REPLACEMENT OF SOYBEAN MEAL PROTEIN BY BROKEN LENTIL SEEDS AS A PLANT PROTEIN SOURCE IN NILE TILAPIA (Oreochromis niloticus) FEEDING

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#### **SUMMARY**

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 of tilapia fries.

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

Chemical analysis of broken lentil seeds showed high contain of CP (26.69%), CF (12.45%), NFE (55.33%), however, its contain of EE (2.45%) and Ash (2.99%). Results indicated that broken lentil seeds contains reasonable amount of CP (amino acids profile, but lower than that present in soybean meal) and its high contain of NFE which is an indicator for its potential value as a source of a plant protein and energy. 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 seeds was nutritionally and economically superior as compared to control as non-conventional plant protein source in Nile tilapia feeding.

**Keywords:** Nile Tilapia fries, broken lentil seeds, growth performance and economical study.

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### 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 Kiyama 1992 and Burel et al., 2000).

Sovbean meal is one of the most commonly used legumes as a major plant protein source in fish diets (Jackson, et al 1982 and El-Sayed 1999), its not only expensive but also difficult to be in steady current competition between fish poultry. For these 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 deterimined 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 investigate the of using broken lentil seeds as a non-conventional plant protein source instead of the common source being soybean meal protein in Nile tilapia diets, on the fish growth, feed and protein utilization

efficiency, digestibility and economic study.

# MATERIALS AND METHODS

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

In this study aimed to evaluate the effect of replacing soybean meal protein by broken lentil seeds in Nile tilapia fries. Chemical composition chemical analysis are presented in Table (1). The control diet (T1) formulated to contain (30%) soybean meal, 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 and extruded through a meat mincer then air dried and broken into small granules (about 0.5mm) to be fed to Nile tilapia fries.

The fish were fed daily at a rate of 1.7% of their metabolic body weight (W<sup>0.8</sup>) 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 days 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 allowances 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

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

Itam	T <sub>1</sub>	Broken lentil seeds (BLS) of soybean meal protein				
Item	(control)	T <sub>2</sub> (25%)	T <sub>3</sub> (50%)	T <sub>4</sub> (75%)	T <sub>5</sub> (100%)	
Composition of the diets						
Yellow corm	44	39.14	34.27	29.41	24.54	
Soybean meal (SBM)	30	22.5	15	7.5	-	
Broken lentil meal (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%	30.9	30.7	30.3	30.0	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	49.7	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	

<sup>\*</sup> 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; lodine 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).

anu soy bean meat (SIMD).		
Item	Broken Lentil Seeds (BLM)	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 <sup>1</sup>	49.84	35.85
$ADF^2$	20.46	27.07
$ADL^3$	0.39	3.73
Hemicellulose <sup>4</sup>	29.38	8.82
Cellulose <sup>5</sup>	20.07	23.30

<sup>\*</sup> Calculated by differences,

3- Acid detergent lignin.,

<sup>\*\*</sup> Estimated by Jobling (1983)

<sup>1-</sup> Neutral detergent fiber,

<sup>2-</sup>Acid detergent fiber,

<sup>4-</sup> Hemicellulose = NDF - ADF, 5- Cellulose = ADF - ADL,

estimated according to Goering and Van Soest, (1970).

Amino acids of the tested material was 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 was 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, CF 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, the high content of CF (12.54%) of BLS may be is the main factor that limits its usage as a feed ingredient for fish.

The gross energy of 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.

Broken lentil seeds (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. The results are in agreement with the findings reported by Frias *et al.*, (1994b), Nwokolo & Smartt (1996) and Sotomayor, (1997), It is clear Table (2) that BLS composition are similar to those 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 with 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).

essential The amino acids composition as percentage of protein of the BLS compared to that of SBM and NRC requirements of Tilapia, (1993) are given in Table (3). The amino acid composition of the feed protein is a good indicator of its nutritive Apparently, there is no information in the literature on amino acids composition of broken lentil seeds (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 NRC (1993), these results are in

Table (3): Amino acids composition of broken Lentil seeds (BLS) protein compared to that of soybean meal (SBM) and requirements of tilapia (1993).

Amino Acid in	Broken lentil seeds	Soybean	NRC (1993) Requirement		
CP%	(26.96%)	meal (44%)	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.			

<sup>\*</sup> Not determined

Table (4): Performance, feed, protein and energy utilization of Nile tilapia fries fed different of Broken lentil seeds.

T4	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>		T <sub>5</sub>
Item	(Control)	(25%)	(50%)	(75%)	(100%)
Initial weight (g/fish)	0.31 <sup>a</sup>	0.30 <sup>a</sup>	0.31 <sup>a</sup>	$0.30^{a}$	0.30 <sup>a</sup>
minai weight (g/11511)	±0.03	±0.03	±0.03	±0.03	±0.03
final weight (g/fish)	1.63 <sup>b</sup>	1.61 <sup>b</sup>	1.82 a	1.60 <sup>b</sup>	1.50°
mai weight (g/115ii)	±0.03	±0.03	±0.03	±0.03	±0.03
Total gain (g/fish)	1.32 b	1.31 <sup>b</sup>	1.51 <sup>a</sup>	1.30 <sup>6</sup>	1.20°
rotar gain (g/11311)	±0.03	±0.03	$\pm 0.03$	±0.03	±0.03
SGR (%d)	2.96 <sup>b</sup>	2.93 <sup>5</sup>	3.16 <sup>a</sup>	$2.99^{b}$	2.87°
Specific growth rate	±0.04	±0.04	±0.04	±0.04	±0.04
Feed fed (g/fish)	2.37 <sup>b</sup>	2.46 <sup>ab</sup>	2.47 <sup>ab</sup>	$2.49^{ab}$	2.64ª
,	±0.08	±0.08	±0.08	±0.08	±0.08
FCR (g feed / gain)	1.80 <sup>b</sup>	1.88 <sup>b</sup>	1.64°	1.91 <sup>ab</sup>	$2.20^{a}$
Feed conversion ratio	±0.06	±0.06	±0.06	±0.06	±0.06
Survival rate (%)	91.10 <sup>a</sup>	93.33°	93.32°	94.44°	91.11 <sup>a</sup>
• •	±2.43	±2.43	±2.43	±2.43	±2.43
(PER)	1.718 <sup>b</sup>	1.769 <sup>b</sup>	1.987ª	1.898 <sup>ab</sup>	1.617°
Protein efficiency ratio	±0.06	±0.06	±0.06	±0.06	±0.06
(NPU%)	25.56 <sup>ab</sup>	23.02 <sup>b</sup>	27.36 <sup>a</sup>	25.21 <sup>ab</sup>	21.50°
Net protein utilization	±1.15	±1.15	±1.15	±1.15	±1.15
(EU%)	16.50 <sup>b</sup>	15.55 <sup>bc</sup>	17.89°	17.03 <sup>ab</sup>	14.50°
Energy utilization	±0.84	±0.84	±0.84	±0.84	±0.84

a,b,c,: Means within raw with different superscripts are significant (P≤0.05).

agreement with 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 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<sub>3</sub> contain 50% BLS being (1.51gm) while the worst value was recorded for T5 contain 100% BLS being (1.20gm). The differences significant (P≤0.05) in total gain and SGR. The results agree with Olvera et al., (1997) 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 may be decreased 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) who 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 & 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%) the best value, 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 Beeker (2003).

There were differences ( $P \le 0.05$ ) 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 may due to lake of required essential amino acids in diets for Nile tilapia according to NRC (1993), and could be attributed to high crude fiber (hemicellulose and cellulose), 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) reported that, when fed the atlantic salmon the lupin meal at 33% had a higher feed consumption and a lower feed conversion

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

mai ker.				
Treatment	DM	CP	EE	NFE
T <sub>1</sub> (control)	85.51ª	79.23ª	92.50 <sup>a</sup>	56.47ª
	±1.62	±0.99	±1.13	±0.96
BLM T <sub>2</sub> (25%)	83.18 <sup>a</sup>	77.64ª	91.72a	56.40 <sup>a</sup>
	±1.62	±0.99	±1.13	±0.96
BLM T <sub>3</sub> (50%)	83.82 <sup>a</sup>	79.33 <sup>a</sup>	93.66ª	55.13 <sup>a</sup>
	±1.62	±0.99	±1.13	±0.96
BLM T <sub>4</sub> (75%)	82.32 <sup>a</sup>	77.87 <sup>a</sup>	92.23 <sup>a</sup>	53.25 <sup>a</sup>
	±1.62	±0.99	±1.13	±0.96
BLM T <sub>5</sub> (100%)	80.16 <sup>a</sup>	72.67 <sup>b</sup>	91.33°	49.80 <sup>b</sup>
	±1.62	±0.99	±1.13	±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.

Dig	Men lentil i	iicai.				
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 <sub>1</sub> (control)	2.64	2438.5	100	1.28	5.03	100
BLM T <sub>2</sub> (25%)	2.46	2327.7	110.8	1.31	437	86.88
BLM T <sub>3</sub> (50%)	2.47	2216.2	221.6	1.51	3.63	72.17
BLM T <sub>4</sub> (75%)	2.49	2106.2	332.3	1.42	3.69	73.36
BLM T <sub>5</sub> (100%)	2.72	1995.4	443.1	1.32	4.11	81.71

Price L.E./ton of:

Soybean meal = 1800 L.E.

Broken lentil meal = 550 L.E.

ratio, protein productive value than the soybean meal diet.

Meanwhile, the results of diets T3 and T4 were 50% and 75% of the soybean meal were replaced by broken lentil seeds respectively in values PER, NPU and EU and no significant differences (( $P \ge 0.05$ ). These results suggest that the protein utilization of the experimental diets was improved as the broken lentil seeds replaced soybean meal in diets Shiau (1997) found that, body protein and fat increased when fish were fed starch rather than simple carbohydrates source.

El sayed (1999) tested alternative protein sources and recommended for tilapia (O.mossambicus) diets such as jack bean, cowpea and green gram legume were useful partial substitute for fish meal in at 25%; 20-30% and 25-37%, respectively inclusion levels.

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 food 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 ≥ 0.05) as the inclusion rate of broken lentil seeds increased from 25 to 75%. These results agree with Olvera et al (1997) found that, the highest digestibility coefficient were observed with 20-30% replacement of fish meal with cowpea protein concentrate for O. niloticus. Ovedapa (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 and Fils et al. (1997) and Booth et al., (2001) reported that dehulling seeds legumes, increase CP and CF digestibility and a higher nutritional value of seeds.

#### Economical study:

The seeds price (L.E.) for one Kg weight gain produced by Nile tilapia fed BLS 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. Aton. By calculation, the control diet showed the highest cost needed for producing one Kg fish gain increased gradually as the substitution level of BLS increased from 25 to 100%

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 (Rumsey 1993).

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

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إجريت تجربة التغذية واستمرت 8 أسابيع في نظام ماني مغلق لإختبار أثر إستخدام مسحوق كسر العدس كمصدر بروتين نباتي كبديل لبروتين كسب فول الصويا بنسب ( ٥، 25، 50، 75،100، وتأثيره على النمو والاستفادة الغذائية والهضم والكفاءة الإقتصادية ليرقات البلطي النيلي.

تم استخدام 450 يرقة من أسماك البلطي النيلي بمتوسط ورن في البداية 0.3جم/يرقة تم توزيعها عشوانيا على 15 حوض (30 يرقة /حوض) وكان معدل التغذية اليومي 1.7% من وزن الجسم التمثيلي مرفوع لأس 0.8 وتمت التغذية على خمس علائق تجريبية وكل معاملة في 3 مكررات وتحتوي العلائق على 30% بروتين و4600 كيلو كالورى طاقة كلية/كجم عليقة.

وجد من التحليل الكيماوي لمسحوق لكسر العدس أنه يحتوي علي نسبة عالية من البروتين الخام 26.69% ونسبة الياف 12.54% ونسبة الياف 12.54% ونسبة الياف 12.54% ونسبة منخفضة في نسبة الدهن 2.45% ونسبة الرماد 2.99%.

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

واوضحت النتانج أيضا أن أعلى وزن حي, معدل النمو النسبي, كفاءة الأستفادة من البروتين والطاقة ومعاملات الهضم للعليقة المحتوية على 50% كسر عدس بالمقارنة بالكنترول بدون تأثير ضار على النمو والاستفاده الغذانية للأسماك ووجد أن استخدام كسربذور العدس حتى 100% من بروتين كسب فول الصويا أعطى أقل تكلفة لطن عليقة الاسماك للحصول على 1 كجم وزن حي .

توصى الدراسة بأنه يمكن استخدام كسر بذور العدس كمصدر بروتين نباتي غير تقليدى واقتصاديا بالمقارنة بالكنترول حتى مستوى 50% من بروتين كسب فول الصويا في علائق اسماك البلطي النيلي.