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EFFECT OF REPLACEMENT OF SOYBEAN MEAL BY LINSEED MEAL ON GROWTH PERFOR MANCE , AND BODY COMPOSITION OF THE NILE TILAPIA, *OREOCHROMIS NILOTICUS* (L) CULTURED IN CONCRETE PONDS.

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Key words: Replacement , soybean , linseed , Nile tilapia , growth rate nutrition.

ABSTRACT

A five-month feeding trial was conducted to evaluate the effect of replacement of soybean meal by linseed meal and adding *Yucca schidigera* powder extract ,methionine and lysine on growth performance and body composition of Nile tilapia, *Oreochromis niloticus* (L) with an average initial weight of 30.8± 1.3 g/fish. The experimental fish were divided into 18 groups and fed on pelleted feed containing each of the plant protein meal supplemented with *Yucca schidigera* powder extract at 750 mg kg^{-1}. Methionine (0.5%) and lysine (0.5%) were added to each diet except the control diet (FMC) which was supplemented with yucca only to examine the effect of replacement soybean with linseed meal on growth rate, feed conversion ratio (FCR), protein efficiency ratio (PER) and body composition of reared fish . Three groups of fish were fed on each of six isonitrogenous (25.2% C.P) and isocaloric (4.3 kcal g^{-1}) feed, where the soybean meal (SBM) was replaced with linseed meal (LSM) at a levels of 0%, 25%, 50%, 75% and 100%.The experiment was performed in three concrete ponds and the valume of each was ($40m^3$) (4 x 10 x 1.0 m , long, width, and height respectively) . Ponds were divided into six units ($6m^2$) by nets. The experimental fish were stocked at a rate of 10 tilapia/m^2 (60 tilapia/unite). After five months of feeding , results revealed that the fish fed on plant protein diets supplemented with *Yucca* exhibited growth performance not differing significantly from that fish fed on FMC. Linseed levels in the diets were significantly influenced the whole body composition of the Nile tilapia. It has been revealed that increasing level of linseed, increased body moisture content and fat content , while protein content decreased. It was concluded that soybean could be completely replaced by linseed in Nile tilapia diets without adverse effect on growth rate, when diets supplemented with *Yucca schidigera.*

INTRODUCTION

Soybean (*Glysine max*, L. Merrill) meal (SBM) is used as major plant protein source in diets for aquatic animals, especially to replace of fish meal (FM) which is costly and less available , less stable in supply and price than soybean. A significant amount of research has been conducted on the replacement of FM with SBM as protein sources in feeds for Nile tilapia (El-Saidy & Gaber 1997, 2002) and SBM is now considered as major ingredient in commercial tilapia feed in Egypt. Numerous studies suggested that linseed meal (LSM). may be a good alternative plant protein source in Nile tilapia feed (El-Saidy & Gaber, 2002). Economically using linseed meal is favored for Nile tilapia and common carp (El-Saidy & Gaber 2001 ; Hossain & Jauncey 1989) compared with SBM, which has the disadvantage of lower protein content, that depending on the processing of LSM (Jauncey, 1982).

The use of *Y. schidigera* extract is useful in reducing ammonia concentration , improving performance and increasing feed efficiency when *Y. schidigera* extract is incorporated into feeds for poultry (Johnston *et al.*,1982) and for Nile tilapia (El-Saidy & Gaber, 2004). Kaneda *et al.* (1987) reported that *Y. schidigera* extract contains at least three steroid saponine. Johnston. *et al.* (1981) suggested that surface properties of component of *Y. schidigera* extract could aid in nutrient absorption. In addition, Jacques & Bastien (1989) suggested modes of action include urease inhibition, increased bacterial use of ammonia and direct binding of ammonia . Therefore, the aim of this work was studying partial to complete substitution of SBM by LSM in diets for Nile tilapia through the examination of growth and feed utilization .

MATERIALS AND METHODS

Experimental diets

Feed grade dehulled and solvent extracted soybean meal (SBM) and linseed meal (LSM) were supplied from Cairo Company for Oil Production (Egypt) . Prior to diet formulation, the plant protein sources were tested and their contents were soybean meal–commercial repressed solvent extracted meal and linseed-commercial repressed solvent extracted meal, total glucosinulate level 1.24 mg g^{-1}.

The proximate composition of the ingredients was determined (Table 1). The diets were formulated, principally to be isonitrogeonus (25.6% crude protein) and isocaloric (4.3 kcal g^{-1} gross energy), (Table 2). but consideration was given to the equivalence of other components in the following order of priority: crude fat, NFE and ash . L-methionine and L- lysine were added to ensure that these essential amino acids were present in excess of requirements for the Nile tilapia in all diets except the control diet (1) (Santiago & Lovell, 1988). *Yucca schidigera* (the Yucca powder from YS, Sigma no. 0.9000.20.8; Sigma, St. Louis, USA), was first dissolved in alcohol, then distilled water was added and mixed thoroughly with all diets, maintaining concentration of 750 mg kg^{-1} diet according to El-Saidy & Gaber (2004). The protein source of the control (1) diet composed mainly from fish meal (FMC) and the protein source of the control (2) diet was composed mainly from SBM without LSM . In the other four diets 25, 50, 75, or 100% of SBM protein was replaced by protein from Linseed meal (LSM) that was extracted and dried for 90°C for 6 h and then ground into meal as particles passing through no.35 mesh (0.50 ma^{-1}). The feeds were manufactured using a pellet mill (California Pellet Mill, San Francisco, CA, USA) with a 3.4 mm die. Feeds were bagged and stored at -18°C.

Growth experiment

The experiment was conducted at the Fish Research Station Delta Barrage, Qaluubia, Egypt. the Nile tilapia, *Oreochromis niloticus* (L), were obtained from the Fish Research Station. They were stocked into three (40 m^3) cement ponds. Each cement pond was divided into six equal parts by nets (6m^3 each) and stocked with 60 fish (stocking density 10 fish/m^3). The fish were acclimatized for two weeks in a fresh water static system. The fish within each pond were redistributed to ensure that there were no significant differences between groups; the fish were divided into 18 groups with three replicate groups per diet. Experimental fish were weighed individually at the beginning of the experiment and every month and ration was adjusted accordingly. A ration (2 % of body weight) was supplied twice a day at 0800-1700 , six days a week and feed allowance was corrected after weighing fish. The experiment continued for five months until fish reached the market size. Also, at the beginning of the experiment ten fish were killed and used for assessment of initial chemical composition. At the end of the experiment, three fish were removed from each replicate to carry out the chemical composition.

Water quality

Water quality parameters (temperature, dissolved oxygen, pH, ammonia, nitrate and nitrite) were monitored to ensure that water quality remained well within limits recommended for Nile tilapia . Water temperature and dissolved oxygen were measured every other day using an YSI Model 58 oxygen meter. Total ammonia and nitrate was measured weekly using spectronic 601 spectrophotometer. Alkalinity was monitored twice weekly using the titration methods of Golterman *et al.* (1978). pH was monitored twice weekly using an electronic pH meter. During the feeding trial , the water quality parameter averaged (±SD): water temperature 27.8±0.8; dissolved oxygen 4.8±0.4; pH 7.4±0.6; ammonia 0.1±0.04 mg/L; nitrite 0.1±0.05 mg/L; nitrate 1.5±.2 mg/L and alkalinity 181±.46 mgL^{-1}

Chemical analysis

At the beginning of the study, 15 fish were sampled from the fish stock and frozen at -18°C for analysis of the whole body composition. At the end of the five months growth study, three fish per group were collected for analysis. Fish were homogenized for whole body composition and frozen at -18°C until analyzed. Samples were analyzed as follows: dry matter after desiccation in an oven (105°C for 24h), crude protein (micro kjeldahl, Nx6.25), crude lipid (ether extraction by soxlhet method), crude fiber according to methods of (AOAC, 1995) and gross energy content were determined using (Ballistic bomb calorimeter, Gallenkamp, England).

Calculations and statistical analysis

Calculations of growth parameters Feed intake (FI); Initial body weight(IBW); final body weight (FBW); specific growth rate (SGR) , Feed conversion ratio (FCR) , Protein efficiency ratio (PER), Energy weight gain (EWG) were conducted according to Cho & Kaushik (1985). Data were analyzed by analysis of variance (ANOVA) using the SAS ANOVA procedure (Statistical analysis system 1987). Duncan's multiple range tests were applied to compare differences among individual means. Treatment effects were considered significant at $P < 0.05$. All percentages and ratios were transformed to arcsine values prior to analysis (Zar, 1984).

RESULTS

Water quality

Water temperature ranged from 26.5°C to 28.8°C, dissolved oxygen from 4.2 to 4.5 mg L^{-1}, total ammonia from 0.2 to 0.18 mgL^{-1}, pH from 7.6 to 7.9 and alkalinity from 176 to 180 mgL^{-1}. There were no significant differences in the water quality parameters among the treatments during the whole experimental period. The water quality parameters were within the acceptable range for tilapia growth (Stickney, 1979).

Growth performance

The proximate composition of the experimental diets is shown in Table (2). There was no remarkable variations in the nutrients contents among the different experimental diets. The calculated essential amino acids (EAA) are presented in Table (3) . These values were calculated based on their levels presented in (Table 1), while for wheat bran and corn meal according to NRC (1993).

There were no differences in the initial weights of fish stocked , but the performances differed significantly ($P \leq 0.05$) in terms of weight gain and specific growth rate (SGR). The performance of Nile tilapia fingerlings fed on the experimental diets are summarized in Table (4). All groups of fish fed on the *Yucca schidigera* supplemented diets exhibited excellent growth rates throughout the study. No mortality was observed during the five months of the feeding experiment in all experimental treatments.

At the end of experiment, the fish fed on diets SBM (containing 100% soybean protein) exhibited no significant differences ($P>0.05$) for final body weight (FBW), body weight gain (BWG) or specific growth rate (SGR) compared with fish fed on diet (FMC) (Table 4).

No significant differences in fish growth performance was found between treatments which indicated that partial and total replacement of SBM by LSM in diets resulted in growth and feed performance comparable to SBM diets. The cumulative weight gains of fish fed the isonitrogenous diets containing different levels of linseed were similar, ranging from 159.9g in the control group to139.3g in groups fed on diets when SBM was totally replaced by linseed meal (Table 4). Although fish fed on soybean based diet (control 2), showed higher feed intake (FI) than those partially or totally replaced by linseed. Statistical analysis showed that there was a significant ($P \leq 0.05$) difference in feed intake between all dietary treatments. It appeared that fish appetite was adversely affected by the replacement soybean with linseed in the diets of Nile tilapia . In fact soybean diets seemed to stimulate fish appetite slightly. Feed intake showed the same trend. Replacement of SBM based protein diet for the Nile tilapia also not influenced on feed conversion ratio , so that weight gain produced per unit of food consumed was not lower than control diets (Table 4).

Protein efficiency ratio (PER) value was calculated to assess the effect of differences in protein intake among fish fed on the soybean based diet or replacement with linseed diets. Like FCR value, differences in PER values between all dietary treatments in Nile tilapia were not significant ($P \geq 0.05$) (Table 4), indicating that weight gain per unit of protein intake is nearly the same in all treatments.

Whole body composition:

The whole body compositions of fish at the end of the experiment are shown in Table (5). Results revealed that by increasing the inclusion LSM in diets, body moisture content and fat content increased, while protein content and ash content values were significantly decreased ($P<0.05$)

DISCUSSION

Dietary protein above 24% (Shiau *et al.*, 1987, 1990 ; Cowey & Cho, 1991 ; Tacon, 1993),satisfied the growth requirement of Nile tilapia the actual crude fat (9.34 %) and the actual energy level (4.3 $kcalg^{-1}$) were similar to the respective level of commercial tilapia feed in Egypt. The highest crude fiber content in diet (6) was the result of the high inclusion of linseed meal (LSM) that contained 7.1% crude fiber (Table 2). In addition the fish were fed at a feeding level of 2% of body weight according to Xie *et al.* (1997) who reported that the proportion of gross energy intake channeled into heat production showed the lowest value at an intermediate ration (2%) and was higher at lower or higher ration levels. Also, they concluded that, the decline in feeding efficiencies at higher ration could be caused by: (1) a decrease in apparent digestibility (2) an increase in the proportion of gross energy intake lost in excretory energy and (3) an increase in the proportion of gross energy intake spent in heat production.

As the protein in our diets was largely from plant protein sources, the methionine and lysine content in both soybean and linseed was deficient. The supplementation of diets with constant levels of methionine 0.5% and lysine 0.5% has been done to cover the requirements as recommended by Santiago & Lovell (1988) for Nile tilapia. Despite supplementation of diets with methionine and lysine the energy weight gain was 517.2% for tilapia and SGR 1.01. This can be attributed to that tilapia full utilized efficiently synthetic amino acids. Also, the sparing effects cystine at low level can not be converted into methionine. However, at the high level of cystine the use of methionine was lowered for protein synthesis (Liou 1989).

In addition, other studies indicate that adequate treatments of synthetic amino acids incorporated into diets improves utilization in several fish including common carp (Murai *et al.*, 1981, 1982 & 1983). The studies of Murai *et al.* (1982 & 1984) show that supplementation of deficient protein with adequate amounts of IAA can indeed improve the use of protein sources known to have recognize amino acid imbalances. Viola *et al.* (1992) observed that supplementation of a diet based on plant protein, with lysine improved efficiency of protein utilization and also resulted in a decrease in nitrogenous losses

In Egypt, the feed does not incorporate FM in tilapia feed because of its high cost and fierce price competition. Although our diets had no inclusion of FM except control diet (1) the fish did not grow of slower than the fish fed on diets with a high inclusion of FM .In the study El-Saidy & Gaber (1997&2003), they reported that soybean meal supplemented with 1% methionine only or 1% methionine plus 0.5 % Lysine can totally replace the FM in Nile tilapia diets

Replacement of soybean meal with linseed meal had variable success. In those study in which growth is little reduced, several hypotheses have been suggested to explain the results:1) suboptimal amino acid balance (NRC 1993) ; 2) inadequate levels of phosphorus in linseed meal (NRC 1993) ; 3) Presence of antinutritional factors (including trypsin inhibitors) (Liener 1980); and 4) inadequate levels of energy in LSM (El-Saidy & Gaber, 2001). LSM has one of the best amino acid after SBM and the composition meet the requirements of amino acid for the Nile tilapia . The calculated amino acid contents (Table 3) indicated that all diets met the amino acid requirements of the Nile tilapia (Santiago & Lovell 1988). However, the biological value of amino acid from linseed meal may be less than indicated. El-Saidy & Gaber (2001) stated that methionine availability may be reduced when linseed meal comprises large percentage (>50%) of diet. However, practical diets for Nile tilapia have percentage of LSM higher than 50% could be fed without adverse effect on growth. The use of plant-derived materials as oil seed cake and leaf meal is limited by the presence of a wide-variety of antinutritional substances, especially cassava leaf and linseed meal, due to cyanogens containing feed materials, have generally shown reduced growth when compared to the respective control (Hossain & Jauncey, 1989). However, dietary cyanide did not depress growth in Nile tilapia (Ng & Wee, 1989). These results agree with our present experiment, when Nile tilapia fed on diet totally replaced with linseed meal, we found that, there is no growth depression.

Also, linseed meal contained mucilage, which lower food digestibility in digestive system of fish (Slominski *et al.,* 1999) however the addition of yucca led to overcome these problem and increased availability of linseed meal according to (Johnston *et al.,* 1982) suggested that surfactant properties of components of *Y. schidigera* extract could aid in nutrient absorption.

Body composition change over a growth period is partly attributable to body size and growth rates and partly to dietary factors (Kaushike, 1995). As presented in Table (5) fat content increases with increasing size as well as growth rates as affected by dietary diets and is inversely related to water content (Zeitler *et al.*, 1983 ; Focken & Becker, 1993). Data obtained in our experiment agree with this trend.

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Table (1) : Proximate composition of protein sources used in the experimental diets (% dry matter)

Ingredients	Moisture	Crude protein	Crude fat	Crude ash	Crude fiber	N-free extract
Fish meal	8.0	60.0	8.7	11.0	0.9	11.4
Soybean meal	9.0	44.0	11.8	6.0	6.9	22.3
Linseed meal	9.6	38.0	12.6	9.7	9.5	20.6

Table (2): Ingredients and composition of the experimental diets for tilapia

Ingredients (%)	Diets					
	(1)	(2)	(3)	(4)	(5)	(6)
Fish meal (66%C.P.)	30.0	0.0	0.0	0.0	0.0	0.0
Soybean (44%C.P.)[1]	0.0	40.0	30.0	20.0	10.0	0.0
Linseed (38%C.P.)	0.0	0.0	12.5	26.0	40.0	52.0
Wheat bran	22.5	26.0	28.0	26.0	15.0	15.0
Corn meal	37.5	25.5	21.0	19.5	26.5	24.5
Sunflower oil	5.0	5.0	5.0	5.0	5.0	5.0
Sodium diphosphate	2.0	2.0	2.0	2.0	2.0	2.0
Vitamins. & minerals. premix [2]	1.0	1.0	1.0	1.0	1.0	1.0
L-methionine [3]	0.0	0.5	0.5	0.5	0.5	0.5
L-Lysine [3]	0.0	0.5	0.5	0.5	0.5	0.5
Molasses	2.0	2.0	2.0	2.0	2.0	2.0
Yucca schidigera(mg/kg)	0.75	0.75	0.75	0.75	0.75	0.75
Total	100	100	100	100	100	100
Chemical composition(%):						
Moisture	9.8	9.79	9.47	9.64	9.38	9.64
Crude protein	25.5	25.6	25.6	25.6	25.6	25.6
Crude fat	9.8	9.29	9.34	9.55	9.55	9.53
Crude fiber	6.2	6.31	6.62	6.68	6.71	7.2
Ash	7.3	7.25	6.71	6.79	7.3	7.3
NFE[4]	41.3	41.76	41.26	41.74	41.46	41.73
Gross Energy (kcal/g)	4.3	4.3	4.3	4.3	4.3	4.3

1-Soybean meal: heated at 110°C for sex h and solvent (hexane extracted)
2- Vitamin and mineral premix According to Xie *et al* (1997).
3-L.methionine and L.lysine: dietary methionine and L.lysine (Commercial products)
4-Nitrogen free extract (NFE) = (moisture + crude protein +crude fiber +ash+ crude fat) – 100

Table (3): Calculated level of amino acids contents in the experimental diets and amino acid requirement for Tilapia. (% of dry diet)

Amino acid	Requirements	Diets					
	Tilapia [a]	(1)	(2)	(3)	(4)	(5)	(6)
Arginine	1.33	1.52	1.56	2.18	2.89	3.60	4.16
Histidine	0.54	0.64	0.60	0.69	0.83	0.97	1.06
Isoleucne	0.99	1.13	1.03	1.03	1.42	1.63	1.78
Leucine	1.09	2.03	1.87	2.09	2.50	2.79	3.00
Lysine	1.63	1.67	1.79	1.92	2.08	2.25	2.35
Methionine [b]	1.02	0.64	0.80	0.85	0.91	0.99	1.03
Phenylalanine [b]	1.32	1.05	1.10	1.32	1.60	1.85	2.08
Threonine	1.15	1.0.	1.37	1.58	1.80	1.85	2.22
Valine	1.09	0.81	1.10	1.06	1.04	1.00	0.95
Cystine		0.34	0.44	0.49	0.57	0.66	0.70
Tyrosine		0.51	0.74	0.85	1.00	1.12	1.28

a- According to Santiago and Lovell (1988)
b-The values of methionine and phenylalanine are the requirement in the presence of 2% cystine and 1% tyrosine of the diet, respectively

Table (4) : Growth performance and nutrient utilization of Nile tilapia fed the experimental diets for five months

Parameters	Diets					
	(1)	(2)	(3)	(4)	(5)	(6)
IBW	30.4±1.25	30.6±1.45	30.5±2.56	31.3±1.46	30.8±1.35	30.8±2.76
FBW	160.4±21.2	159.9±19.2	151.8±40.8	147.07±15.1	143.9±18.1	139.3±34.61
SGR	1.16±0.16	1.1±0.06	1.06±0.16	1.03±0.06	1.02±0.12	0.99±0.15
FI	201.14±0.4^{a}	196.17±0.5^{a}	185.04±1.2^{b}	176.5±0.8^{c}	170.17±0.65^{d}	160.94±1.3^{e}
FCR	1.57±0.62	1.54±0.22	1.65±0.56	1.54±0.18	1.54±0.3	1.6±0.57
PER	2.7±0.31	2.64±0.4	2.62±0.58	2.62±0.32	2.66±0.47	2.69±0.8
EWG	628.4±87.8	628.4±87.8	605.5±189.6	575.5±57.2	528.0±45.2	422.8±152.8

a,b,c..Values in the same row with same superscripts are not significantly (P>0.05) differences.
Feed intake (FI); IBW=Initial weight; FBW=final body weight;
specific growth rate (SGR) = (Ln FBW–Ln IBW) /150 days x 100;
Feed conversion ratio (FCR) =dry feed fed/live body weight ;
Protein efficiency ratio (PER) =body weight gain /crude protein fed ;
Energy weight gain (EWG) =energy body weight gain /initial energy body weight X100.

Table (5) : Whole fish body (%wet weight bases) composition (mean ±SD) of Nile tilapia fed diets containing different percentage of linseed meal.

Parameters	Diets					
	(1)	(2)	(3)	(4)	(5)	(6)
Moisture	73.98±3.21^{c}	73.12±1.01^{c}	74.17±1.25^{bc}	75.18±1.11^{ab}	76.04±0.67^{a}	76.69±0.61^{a}
Crude fat	3.71±0.3^{a}	2.64±0.3^{bc}	2.91±0.09^{c}	3.14±0.16^{bc}	3.31±0.07^{b}	3.64±0.64^{a}
Ash	4.5±0.4^{c}	6.02±0.42^{a}	4.65±o.35^{b}	4.9±0.34^{b}	5.02±0.15^{b}	4.51±0.1^{c}
Crude protein	17.3±0.2	17.78±0.42^{a}	17.76±1.04^{ab}	16.49±0.78^{b}	15.4±0.45^{bc}	14.97±0.1^{d}
Energy (kcal/100g)*	125.5±5.3	125.5±5.3	127.9±6.6	122.8±4.5	118.1±3.3	118.8±2.9

*a,b,c....Values in the same row with the same superscripts are not significantly different (P>0.05). *Values in the parentheses are ± standard deviation of means.
*Initial weight, moisture 78 79, protein 12.43, fat 2.09, ash 4.42, energy 90.01 kcal/100g.