, respectively. Experimental diets were prepared by fine grinding of the dietary ingredients. After that, all ingredients of each experimental diets were mixed toughly and produced in pellets form (1mm, in diameter) using pelleting machine, thereafter dried and stored in a freezer (-20°C) until use. The diets were offered two times at daily (9.00 and 13.00) at equal portions. Fish were bulk weighed (one aquarium at a time) biweekly and feed amounts were adjusted accordingly. Growth performances were determined according to Cho and Kaushik (1985) as follows:

Weight gain (WG) (g) = Final body weight (g) – initial body weight (g).

Feed conversion ratio (FCR) = Dry feed intake (g)/ weight gain (g).

Specific growth rate (SGR) = 100 (LnW2-LnW1) / experimental days.

Protein efficiency ratio (PER) = Weight gain (g)/protein intake (g).

Relative growth rate (RGR) =100*(Final body weight (g)/ initial body weight (g)).

Protein productive value (PPV) = 100 (Protein gain (g)/protein intake (g)).

Chemical and statistical analysis:

Diets and fish samples were analyzed according to AOAC (1989) for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash. Gross energy values were calculated by using factors of 5.65, 9.45, 4.00 and 4.00 for CP, EE, CF and NFE, respectively (Jobling, 1973). Diets ingredients and chemical analysis (Dry matter basis) of the experimental diets are shown in Table (2). The economic efficiency was calculated as the cost of feed required for producing one kg of fish weight gain. Data obtained were statistically analyzed using SAS program (1997) and the significant differences among means were evaluated by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSION

Chemical composition of the soybean meal (SBM) and black seed meal (BSM) were shown in Table (1). Black seed meal (BSM) had relatively lower in crude protein and crude fiber (34.40% and 3.60%) compared to soybean meal (SBM) (44.80% and 7.30%), respectively. In contrast, BSM has higher content of ash (12.70%) compared with (6.50 %) in SBM. Results of chemical composition of the soybean meal and black seed meal are nearly similar to obtained in previous studies Soliman et al., (2000), Hassan et al., (2009) and Taha. (2009). Experimental diets were formulated to contain different levels of black seed meal (control, 0%) and the tested levels

(25%, 50%, 75% and 100%) as a partial or complete replacement of soybean meal in diets of Nile tilapia (*Oreochromis niloticus*). All experimental diets were formulated to be isocaloric and isonitrogenous (Table 2).

Growth performance:

Growth performance of Nile tilapia fingerlings fed the experimental diets are shown in Table (3). Initial weight of fish in different treatments diets were not differ significantly (P<0.05), indicating that all experimental groups were homogenous. At the end of the feeding trial (60 days), fish fed diets T1 (control, 0%), T2 (25%) and T3 (50%) were not exhibited significant differences in feed intake (FI) and weight gain (WG). While increase substitution levels of BSM more than 50% in T3 (75%) and T4 (100%) led to decrease weight gain (WG) and feed conversion ratio (FCR%).

The low values of body weight and SGR of fish fed the diets containing the high levels of BSM are possibly led to low essential amino acid levels provided by BSM when compared with those provided by SBM apparent amino acid availabilities for SBM were higher than that for BSM with the exception of methionine by Khalifah, 1995 (Table 7), and possible existing toxic substances at high level of BSM (Tennekoon et al., (1991) and Solimanet al., 2000). Best feed conversion ratio (FCR%) was observed in control groups (4.06%) and followed by T3 (4.33%) and T2 (4.44%) without significant differences among control (0%), T2 (25%) and T3(50%). High inclusion levels of BSM in diets led to gradually reduction in feed conversion ratio in T4 (5.75%) and T5 (6.19%). Reduction in FCR% could be related to high ash content in BSM which might be reflect on fish palatability to diets the same results were noted by Hassan et al., (2009).Survival rates (SR %) were 100% for all experimental groups showing clearly that toxic substances in BSM are not lethal for the fish at any substitution levels similar to reported by Solimanet al., (2000). Results of survival rate are complete agreement with observations in previous studies, whose found that, using BSM in Nile tilapia diets did not effect on the survival rates of Atwa. (1997), Abd Elmonem et al., (2002) and Hassan et al., (2009) Generally, results obtained from growth performance revealed that, the values of all the growth parameters were decreased with increase the substitution levels than 50% of soybean meal. which could be refer to increasing the level of some anti-nutritional factors or deficiencies of some essential amino acid when using plant protein source in fish diets might be reflect on growth performance (Wee, 1991).

Feed utilization parameters:

Feed utilization parameters of Nile tilapia (specific growth rate (SGR %), relative growth rate (RGR%), protein efficiency ratio (PER%) and protein productive value (PPV%) take the same trend of growth parameters where no significant differences observed among T1 (control, 0%), T2 (25%) and T3 (50%) in feed utilization parameters. Increase the replacement levels at 75% and 100% in T4 and T5, respectively, gradually reduce SGR%, RGR%, PER% and PPV% (Table 4). Incorporation of BSM in Nile tilapia fingerlings diets at 25% and 50% in T2 and T3, respectively, had no negative effect on growth performance or feed utilization, this results are in complete agreement with the findings , whose reported that partial substituted of soybean meal by black seed meal had no significant effects on feed utilization parameters Soliman et al., (2000), Hassan et al., (2009) and Taha, (2009), whose reported that partial substituted of soybean meal by black seed meal had no significant effects on feed utilization parameters. In contrast, Soliman et al., 2000 found 10% inclusion level of BSM is recommended as the maximum in Nile tilapia diets and and Zewil (1996) suggested an inclusion level of (13.34%) BSM is recommended in Japanese quail diet. Generally, high inclusion levels of plant as a protein source in fish diets led to reduce the growth performance and feed efficiency. That might be due to the presence of some toxic substances or anti-nutritional factors reflect on feed utilization and growth performance of fish (Lim and Dominy, 1989, De Silva and Aderson, 1995, Siddhuraju et al., 2000 and Francis et al., 2001).

On the other hand, Khalifa, 1995 reported that, several essential amino acid levels and apparent amino acid availabilities seem to decrease in SBM compared with BSM contents (Table 7).

Table (1): Proximate composition of the soybean meal and black seed meal used in the formulation of the experimental diets (on DM basis).

Ingredients	Moisture	CP	EE	CF	Ash	NFE*
Soybean meal (SBM)	11.97	44.80	3.20	7.30	6.50	38.20
Black seed meal(BSM)	8.11	34.40	14.20	3.60	12.70	35.10

* NFE = 100-(CP + EE + CF + ash).

Table (2): Feed ingredients and proximate chemical analysis of experimental diets (DM %).

Ingredients	T1	T2	T3	T4	T5
	(0%)	(25%)	(50%)	(75%)	(100%)
Concentration	17	19	20	22	22.5
Soybean meal	40	30	20	10	0
Black seed meal	0	10	20	30	40
Corn	23	21	20	20	20
Wheat bran	15	15	15	13	12.5
Oil	3	3	3	3	3
Vit., & min.*	2	2	2	2	2
Chemical composition of diets basis on dry matter:					
OM	91.92	90.19	89.07	88.77	89.03
CP	30.9	30.55	30.29	29.82	29.78
CF	9.47	9.29	8.86	9.28	10.34
EE	5.91	7.4	7.78	8.21	9.03
Ash	8.08	9.81	8.93	8.23	8.97
NFE**	45.64	42.95	44.14	44.46	41.88
GE(kcal/100g)***	449.33	449.97	455.15	459.54	460.98
P/E ratio****	68.77	67.89	66.55	64.89	64.60

*Vit. & min. mixture/kg premix: Vitamin D3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g, riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; pantothenic acid, 4 g; nicotinic acid, 8 g; folic acid, 0.4 g biotin,20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, selenium, 0.4 g and Co, 4.8 mg.
** (NFE) = 100-(CP + EE + CF + ash).
***GE (Gross energy value) was calculated from their chemical composition, using the factors 5.65, 9.45, 4.00 and 4.00 (k cal/g) for protein, fat, fiber and NFE, respectively. (Jobling, 1973).
**** P/E ratio (protein to energy ratio) = CP mg /GE kcal.

Table (3): Growth performance parameters of Nile tilapia fed the experimental diets.

Treatments		IW(g/fish)	FW(g/fish)	WG(g/fish)	FI(g/fish)	FCR(g)	SR%
T1 (0%)	Mean	6	13.17^a	7.17^a	0.48^a	4.06^d	100
	SE	0.00	±1.23	±1.23	±0.02	±0.22	0.00
T2 (25%)	Mean	6	12.13^a	6.13^a	0.45^a	4.44^c	100
	SE	0.00	±0.2	±0.12	±0.07	±0.05	00
T3 (50%)	Mean	6	12.40^a	6.40^a	0.46^a	4.33^c	100
	SE	0.00	±0.17	±0.17	±0.27	±0.04	00
T4 (75%)	Mean	6	10.10^b	4.10^c	0.39^b	5.75^b	100
	SE	0.00	±0.17	±0.17	±0.31	±0.1	00
T5 (100%)	Mean	6	9.69^b	3.69^d	0.38^b	6.19^a	100
	SE	0.00	±0.1	±0.13	±0.11	±0.07	00

Values in the same column with different superscripts are significantly different at P<0.05.
SE: Standard error.

IW: Initial weight, FW: Final weight, WG: Weight gain, FI: Feed intake, FCR: Feed conversion ratio, SR: Survival rate

Table (4): Feed utilization parameters of Nile tilapia fed tested diets

Treatments		SGR%	RGR%	PER(g)	PPV%
T1 (0%)	Mean	1.11[a]	2.20[a]	0.79[a]	0.42[a]
	SE	±0.16	±1.81	±0.15	±0.19
T2 (25%)	Mean	1.07[a]	2.02[a]	0.65[a]	0.33[a]
	SE	±0.03	±0.29	±0.03	±1.13
T3 (50%)	Mean	1.08[a]	2.07[a]	0.80[a]	0.41[a]
	SE	±0.28	±0.25	±0.02	±0.21
T4 (75%)	Mean	0.99[b]	1.68[b]	0.53[b]	0.26[b]
	SE	±0.13	±0.25	±0.03	±1.06
T5(100%)	Mean	0.97[b]	1.62[b]	0.44[b]	0.23[b]
	SE	±0.02	±0.15	±0.05	±0.12

Values in the same column with different superscripts are significantly different at P<0.05.
SE: Standard error.
SGR: Specific growth rate, RGR: Relative growth rate, PER: Protein efficiency ratio, PPV: Protein productive value

Table (5): Body composition on dry matter basis of Nile tilapia fed the tested diets.

Treatments		DM(%)	CP(%)	EE(%)	Ash(%)
T1 (0%)	Mean	21.10	51.50	31.50	16.25[a]
	SE	±0.06	±0.11	±0.15	±0.18
T2 (25%)	Mean	21.60	51.30	31.00	17.25[b]
	SE	±0.22	±0.27	±0.16	±0.23
T3 (50%)	Mean	21.80	51.40	31.57	17.49[b]
	SE	±0.16	±0.19	±0.21	±0.13
T4 (75%)	Mean	21.70	51.44	31.38	17.50[b]
	SE	±0.21	±0.17	±0.04	±0.26
T5 (100%)	Mean	21.20	51.76	31.70	17.90[c]
	SE	±0.15	±0.31	±0.23	±0.19

Values in the same column with different superscripts are significantly different at P<0.05.

Table (6): Feed costs (L.E) for producing one experimental diets kg by fish fed the

Treatment	Cost (L.E.*)[1] /ton	Relative to control (%)	Decrease in feed cost (%)	FCR	Feed cost (L.E.)[2] /kg weight gain	Relative to control (%)[3]
T1 (0%)	6898.4	100	0	4.06	28.01	100
T2 (25%)	6519.8	94.51	5.49	4.44	28.95	103.36
T3 (50%)	6088.0	88.25	11.75	4.33	26.36	94.12
T4 (75%)	5717.4	82.88	17.12	5.75	32.88	117.38
T5(100%)	5261.0	76.26	23.74	6.19	32.57	116.27

*L.E: Egyptian pound
1: Calculated according to the local price at 2016(9000, 7150, 2300, 3680, 3280, 19000, and 30000 L.E. for concentration, soybean meal, black seed meal, corn, wheat bran, oil and vit. & min. mixture, respectively.
2: Feed cost (L.E.)/kg= FCR X F. cost/kg
3: Feed cost (L.E.)/kg weight gain for treatment/ Feed costs (L.E.)/kg weight gain for control, as assuming that relative Feed cost (L.E.)/kg weight gain of the control group equal 100.

Table (7). Apparent Amino Acid Availability of Black Seed Meal and Soybean Meal.

Amino Acid	Apparent Amino Acid Availability1 (%)	
	black seed meal	Soybean meal
Arginine	60.31	91.00
Histidine	80.06	91.00
Isoleucine	78.30	93.00
Leucine	62.28	92.00
Lysine	77.96	92.00
Methionime	98.33	90.00
Phenylalane	87.35	94.00
Threonine	81.65	90.00
Valine	79.14	91.00

Khalifah (1995)

Body composition:

Body compositions of fish fed the experimental diets are presented in Table (5). Generally, chemical composition of fish has not a great influence by inclusion levels of BSM. Results showed that, no significant differences in the crude protein (CP) and ether extract (EE) among control (T1) and treatments (T2, T3, T4 and T5) ($P<0.05$). In contrast, body ash content which significantly increased gradually with increasing BSM levels in diets with ($P<0.05$). Results of body composition were complete agreement with obtained by Soliman *et al*. (2000) and Hassan et al., (2009).

From the present study, BSM could be used in Nile tilapia diets up 50% levels (replacing partially SBM), without adverse effect on growth performance, nutrients utilization and protein content in whole body of Nile tilapia fingerling. However, SBM contains many essential nutrients such as thiamin, riboflavin, pyridoxine, niacin, Ca, Fe, Cu, Zn and P which effect on feed utilization and increase growth performance (Khalifeh, 1995). Increase substitution level than 50% might be reduce the digestibility and reflect on growth performance and also increase the ash content in BSM 12.7% compared with 6.5% in SBM reflect on feed utilization

Economical efficiency:

Current study highlights the potential of using BSM as partial (25%, 50% and 75%) in T2, T3 and T4 or complete replacement (100%) of SBM in T5, respectively.

However, nutritional costs are considered to be the highest recurrent agent in aquaculture, often ranging about 30 to 70%, depending on the intensity culture systems. Reduction the nutritional costs either through diets development (using untraditional feed ingredients such as BSM), improved husbandry or other direct or indirect means led to reduce the production investments and increased the net return gain (Collins et al., 2013). However, rest costs usually constant, therefore, nutritional costs required to produce one kg gain of weight could be used to compare the economical efficiency of different treatments. As shown in Table (6), prices of experimental diets based on feed ingredients in the local market during 2016 were 6898.4, 6519.8, 6088.0, 5717.4 and 5261.0 L.E./ton diet for the T1(0%), T2(25%), T3(50%), T4(75%) and T5(100%), respectively.

Diet ingredients costs (LE/ton) decreased gradually with increasing substitution levels of SBM by BSM up to 100% (T5). Data presented in the same table showed that, increasing substitution levels of SBM by BSM at 25%, 50%, 75% and 100% decreased total diets costs by 5.49%, ,11.75%, 17.12%, and 23.74%, respectively, compared to the control diet, feed costs (L.E./kg WG) decreased in T3(50%) to get the best economic efficiency (94.12%) compare to the control while worst economic efficiency were observed in the high inclusion levels in T4(75%) and T5(100%) which were 117.38% and 116.27%, respectively. Economic efficiency nearly take the same trend of growth performance and feed utilization where increase the inclusion level of BSM more than 50% had negative of feed utilization and growth rates and led to increase the feeding cost/kg in T4(75%) and T5(100%).

CONCLUSION

Black seed meal could be incorporated in Nile tilapia diets not more 50% of soybean meal as an alternative without any adverse effect on growth performance or feed utilization and improve the economical efficiency.

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

H. Abozaid designed and performleed the experiments and also wrote the manuscript. H. Abozaid ,Ghada Abusina, A. S. M. El-Nadi, performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. H. H. Abd El-Rahman, A. A. Abedo designed experiments and reviewed the manuscript. All authors read and approved the final version.

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