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EFFECT OF DIFFERENT TREATMENTS OF SOYBEAN SEEDS ON THE PRODUCTIVE PERFORMANCE, FEED UTILIZATION AND BODY COMPOSITION OF NILE TILAPIA *(Oreochromis niloticus)* FINGERLINGS

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ABSTRACT

A fish feeding study was conducted in a closed recirculation water system for 12 weeks; to evaluate the effect of feeding Nile tilapia *(Oreochromis niloticus)* on treated soybean products. Extruded full fat soybean (EFFSB), Germinated Soybean seeds (GSB), Germinated and heated soybean seeds (GHSB) replaced 50 and 100% of the Soybean meal in the control diet. Growth performance, feed utilization and body composition of 315 tilapia fingerlings (an average initial weight of 1.2 *gl* fish) were used. Fish were randomly distributed into 21 aquaria (15 *fingerlingsl* aquarium); the water volume in each aquarium was 60 liters. Fish were fed daily at a rate of 1.7 % of fish metabolic body size ($w^{0.8}$) with isonitrogenous (30% CP) and isocaloric (4200 kcal *GEl* kg diet). Each diet was given to three replicate aquaria. The following experimental treatments were tested TI (100% soybean meal (Control), T2 (50 % of the soybean meal was replaced by EFFSB), T3 (100% EFFSB), T4 (50 % of the soybean meal was replaced by GSB), T5 (100% GSB), T6 (50 % of the soybean meal was replaced by (GHSB), T7 (100% GHSB).

Results revealed that the highest specific growth rate (3.1 *%/day)* was recorded by T2, whereas the lowest value (1.61 *%1* day) was recorded by treatment 5. The survival rates of treatments 2, 3, I were 97.78, 95.55 and 95.55%, respectively. The worst survival rate (51.1 %) was obtained by T5. Better feed conversion ratio (FCR) was occurred when fish was fed treatments I (control), 2, 3, 6 and 7 than fish groups fed treatments 4 and 5 (P>0.05). Protein efficiency ratio (PER) were found to be high when fish were fed diets containing EFFSB. The highest value of dry matter in whole fish body was recorded for T3, whereas the lowest value was noticed for T5. Body crude protein content of treatments I, 2 and 4 and 6 were significantly higher than the other treatments. Body fat content of fish fed T3 was higher significantly than the other treatments (P<0.05). From the previous results we can conclude that germination of soy seeds without heating had minor effect on trypsin inhibitor which affected negatively the growth performance, feed utilization and carcass composition of tilapia fish when included in its diets.

Keywords: Nile tilapia, soybean, extruded, germination, heat treatment.

INTRODUCTION

Soybeans are available in many places of the world at a lower cost than animal protein sources. Among plant proteins, soybeans have high protein quality with the best amino acid profile suitable for aquatic species. Whole soybeans also contain high level of oil, which is a concentrated source of highly digestible energy source (Lim and Akiyama., 1991). Soybean products have been used successfully as a protein supplement in the diet of trout (Reinitz *et al., 1978;* Tacon *et al.,* 1983), catfish (Wilson and Poe, 1985), common carp (Viola *et al.,* 1983; Abel *et al.,* 1984; Muria *et al.,* 1989) and tilapia (Davies *et al*, 1989; Shiau *et al.,* 1990; Goda *et al.,* 2007; Gaber and Hanfey., 2008). Two major types of protease inhibitors have been described in soybeans; a "Kunitz type" inhibitor, which interacts mainly with trypsin and a "Bowman-Brik type", which inhibits both trypsin and chymotrypsin. In comparison with various mammalian and avian species, trout proteases are the most sensitive among the pancreatic proteinases, inhibition of the trout enzymes being 15 times greater than that of the same amount of human enzymes (Krogdahl and Holm, 1983). Heat treatment is therefore indispensable in processing soybean products for use in fish diets Abel *et al.,* (1984), and Sandholm *et al.,* (1976) had shown a negative correlation between the anti trypsin content in soybeans and the protein and energy value of the diet for common carp and rainbow trout. However, excessive heating may cause loss of essential amino acids and lower their availability due to Maillard reaction products (Plakas *et al., 1985).*

Egyptian aquaculture has developed rapidly in recent years. Tilapia is one of the most widely cultured species in Egypt. The total aquaculture production of tilapia increased from 24916 metric tones in 1990 to 635000 metric tones year^{-1} (GAFRD, 2008) and accounted for 63% of the total production (One million tone year^{-1}

The present study was conducted to evaluate the effect of different treatments of soybean products on growth performance, feed utilization and body composition of Nile tilapia *(Oreochromis niloticus) .*

MATERIALS AND METHODS

Experimental fish

Tilapia fingerlings *(Oreochromis niloticus)* with an average initial weight of 1.20 gram per fish were purchased from the hatchery of the Arabic company farm, Abbassa, Sharkia, Egypt. Three hundred and fifteen fish with approximately the same weight were randomly distributed into 7 dietary treatment groups (each group 3 aquaria). The first week of the experimental period was considered as an acclimation period. During this period dead fish were replaced by healthy fish of the same weights. Fish were daily fed at a rate of 1.7 % of fish metabolic body size ($W^{0.8}$) according to Huisman (1976), Abo El-makarem *et al.* (1996) and Osman *et al.* (2003). Daily feed allowance was divided into 4 portions and was offered at 8:00, 11:00, 14:00 and 17:00 h. (Gaber and Hanafy, 2008).

Germination and heat treatment of raw soybean

1. Germination

Germination was performed by a modification of the methods of Khaleque *et al*., (1985). Seeds of the soybeans were initially washed in distilled water and soaked for 10 min in 70% ethanol containing 3% CaCI2. after that the seeds were thoroughly rinsed in distilled water and soaked in water for 4 h (seed/water proportion of 1:2, w/v). Extraction of low-molecularweight (LMW). carbohydrates by this procedure was negligible. The seeds were then allowed to germinate in the dark at 28^0 C for 48 h and finally seeds were milled to pass a 0.75 mm sieve and kept frozen until analyses and formulation of diets.

2. Heat treatment

After seeds were germinated they were milled to pass a 0.75 mm sieve then the milled seeds, were incubated for 10 min. at temperature 100° C (Rackis, 1966). After that the Urease enzyme activity was determined in diets.

Feed ingredients:

Feed ingredients used to formulate the experimental diets were bought from the local market, as shown in (Table 1 and 2).

Table 1: Formulation of the experimental diets (g/ 100 g diet)

Ingredient	Diet 1	Diet2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
	(Tl)	(T2)	(T3)	(T4)	(T5)	(T6)	(T7)
Yellow corn	20	20	18	20	19	20	19
Fish meal (65% CP)	13.5	13.5	13.5	13.5	13.5	13.5	13.5
Corn gluten	5	7	9	7	8	7	8
Rice bran	14.5	14.5	14.5	14.5	14.5	14.5	14.5
Wheat flour meddling	11	11	11	11	11	11	11
Fish oil	I	1	1	1	1	I	1
Soybean oil	2	-	-	-	-	-	-
Soybean meal	30	15	-	15	-	15	-
EFFSB	-	15	30	-	-	-	-
GSB	-	-	-	15	30	-	-
GHSB	-	-	-	-	-	15	30
Di-calcium phosphate	2	2	2	2	2	2	2
* Vitamins and minerals mixture	1	1	1	1	1	1	1

- **Vitamins and minerals premix at 1% of the following per kg of this mixture continents: Vit. A 75000 IU, vit. D 9000 IU. vit. E 150mg, vit K 30mg, vit 8, 26.7mg, vit. 8,30mg, vit 8_6 24.7mg. vit. 8" 75 mg, niacin 225mg, pantothenic acid 69mg, folic acid 7.5mg, vit. C 150mg, choline chloride 500mg, DL-methionine 300mg, Mil 204mg. Fe 93: Zn 210mg, Cll 11.25mg, 11.02mg. (NRC, 1993).**

Table 2: Chemical composition of the seven experimental diets

	DM%	CP%	EE%	CF%	Ash%	NFE%*
T1	86.68	30.14	9.77	5.72	8.30	32.75
T2	87.40	30.30	9.67	5.32	8.10	34.01
T3	87.33	29.83	10.21	5.04	8.14	34.11
T4	87.18	30.48	9.19	5.26	7.98	34.27
T5	86.94	30.02	10.14	4.8	8.16	33.82
T6	86.52	30.07	9.32	5.27	7.95	33.91
T7	86.55	30.30	10.24	4.86	7.89	33.26

*** NFE=100-(CP+EE+CF+Ash)**

Experimental Design:

During the experimental period, seven diets were tested as following:

(Tl) 100% soybean meal (Control)

(T2) 50% soybean meal + 50% extruded full fat soybean (EFFSB).

(T3) 100% EFFSB.
(T4) 50% soybean meal + 50% germinated soybean seeds (GSB).
(T5) 100% GSB.
(T6) 50% soybean meal + 50% germinated and heated soybean seeds (GHSB).
(T7) 100% GHSB.

Chemical Analysis

All ingredients and all diets were analysed for proximate analysis. Moisture (M), Crude protein (CP), Ash and Crude fiber (CF) according to the AOAC (2005) and Crude lipid (EE) (FoIch, Lees & Stanley, 1957). The amino acid profile of soybean product was determined and Urease activity was measured by the rise in pH according to the AOAC (2005).

Parameters measured

The survival parameter employed was the final survival rate (%) (SR = 100(the number of fish at experimental end/ the number of fish at stocking). The growth parameters employed were final weight (g/ fish), Average Daily Gain (ADG) g/ fish/ day and specific growth rate (%/ day), where SGR = 100 x (log_e final weight- log_e initial weight)/ rearing period in days). The feed performance parameters employed were feed conversion ratio (FCR = total amount of feed provided/ final increment of fish biomass), and protein efficiency ratio (PER final increment of fish biomass/total amount of protein in feed consumed).

Statistical analysis

The statistical analysis was applied according to Steel and Torrie (1980) on the collected data using a SAS program (2006). Differences between means were tested for significance according to Duncan's multiple rang test (Duncan, 1955).

Table 3 : Essential amino acid profile of soybean products(g/100g).

	Soybean seeds	GSB	GHSB	EFFSB
Threonine	1.45	1.44	1.43	1.47
Valine	1.53	1.51	1.58	2.07
Glysine	1.60	1.59	1.63	1.68
Isoleucine	1.48	1.45	1.48	1.64
Leucine	2.58	2.53	2.60	2.54
Phenyl alanine	1.91	1.87	1.89	1.96
Histadine	1.45	1.41	1.48	1.92
Arginine	2.16	2.06	2.10	2.86
Cystine	0.49	0.35	0.42	0.64
Methionine	0.47	0.45	0.49	0.66
Lysine	2.36	2.31	2.35	2.82

The following model was used to analyze the obtained data:

Yij = u + Ti + eij Where:
Yij = observation
U = the overall mean
Ti= the effect of treatment.
Eij = random error.

RESULTS AND DISCUSSION

Chemical composition of experimental diets

The Chemical compositions of the experimental diets used are shown in (Table 2). No variations were observed among diets in their Chemical compositions. Also essential amino acid profile of soybean product is shown in (Table 3). The best essential amino acid profile was recorded by EFFSB. Limited variations were detected among soybean seeds, GSB and GHSB. Table (4) shows the content of Urease enzyme activity of the different soybean types used in the experiment. Soybean seeds showed the highest Urease activity followed by germinated soybean seeds (Germination made very small effect on trypsin inhibitor), whereas germinated heated soybean seeds and soybean meal (commercial available) and extruded full fat soy has neglictable Urease activity. Boonyaratpalin *et al.* (1998) found that only heat treatment destroy trypsin inhibitor in soy seeds.

Table 4: Urease activity (pH change unit).

Soybean product	Urease activity (pH change)
Soybean seeds	2.47
GSB	2.41
GHSB	0.06
Soybean meals	0.05
EFFSB	0.05

Growth performance, feed utilization parameters and survival rate

Growth performance, feed utilization parameters and fish survivability, for each of the treatments are shown in Table (5).

There were no significant differences (P>0.05) among the initial individual weights of the all experimental groups. These indicated that fish at stocking were homogeneously distributed among treatments and replicates. Significant variations (P<0.05) were observed in the final body weight and average daily gain among the different treatments in Table (5). The highest significant (P<0.05) final body weight and average daily gain were recorded by fish in treatments 2 and 3 followed, without significant differences by *T6,* where they were fed diets T2 (50% of the soybean meal was replaced by EFFSB), T3 (100% of the soybean meal was replaced by EFFSP) and T6 (50% of the soybean meal was replaced by GHSB), respectively. These results were higher than that of the control treatment which was fed the diet contained soybean meal as a source of plant protein. T4 and T5 (which contained germinated soybean seeds without heating) showed the lowest growth performance values; these may be due to the higher contents of tryp inhibitor present in GSB as shown Table (4). We and Shu (1989) found that Nile tilapia grew well at low levels trypsin inhibitor «0.09 % trypsin inhibitor). Also Boonyaratpalin *et al.* (1998) found that Asian sea bass showed significant decrease in final weight and average daily gain when soybean meal was replaced by socked raw soybean meal in its diets. The results obtained the present study are in agreement with that obtained by Smith

(1977), Wilson, and Poe (1985) for catfish and Abel *al.,* (1984) for common carp.

Specific growth rate (SGR) of T2 andT6 were significantly higher than that the all other treatments (P<0.05) follow, by T3 and the control (T 1), respectively. The lowest SGR values were obtained by T5 where, Germinated soybean seed, were included in the diet. In this connection Berg-lea *et al.* (1989) found that Rainbow trout fed on diets containing purified kunitz soybean trypsin inhibitor showed diminished growth and decreased intestinal trypsin activity. It has been speculated that about 30-50% of the inhibitory effect of soybean on growth of hometherms animals can be accounted for by the soybean trypsin inhibitor (Kakade *et al.,* 1973).To the same results came Smith (1977), Wilson and Poe (1985) Abel *et al.* (1984),Wee and Shu. *(1989)* and Boonyaratpalin *et al., (1998).*

Results of Table (5) revealed the better feed conversion ratio (FCR) was detected for fish in treatments 1 (control: 2, 3, 6 and 7 than fish in treatments 4 and 5 (P>0.05). Similar results were obtained, by El Sayed *et al.* (2000), who found significant decrease (P<0.05) in growth rate and FCR when he used untreated soybean in tilapia diets.

No significant differences (P>0.05) in protein efficiency ratio (PER) were found among treatments except treatments 4 and 5, which recorded lower protein efficiency values compared with the other treatments. These results are in agreement with the results of Wee and Shu. (1989) and El Sayed *et at.* (2000). The Later suggested that a modification in the composition of secreted proteases has been occurred when unheated soybean was used in tilapia diets.

As given Table (5) treatments 4 and 5 showed lower significant survival rate (P<0.05) than the other feeding treatments. In treatment 5 where 100% of the soybean meal was replaced by GSB, 49% of the fish were died; this may due to the higher percentage of trypsin inhibitor which was not destroyed by germination.

Table 5: Growth rate, feed utilization parameters and survival rate of fish fed the different experimental diets.

	Treatments						
	I	2	3	4	5	6	7
Initial weight (g)	1.20	1.20	1.19	1.20	1.19	1.19	1.19
Final weight (g)	13.47^{b}	16.28^{a}	16.03^{a}	6.37^{c}	4.66^{d}	15.72^{a}	13.24^{b}
ADG	0.146^{b}	0.179^{a}	0.169^{a}	0.062^{c}	0.041^{d}	0.173^{a}	0.143^{b}
SGR	2.88^{bc}	3.1 a	3.04^{bac}	1.98^{d}	1.61 e	3.07^{ba}	2.86^{C}
FCR	1.13^{C}	1.06^{c}	1.09^{c}	1.66^{b}	2.19^{a}	1.07^{c}	1.13^{c}
PER	2.94^{a}	3.12^{a}	3.08^{a}	1.98^{b}	1.53^{c}	3.11^{a}	2.94^{a}
Survival (%)	95.55^{a}	97.78^{a}	95.55^{a}	71.1l^{b}	51.11^{c}	93.33^{a}	93.33^{a}

Means within the same row having different superscript are significantly different (p<0.05).

Chemical composition of whole Tilapia fish at the end of the experiment.

Dry matter, crude protein, ether extract, ash and energy content for Nile tilapia *(Oreochromis, niloticus)* during the experiments are presented in Table

(6). The highest value of dry matter was recorded for T3 (28.61 %), whereas the lowest value was noticed for T5 (21.29 %). Body crude protein content of treatments 1,2 and 4 and 6 were significantly higher (p<0.05) than that of the other experimental treatments. The highest value of crude protein content was recorded for Tl (62.53 %) followed in descending order by T6 (61.71%), T4 (61.68%),T2 (61.42%) then T3 (60.66%), respectively. T5, where no heat treatment for germinated soy seeds was applied, this treatments showed the lowest significant (P<0.05) body protein content (58.6%). Body fat content of fish in T3 was significantly higher than that of the other treatments (P<0.05), while the lowest value was found in T4 which was fed diet 50% of its soybean meal was replaced by GSB.

Table 6: Chemical composition of whole Tilapia fish fed the different experimental diets.

Treatments	DM%	CP%	EE%	ASH%
T1	26.53^{b}	62.53^{a}	19.56°	14.13^{e}
T2	25.93^{b}	61.42^{ab}	21.48^{b}	15.2^{d}
T3	28.61^{a}	60.66^{b}	22.94^{a}	15.73^{c}
T4	23.56^{c}	61.68^{ab}	16.4^{g}	16.05^{b}
T5	21.29^{d}	58.6^{c}	17.51 f	16.3^{b}
T6	25.88^{b}	61.71^{ab}	18.87e	17.17a
T7	25.73^{b}	60.93^{b}	20.09^{c}	16.13^{b}

Means within the same row having different superscript are significantly different (p < 0.05) .

From the results of the present study we can conclude that including extruded full fat soy as plant protein source compared with other treatments procedure showed higher growth rate and better utilization parameters when it was fed to tilapia fish(*O niloticus*).

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تأثير المعاملات المختلفة لبذور فول الصويا على الأداء الإنتاجي والاستفادة من الغذاء وصفات الجسم لأصبعيات أسماك البلطي النيلى

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أجريت تجربة التغذية داخل نظام مغلق وذلك لفترة 12 أسبوع بغرض تقييم تغذية اصبعيات أسماك البلطي النيلي على صور مختلفة من منتجات الصويا المعاملة على صور مختلفة من منتجات الصويا المعاملة وهي:
الصويا كاملة الدهن والتي سبق أن تمت عليها عملية البثق الحراري، بذور فول الصويا المنبتة، بذور فول الصويا التي تم استنباتها ثم عوملت حراريا وذلك بنسب احلال مقدارها 100، 50% من كسب فول الصويا (العليقة القياسية).
وقد تم أخذ القياسات التالية لعدد 315 أصبعية بلطي نيلي متوسط وزن البداية (1.2 جم/سمك) وهي : معدلات النمو، معدل الاستفادة من الغذاء وصفات الجسم للأسماك.
تم توزيع الأصبعيات عشوائيا بحيث احتوى الحوض الواحد سعة س 60 لتر على 15 أصبعية وذلك ضمن 21 حوض بحيث اشتملت المعاملة الواحدة على ثلاث مكررات استخدم معدل تغذية قدرة 1.7% من وزن الجسم التمثيلي لعليقة متزنة في محتواها من البروتين (30%) والطاقة الكلية (4200 كيلو كالوري طاقة كلية / كج عليقة) وتم تكوين علائق (معاملات) لاجراء التجربة وكانت كالتالي:

١ - المعاملة الأولى قياسية وهي 100% كسب فول صويا.
٢ - المعاملة الثانية استبدال 50% كسب فول صويا بـ 50% الصويا كاملة الدهن والتي سبق أن تمت عليها عملية البثق.
٣ - المعاملة الثالثة 100% الصويا كاملة الدهن والتي سبق أن تمت عليها عملية البثق.
٤ - المعاملة الرابعة استبدال 50% كسب فول صويا بـ 50% بذور فول صويا منبتة.
٥ - المعاملة الخامسة 100% بذور فول صويا منبتة.
٦ - المعاملة السادسة استبدال 50% كسب فول صويا بـ 50% بذور فول صويا منبتة ومعاملة حرارياً.
٧ - المعاملة السابعة 100% بذور فول صويا منبتة ومعاملة حرارياً.

أظهرت النتائج أن أعلى معدل نمو نوعي (3.1% يومياً) قد سجل في المعاملة رقم (2) وهي احلال 50% من كسب فول الصويا بالصويا كاملة الدهن والتي تمت عليها عملية بثق بينما كان أقل معدل نمو نوعي (1.61% يومياً) من نصيب المعاملة رقم (5) وهي 100% بذور فول صويا منبتة. وكانت نسبة النفوق منخفضة في المعاملات رقم 2، 3، 1 حيث كانت نسبة الأسماك الحية 97.78 ، 95.55 و 95.55% على التوالي بينما كانت المعاملة رقم (5) أقلهم حيث وصلت نسبة الأسماك الحية إلى 51.1% .
لوحظ تحسن معدل التحويل الغذائي للأسماك المغذاة في المعاملات رقم 1، 2، 3، 6 و 7 مقارنة بتلك في المعاملات رقم 4 و 5 (P<0.05) .
تحسن معامل الاستفادة النسبي من البروتين عند تغذية الأسماك على علائق احتوت على صويا كاملة الدهن تمت عليها عملية الاكستروجين كانت أعلى نسبة للمادة الجافة في الأسماك التي تغذت على المعاملة رقم (3) بينما كانت أقل ما يكون في الأسماك المغذاة على المعاملة رقم (5).
زاد محتوى الجسم في البروتين زيادة معنوية عند تغذية الأسماك على معاملات رقم 1، 2، 4 و6 مقارنة بباقي المعاملات في التجربة، بينما كانت الزيادة معنوية (P<0.05) . في محتوى الجسم من الدهون من نصيب الأسماك التي تغذيت على المعاملة رقم (3).
وعليه فإنه من خلال النتائج يمكن استنتاج ان استخدام بذور فول الصويا المنبتة دون معاملتها حراريا يكون له تأثير ضعيف على مثبطات انزيم التربسين وبالتالي ينتج تأثير سلبي على معدلات نمو الأسماك، معدلات التحويل الغذائي وصفات الجسم للأسماك البلطي النيلي.

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