Effect of replacement of fish meal protein with boiled full fat soybean seeds and dried algae on growth performance, nutrient utilization and some blood parameters of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Mixture of boiled full fat soybean seeds and dried algae (*Dunaliella* spp.) at mixing level of 1:1 was tested to replace fish meal protein at 50% (soybean); 50% mixture ; 75% mixture ; 100% mixture and 100% soybean, on Nile tilapia fry of initial weight 1.2 ± 0.05 g. Results obtained are summarized as follows :

1- Incorporation of the mixture of soybean seeds with dried algae in diets of Nile tilapia fry at 50 and 75% levels released no significant effects on final weight average weight gain, daily gain and survival rates, compared to the control groups, while the same parameters were decreased significantly in the other treatment groups.

2- Replacing fish meal protein with the mixture of soybean seeds plus dried algae (1:1) at 50% level had insignificant effects on feed conversion and protein efficiency ratios compared to the control groups, while the same parameters were negatively affected in the other group.

Key words: Nile tilapia, growth performance, algae, survival rates, fish protein

INTRODUCTION

With respect to practical diets, protein is one of the key components when considering the nutritive requirements of any fish species. Fishmeal (FM) is an excellent source of protein and other essential nutrients for aquaculture feeds. The uncertain supply and variable price of FM warrant the investigation of other sources of energy in tilapia feeds The prime targets for cost reduction in formulated diets are the source and level of protein. Replacement of FM and other marine proteins with alternative sources of protein has been encouraged (Tacon & Akiyama, 1997). With suitable considerations of nutrient profiles and palatability, a variety of animal and plant proteins have been used as substitutes for marine protein sources with good success (e.g. Olvera-Nova & Olivera-Castillo, 2000; Forster *et al.,* 2003; Samocha, *et al.*,2004). FM can be replaced either singularly or in combination with plant protein sources without affecting the physical and nutritional quality of the feeds (Viola *et al*.,1988; Samocha *et al*., 2004).

Among different sources of vegetable proteins; soybean meal is the most commonly used as replacement or complement to marine proteins (Olvera-Nova & Olivera-Castillo, 2000). Soybean meal (SBM) is the commonly utilized plant protein in fish feeds, for many reasons, such as its availability, palatability, and its inexpensive cost. SBM is also known for its balanced amino acid profile and high nutritional value (Tacon, 2000; Samocha *et al*., 2004). However, a reduction of the fish growth has been reported when FM was replaced in increasing levels by SBM (Robaina *et al*., 1995; Raky *et al*., 2005) or fish silage (Wassef *et al*., 2003).

Raw SBM contains a wide variety of endogenous antinutrients, which require removal or inactivation through processing prior to usage within aqua feeds (Tacon, 1995). Wilson and Poe (1985) stated that raw SBM or inadequately heated SBM contains highly active trypsin inhibitors that causes growth reduction. Therefore, soybean should be treated by boiling (Raky, 2001; Raky *et al*., 2005) or defatted (Wee & Shu, 1989) or germinated prior roasting (Wassef *et al*., 1988) and prior to inclusion as meals in aqua feeds.

SBM is currently comprised up to 50% of the diet of tilapia. Numerous studies have been conducted using processed soybean as a FM replacement in tilapia feeds, successful trials were achieved by replacing pre-pressed solvent extract as for full fat SBM with or without methionine supplementation up to 75% of FM in feed of O. *niloticus* (Pantha, 1982). Viola *et al*. (1988) studied the limiting factors in SBM for tilapia hybrids (*O. niloticus* x *O. aureus*) reared within outdoor ponds and noticed that fish fed a SBM based diet supplemented with lysine, methionine, lipid and di-calcium phosphate (DCP) had the same performance as fish fed FM based diet (at 100% substitution level) without negative effects on fish growth. However, SBM when compared to FM is characterized by a lower composition of essential amino acids (EAA), mainly methionine, lysine and threonine (NRC, 1993). Methionine is sulphur containing amino acid, plays an important role in the process of metabolism. The addition of methionine to plant protein, which contains low amounts of EAA, is a proper diet for fish feeding (Wang & Shao, 2007).

Single cell protein (SCP) is a term applied to wide range of unicellular and filamentous algae, fungi and bacteria, which can be produced by controlled fermentation processes for use as animal feed. In general, these microbial products are good source of dietary protein, with methionine, being the first limiting EAA within algae (Tacon, 1987).

Tiews *et al.* (1979) carried out extensive research on the yeast, single cell protein and alkaline/petrochemical yeast (*Candida lipolytica*) i.e. Toprina and obtained equivalent growth to FM based rations.

Relatively few studies have been reported on the direct use of dried algae. Single cell protein, *Spirulina maxima*, has been found to have a lower feed value for fish than higher yeast, bacterial single cell protein or FM (Atack *et al.,* 1979). Lu *et al.* (2002) reported that raw *Spirulina platensis* appears to be an effective uni-feed for larval tilapia (*Oreochromis niloticus*) when developing a food chain of phytoplankton-fish for a closed ecological re-circulating aquaculture system (CERAS).Lu *et al.* (2002) added that juvenile tilapia fed *Spirulina* had higher protein content, polar lipid content and lower ash content than fish fed the commercial diet. No significant differences were observed in the mineral and EAA contents in the fish at the experiment's completion.

Appler and Jauncey (1983) with *O. niloticus* indicated that certain dried algae meals (*Cladophora glomerata, Scenedesmus obliques, Chlorella* spp., *Euglena* spp.) may offer particular promise as a partial dietary replacement for FM within rations at relatively low dietary inclusion levels (20% algae, SCP). Relatively few studies have been carried out concerning the use of aquatic plants as feed ingredients in tilapia feeds (e.g. Journey *et al.,* 1990; Wee, 1991; El-Sayed, 1992; Raky, 1995).

Dunaliella is a unicellular, bi-flagellate, naked green alga (Chlorophyta, Chlorophyceae) which is morphologically similar to *Chlamydomonas,* with the main difference being the absence of a cell wall (Borowitzka, 1990). Thus the dried *Dunnaliella* is easily and fully digestible by animals (as fish). The green halophilic unicellular flagellate *Dunaliella salina* Teodoresco is a rich natural source of β-carotene (Borowitzka & Borowitzka, 1988). Under non-inducing non-accumulating conditions *D. salina* cells are green and contain only 0.3% of β-carotene, while under extreme environmental conditions (high salinity, high temperature, high light and low nitrogen supply) concentrations up to 14% of dry weight have been reported and the cells appear orange-red. In general, carotene is greatest under sub-optimal growth conditions when the specific growth rate is low (Borowitzka *et al.,* 1984).

Parsons *et al.* (1961) found that protein was the principal organic constituent of the algal cells of *D. Salina* (57% dry weight of cells).

D. salina is one of the most environmentally tolerant eukaryotic organisms known and can cope with a salinity range from seawater (= 3% NaCl) to NaCl saturation (= 31% NaCl), and a temperature range from <0 °C to >38 °C (Ginzburg, 1987).

The physiology of *Dunaliella,* especially *D. salina* is reviewed in details by Ginzburg (1987). The most commonly used medium for culture of *Dunaliella* is modified by Johnsons medium (Borowitzka, 1988). However, these algae can also be grown in a wide range of other media including Guillard's f/2 medium (Guillard & Ryther, 1962) and enriched seawater (Rao & Chauhan, 1984).

The objective of the present study is to evaluate the effect of the fishmeal replacement with plant proteins (boiled soybean, BSB) supplemented with dried algae to spare the deficiency in some amino acids in Soya, and the subsequent influence on growth and survival of tilapia.

MATERIALS AND METHODS

Algal preparation

Dried *Dunaliella* salina and Bardawil Algae collected from water near salt marshes were used in the present study .The aim of the present work is not only to remove these impurities, but also to make use of them in obtaining a supplemented food for fish. Algal samples were collected during summer time. Algal suspension was filtered through 100µm mesh size net to remove macro zooplankton.

Experimental Diets

1- Experimental system and animals:

Nile tilapia, *Oreochromis niloticus* (L) fry derived from stock ponds, reared at El-Kanater El-Khairia experimental fish farm (30 km. North of Cairo, NIOF, Inland Water Branch, Egypt), were transferred to the Nutrition Laboratory. The fry of initial weight ranged from 1.1 – 1.3 g were stocked thereafter into 12 glass aquaria (100 L each), filled with natural fresh (de-chlorinated) water at a rate of 100 fish/aquaria in duplicate groups for each dietary treatment. Water quality parameters were within the acceptable ranges for tilapia growth throughout the feeding trial (APHA, 1995), and values ranged between 5.1-6.5 mg l^{-1} for dissolved oxygen, 0.06-0.09 mg l^{-1} for nitrites and 0.16-0.29 mg l^{-1} for phosphate.

2- Preparation of Experimental Diets:

A basal (CTR) control diet (35% crude protein and 15.61 mg protein/Kcal) containing fishmeal and dried fish silage (Fagbenro *et al*., 1994 ; Raky, 2001) as the animal protein source (diet,1) was firstly formulated to fulfil the nutritional requirements of the species, particularly amino acids according to Santiago and Lovell (1988). Five experimental diets were formulated, in which fishmeal was replaced by 50% and 100% boiled, full fat, soybean meal (diets, 2 and 6 respectively). A mixture of dried algae (DA) and BSB (1:1 w/w) was used as fishmeal replacement at 50, 75 and 100% levels on weight basis (diets 3, 4 & 5). All diets were formulated to be isonitrogenous and isocaloric in terms of protein to energy ratio.

The ingredients were mixed and supplied as pellets by using California Pellet Meal (CPM) machine. Prior to diet formulation, the proximate composition and amino acid analysis of the ingredients were determined by using Beckman amino acid analyzer model 19 Cl**.** (Tables 1 & 2).

Table (1). Proximate composition of dietary protein sources used in the experimental diets fed to *Oreochromis niloticus* fry

Component	Fish meal (FM)	Boiled soybean meal (BSB)	Dried Fermented Fish silage (DFFS)	Wheat bran
Dry matter	90.70	90.20	96.90	85.80
Crude protein	60.50	38.90	52.90	14.00
Crude lipid	9.80	2.10	12.70	2.50
Ash	19.00	8.00	20.04	1.90
Crude fiber	0.61	4.00	2.16	4.90
NFE*	10.09	47.00	12.20	36.70
Gross energy**	4.34	2.40	4.20	1.03
Metabolized energy***	3.14	1.70	1.02	0.75

* NEF, Nitrogen free extract: 100 – (Crude protein + crude lipid + ash + crude fiber).
** Gross energy = (5.65 x protein %) + (9.45 x Fat %) /100.
***Metabolizable energy: (3.9 x protein % + 8 x Fat %) /100
According to NRC (1993)
a,b,c and d values in the same row bearing different superscript letters are significantly different ($p<0.05$).

Table (2). Essential amino (α- amino nitrogen) acids composition of feed ingredients.

Amino acid (%)	FM	DFFS	BSB	Wheat bran	Dunaliella salina	Dunaliella bardawil
Leucine	5.90	3.86	3.50	0.98		
Methionine	1.35	2.35	0.72	0.27	0.78	0.60
Arginine	5.86	2.79	4.10	0.72		
Isoleucine	4.56	1.90	2.37	0.60		
Phenylalanine	3.65	2.64	2.70	0.80		
Valine	4.81	2.50	0.62	0.68	2.11	1.85
Threonine	3.76	1.42	1.90	0.46		
Lycine	4.50	4.01	3.20	0.45		
Histidine	2.61	1.35	1.31	0.4	0.69	1.20
Tryptophane	0.84	0.40	2.40	0.21		
Total %	**37.84**	**23.22**	**22.82**	**5.57**		

The proximate composition of fish and diets was determined at the start and the end of the experiment according to AOAC standard methods (AOAC, 1995). Formulation of the test diets and the proximate composition are shown in Tables (3 & 4).

Table (3). Formulation (%) of the test diets used for feeding *Oreochromis niloticus* fry for 20 weeks.

Diets	[1]	[2]	[3]	[4]	[5]	[6]
Component Ingredients	Control FM based diet	50% FM+ 50%BSB	50% FM+ 50% Mixture*	25% FM+ 75% Mixture*	100% Mixture* (BSB + DA)	100% soybean based diets
Fish meal normal type (FM)	30.00	15.00	15.00	7.50	0.00	0.00
Dried Fermented Fish Silage (DFFS)	15.00	15.00	15.00	15.00	15.00	15.00
Treated soybean meal (BSB)	15.00	30.00	15.00	18.75	22.50	45.00
Dried Algae (DA)	0.00	0.00	15.00	18.75	22.50	0.00
Wheat bran	30.00	30.00	30.00	30.00	30.00	30.00
Mineral Premix	1.00	1.00	1.00	1.00	1.00	1.00
Vitamine Premix	1.00	1.00	1.00	1.00	1.00	1.00
Sun flower oil	8.00	8.00	8.00	8.00	8.00	8.00
Total (%)	100.0	100.0	100.0	100.0	100.0	100.0

***** Mixture is (BSB) boiled soybean + (DA) dried algae (1:1).

▪ Fermented fish silage was prepared by using culture media of *Lactobacillus plantarum* (Fagbenro, et al., 1994).

▪ Treatment of Soya by boiling for one hour and dried in oven at 48ºC for 20 minutes (Wee and Shu, 1989).

▪ Dried algae: is a mixture of some fresh and marine algae.

▪ **Mineral premix** (g/kg premix): $CaCO_3$: 336, KH_2PO_4: 502, $MgSO_4.7H_2O$: 162, NaCl, 50, Fe(II) gluconate: 10.9, $MnSO_4$: 3.12, $ZnSO_4.7H_2O$: 4.67, $CuSO_4.5H_2O$: 0.62, KI: 0.16, $CoCl_2.6H_2O$: 0.08, NH_4-molybdate: 0.06, $NaSeO_3$: 0.02.

▪**Vitamins premix** (g/kg premix): Ascorbic acid: 10, α-tocoferol: 2.5, Cyanocobalamine: 5, Pyridoxine: 5, Folic acid: 1, Pantothemic acid: 10, Choline Chloride: 100, Biotin: 0.25, niacin: 25, riboflavin: 5, Thiamine: 5, inositol:

Table (4). Proximate composition of the experimental diets used for feeding *Oreochromis niloticus* fry for 20 weeks.

Diets	[1]	[2]	[3]	[4]	[5]	[6]
Component Ingredients	Control FM based diet	50% FM+ 50%BSB	50% FM+ 50% Mixture	25% FM+ 75% Mixture	100% Mixture (BSB + DA)	100% soybean based diets
Proximate analysis:						
Dry matter (%)	90.00	91.20	90.60	92.00	92.00	90.90
Crude Protein (%)	35.00	32.90	32.90	31.30	29.70	29.70
Crude Lipid (%)	2.80	3.30	3.00	2.95	3.10	2.87
Crude Fiber (%)	4.00	4.60	4.11	4.13	4.75	4.81
Ash (%)	8.91	9.80	8.76	8.41	8.40	8.39
Nitrogen free extract (%)	49.29	49.40	51.33	53.41	84.50	54.43
Gross Energy	224.2	217.0	214.4	205.0	197.1	195.0
Protein/Energy ratio (mg protein/Kcal)	15.61	15.70	15.35	15.30	15.10	15.23

NRC (1993) factors , Gross Energy ;= (5.65 × % Protein) + (9.45 × % Fat) / (100) × 1000

Fish were acclimated for two weeks and fed commercial tilapia feed at a daily rate of 3% of biomass, thereafter, each experimental diet was fed to randomly duplicate aquaria. The feeding trial started on May 2^{nd} and lasted for 20 weeks. A fixed feeding regime of 5% of the body weight/day (dry food/whole fish) was employed and fish were fed three times daily in equal proportions, at ambient temperature (mean 27ºC). Feeding was performed for six consecutive days with no food being given on the 7^{th} day when the fish were weighed. The necessary adjustment in the quantity of food intake was carried out at the end of every weighing period. Fish health and mortalities were observed during the feeding period.

3- Analytical procedure:

Over the experimental period (140 days) the growth rate was measured, amounts of food given and mortality were recorded daily. The feed utilization and growth parameters were calculated using the following formulas**:**

Feed conversion ratio (FCR) = feed intake (g) /wet weight gain (g)
Specific growth rate (SGR %) = 100× ln [final weight / initial weight] / days
Protein efficiency ratio (PER) = total weight gain (g) / amount of protein fed (g)
Hepatic protein, lipids and glycogen were measured by the standard methodology of AOAC (1995),

4- Biological parameters:

Hepatosomatic index % (HIS) = 100 x liver weight (g) / body weight (g)Gonadosomatic index % (GSI) = 100 x ovary weight (g) / body weight (g)Gastrosomatic index % (GI) = 100 x Gut weight (g) / body weight (g)

5- Hematological parameters:

Blood was collected using heparinized syringes from the caudal peduncle of 10 randomly sampled fish from each treatment at the end of the experiment. Haematocrit (HI) was determined according to Baker, *et al.* (1966). Blood was centrifuged at 3000 rpm for 5 minutes to allow separation of plasma which was subjected to determination of (PTP) plasma total protein (Protein-Kit produced by Bio Merieux, France). Glucose (glucose-enzymatic PAP-Kit produced by Bio Merieux Vitek Inc., USA), Glutamic Oxaloacetic Transaminase (AST) and Glutamic Pyruvic Transaminase (ALT) were determined using Reitmen and Frankel (1957) method.

6- Statistical Analysis:

The data were subjected to one way analysis of variance (ANOVA) to test the significance among treatments. Duncan multiple range test was applied to rank treatments means ($P < 0.05$). All statistical analyses were performed with SAS software system, Version 6 for Windows (SAS Institute, Inc., Cary, North Carolina, USA, 1990).

RESULTS

1-Growth performance and feed utilization

After 20 weeks of the experiment , results of Table (3) show the effect of fish meal based diet FM ; the 50% FM + BSB ; 50% FM + 50% Mixture ; the 25% FM + 75% Mixture ; 100% Mixture and 100% BSB based diet on growth performance of Nile tilapia . Results of the same Table revealed that the average of initial weights was 1.2 g. in all experimental groups which indicate the complete random distribution of fish into experimental groups.

As presented in Table (6) groups fed FM base diet; 50% FM + 50% Mixture and 25%FM +75% Mixture had significantly (p< 0.05) higher final weights followed in a decreasing order by 50% FM + 50% BSB; 100% Mixture and 100% BSB groups, (30.6 g., 31.1 g. & 28.9) respectively.

The two diets showed; the best SGR (2.33% and 2.27%, respectively), without significant difference from the CTR group (2.31%);and showed also higher survival rates, protein efficiency ratios and protein productive value (PPV); and the optimum food conversion ratio. Fish fed the diet (3) exhibited the best results of growth and feed utilization followed by diet (4), as shown in Table (5).

Table(5). Growth parameters, nutrients utilization ratio for fish fed experimental diets reared in aquaria for 20 weeks.

Items \ Test Diets	[1] Control FM based diet	[2] 50% FM+ 50%BSB	[3] 50% FM+ 50% Mixture*	[4] 25% FM+ 75% Mixture*	[5] 100% Mixture* (BSB + DA)	[6] 100% soybean based diets
Initial weight (g)	1.2 ±0.05	1.2 ± 0.05	1.2 ± 0.07	1.2 ± 0.1	1.2 ± 0.04	1.2 ± 0.02
Final weight (g)	30.6 ±1.02 A	25.7 ± 0.9 B	31.1 ± 1.02 A	28.9 ± 1.0 A	24.6 ±0.81 B	15.1 ± 0.5 C
Mean weight gain (g)	29.4 ± 1.3 A	24.5 ± 1.0 B	29.9 ± 0.95 A	27.7 ± 0.8 A	23.4 ±0.75 B	13.9 ±0.62 C
Mean daily weight gain (g/f/day)	0.21 A	0.175 B	0.214 A	0.198 A	0.167 B	0.099 C
Specific growth rate (SGR %)	2.31 A	2.19 B	2.33 A	2.27 A	2.16 B	1.81 C
Survival rate (%)	90 A	85 B	92 A	88 A	80 B	65 C
Protein effeciency ratio (PER %)	1.88 A	1.74 B	1.87 A	1.80 B	1.63 C	1.30 D
Food conversion ratio (FCR %)	1.40 C	1.56 C	1.46 C	1.52 C	1.86 B	2.62 A
Protein production value (PPV)	18.57 A	11.55 B	18.24 A	18.05 A	7.27 C	5.39 C

* Mixture is (BSB) boiled soybean + (DA) dried algae (1:1).

* Dried algae: is a mixture of *Dunaliella salina and Dunaliella bardawil*

* Protein productive value = Final body protein – initial body protein / crude protein intake x 100

a,b,c and d values in the same row bearing different superscript letters are significantly

2- Body composition

Averages of proximate muscle and liver composition as affected with the dietary treatments are presented in Table (6). Results revealed that the highest crude protein percentage (DM bases) were recorded (P < 0.05) by diets 1,3 & 4, followed in decreasing order by diets 2, 5 & 6. On the other hand, diet 6 showed the highest lipid contents in muscle and liver followed in a decreasing order by diets 3; 5; 2; 4&1 respectively . As presented in the same Table, ash percentages fluctuated between 21.00 % (diet 5) and 19.71% (diet 1) with significant differences among the dietary groups. Similar trend was observed in moisture percentage, where groups fed diets 4 and 5 showed the highest moisture contents compared to diets 1, 2, 3 and 6.

Table (6). Liver and muscle composition of *Oreochromis niloticus* fry fed the experimental diets (% Fresh weight basis) at beginning and at the end of the experiment (20 weeks).

Items	Initial	After 140 days					
		Diet (1)	Diet (2)	Diet (3)	Diet (4)	Diet (5)	Diet (6)
Carcass Composition:							
Crude Protein	56.00	62.50 A	58.80 B	62.00 A	61.65 A	57.11 B	54.00 C
Total lipid %	15.90	15.80 C	17.50 B	18.91 B	16.12 A	18.18 B	22.44 D
Ash %	20.00	19.71 B	20.20 A	19.80 B	20.86 A	21.00 A	20.64 A
Moisture %	72.13	74.10 C	74.90 B	75.80 A	76.00 A	76.11 A	75.91 A
Liver metabolites:							
Crude Protein (%)	18.07	20.16 A	18.38 C	20.09 A	19.91 A	19.07 B	17.20 C
Total lipid (%)	11.80	10.72 C	11.30 B	10.83 B	11.00 B	11.80 A	11.81 A
Hepatic glycogen (%)	1.85	2.16 A	1.96 B	2.00 A	1.96 B	2.13 A	1.95 B

a,b,c and d values in the same row with different superscript letters are significantly different ($P<0.05$).

* Muscle gross energy (K cal/100g muscle) by NRC (1983) = 5.7 x % crude protein + 9.5 x % crude lipid. (Kj = 4.186 K cal)

3-Biological properties

Averages of biological indices as affected with dietary treatments are presented in Table (7) .Results revealed that the highest hepatosomatic percentages were recorded (P<0.05) by diets 1, 4 and 3, followed in decreasing order by diets 2, 5 and 6 respectively. Similar trend was observed in gonadosomatic index, where groups fed diets 2 and 5 showed the lowest index, compared to diets 2, 4,3 and 1 respectively. On the other hand, diets 6 an d5 showed the highest gastrosomatic index followed in decreasing order by diets 3, 2, 1 and 4 respectively.

4 - Haematological parameters

One of the most sensitive means of evaluating liver function is measuring of appropriate serum parameters AST and ALT; haematological parameters are shown in Table (7). Diets 6 and 5 showed the highest AST, ALT (258; 36 and 250; 30) respectively and the lowest AST values recorded in diet 3 followed by diets 4 and 2 .

Values recorded in Table 8 were reported for fish fed on diets 1, 3, 2 and 4 respectively. However, the highest values of AST/ALT ratio were recorded for the fish fed diets 6 and 5 respectively with insignificant difference compared with control groups as presented in the same table. Plasma total protein (PTP) fluctuated between 5.19 g/dl diet (4) and 4.1g/dl diet (6) in significant differences among the dietary groups.

Similar trend was observed in groups fed diets (3) and (4) with the highest blood glucose contents compared to 1, 2, 5 and 6. Results revealed that the highest hematocrit (HI) g/dl were recorded ($p<0.05$) by diets 1,3 and 4, followed in decreasing order by diets 2,5,and 6 respectively.

Table (7). Biological and Haematological parameters of *Oreochromis niloticus* fry fed test diets for 20 weeks.

Parameters	Diet (1)	Diet (2)	Diet (3)	Diet (4)	Diet (5)	Diet (6)
Biological indics						
Hepatosomatic index (HIS %)	2.5 *A*	2.1 *B*	2.4 *A*	2.5 *A*	1.95 *B*	1.86 *B*
Gastrosomatic index (GI %)	5 *B*	5 *B*	5.1 *B*	4.67 *B*	5.8 *A*	5.93 *A*
Gonadosomatic index (GSI %)	3.32*A*	3.87 *A*	3.62 A	3.76 *A*	3.15 *B*	3 *B*
Haematological Parameters						
Hematocrit (HI) g/d l	46.6*A*	42 *A*	43 *A*	44 *A*	38.9 *B*	37 *B*
Plasma total protein (PTP)g/d l	4.6 *A*	4.4 *B*	4.8 *A*	5.19 *A*	3.97 *B*	4.1 *B*
Glucose mg/d l	79 *B*	82.2 *B*	88.7 *A*	87.4 *A*	76.9 *B*	69.2 *C*
Glutamic oxaloacetic Transaminase (AST) µ/d l	250 *A*	239. *B*	226 *C*	230 *C*	250 *A*	258 *A*
Glutamic pyruvic Transaminase (ALT) µ/d l	26 *B*	27 *B*	26.3 *B*	28 *B*	30 *B*	36 *A*
AST/ALT	9.62	8.86	8.6	8.21	8.33	7.17

A,B,C and D values in the same row bearing different superscript letters are significantly different ($p<0.05$).

This may be due to the deficiency of one or more of the limiting amino acid in diet (6) as previously reported. On the other hand, the lowest values (226.2 & 26.3 µ/d l) obtained from those fed diet (3) with the highest body weight (31.1 g/fish). A gradual replacement of FM by the mixture (BSB + DA) showed highest level of plasma AST & ALT. Lemaire *et al*. (1991) reported that a rise in the activity of serum GPT might suggest a defect in liver function. AST/ALT ratios were within normal values, that ranged from a maximum of 8.86 (diet 2) to a minimum of 17.7 (diet 6).

Haematocrit and plasma protein levels tended to decrease in fish fed diets (5) and (6) in which the highest levels of the mixtures (BSB + DA) or boiled Soya based diets were incorporated, the lowest (3.97 g/dl) was of diet (5). Fish fed diet (4) obtained highest values of HI and PTP (44 &5.19 g/dl, respectively).

DISCUSSION

The results proved that partial replacement (50% and 75%) of FM with a mixture of BSB and DA (diets 3 & 4) produced nearly similar growth performance to those fish fed diet (1) (control group). These results may be due to the fact that algal meal (DA) and boiled Soya meal (BSB) had better palatability and better amino acids profile

The current study showed that up to 50% of the FM in a practical diet, for tilapia fry, could be effectively replaced by a mixture of BSB and DA without a significant reduction in growth performance. These results are in agreement with those of Viola and Zohar (1984), who showed that the bacterial single cell protein "Pruteen" (SCP) successfully replaced 50% of the FM protein in diets for hybrid tilapia (*O. niloticus* x *O. aureus*).

Fish fed diets (5 & 6) exhibited the lowest survival rate, PER (1.63 & 1.3, respectively) and PPV (7.27 & 5.39), differ significantly from the same values of 1.86 & 2.62, respectively. However, fish fed diet (5) in which the FM was completely replaced with the mixture (BSB + DA, 1:1 w/w) showed better results than those fed diet (6) with 100% SBS (Table 8). Deficiencies or excess of one or more of essential amino acids are known to limit protein synthesis, and/or growth, EAA deficiency has been widely demonstrated to reduce feed intake (Murai, 1992). Parameters of control group, and FCR showed highest value compared with CTR group. Thus the results in Table (2) revealed that the percentages of lucine, methionine and valine in soybean were lower than those of other ingredients tested. The present results showed the sensitivity of tilapia fry to the shortage in some α-amino nitrogen contained in Soya. Fry fed 100% soybean (diet 6) produced the lowest growth performance (SGR% 1.81) and the highest FCR% (Table 8). This finding is in agreement with that of Hung (2004). The reduced growth performance for fish fed diets (5 & 6) might be attributed to several contributing factors: (a) a dietary imbalance as defined by a deficiency of one or more limiting amino acids in the plant protein (e.g. Methionine, Valine, Histadine) and/or (b) the presence of various antineutrients in Soya. These factors cause either a reduction in the efficiency of protein utilization, FCR, PER, PPV as previously proved by Atack *et al*. (1979) and Raky (2001) and/or alternatively leading to a depression in daily weight gain (Raky 2001). The present results partially agree with those obtained by Gómez-Requeni *et al.* (2003) and Gómez-*Requeni et al.* (*2004*) who noticed that a reduction in indispensible amino acids such as lycine, in experimental diets produced a lower level of these amino acids in muscle of gilthead sea bream (*Sparus aurata* L.). These results are similar to those reported by Yamamoto *et al.* (2002), who replaced all FM in diets for rainbow trout and observed that fish fed diets based on FM had higher lysine content in their bodies than fish fed soybean meal diets. Martínez-Llorens *et al.* (2007) reported the possibility of feeding sea bream weighing less than 80 g with 30% soybean meal, and for fish weighing more than 80 g, a 50% dietary soybean meal can be used until the fish reach commercial weight, with no negative effects on growth or feed efficiency. Nevertheless, when sensory analysis and economic aspects are considered, the maximum inclusion level of soybean was 20-22%.

On the other hand, Bonaldo *et al.* (2006) determined the effect of three isoproteic and isolipidic extruded diets containing 18 and 30% of soybean for 87 days on growth performance and nutrient utilization of Egyptian sole (*Solea aegypttiaca*). They reported that no differences in performance or nutrient retention were found. Based on their results, fermented soybean meal (FSBM) seems to be a good protein source for Egyptian sole and can be added in the diet up to 30% without any reduction in growth rate. In contrast, Kaushik and Luquet (1980) found that 80-100% replacement of FM with various SCP sources was possible in experimental diet for rainbow trout (*Salmo gaudneri*).

Refstie *et al.* (1999) observed negative effects from low protein soy products (deficiency in some EAA) on the digestibility of nutrients in Salmon and concluded that the negative effect on feed utilization was probably an effect of the viscosity caused by the non-starch polysaccharides in soybean products. There is a significant interaction between methionine and choline to tilapia (Kasper *et al.*, 2000). Oxidation of choline to betaine is considered a one-way reaction in vertebrates and there is no evidence that tilapia is able to synthesize choline from betaine, and then we might speculate that methionine contributed to choline biosynthesis in our study

Similar to the present results, Liener (1994 a & b) stated that protein inhibitors include both Trypsin and Chemotrypsin addition, Makkar and Becker (1999) added that there were no significant differences between the growth performance of common carp fed diets containing raw and heated *Jalropha* meal (25%) in diets with high level of trypsin inhibitor. Significant growth reduction in common carp (*Cyprinus carpio* L.) was observed by Siddhuraju and Becker (2001)) and phytohaemaglutinins particularly in legumes, are considered as potential antinuntrients and are known to decrease the growth performance of animals. Even though the mucuna samples (raw and autoclaved) plant source used contained a sufficient quantity of essential amino acids, the complex formation in the oxidized product of phenolic non-protein amino acid, 3, 4-dihydroxyphenylalanine, with peptide sulphur amino acids (Siddhuraju *et al.,* 2000) may reduce the availability of cystein and methionine in the diets. These two factors might also have been involved in the reduction of growth performance and feed utilization. A reduction of the carcass crude protein content in tilapia with respect to higher inclusion of sesbania seed meal in the diets was reported by Olvera-Novoa *et al*. (1990). However, interestingly, results of the present study revealed no significant variation between those fed the control diet (1) and different inclusion levels of BSB with DA addition. Robaina *et al*. (1995) stated that phytic acid has been implicated for the lower availability of phosphorus in gilthead seabream fed diets with 30% heated liver. Higher lipid deposition and lower glycogen level in liver content was found with fish fed diet

(6) which contains 100% BSB (Table 3). This result is in agreement with Abdelghany (2003) who reported that soybean flour (SBF) has good potential, as a substitute protein source, for up to 75% of herring fishmeal (HFM) in red tilapia diets with no significant ($P > 0.05$) effects. Adverse effects on growth, feed efficiency, body composition, and the growth performance were obtained with fish fed diet contained 50% HFM and 50% SBF. The author also added that, lipid content was significantly higher ($P < 0.05$) in fish fed diets in which SBF substituted HFM for 50% or more and hematological values of test fishes were within normal values, which were also proved in the current study (Table 10). Cañavate *et al*.,(2007) reported that growth of Senegal sole larval and metamorphic stages was not affected by supplementing the diet with *D. salina* as a dried algae and did not affect antioxidant biomarkers; malondialdehyde (MDA) which was the only biomarker whose activity was significantly reduced when *D. salina* was supplemented to the larval rearing tanks. These results are evidence of the antiperoxidative effect of β-carotene from algae in the larval rearing process of fishes. BSB (diets 5 & 6, respectively) obtained highest gut index (5.8 & 5.93, Table 7). The fish in these groups also showed the lowest hepatosomatic (1.95 & 1.86) and gonadosomatic (3.15 & 3.0, respectively) indices. In this respect, Santiago and Lovell (1988) reported that gonadosomatic indices of Nile tilapia fed diets containing 0, 20, 40 and 80% Leucaena leaf meal were not markedly affected by the diets.

CONCLUSION

The present study confirms the boiling technique of raw soybean as proper treatment inclusion-Nile tilapia feeds, also the mixing with single cell protein (SCP), (marine and freshwater algae) which contain higher percent of Methionine, Valine and Histadine for supplying the shortage of these EAAS in the treated Soya. From the results of the present investigation it can be concluded that up to 50 % treated Soya and dried algae can be incorporated safely and economically in diets of Nile tilapia.

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