Egypt J. Aquat. Biol. & Fish., Vol. 13, No. 2:35 - 52 (2009) ISSN 1110 –1131

Effect of partial replacement of soybean meal protein with dehydrated alfalfa meal (*Medicago sativa* L.) on growth performance and feed utilization of male Nile tilapia (*Oreochromis niloticus* L.) fingerlings reared in tanks.

Nabil F. Abd El-Hakim, Mohsen S. Hussein and Hassaein A. Abdel-Halim.
Al-Azhar University, Faculty of Agriculture, Nasr City, Cairo, Egypt.

ABSTRACT

The present study was conducted to investigate the effect of replacement of soybean meal with alfalfa meat at 0.25.50 and 75% levels on growth performance, feed utilization and the whole body chemical composition. The experiment was conducted in fiberglass tanks belouging to the Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Four dietary treatments were performed, each in three replicates, where male Nile tilapia with an initial weight of 27.50g were stocked in fiber glass tanks 50 fish / tank (1m^3 total volume) and fed on diets contianing 0.25.50 and 75% alfalfameat in replacement with soybean meal. The experiment lasted 16 weeks after start. Fish were fed on the experiment diets at a rate of 3 % of the tank biomass. Results obtained are summarized as follows:

1-Final weight decreased significantly at substitution levels 50 and 75% of Alfalfa while the substitution level of 25% released no significant effects on final weight compared to the control group.

2-The same trend was observed with weight gain daily gain, specific growth rate and condition factor.

3-Final body length, protein efficiency ratio, energy efficiency ratio, protein productive value, total feed intake; protein intake and energy intake were significantly (P<005) decreased as the increase substitution levels of alfafa meal in the diets.

4-Feed conversion ratio was best for control group, followed the group fed 25.0% (T2) alfalfa meal; while groups fed T3 (50%) and T4 (75%) showed the worst feed conversion ratio.

5-Insignificant differences were observed for mortality rate of fishes fed different levels of alfalfa meal or control group.

6-The group fed 25.0 % of alfalfa meal consumed more feed as compared with control or other dietary treatments.

7-Insignificant differences were observed for water temperature, pH value for groups of fishes fed different levels of alfalfa meal, while dissolved oxygen was significantly (P<0.05) decreased by the increase of substitution levels in the diets.

8-Ammonium, nitrite and nitrate concentrations mg/L significantly ($P<0.05$) increased by the increase of alfalfa meal in diets.
9-Crude protein was increased in flesh of fish by the increase of alfalfa meal in the diet; while the opposite trend was observed for ether extract; ash % and gross energy (Kcal/kg) where they decrease as the substitution levels of alfalfa meal increased in the diets.
10-The profit index was higher for group of fish fed (75.0%) substitution level in compared with other treatments including control group.

Key words: Soybean meal, protein, dehydrated alfalfa meal**,** growth performance, male Nile tilapia

INTRODUCTION

Fish nutritionists have tried to use less expensive plant protein sources to partially or totally replace fish meal and soybean meal. The intensive use of soybean meal in poultry and fish feeds led to increasing price of soybean meal with its unavailability (Osman and Sadek, 2004, & Abou-Zead *et al.,* 2008).

In this respect, research efforts have been directed to identify novel; alternative and economically viable plant protein sources for partially or totally replacing soybean meal in the fish feed formulation. One of the possible alternative plant protein sources is dehydrated alfalfa meal.

Male Nile tilapia (*Oreochromis niloticus*) was chosen to carry out study in order that Nile tilapia fish have become a top priority fish for culture in Egypt because of its fast growth, efficient use of natural aquatic foods, propensity to consume a variety of supplemented feeds, resistance to diseases and handling, ease of reproduction in captivity, tolerance to wide range of environmental conditions.

Feed comprises the major cost in fish production and accurate supply of the nutritional requirements increases the efficiency of production (*Sklan et al.,* 2004). Protein is the most expensive component in most fish feeds and accounts for as much as 40-70% of the aquaculture operation (*Pathmasothy,* 1983). Use of relatively expensive feed ingredients for fish production in Egyptian fish farming is limited by the low traditional market value of fish and fish production in such farms. Many studies have been carried out on the substitution of fish meal by alternate less expensive protein sources, mainly from plants, however, due to the presence of some antinutrional factors, most of these ingredients can only be used in tilapia diets after preparatory treatments (*Antoine et al.,* 1987; *Olvera-Novoa et al.,* 1990*; Yousif et al.,* 1994 and *Pouomogne et al.,* 1997).

Studies on green plants leaves as dietary sources for fish have focused on the use of leaf protein concentrates such as rye grass and alfalfa leaf protein concentrate (*Ogino et al.,* 1978; *Olvera-Novoa et al.,* 1990, *Miquel et al.,* 1990, *Omer et al.,* 1994 and *Ali, et al.,* 2003).

The use of leaf protein concentrates as dietary feed ingredients has great potential because of the feasibility of producing good quality protein from tropical and subtropical plant leaves (Nagy *et al.,* 1978*;* Pirie, 1979*;* and Vinconneau, 1979). The alfalfa protein concentrate is a multi-species interacting protein system, since it has solubility characteristics similar to casein and on the basis of its amino acid profile, is of good nutritional quality *(Fiorentini and Galoppini,* 1981 *and Hood et al.,* 1981)*. Miguel et al.,* (1990) concluded that the alfalfa leaf protein could be included at levels of up to 35% of the dietary protein in feeds for tilapia without any adverse effects on survival or appetite.

The growth depression of fish fed diets supplemented with dehydrated alfalfa leaves could be attributed to the presence of growth inhibitors such as trypsin inhibitor which is reported to occur in the vegetative tissues of several plants *(Soto & Mitchell,* 1960*; Humphries,* 1980 *and Omer et al.,* 1994)*. Olvera-Novoa et al.,* (1990) evaluated the use of purified alfalfa leaf protein concentrate (LPC) in diets of *O. mossambicus*. They reported reduced performance at higher inclusion levels of LPC (>45%). To explain this reduction in growth, they suggested to be a deficiency of the essential amino acids, in particular methionine and lysine which are the first limiting amino acids in alfalfa protein concentrate *(Omer et al.,* 1994). As suggested by the same authors, the supplementation of crude alfalfa and Atriplex leaves in diets for male tilapia can not be recommended. *Ali et al.,* (2003) reported that up to 5% level of alfalfa meal could be used in the diets of *Oreochromis niloticus* without any adverse effects on growth performance and nutrient utilization in fish.

The objective of this study was to determine the growth performance, feed utilization efficiency and economical evaluation of male Nile tilapia fed formulated diets containing varying levels of dehydrated alfalfa meal.

MATERIALS AND METHODS

This experiment was performed at a fish culture tanks system with closed water recirculation belonging to the fish farm experimental system, Fish Production Branch, Department of Animal Production Faculty of Agriculture, Al-Azhar University, Nasr City Cairo, Egypt. The experimental Period lasted 115 days (16 weeks) after start. The experiment started at July first, 2004 and lasted to 30th of October, 2004.

Experimental System

The experimental rearing system consisted of a series of twelve rectangular fiberglass tanks each of one cubic meter water volume (1mx2mx0.5m). The experimental tanks represented four nutritional treatments (three replicated) including the control soybean meal diet where dehydrated alfalfa meal contents replaced of 25% (T2), 50% (T3) and 75% (T4) of soybean meal protein.

Experimental tanks

The water supply of these tanks was the drinking tap water derived from the mechanical filter reservoir via a pump to another two fiberglass tanks of 5 m^3 capacity.

The series of the experimental fiberglass tanks are connected together with a tap dechlorinated water supply as well as a drainage system which is connected with the mechanical filter. All experimental series fiberglass tanks were supplied with air through an aeration system connected with an oil free compress on.

Experimental fish and stocking rate:

Nile tilapia (*Oreochromus niloticus*) male fingerlings (sex reversed with hormone treatment) were used in the present study. The fish were purchased from a private hatchery at Kafr-El-Sheikh Governorate, and transported at early morning using a special fish transport car with aeration facilities. Fish were acclimated to the experimental system for 15 days and were fed the control diet as a conditioning period before starting the experiment. Thereafter, the fish were randomly distributed into four groups (3 replicates) representing one of the dietary treatments cited above and stocked in the experimental tanks at a rate of 50 fish/m^3. The average initial weights were 27.50, 27.40, 27.45 and 27.41g/fish (mean±1.14), respectively, and the average initial lengths were 8.0, 8.40, 8.1 and 8.30cm/fish, (mean±0.04), respectively. The experimental period lasted 16 weeks. (115 days) from the start.

Experimental diets

Chemical proximate analysis of feed ingredients used in the present study are presented in Table (1). Four iso-nitrogenous and isoenergetic experimental diets were formulated to provide 25% crude protein and almost. 3235 kcal/kg ME, (metabolic energy) (Table 2). The first diet served as a control diet (T_1) containing protein from soybean meal. The soybean meal protein was substituted at a rate of 25, 50 and 75% protein from dehydrated alfalfa meal (second (T_2), third (T_3) and fourth (T_4) diets, respectively). These diets were compared with the control diet (first diet) in which soybean meal was the sole protein source.

All the experimental diets were prepared by fine grinding of the dietary ingredients. Thereafter all ingredients included in each experimental diet were hand-mixed thorough by, crushed, graded and produced in a pelleted form (0.2cm in diameter) using a mincing machine after mixing with 25% of water. The experimental pellets were sun dried in good storage conditions till the experimental start.

All the experiment diets were tested simultaneously in triplicates. The fish were fed by hand at a rate of 3% of the tank fish biomass twice daily at 9.00 am and 300 pm. Daily, the quantities of food fed were adjusted every two weeks according to the last total fish biomass weighed and counted. Fish were fed for six consecutive days.

Table (1): Proximate chemical analysis of the tested ingredients used in the experimental diets (on DM basis).

Ingredients	DM%	Moisture%	CP%	EE%	CF%	Ash %	NFE%*	GE kcal**	MEKg DM***
Soybean meal	40.57	9.43	44.0	2.1	7.4	6.5	40.0	4580.5	3950
Dehydrated alfalfa	93.04	6.96	21.41	4.57	15.38	16.41	35.27	3065	2921
Ground yellow corn	87.30	12.7	7.7	4.1	2.5	1.3	84.4	4204	4477
Corn gluten	91.26	8.74	60.0	2.9	1.6	2.0	33.5	5035	4442
Wheat bran	88.2	11.8	11.9	3.0	11.0	5.0	69.1	3782	3888
Moles	75.0	25.0	4.4	0.1	-	9.8	60.7	2851	193

* Nitrogen free extract (NFE) =100-(CP+EE+CF+Ash).
** Gross Energy (GE) Kcal/Kg GM;
*** Metabolic Energy (ME) Kcal/Kg DM,

Table (2): Formulation and proximate chemical composition of the experimental diets (on DM basis).

Ingredients / Diets	Diet control 0.0%	Diet 2 25%	Diet 3 50%	Diet 4 75%
Soybean meal	30.0	22.0	15.0	10.0
Dehydrated alfalfa	-	7.5	15.0	20.0
Ground yellow corn	47.5	46.5	44.80	41.0
Corn gluten	12.5	16.30	18.3	20.0
Wheat bran	3.0	4.0	2.4	6.0
Moles	3.0	1.0	2.5	1.0
Min. and Vit. Mixture	0.5	0.5	0.5	0.5
Sun flower oil	2.5	0.8	0.5	0.5
DL-Methionine	0.5	0.5	0.5	0.5
Lystine HCL g/100g DM	0.5	0.5	0.5	0.5
Total	100	100	100	100
Chemical analysis (on DM basis):				
Dry matter %	92.41	90.65	91.36	91.74
Crud protein%	24.85	25.05	24.94	24.99
Ether extract %	8.22	7.75	8.03	8.27
Crude fiber%	4.44	5.64	6.09	6.85
Ash%	9.23	9.07	9.24	9.25
Nitrogen free extract %	52.76	52.49	51.7	49.64
Gross Energy Kcal/kg	429.12	424.73	423.59	417.90
Metabolizable energy Kcal/kg	415.83	411.72	409.92	402.83
P:E ratio	57.91	58.98	58.88	59.80

Diet 1 (control = 100% Soybean meal
Diet 2 = Dehydrated alfalfa replace 25% from Soybean meal Protein.
Diet 3 = Dehydrated alfalfa replace 50% from Soybean meal Protein.
Diet 4 = Dehydrated alfalfa replace 75% from Soybean meal Protein.
Each kg of Min. X vit. Mixt. Content Vit A 12000 IU; Vit. D
NFE= Nitrogen Free extract = 100 – (moisture + Protein +
Gross energy (Kcal/100g) estimated according Jobling (1983) based on 5.7 Kcal/g Protein, 9.5 Kcal/g Lipid and 4.0 Kcal/g carbohydrate.
Metabolizable energy (ME) calculated using the value of 4.5 Kcal/g according to Pantha (1982). Protein 8.1 Kcal/g lipid and 4.5 Kcal/g carbohydrate
*Each kg of mixture contains: Vit. A, 12000 IU; Vit. D32000IU; Vit. E10 mg; Vita k 2 mg; Vit B6 1.5 mg; Vit B12 10mcg; Vit. B2 4mg; Pantothenic acid 10 mg; Nicotinic acid 20 mg; Folic acid 1mg; Biotin 50 mcg; Choline chloride 5mg; Copper 10 mg; Iron 30 mg; Manganese 55 mg; Zinc 55 mg and Selenium. 1 mg.

Growth Performance and Feed Utilization Parameters:

Live body weight and body length:

Live body weight (LBW)g, and body length (BL) cm of individual fish of each experimental tank were measured and registered at the beginning and every two weeks (14 days) during the experimental period.

Growth performance parameters were computed by methods described by *Olvera- Novoa et al.,* (1990) as the following equation:

-Average Total body weight Gain (g/fish) (%):

Total body weight gain was estimated according to the following formula:

$$\text{ATWG (g/Fish)} = \frac{\text{Final body weight} - \text{initial body weight}}{\text{body weight}} \times 100$$

- Average Daily Body Gain (g/fish/day):

$$\text{- ADG (g/fish/day)} = \frac{\text{Average total body weight gain (g)}}{\text{Period (days)}}$$

- Condition Factor (k):

The condition factor value was calculated according to *Lagler (1959)*

$$K = \left[\text{weight (g)/length3 (cm)}\right] \times 100$$

$$\text{Growth rate (GR)} = \frac{Wt - Wo}{T}$$ ***(Hopkins, 1992)***

- Specific growth Rate (SGR): Specific growth rate was estimated according to ***Jauncey and Rose (1982)***

$$\text{SGR (\% day)} = \frac{100 \times (\text{log. Final body weight} - \text{Log. Initial body weight})}{\text{Experimental period (days)}}$$

- Feed Conversion Ratio (FCR):

$$\text{FCR} = \frac{\text{Total feed consumption (dry wt., g)}}{\text{Final body wt. (g)-initial body wt. (Fresh wt.(g)}}$$

Protein efficiency Ration (PER):

$$PER = 100 \times \frac{\text{Final body wt.(Fresh wt. g)-Initial body wt.(Fresh wt., g)}}{\text{Protein intake (g)}}$$

Protein Productive Value:

$$PPV = 100 \times \frac{\text{Protein retained in Tissue (g)}}{\text{Protein intake (g)}}$$

Survival Rate (%) as estimated according to *Hopkins* (1992)

$$SR(\%) = \frac{\text{Total number of fish at the end of the exp.- total No. of fish at the start}}{\text{Total No. of Fish at the start of the exp.}} \times 100$$

Feed intake: Calculated as the amount of consumed feed (g) during the experimental period (day).

Fed efficiency ratio (FER): $\frac{\text{Weight gain (g)}}{\text{Feed intake (g)}}$

Energy Efficiency Ratio (RGR) was estimated as follows:

$$(RGR) = \frac{}{\text{Final weight (g) – initial weight (g)}}$$

Water characteristics

Water temperature, pH, dissolved oxygen, ammonia (NH_3), nitrate (NO_3) and Nitrite (NO_2) were all periodically measured during the feeding trials. Water temperature ($^{\circ}C$) was reassured daily at 8.00 a.m by using a thermometer, while pH was monitored once per day using ORIOW pH /ISE meter, model EA 940 according to official methods of Analysis (1993). Dissoloved oxygen was measured two times a day (8.00 and 16.00 O'clock a.m.,) at a depth of 30 cm with an oxygen meter (WPA 20 scientific Instrument).

Also water samples were taken weekly for analysis of ammonia, nitrate and nitrite. Analytical methods were carried out according to the American Public Health Association (APHA, 1992).

Proximate analysis of fish and experimental diets:

At the start of the experiment, 10 fish were taken and kept frozen for chemical analysis. At the end, the basal diets and a random sample of five fish collected from each treatment were weighed and dried, then grounded before being assayed for moisture, crude protean, crude fat, crude fiber and ash while using standard methods (AOAC, 1990) the proximate chemical analysis of whole- fish body were done. Moisture content was estimated by drying the samples to constant weight at 85°C in a drying Oven (GCA, model 18 EM,) and calculating the weight loss. Nitrogen content was measured using a microkjeldahl nitrogen content by 6.25. Lipid content was determined by ether extraction in multi- unit extraction Soxhlet apparatus for 16 hours and ash was determined by combusting dry samples in a muffle furnace at 550°C for 6 hours. Crude fiber was estimated according to Goering (NFE%) and calculated by difference. Gross energy (Kcal GE/kg) contents of all the samples were calculated according to Jobling (1983).

The cost of feed to raise unit biomass of fish was estimated by a simple economic analysis. The estimation was based on local retail sole market price of all the dietary ingredients at the time of the study (in LE/kg).

At the end of the experiment, an economical efficiency has been conducted to determine the cost of feed & tilapia fingerlings to produce one Kg market fish wet weight according to Miller (1992).

$$EE\% = \frac{\text{Total income (LE)}}{\text{Total cost (LE)}} \times 100$$

Statistical analysis

The obtained date of fish growth. Feed utilization and survival rate, proximate and chemical composition were statisticaly analysed using SPSS computer program version 10 as described by Dytham (1999). Differences between means and comparison between treatments were tested at the 5% probability level using *Duncan test* (1955).

RESULTS AND DISCUSSION

The mean growth response and feed utilization efficiency of mono sex male tilapia fed the experimental diets are shown in Table 3.

The fish fed the control diet (T_1) and 25% of the protein as dehydrated alfalfa protein (T_2) had significantly ($P<0.05$) better weight gain, daily weight gain on the basis of final body weight, specific growth rate, condition factor, feed conversion ratio, survival rate, feed consumption, protein efficiency, energy efficiency ratio, protein productive value and feed efficiency compared with fish fed diets supplemented by increasing the substitution levels of dehydrated alfalfa protein (T_3 and T_4). The lowest results were observed for fish fed diets supplemented with 50 and 75% dehydrated alfalfa. No significant differences were found in average body weight , daily gain, specific growth rate, feed conversion ratio, survival rate, protein efficiency ratio , energy efficiency ratio, protein productive value, feed efficiency and total feed intake of fish fed diets containing 0.0 and 25% of dehydrated alfalfa (T_1 and T_2).

Diets 0.0 and 25% dehydrated alfalfa protein gave the highest value of the growth performance and feed utilization efficiency, statistically better than the 50 and 75% diets . Fish fed diet75% exhibited the lowest mean performance data, suggesting an adverse effect of the protein used. There was a good growth response in all the treatments, but a significant deleterious effect in the fish fed the diets with higher levels of plant protein was recorded, in terms of both growth performance and feed utilization.

Table (3): Effect of partial replacement of soybean meal Protein with dehydrated alfalfa meal on growth performance, feed utilization efficiency and production of male Nile tilapia fed experimental diet (mean ± S.E.)

Treatment \ Diets	T_1 control	T_2 25%	T_3 50%	T_4 75%
Initial bodyweight (g)	27.50±1.21^{a}	27.40±1.32^{a}	27.45±1.03^{a}	27.41±1.0^{a}
Final body weight (g)	112.66±2.38^{a}	112.0±2.66^{a}	88.10±2.71^{b}	82.75±2.31^{c}
Initial body length(cm)	8.0±0.03^{a}	8.4±0.06^{a}	8.1±0.03^{a}	8.3±0.06^{a}
Final body length (cm)	15.7 ± 1.22^{a}	15.4±2.36^{a}	13.4±1.41^{b}	13.3±1.31^{b}
Weight gain (g/fish)	85.16 ± 5.03^{a}	84.60±4.22^{a}	63.65±2.56^{b}	55.34±2.31^{c}
Daily gain (g/fish/day)	0.760 ± 0.17^{a}	0.755±0.31^{a}	0.568±.08^{b}	0.494±0.09^{c}
Specific growth rate (SGR%/day)	6.60 ± 0.11^{a}	7.60±0.1^{a}	5.68±0.20^{b}	4.94±0.22^{c}
Condition factor (K)	2.39 ± 0.91^{c}	2.55±0.87^{a}	2.20±0.7^{b}	2.07±.99^{d}
Feed conversion ratio (FCR)	2.47 ± 0.75^{c}	2.64±096^{b}	3.19±0.88^{a}	3.10±1.04^{a}
Survival rate (%)	90.90 ± 1.24^{a}	90.90±1.28^{a}	89.09±3.13^{a}	90.90±1.99^{a}
Feed consumption (Fc)	211.0 ± 3.13^{b}	224.0±2.87^{a}	203.0±3.33^{c}	172.0±4.12^{d}
Protein efficiency ratio (PER)	1.61 ± 0.62^{a}	1.51±0.66^{a}	1.25±0.36^{b}	1.29±0.28^{b}
Energy efficiency ratio (EER)	11.23 ± 3.32^{a}	10.55±2.16^{a}	8.70±3.11^{b}	8.99±3.0^{b}
Protein productive value (PPV)	51.74 ± 2.08^{a}	64.05±1.39^{a}	41.51±1.09^{b}	41.12±0.99^{b}
Feed Efficiency (FE)	385.34^{a}	279.37^{c}	313.55^{b}	321.74^{b}
Total feed intake (kg/day)	0.221^{a}	0.223^{a}	0.203^{b}	0.172^{c}
Experimental period (days)	112	112	112	112
Protein intake (g)	52.75 ±5.02^{a}	56.0±6.05^{a}	50.75±5.^{a}	43.0±6.02^{b}
Energy intake (Kcal/g)	757.70 ± 2.05^{b}	802.14±3.7^{a}	731.99±7.0^{b}	614.9±5.36^{c}

a,b,c,d, means in each row having the same super script are not significantly different ($P < 0.05$).

Protein efficiency ratio, energy efficiency ratio, protein productive value, feed efficiency , feed intake , protein intake and energy intake from fish fed diets T_1 and T_2 were significantly higher than from any of the other treatments .

Effect of partial replacement of soy been meal protein with dehydrated alfalfa meal on body chemical composition of male Nile tilapia fed experimental diets is shown in Table 4. The crude protein content was variable between treatments. The highest was observed in fish fed 75% dehydrated alfalfa meal diet (T_4), while the lowest was observed in fish fed the control diet (T_1) and diet T_2 supplemented with 25% dehydrated alfalfa meal. The carcass protein content of the control fish was significantly lower than that from any other treatments. The carcass moisture was inversely related to fat content. Similar relationships have been previously reported for tilapia by many workers (Winfree and Stickney, 1981, Jauncey, 1981, Siddiqui *et al*., 1998, Olvera–Novoa *et al*., 1990, Yousif *et al*., 1994 and Ali *et al*., 2003).

Body moisture content of fish increased whereas fat gross energy contents decreased when the level of alfalfa meal in diets was either 0.0 & 25%. The lipid values were higher than expected. These high values may be due to the higher deposition of fat in the fish body prior to the initiation of this experiment (Yousif *et al.*, 1994). No significant differences were however observed in the body moisture, fat and gross energy content of fish fed either 0.0 & 25% of alfalfa meal.

Table (4) Effect of Partial replacement of sybean meal protein with dehydrated alfalfa meal on body chemical composition of male Nile Tilapia fed the experimental diets. (mean ± S.E.)

Parameters	Levels of alfalfa Meal			
	Control	DI (25.0%)	D2 (50.0%)	D3 (75.0%)
Moisture, (MO)%	76.0±0.72[b]	78.30±0.43[a]	76.80±0.70[b]	78.9±0.80[a]
Dry matter, (DM)%	24.0±1.1[a]	21.70±1.06[c]	23.20±0.89[b]	21.10±0.92[c]
Crude protein, (CP)%.	60.8±0.89[c]	61.63±0.91[c]	63.33±0.83[b]	66.83±0.87[a]
Ether extract, (EE)%	16.20±0.56[a]	16.50±0.55[a]	13.0±0.57[b]	11.9±0.59[c]
Ash, %.	15.30±0.17[b]	14.50±0.10[c]	16.0±0.15[a]	14.50±0.16[c]
Gross energy, (Kcal/Kg).	4.110±1.6[a]	3.919±1.1[b]	3,.996±1.0[b]	3.956±1.08[b]

a-d …. means in each row have the different litter are significantly different ($P < 0.05$).

The growth performance parameters and fed utilization efficiency of the fish fed the diets supplemented with different substitution levels of dehydrated alfalfa protein were inferior ($P<0.05$) to that of the fish fed the control diet (T_1). The fish demonstrated decreasing rates of growth performance and feed utilization efficiency as a function of increasing levels of dehydrated alfalfa meal in the experimental diets.

Ali *et al.*, (2003) reported that the decrease in growth performance parameters of male Nile tilapia was linearly correlated with level of dehydrated alfalfa meal in the diet Soto and Mitchell, (1960). Humphries, (1980) and yousif *et al* (1994) indicated that the growth depression of fish fed diets supplemented with dehydrated alfalfa meal could be attributed to the presence of growth inhibitors such as a trypsin inhibitor which is reported to occur in vegetative tissues of several plants such as dehydrated alfalfa. The trypsin inhibitor activity impairs the digestion and absorption of protein (Jauncey and Ross, 1982 and Yousif *et al.*, 1994). Olvera-Novoa *et al.* (1990) and Yousif *et al.* (1994) evaluated the use of purified alfalfa protein in diets for tilapias fish. They reported reduced performance at higher inclusion levels of LPC (>45%). A further cause was suggested to be a deficiency of the essential amino acids, in particular methionine and lysine which are the first limiting amino acid in alfalfa protein concentrate (Yousif *et al.*, 1994)

However, Yousif *et al.*, (1994) reported that the poor growth and low feed utilization of fish at increasing inclusion levels of dehydrated alfalfa meal was probably due to the high content of non-protein nitrogen which amounted to 43.98%.

Oliver-Novoa, *et al*. (1990) Attributed the values for weight gain, SGR, Feed intake and carcass nitrogen deposition were best at low levels (15-35%) of alfalfa protein inclusion. The reduced growth performance at the higher inclusion levels of LPC was probably due to EAA deficiencies, in particular the sulphur – containing amino acids, which are the first limiting amino acids in alfalfa protein concentrates (Fiorentine and Galloping 1981, Hood *et al*., 1981).

Olvera – Novoa *et al*., (1990) found that at high dietary inclusion levels of alfalfa protein, the apparent digestibility was adversely affected. This effect may have been due to the presence of protease inhibitory activity within the concentrates tested. Soto and Mitchell (1960) and Humphries (1980) reported the presence of trypsin inhibitor in both alfalfa meal and protein concentrates. Humphries (1980) found high trypsin inhibitory activity in alfalfa LPC. The presence of this inhibitor may thus have contributed to the growth depression noted in the present study with the diets containing higher levels of alfalfa (T_3 and T_4).

The effect of varying inclusion levels of dehydrated alfalfa on carcass composition was minor. The values for carcass protein were higher than those obtained with other plant protein (Martinez – Palacios *et al*., 1988; Olvera-Novoa *et al*.; Olvera *et al*., 1988; 1990, Yousif *et al*., 1994). Ali etal., (2003) reported that the higher value for carcass protein was observed in fish fed the control diet , where the lowest was observed in fish fed diet supplemented with 30% dehydrated Artiplex leaves .

Ali *et al*. (2003) stated that body moisture content of fish increased whereas fat and gross energy contents decreased when the level of alfalfa meal in diets was either 15 or 20%. No significant differences were however observed in the body moisture, fat and gross energy contents of fish fed either 15 or 20% of alfalfa meal. Olvera – Novoa *et al*. (1990) found that there was a slight reduction in body fat with increasing levels of chloroplastic alfalfa protein, which could have been due to dietary energy reduction, The same authors suggested that the nutitional quality of alfalfa LPC was adequate with respect for fish meal, particularly when cytoplasm protein was used.

Results of water quality parameters measured during the whole experimental period of Nile tilapia reared in tanks and fed different levels of alfalfa meal are presented in Table(5). The average values of water temperature were found to be 24.5; 42.8; 253and 24.0ºC, respectively for groups fed C; Dl; D2 and D3 respectively. The analysis of variance indicated that insignificant differences were detected among control and other dietary treatments for temperature levels. Concerning the pH of water, the values ranged between 7.07 to 7.18. The analysis of variance showed that also insignificant differences among the experimental groups.

As presented in Table (5), the values of dissolved oxygen (DO) were 5.93; 5.83; 4.98 and 4.88 mg/l respectively for the above groups cited before. The analysis of variance indicated that groups fed C and D 1 had a higher DO as compared with D2and D3, although insignificant difference was observed between D2and D3. The results of ammonium (NH3) concentrate indicated that the values had ranged between 0.15 to 0.73 mg/l, where the analysis of variance indicated that NH3 significantly (P<0.05) increased as the alfalfa meal increased in the diet. The results of Nitrite (N02) were 0.l5:0.34; 0.43 and 0.43 mg/l respectively for the above mentioned groups. The statistical evaluation indicated that NO2 significantly (P< 0.05) increased when alfalfa meal increased in the diet, although insignificant differences were observed between D2 ana D3 in their values. The same trend was observed for the nitrate (NO3), since the values had ranged between 0.33 to 0.55 mg/l. It can be noticed that the water quality parameters were within the acceptable levels required for normal growth and physiological activities of Nile tilapia (Boyd, 1990), although there was an increased level in some gases as the levels of alfalfa meal increased.

Table (5) Average of water quality parameters of the rearing units during the experimental period (18 weeks) (Means ± SE)

Parameters	Levels of alfalfa Meal			
	Control	DI (25.0%)	D2 (50.0%)	D3 (75.0%)
Water Temperature (ºC)	24.5±1.26[a]	24.8±1.85[a]	25.0±2.12[a]	24.0±2.0[a]
pH	7.07±0.1[a]	7.18±0.15[a]	7.07±0.16[a]	7.13±0.14[a]
Dissolved oxygen (DOmg/L.)	5.93±3.12[a]	5.83±4.12[a]	4.98±2.8[b]	4.88±3.0[b]
Ammonium (NH3) mg/L.	0.15±0.03[c]	0.52±0.4[b]	0.63±0.5[b]	0.73±.08[a]
Nitrite (NO2) mg/L.	0.15±006[c]	0.34±0.008[b]	0.43±005[a]	0.43±0.007[a]
Nitrate (No3) mg/L.	0.33±0.022[d]	0.38±0.020[c]	0.40±0.017[b]	0.55±0.016[a]

a-d …. means in each row have the different litter are significantly different (P < 0.05).

These results agree with those of Hussein (2004) who found that young Nile tilapia (size from 22 to 25 mm) tolerate temperature range from 16 to 40ºC. Also, Piper *et al*. (1982) showed that the optimum oxygen for growth of all fish species ranged between 5.0 to 8.0 mg/L. Most fishes may live but don`t eat or grow at a level below 4.0 mg/L. The results reported by Huet 1(972) showed that the optimum pH for fish growth ranges between 6.5 and 9.0. High pH inhibits growth or induces mortality, since water tends to alkalinity associated with sodium or potassium rather than calcium (Boyd, 1983).

Economical evaluation of diets

Averages of cost per kilogram of diets, incidence cost and profit index of diet control and groups fed on 25.0: 50.0 and 75.0 of alfalfa meal are presented in Table (6).

Table (6): Economical evaluation of experimental diets inclusion different levels of alfalfa meal

Parameters	Levels of alfalfa Meal			
	Control	DI (25.0%)	D2 (50.0%)	D3 (75.0%)
Cost / kg feed	1.42	1.32	1.20	0.94
Incidence cost*	0.30	0.30	0.24	0.16
Profit Index**	2.25	2.24	2.20	3.10

* Incidence cost = Cost of food consumed / Kg of fish produce.
** Profit index = Price of fish produced / feed cost.

The average cost of diet per Kg gain were found to be 1.42; 1.32; 1.20 and 0.94 respectively for groups fed C; Dl: D2 and D3 .While the incidence cost calculated by dividing the price of fish produced on feed cost were 0.30; 0.30:0.24 and 0.16 respectively for the above groups. The results indicated that both cost of Kg of feed and incidence cost decreased as levels of alfalfa meal increased in the diet, due to amount of yellow corn and soybean meal which were decreased in the diet. However, the best profit index was obtained by fish fed diet fed D3 (3.10), where alfalfa meal was added by 75.0% level. Jauncey and Ross (1982) indicated that some of the most promising alternatives of fish meal are the plant proteins of oilseed Cakes and meals which are by-products of extraction of the greater part of the oil from oilseeds. Most of these cakes are ready available and rich in protein (20-50%). Hanely (1991) reported that because of the high cost of fish meal of Tilapia diets, most studies on their nutrition have been concerned with substitution of this component with lower cost protein sources and byproduct material. Ayman (2004) reported that net revenue was highest in Nile Tilapia when fed diets containing Medicago saliva, followed by the group fed different sources of plant protein. Furthermore, Email (2001) indicated, that economical evaluation revealed that feed cost /kg fish decreased with feeding of 15% *Azolla* meal as compared with control group. However, he indicated also that the economical study indicated that feed cost /kg fish decreased with groups that used berssem meal and mung bean seeds as compared with control group.

Recommendation

Based on the present results incorporation of alfalfa meal to replace soybean meal safety at 25 % level in Nile tilapia diets without any adverse effects on growth performance is recommened.

The potential for the utilization of alfalfa protein concentrates in feeds for fish will be dependent upon the cost of production of this protein source at an industrial level.

REFERENCES

Abou – Zead, M.Y., Soltan; M.A. and Ibrahim, M.S. (2008). Effect of Replacing Soybean meal by sunflower meal in the diets for Nile tilapia, *Oreochromis niloticus* (L.).8th International Samposium on Tilapia in Aquacult., Cairo International convention Center (CICC), Egypt, 2: 787-799.

Antoine, T.; Wery, P.; Micha, J.c. and Van Hove, C. (1987). Comparision de la crossance et de la compostion chimque *d'oreochromis* (Tilapia) *niloticus* L. et de Cichlosoma (Theraps) mealnurum Gth. Nourris avec Azolla. Aquacult., 66: 181 – 196.

Ali, A.; Al-Asgah, N.A.; Al-Ogaily S.M. and Ali, S. (2003). Effect of feeding different levels of alfalfa meal on the growth performance and body composition of Nile Tilapia (*Oreochromis niloticus*) fingerlings. Asian Fish. Soci- 16 : 1-2.

AOAC, (1990). American of Official Analytical Chemists. Methods of Analysis. 15th edn.K.Heldrich. Association of Aquacult. Chemists. Washington, DC. Vol.1 and 2, 1298 PP.

APHA, (1992). American Public Health Association standard Methods for the Examination of Water and Waste water, 22nd ed. Washinton.

Boyd, C. E. (1982). Water quality managent for pond fish culture. Elsevier Scientific Publishing Company, New York, N.Y, 318 PP.

Boyd, C.E., (1990). Water Quality in ponds for Aquaculture Alabama Agricutlural Expeirmental Station, Auburn Univrsity, Alabana.

Duncan, D. B. (1955). Multiple range and multiple F-test Biometrics. 11:1 – 42.

Fiorentini, R. and Galoppini, C. (1981). Pilot plant production of an edible alfalfa protein concentrate J.Food Sci. 46: 1514 – 1517.

Hood, L.L.; Cheng. S. G.; Koch, U. and Brunner, J.R. (1981). Alfalfa proteins:isolation and practical characterization of the major component–fraction 1 protein J. Food Sci., 46 : 1843 – 1850.

Hopkins, K. D. (1992). Rporting fish growth. A review of the basics. J. World Aque. Soc. 23 (3): 173 – 179.

Heut, M. (1972). Text book of culture breeding and cultivation of fish. Fishing News. Limited, Farnham, Surrey, England.

Humphries, C. (1980). Trypsin inhibitors in leaf protein concentrate. J.Sci. Food Agric. 31: 1225 – 1230.

Hussein, M.S. (2004). Effect of Chicken manure and overwintering culture performance of mixed – sex Nile tilapia *Oreochromis niloticus* (L.) and common carp, *Cyprinus carpio* (L.) fingerlings at different stocking densties in earthen ponds. J. Product..& Dev., 9 (1): 17 – 24.

Jauncey, K. (1982). The effects of varying dietary protein level on the growth. Food conversion, protein utilization and body composition of juvenile tilapia (*Sarotherodon mossambicus*). Aquacult., 27: 43 – 54.

Jauncey, K. and Ross, B. (1982). A guide to Tilapia Feeds and Feeding. Inst. Aquacult., Univ. Stirling, Scotland, 111 pp.

Jobling, M. (1983). A short review and critique of methodology used in fish growth and nutrition studies. J. Fish Biology, 23: 685 – 703.

Lagler, K.F. (1959). Fresh Fisheries Biology. 2^{nd} (ed.), Publ. by Dubauque, Kowa, U.S.A. pp. 66 – 85.

Martinez – Palacios, C.A.; Galvan – Cruz, R.; Olvera – Novoa, M.A. and Chavez – Martines, C. (1988). The use of jack bean (*Canavalia ensiformis*: Leguminosae) meal as a partial substitute for fish meal in diets for tilapia (*Oreochromis mossambicus*) (Cichlidae). Aquacult., 68: 165 – 175.

Miguel A. Olvera – Novoa, Silvia G. Campos, Mirna G. Sabido and Carlos A., Martinez Palacios (1990). The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oreachromis mossambicus*). Aquacult., 90: 291 – 302.

Nagy, S.; Telek, L.; Hall, N. T. and Berry, R.E. (1978). Potential food uses for protein from tropical and subtropical plant leaves. J. Agric. Food Chem. 26 (5): 1016 – 1028.

Ogino, C.; Cowey, C.B. and Chiou, J.Y. (1978). Leaf protein concentrates and protein source in diets for carp and rainbow trout. Bull. Jpn. Soc. Sci. Fish, 44: 49 – 52.

Olvera – Novoa, M.A.; Campos G.; Sabido G.M. and Martinez Palacios, C.A. (1990). The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oroachromis mossabicus*). Aquacult., 90: 291– 302.

Olvera Novoa, M. A.; Martinez, P.C.A.; Galvan, C.A. and Chavezs, S.C. (1988). The use of the leguminous plant sesbania grand flora as a partial replacement for fish meal in diets for tilapia (*Oreochromis mossambicus*) Aquacult. 71:51-60.

Osman, M. F. and Sadek, S.S. (2004). An overview of fish feed industry in Egypt. Challenges and opportunities. Expert consultation on Fish Nutrition Research and Feed Techology in Egypt. World fish Culture Center, Abbassa, Sharkia, Egypt, 2^{nd} December, 2004.

Pantha, M.B. (1982). The use of soybean in practical feeds for tilapia (Oreochromis niloticus L) M. Sci. Thesis Univ. of Sterling.

Pathmasothy, S. (1983). A review of feed, their processing and feeding techniques in Malaysian aquaculture system. In: Proceedings of the International Conference on Development and Management of Tropical Living Aquatic Resources. (ed. By H.H. Chan. K. J. Ang. H.M. Ibrahim M.A. Ambak). Pp. 126 – 131. Univ – Pertanian Malaysia. Serdang. Selango. Malaysia.

Pirie, N.W. (1979). Potential for leaf protein as human food. J. Am. Oil Chem. Soc. 56 : 472.

Pouomogne, V.; Takam, G. and Pouemegne, J.B. (1997). A preliminary evaluation of cacao husks in practical diets for juvenile Nile Tilapia (*Oreochromis niloticus*) Aquacult., 156 : 211 – 219.

SAS (2005). Statistical Analysis System, SAS program , ver. 9.1. SAS institute incorporation, Cary, NC 27513 USA.

Siddiqui, A. Q.; Howlader, M.S. and Adm, A.A. (1988). Effects of dietary protein levels on growth, feed conversion and protein utilization on fry and young Nile tilapia, *Oreochromis niloticus*. Aquacult., 70: 63 – 73.

Sklan, D.; Prag, T. and lupatsch, I. (2004). Apparent digestibility cofefficients of feed ingredients and their prediction in diets for tilapia *Oreochromis niloticus* x *Oreochromis aureus* (Teleostei, Cichlidae) Aquacult., Res. 358 – 364.

Soto, J.R. and Mitchell, H.L. (1960). The trypsin inhibitor of alfalfa. J. Agric. Food Chem., 8: 393 – 395.

Vinconneau, H.F. (1979). Procesing of leaf protein into food ingredients. J. Am. Oil Chem. Sol., 56: 469 – 470.

Winfree, R.A. and Stickney, R.R. (1981). Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of *Tilapia aurea*. J. Nutr. 111: 1001 – 1012. M., 1994.

Yousif, O.M.; Alhadhrami, G.A. and Pessarakli, M. (1994). Evaluation of dehydrated alfalfa and salt bush (Atriplex) leaves in diets for tilapia (*Oreochromis aureus L*.).Aquacult., 126:341– 347.