REPLACEMENT OF SOYBEAN MEAL PROTEIN BY BROKEN LENTIL SEEDS AS A PLANT PROTEIN SOURCE IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FEEDING

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Abstract

A fish feeding trial was conducted in a closed recirculation water system for 8 weeks to study the effect of broken lentil seeds as a dietary plant protein instead of soybean meal protein (0, 25, 50, 75 and 100%) on growth performance, feed utilization digestibility and economical study.

Four hundred and fifty tilapia fries (*Oreochromis niloticus*) with an average initial weight of 0.3g/fish were randomly distributed into fifteen aquaria (30fry/aquarium) water volume in each aquarium was 70 litters. The fish were fed daily at a rate of 1.7% of their metabolic body weight $(W)^{0.8}$ with isonitrogenous (30% CP) and isocaloric (4600Kcal GE/kg) diets. Each diet was given to three replicate aquaria.

Chemical analysis showed that broken lentil seeds, contain high percent of CP (26.69%), CF (12.45%) and NFE (55.33%), however, it contains low percent of EE (2.45%) and Ash (2.99%).

The results showed that the highest weight gain, SGR, PER, NPU, EU and digestibility coefficient was achieved by 50% broken lentil seeds compared with the control group without harmful effect on the performance and feed utilization of fish. Moreover, such substitution of broken lentil seeds levels recorded the least feed cost needed to obtain one kilogram of live weight gain up to 100%.

The broken lentil seed was nutritionally and economically superior as compared to control as non-conventional plant protein source in Nile tilapia feeding.

Key words: Nile Tilapia fries, broken lentil seeds, growth performance and economical study.

INTRODUCTION

Leguminous plants which have been found to be suitable for incorporation into fish diets are clover, lucerne, groundnut (peanut), locust beans, chickpea, guar, ipil-ipil, lima beans, field mung bean, cow peas, lupin, soybean and Lentil (Akiyama 1988, Lim and Akiyama 1992 and Burel *et al.*, 2000).

Soybean meal is one of the most commonly used legumes as a major plant protein source in fish diets (Jackson, *et al.*, 1984 and El-Sayed 1999), its not only expensive but also difficult to be in steady current competition between fish and poultry. For these reasons, nutritionists try to replace part of soybean meal as plant protein by another unconventional plant protein sources. In Egypt, the yield from lentil crop (*lentila lens*) is about 2835 ton, and the waste (part of seed and the hull) was determined by 40% producing about 1134 ton (Ministry of Agriculture, 2003).

The nutritional value of the lentil crop is high with low levels of toxic and anti-metabolic materials, (Cubero 1981 and Ladizinsky 1986).

Composition of lentil seeds depends on many factors including the species and the variety and the information found in the literature converted about 20 varieties by Frias *et al.*, (1994 a, b), Urbano *et al.* (1995), Nwokolo and Smartl, (1996). Sotomayor (1997) reported that, lentil seeds CP ranged from (23-32%), EE (0.8-2.0%) starch (40-57%), CF (10-12%) cellulose (3.5-14.8%), hemicellulose (1.2-15.7%) and lignin (trace-2.6%).

This study aimed to evaluate the effect of replacing soybean meal protein by broken lentil seeds in Nile tilapia fries. Also to investigate the effect of using broken lentil seeds as a non-conventional plant protein source instead soybean meal protein in Nile tilapia diets and its effects on the fish growth, feed efficiency and protein utilization, digestibility and economical study.

MATERIALS AND METHODS

The experiment was carried out in a recirculation water system with biological filtration in the wet lab of Animal Production Research Institute, By-products Utilization Department, Ministry of Agriculture, Dokki, Giza, Egypt.

The control diet (T1) formulated to contain (30%) soybean meal, whereas 25, 50, 75 and 100% of soybean meal protein were replaced by broken lentil seeds in diets T2, T3, T4 and T5, respectively. Diets were formulated to be isonitrogenous (30%CP) and isocaloric (4600 Kcal\kg). Each of the experimental diets was mixed with water then pelleted through a meat mincer then air dried and broken into small granules (about 0.5mm) to be fed to Nile tilapia (*O. niloticus*) fries.

The fish were fed daily at a rate of 1.7% of their metabolic body weight ($W^{0.8}$) according to Osman *et al.* (2003). The tested diets were provided three times daily at 9.00, 12.00 and 15.00 O'clock for 6 day a week.

Tilapia fries (*O. niloticus*), brought from Abassa hatchery, Sharkia Governorate. The average initial live body weight was 0.30g/fish. Fries were weighed every two weeks and the daily feed allowance was corrected according to new total biomass.

The chemical composition of the tested material, diets, feces and fish body were carried according to the procedures of A.O.A.C. (1990), while the crude fiber fractions (NDF, ADF and ADL) were estimated according to Goering and Van Soest, (1970).

Composition and chemical analysis of the experimental diets are presented in Table (1)

Amino acids of the tested material were estimated using amino acid analyzer (LKB Alpha Plus High Performance Amino Acid Analyzer LKB Biochrom LTD, England) according to methods of (Winder and Eggum, 1966). Moreover, the broken lentil seeds were assigned for determination of calcium (Lehman and Henry, 1984) and inorganic phosphorus (Martinek, 1970).

After 8 weeks of running the feeding experiment, digestibility trial was conducted and the apparent digestibility coefficients of nutrients were estimated by using ash as an inert marker as described by Tacon and Rodrigus (1984).

The economical efficiency of treatments was done by calculating the cost of feed required producing one kg of fish weight gain.

Data obtained were statistically analyzed using the SAS program (1990) and the significant differences among means were evaluated by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical analysis, crude fiber fractions and amino acids profile:

Results of Table (2) indicated that broken lentil seeds (BLS) contains reasonable amount of CP (26.69%) which is an indicator for its potential value as a source of plant protein, low content of ash and also, it is rich in NFE (55.33%), compared with soybean meal (SBM) (40.82%). However, it contains higher CF (12.54%) compared with soybean meal (5.0%).

The gross energy of broken lentil seeds (BLS) source was nearly similar to SBM, being (4424 and 4570.75 Kcal GE /kg), respectively (Table 2).

The lower amount of both cellulose and lignin of BLS being 20.07 and 0.39%, respectively, may cause a high digestible and high utilization values compared with SBM.

BLS of different species was analyzed by many authors who stated that its content of CP ranged from 23 to 32%; EE between 0.5 and 2.0%; CF between 10 and 12 % ; starch between 40 and 57%. In addition, it has from 3.5 to 14% cellulose; 1.2 to 15.7% hemicellulose and trace to 2.6% lignine (Frias *et al.*, 1994b, Nwokolo and Smartt 1996 and Sotomayor, 1997), It is clear from (Table 2) that BLS composition are similar to those obtained by the previous authors

In this study, (Table 2) the Ca and P in SBM was 0.30 and 0.65% respectively, while BLS contains higher Ca and P, being 0.62 and 1.8%, respectively. These results are in agreements found by Viola *et al.*, (1988) who concluded that, phosphorus was the limiting factor in SBM. The availability of phosphorus was higher for BLS compared to the SMB, (MC curdy and March 1992).

The essential amino acids composition as percentage of protein of the BLS compared to that of SBM are given in Table (3). The amino acids composition of the feed protein is a good indicator of its nutritive value. Apparently, there is no information in the literature on amino acids composition of BLS however, the essential amino acids content of soybean meal (SBM) are higher than the corresponding values in BLS. It is clear from out finding that methionine was the first limiting amino acids in both BLS and SBM compared with requirement of Tilapia (NRC, 1993). These results are in agreement with those of Jackson *et al.*, (1984) and Chien and Chiu (2003).

Tacon (1993) reported that, soybean meal is the best plant protein source in terms of protein content and essential amino acids profile. However, it is limiting in sulfur containing amino acids and contains many endogenous anti-nutrients and can be destroyed or inactivated during thermal processing.

Fish performance and feed, protein and energy utilization:

The performance, feed, protein and energy utilization of Nile tilapia fries fed different levels of BLS, are presented in Table (4). The results clearly showed that, the best total weight gain value was found in diet T_3 (contain 50% BLS) being (1.51gm) while the worst value was recorded for T5 (contain 100% BLS) being (1.20gm). The differences were significant ($P \leq 0.05$) in total gain and SGR. These results agree with Olvera *et al.*, (1997) who found that, the highest growth rate and feed utilization were observed with 20-30% replacement of fish meal with cowpea concentrate for *O. niloticus*.

Higher growth performance achieved in the present study when BLS was incorporated in tilapia diets may be explained by the higher carbohydrates (starch and other saccharides), low ash content in BLS and/or go the reduction in the level of yellow corn in the diets along with increase the level of BLS. Arnesen (1993) suggested that corn starch when compared with other carbohydrate sources is rapidly absorbed, which may cause hyperglycemia. Also, Osman *et al.* (2004) reported that, broken rice meal can replace up to 75% of yellow corn meal in tilapia fry diet without any negative effect on growth parameters. The carbohydrates in the legumes such as lentil spare some protein when the dietary protein level was low according to Shiau and Peng (1993). Also, to the same Carter and Hauler (2000) found no differences in gross feed consumption and FCR between SBM and some legumes such as lupine meal at the 25% replacement level in the diet. However, when lupine meal was included at a rate of 33% in the diet a higher feed consumption, lower feed efficiency ratio and lower PER values were obtained. Chien and Chiu (2003) reported that, no difference in fish growth when using SMB and lupine meal as a major plant protein source in the diet. Fils *et al.* (1997) and Christine et al. (2000) suggested that, the extrusion of lupine improved the utilization of NFE and CP in rainbow trout.

Feed conversion ratio (FCR) showed that, T3 (50%) recorded the best value in which only 1.64g feed was required to produce 1g fresh weight gain. This might be due to the relative reduction of anti-nutrients and increased palatability and nutrient availability of proceed lentil when remove the hull (Siddhuraju and Beckeer 2003).

There were differences ($P \leq 0.05$) in FCR when increase the replacement of SBM by BLS in the diets up to 100% being (2.20). The same trend was observed with the values of PER, NPU and EU in the T5 (100%), the lowest values in protein and energy utilization (T5) may due to lake of essential amino acids required in diets for Nile tilapia according to NRC (1993), and could be attributed to high crude fiber (hemicellulose and cellulose) hence, higher fiber levels has binding capability in fish gut, therefore it has been reported to bind nutrients including protein and lead to decreasing carbohydrate absorption (Shiau, 1997).

Carter and Hauler (2000) fed the Atlantic salmon the lupin meal at 33% they found had a higher feed consumption and a lower feed conversion ratio, protein productive value than the soybean meal diet.

Meanwhile, the results clearly showed that increase in the level of broken lentil seeds up to 75% in place of SBM improved PER, NPU and EU. These results suggest that the protein utilization of the experimental diets was improved as the broken lentil seeds replaced soybean meal in diets up to 75% in place of SBM.

El sayed (1999) tested alternative protein sources for tilapia (*O. mossambicus*) diets and found that, jack bean, cowpea and green gram legume were useful partial substitute for fishmeal at 25%; 20-30% and 25-37%, respectively.

These results suggest that the digestive tract of tilapia is very long possible the bacterial population of the tilapia gut is able to decompose part of the more complex carbohydrates and to derive energy from them, this hypothesis is explaining the energy value of feed-stuffs rich in hemicellulose in legumes (Viola and Arieli, 1983).

Apparent digestibility trail:

Data presented in Table (5) showed that, the digestibility coefficient of all nutrients did not differ significantly ($P \geq 0.05$) as the inclusion rate of broken lentil seeds increased from 25 to 75% .These results agree with Olvera *et al.* (1997) who found that, the highest digestibility coefficient were observed with 20-30% replacement of fish meal with cowpea protein concentrate for *O. niloticus.* Oyedapa (1998) suggested that Nile tilapia may utilize legumes seed as protein sources and no significant difference occurred among apparent protein digestibility values of various seeds. Heat treatments for legumes are known to break the structure of starch and thus greatly improve its digestibility (Christine *et al.*, 2000).

These results agree with Fils *et al.* (1997) and Booth *et al.*, (2001) who reported that de-hulling seeds legumes, increase CP and CF digestibility and increased the nutritional value of seeds.

Economical study:

The seeds price (L.E.) for one Kg weight gain produced by Nile tilapia fed BLS is shown in Table (6).

Feeding costs in fish production is about 50% of total production (Collins and Delmendo 1979).

The control diet recorded the highest price being 2438.5 L.E.\ton. However, the price of one ton feed mixture reduced in all levels of SBM substitution by BLS. By calculation, the 50% BLS diet showed the lowest feed price needed for producing one Kg gain in fish weight, i.e. substitution SBM by 50% BLS gave the highest economical efficiency.

Generally, the results of the present study indicated that broken lentil seeds may be possible to replace a significant amount of soybean meal protein up to 50% in commercial feeds for Nile tilapia safely and to reduce feed costs assist in reducing the dependence an soybean meal as the primary dietary plant protein source for fish and result in lower cost of fish production.

Table 1. Composition and chemical proximate analysis of the experimental diets (on DM basis).

Item	T_1 (control)	lentil seeds (BLS) of soybean meal protein			
		T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
Composition of the diets					
Yellow corm	44	39.14	34.27	29.43	24.54
Soybean meal (SBM)	30	22.5	15	7.5	-
Broken lentil seeds (BLS)	-	12.36	24.73	37.07	49.46
Fish meal	20	20	20	20	20
Soy oil	5	5	5	5	5
Vitamin and Mineral Premix*	1	1	1	1	1
DM%	93.88	92.45	91.72	92.31	91.74
CP%	30.90	30.70	30.30	30.00	29.84
EE%	8.11	8.22	8.45	8.34	8.57
CF%	2.53	3.59	4.66	5.72	6.78
Ash%	6.95	6.71	6.48	6.24	5.87
NFE**	51.51	50.78	50.11	49.70	48.94
GE(kcal/kg)***	4630	4627.14	4624.29	4621.43	4618.57
Price/ ton L.E.	2438.5	2327.7	2216.9	2106.2	1995.4

* Each Kg contains: vit a 4.8 mIU, D3 0.8 mID , E4g, K0.8g, B10.4g, B2 1.6g, B6 0.6g, B12 4g, pantothenic acid 4g, Nicotinic acid 8g, folic acid 400mg, biotin 20mg, cholin chloride 9g, copper 4g, Iodine 0.4g, Iron 12g, Manganse 22 g, Zinc 22g and selenium 0.04g.

** Calculated by differences.

*** Estimated by Jobling (1983)

Table 2. Chemical analysis and crude fiber fractions of broken lentil seeds (BLS) and soybean meal (SMB).

Item	Broken Lentil Seeds (BLS)	Soybean Meal (SBM)
Chemical analysis:		
DM%	90.4	90.33
CP%	26.69	44.0
EE%	2.45	3.13
CF%	12.54	5.0
Ash%	2.99	7.05
NFE*	55.33	40.82
GE(kal/kg)**	4424.0	4570.75
Ca%	0.62	0.30
P%	1.80	0.65
Cell wall constituents:		
NDF[1]	49.84	35.85
ADF[2]	20.46	27.07
ADL[3]	0.39	3.73
Hemicellulose[4]	29.38	8.82
Cellulose[5]	20.07	23.30

* Calculated by differences. ** Estimated by Jobling (1983)

1- Neutral detergent fiber. 2-Acid detergent fiber. 3- Acid detergent lignin.

4- Hemicellulose = NDF – ADF. 5- Cellulose = ADF – ADL.

Table 3. Amino acids composition of broken Lentil seeds (BLS) protein compared to that of soybean meal (SBM) .

Amino Acid in CP%	Broken lentil seeds (26.96%)	Soybean meal (44%)	NRC (1993) Requirement of tilapia
Arginine	4.53	7.16	4.20
Histidine	3.04	2.66	1.72
Isoleucine	3.56	4.43	3.11
Leucine	1.52	7.70	3.39
Lysine	4.53	6.11	5.12
Methionine	0.63	1.41	2.68
Phenylanine	0.96	4.90	3.75
Threonine	2.08	3.86	3.75
Tryptophon*	-	-	1.00
Valine	2.08	4.70	2.80
Total E.A.A.	22.93	42.93	31.52
FLAA	Methio.	Methio.	
SLAA	Pheny.	Threon.	
TLAA	Leuci .	Lysin.	

* Not determined

Table 4. Performance, feed, protein and energy utilization of Nile tilapia fries fed different of broken lentil seeds.

Item	T_1 (Control)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
Initial weight (g/fish)	0.31[a] ±0.03	0.30[a] ±0.03	0.31[a] ±0.03	0.30[a] ±0.03	0.30[a] ±0.03
final weight (g/fish)	1.63[b] ±0.03	1.61[b] ±0.03	1.82[a] ±0.03	1.60[b] ±0.03	1.50[c] ±0.03
Total gain (g/fish)	1.32[b] ±0.03	1.31[b] ±0.03	1.51[a] ±0.03	1.30[b] ±0.03	1.20[c] ±0.03
SGR (%d) Specific growth rate	2.96[b] ±0.04	2.93[b] ±0.04	3.16[a] ±0.04	2.99[b] ±0.04	2.87[c] ±0.04
Feed fed (g/fish)	2.37[b] ±0.08	2.46[ab] ±0.08	2.47[ab] ±0.08	2.49[ab] ±0.08	2.64[a] ±0.08
FCR (g feed / gain) Feed conversion ratio	1.80[b] ±0.06	1.88[b] ±0.06	1.64[c] ±0.06	1.91[ab] ±0.06	2.20[a] ±0.06
Survival rate (%)	91.10[a] ±2.43	93.33[a] ±2.43	93.32[a] ±2.43	94.44[a] ±2.43	91.11[a] ±2.43
(PER) Protein efficiency ratio	1.718[b] ±0.06	1.769[b] ±0.06	1.987[a] ±0.06	1.898[ab] ±0.06	1.617[c] ±0.06
(NPU%) Net protein utilization	25.56[ab] ±1.15	23.02[b] ±1.15	27.36[a] ±1.15	25.21[ab] ±1.15	21.50[c] ±1.15
(EU%) Energy utilization	16.50[b] ±0.84	15.55[bc] ±0.84	17.89[a] ±0.84	17.03[ab] ±0.84	14.50[c] ±0.84

A,B,C,: Means within raw with different superscripts are significant ($P \leq 0.05$).

Table 5. Apparent digestibility coefficient (ADC%) of nutrients using ash internal marker.

Treatment	DM	CP	EE	NFE
T_1 (control)	85.51[a] ±1.62	79.23[a] ±0.99	92.50[a] ±1.13	56.47[a] ±0.96
BLM T_2 (25%)	83.18[a] ±1.62	77.64[a] ±0.99	91.72[a] ±1.13	56.40[a] ±0.96
BLM T_3 (50%)	83.82[a] ±1.62	79.33[a] ±0.99	93.66[a] ±1.13	55.13[a] ±0.96
BLM T_4 (75%)	82.32[a] ±1.62	77.87[a] ±0.99	92.23[a] ±1.13	53.25[a] ±0.96
BLM T_5 (100%)	80.16[a] ±1.62	72.67[b] ±0.99	91.33[a] ±1.13	49.80[b] ±0.96

A,B,: Means within raw with different superscripts are not significant ($P>0.05$).

Table 6. Feed price (L.E) for one Kg weight gain produced by Nile tilapia fed broken lentil meal.

Treatment	Feed intake (g/fish)	Price (L.E.)of one ton	Decrease in price L.E/ton	Total gains (g)	Feed Price/kg L.E	Relative to control
T_1 (control)	2.37	2438.5	100	1.32	4.38	100
BLM T_2 (25%)	2.46	2327.7	110.8	1.31	4.37	99.77
BLM T_3 (50%)	2.47	2216.2	221.6	1.51	3.63	82.88
BLM T_4 (75%)	2.49	2106.2	332.3	1.30	4.03	92.01
BLM T_5 (100%)	2.64	1995.4	443.1	1.20	4.39	100.22

Price L.E./ton of: Soybean meal 1800 L. E., Broken lentil meal 550 L.E

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استبدال بروتين كسب فول الصويا بكسر بذور العدس كمصدر بروتين نباتي في تغذية البلطي النيلي

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أجريت تجربة التغذية واستمرت ٨ أسابيع في نظام مائي مغلق لإختبار أثر إستخدام مسحوق كسر العدس كمصدر بروتين نباتي كبديل لبروتين كسب فول الصويا بنسب (٠، ٢٥، ٥٠ ،٧٥،١٠٠%) وتأثيره علي النمو والإستفادة الغذائية والهضم والكفاءة الإقتصادية ليرقات البلطي النيلي.

تم إستخدام ٤٥٠ يرقة من أسماك البلطي النيلي بمتوسط وزن في البداية ٠,٣جم/يرقة تم توزيعها عشوائيا علي ١٥ حوض (٣٠ يرقة /حوض) وكان معدل التغذية اليومي ١,٧% من وزن الجسم التمثيلي مرفوع لأس ٠,٨ وتمت التغذية علي خمس علائق تجريبية وكل معاملة في ٣ مكررات وتحتوي العلائق علي ٣٠% بروتين و٤٦٠٠ كيلو كالوري طاقة كلية/كجم عليقة.

وجد من التحليل الكيماوي لمسحوق كسر العدس أنه يحتوي علي نسبة عالية من البروتين الخام ٢٦,٦٩% ونسبة ألياف ١٢,٥٤% ونسبة كربوهيدرات ٥٥,٣٣% بينما يحتوي علي نسبة منخفضة في نسبة الدهن ٢,٤٥% والرماد ٢,٩٩%.

ودلت النتائج أن كسر بذور العدس تحتوي علي كمية مناسبة من البروتين الخام ومحتواه من الأحماض الأمينية ولكن أقل من الموجود في كسب فول الصويا ويحتوي كسر العدس أيضا علي نسبة عالية من الكربوهيدرات الذائبة أي أنه مصدر جيد للبروتين النباتي والطاقة.

واوضحت النتائج أيضا ان أعلي وزن حي، معدل النمو النسبي، كفاءة الأستفادة من البروتين والطاقة ومعاملات الهضم كان للعليقة المحتوية علي ٥٠% كسر عدس بالمقارنة بالكنترول بدون تأثير ضار علي النمو والاستفاده الغذائية للأسماك ووجد ان استخدام كسربذور العدس حتى ١٠٠% من بروتين كســـــب فول الصويا اعطى اقل تكلفة لطن عليقة الاسماك للحصول علي ١ كجم وزن حي .

أوضحت النتائج إمكانية استخدام كسر بذور العدس كمصدر بروتين نباتي غير تقليدى واقتصادي بالمقارنة بالكنترول فى تغذية اسماك البلطي النيلي.