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Dietary Supplementation of Spirulina, *Arthrospira platensis*, With Plant Protein Sources and their Effects on Growth, Feed Utilization and Histological Changes in Nile Tilapia, *Oreochromis niloticus*

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Abstract

This experiment was designed to assess the effect of alternative plant protein sources (soya bean meal (SBM), corn gluten meal (CGM), distiller dried grains (DDG)), with or without spirulina (*Arthrospira platensis*) supplementation, on growth, feed utilization, body composition, histological (liver and intestine) changes of Nile tilapia, *Oreochromis niloticus*. A total of 180 Nile tilapia, *O. niloticus*, fingerlings (3.78 ± 0.02 g) were assigned to six treatments (three replicates each, 10 fish each), and were stocked in 100 L glass aquarium. Fish were fed with six experimental diets using SBM, CGM, and DDG with or without spirulina supplementation at dose of 0.5%. The feeding experiment lasted for 84 days. The results indicated that tilapia fed on SBM based diet with or without spirulina supplementation have significantly improved growth performance, survival, feed conversion ratio and nutrient utilization compared to other treatments. Serum protein profile showed an increase of significant globulin with SBM and *A. platensis* supplemented diets. Moreover, the SBM based diet alone or supplemented with *A. platensis* improved both liver histopathological featuring and glycogen contents, and increased intestinal villi length and area of absorption values. It is recommended that SBM should be used as plant protein source, supplemented with 5 kg^{-1} diet spirulina in Nile tilapia diet.

Keywords: Alternative protein source; *A. platensis; Oreochromis niloticus*; Growth; Nutrient utilization; Liver and intestine histology

Introduction

Tilapia is a highly popular aquaculture product for its fast growth rate and ability to grow in extremely diverse and adverse conditions, so it is considered the most intensive cultivated freshwater fish in Egypt [1]. Tilapias are therefore likely to occupy a higher rank in global aquaculture production next to carp production. According to [2], the attributes that make tilapia as an ideal candidate for aquaculture production, especially in developing countries, are: rapid growth (as omnivorous fish can use high proportion of inexpensive plant sources in their feeds), high survival rate in a wide range of environmental conditions (Such as temperature, salinity, low dissolve oxygen, etc.), resistance against stress and diseases, short generation interval, low supplementary feed required in natural environment and the ability to use the commercial feed immediately after yolk-sac absorption.

With the continuous growth of tilapia production, the need for suitable diets of local ingredients produced within each country has become a necessity. Feed is the largest expenditure item in both semi-intensive and intensive fish cultures, as it stands for about 30% to 70% of the total operational cost in a fish farm [3]. The replacement of fishmeal with locally available cheaper plant feedstuffs is proved to be very essential for the future development of aquaculture sector [4]. For culturing fish in captivity, nothing is more important than sound nutrition and adequate feed. Tilapia, as an economic product, requires minimum fish meal [5], and Nile tilapia, in particular, is naturally accustomed to eating plant ingredients [6]. Such plant protein is not only considered cheaper than fish meal, but it also enjoys high availability and may contain valuable antioxidants as well [7].

A substantial amount of research has been underway for testing potential protein sources that can replace fish meal in tilapia diets. Alternative protein sources include: soybean meal (SBM) [8], corn gluten meal (CGM) [9] as these feed stuffs have good palatability and high nutritional quality. However, the major problem confronting fish farming industry is the increasing cost of feed ingredients in the local market. Therefore, the selection of ingredients for the formulation of fish feed is very crucial as they should be cheap and available in the local market [10]. Soybean meal appeared to be better utilized by most fish species due to their nutritional quality, lower cost and high availability, as compared to other plant protein sources [11]. Soy protein is considered the best plant protein source for meeting the essential amino acid requirements of tilapia and other fish species [12]. It is highly digestible by fish, and their digestion coefficients are comparable or even higher than those of fish meal protein [13].

Corn gluten meal (CGM) was found to be a suitable protein source in a fish meal-free diet if the proper amino acid balance was maintained [14]. An additional protein source available for aquaculture feeds is distillers dried grains with soluble (DDG). Due to the relative availability, low cost, and adequate nutrient composition, DDG may be an inexpensive protein supplement to provide lower-cost diet formulations, especially for omnivorous species [15].

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The use of feed additives has proven to be a successful application in aquaculture without adverse effects on the environment as the using of antibiotics [16]. Algae have emerged as a feed additive source for its content of protein, lipid, carbohydrate and high amount of carotenoids with antioxidant property [17]. Spirulina has been used at a low-level as feed additive in order to improve the taste, texture and color of the fish. Moreover, it is recommended for its positive effects on growth, feed utilization, physiological condition, stress and disease resistance [18]. Spirulina, *Arthrospira platensis*, is a freshwater blue green filamentous alga that contains protein (60- 70%), vitamins, minerals and essential fatty acids such as palmitic acid, linolenic acid and linoleic acid. Hence, it has been used as a nutrient for fish larvae [19] and as an ingredient in fish diets for juveniles and adults [20].

Also, it can partially replace fishmeal protein in fish feeds, and can be manipulated to produce essential amino acids, vitamins, natural B-carotene and antibacterial substances of better quality and quantity. Some reports showed improved growth activity with the inclusion of spirulina in fish diets [21]. Therefore, the aim of the present study was to investigate the efficiency of using SBM, CGM, DDG as plant protein supplement with or without spirulina, *Arthrospira platensis,* on growth, feed utilization, body composition and some histological changes of Nile tilapia, *Oreochromis niloticus* and fingerlings.

Materials and Methods

Experimental fish and raring facility

A total of 180 apparently healthy Nile tilapia, *Oreochromis niloticus* and fingerlings with an average initial body weight of 3.78 ± 0.02 g $fish^{-1}$ obtained from a private commercial freshwater fish farm in Motobas, Kafr-El Sheikh Governorate, Egypt. Fish were kept in circular fiberglass tanks at Fish Nutrition Laboratory, Department of Animal and Fish Production, Faculty of Agriculture (Saba Basha), Alexandria University as an acclimation period for two weeks. Fish were fed on diet contained 30% crude protein prior to the start of the experiment. Afterwards, fish were randomly placed in eighteen glass aquaria (six treatments each with triplicates) with dimensions of 100 × 40 × 30 cm each, and 100 L water volume $aquarium^{-1}$ at a stocking density of 10 fish per aquarium. The daily water exchange rate was 30%, and excreta were removed by manual siphoning. All water quality parameters were maintained at acceptable range for *O. niloticus*.

Experimental design and diets

Six isonitrogenous (31.05%) and isocaloric (1891 kj 100 g^{-1} DM) experimental diets were formulated and introduced to the fish in a completely randomized design. The diets were formulated using SBM, CGM, DDG as plant protein with or without spirulina (SP), *Arthrospira platensis* supplementation at dose of 0.5%. The experimental diets were prepared by grinding all the ingredients and thoroughly mixing them with the other dietary ingredients, vitamins and minerals mixture. Spirulina was added to respect diet (0.5%), then warm distilled water (35°C) was slowly added until the diets began clumping. The diets were then extruded by a mill machine and dried in an electric-oven before storage in plastic containers at -20°C. The resulting pellet size was 0.6 mm in diameter and 2 mm in length. Fish were fed one of the experimental diets at rate of 3% of live body weight two times a day for 84 days.

Measured Parameters

Growth and nutrient utilization

The final body weight (FBW) of each experimental treatment was determined by dividing the total fish weight (weighing each individual separately) in each aquarium by the number of fishes. The weight gain (WG), average daily gain (ADG), and specific growth rate (SGR (%), survival rate (%), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV (%), energy gain (ER (%), and energy utilization were calculated using the following equations:

$$\text{WG (g fish}^{-1}) = W_2 - W_1$$

where W_1: Initial weight of the fish (g), and W_2: FBW of fish (g).

$$\text{ADG (g fish}^{-1}\text{ day}^{-1}) = (W_2 - W_1)/n$$

where W_1: Initial weight of fish (g); W_2: FBW of fish (g); and n=days.

$$\text{SGR (\% day}^{-1}) = 100 \times (\ln W_2 - \ln W_1)/\text{days}$$

Where ln is the natural log. Survival (%) = 100 × (Final number of fish/initial number of fish).

$$\text{FCR} = \text{feed intake (g)/weight gain (g).}$$

$$\text{PER} = \text{WG (g)/protein intake (g).}$$

$$\text{PPV (\%)} = \text{Protein gain (g)/protein intake (g)} \times 100.$$

$$\text{EU (\%)} = 100 \times (E_T - E_I)/\text{energy intake (kj)}$$

where E_T: Energy in fish carcass (kj) at the end of the experiment, and E_I: energy in fish carcass at the start of the experiment.

Diets and whole-body proximate chemical compositions

The diets and the chemical compositions of whole-body fish samples were assessed according to procedures [22]. Approximately five fish per treatment were used in the analysis. Dry matter (DM) was determined after drying the samples in an oven (105°C) for 24 h, and ash was measured following incineration at 550°C for 12 h. Crude protein was determined by the micro-Kjeldhal method, with N% × 6.25 (using a Kjeltech autoanalyzer, Model VELP Scientifica, UDK 127), and crude fat was assessed by Soxhlet extraction (Model VELP Scientifica, SER 148) with diethyl ether (40-60°C). Crude fibre (CF) was determined after digestion with 5% sulfuric acid and 5% sodium hydroxide for 15 min; the residues were then dried and ashed. The nitrogen-free extract (NFE) was calculated using the following equation: NFE = 100 – (CP+EE+CF+ash).

Serum protein profile

Blood was collected from the caudal vein of previously anaesthetized fish (50 mg clove oil L^{-1}) using a sterile syringe. Blood samples were taken from six fish per treatment and kept for cloating in refrigerator for four hours, then centrifuged (1075× *g*, 10 min., 4°C) to obtain serum. The serum samples were stored at -80°C until used in the biochemical assays.

The total protein (g dL^{-1}) was determined in plasma samples of fish from the different experimental groups by the Biuret method according to Bardawill et al. [23]. Albumin (g dL^{-1}) was determined by the bromocresol green method [24] and globulin (g dL^{-1}) was calculated as the difference between total protein and albumin. Albumin to globulin ratio (A/G ratio) was calculated via dividing Albumin by globulin.

Histological investigation

At the end of the experiment, three fish were randomly selected for dissection, liver and intestine were removed, thoroughly washed with a physiological saline (0.9% NaCl) and blotted buffered formalin 10%. The fixed specimens were processed using a conventional paraffin embedding technique. From the prepared paraffin blocks, 5 mm thick sections were obtained and stained with hematoxyline and eosin (H and E) for light microscopic examination according to the method described by Culling [25]. Measurements of villi length and width were taken using microscope with a micrometer rule as described by Spadoni et al. and Eyarefe et al. [26,27].

Statistical analysis

All statistical analyses were performed using SPSS (Standard Version 17.0 SPSS Inc. Chicago, Illinois). Data were subjected to two-way ANOVA to test the effect of plant protein source and spirulina supplementation. Duncan's multiple range test was used as a post hoc test to compare means at $P<0.05$ [28]. The results were presented as means ± standard error. The following general liner model was used:

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

Where Y_{ijkis} is the mean value of the aquaria, μ is the mean population, a_i is the fixed effect of plant protein source, b_j is the fixed effect of spirulina supplementation, $(ab)_{ij}$ is the interaction between the fixed effects, and e_{ijk} is the random error.

Results

Growth performance

The growth performance of *O. niloticus* fingerlings fed different protein sources showed a significant increase of FBW, WG, ADG, SGR in fish fed SBM based diets compared to Fish fed on CGM and DDG based diets (Table 1). Furthermore, the interaction of both protein sources and spirulina supplementation significantly increased all growth performance criteria of fish fed each protein sources with spirulina supplementation, yet the highest result was recorded with fish fed SBM based diet and supplemented with spirulina (Table 2). Spirulina supplementation-maintained survival at 100% during the study comparing with 87.5% in the non-supplemented diet (Table 2).

Feed and nutrients utilization

The FCR, PER (%), PPV(%) and EU(%) were improved with fish fed on SBM and CGM based diets compared to fish fed DDG based diets (Table 3). Moreover, spirulina supplementation significantly improved feed and nutrients utilization compared to non-supplemented fish (Table 3). The interaction between protein sources and algae supplementation showed a significant improvement of FCR, and the best result was recorded with SBM supplemented with spirulina-based t.

Whole-body chemical composition

The whole-body chemical composition of tilapia fingerlings at the end of the feeding experiment indicated that there was no significant difference in crude protein (%) nor in ether extract (%) in fish fed plant protein sources. Yet a significant increase of dry matter (DM) with fish fed CGM and DDG based diet was detected, and ash content of fish fed SBM recorded the highest value (Table 4). Spirulina supplementation increased DM. Furthermore, the significant interaction between plant protein sources and spirulina supplementation were presented in DM and ash where the highest values were recorded with CGM and SBM supplemented with spirulina, respectively (Table 4).

Protein profile parameters

The serum protein profile did not show any significant differences in total protein and albumin. Meanwhile, globulin and A/G ratio levels were significantly increased in fish fed SBM and CGM based diet compared to those fed DDG based diet (Table 5). Also, Spirulina supplementation significantly improved globulin and A/G ratio levels compared to fish fed non-supplemented diet. The same trend was observed in the interaction of protein source and algal supplementation where the highest results were recoded with fish fed SBM and CGM based diet supplemented with spirulina (Table 5).

Ingredients (g 100 kg^{-1} diet)	Experimental Diets					
	SBM	SBM+SP	CGM	CGM+SP	DDGs	DDGs+SP
Fish meal, 60%	22.5	22.5	22.5	22.5	22.5	22.5
Soybean meal, 44%	33.5	33.5	-	-	-	-
Corn gluten meal, 42%	-	-	34.5	34.5	-	-
distillers dried grains, 28%	-	-	-	-	45	45
Rice bran	6	6	6	6	6	6
What bran	10	10	10	10	10	10
Yellow corn	24	23.5	23	22.5	12.5	12
Spirulina meal	-	0.5	-	0.5	-	0.5
Fish oil	2	2	2	2	2	2
Vit. and Min. mix[1]	2	2	2	2	2	2
Proximate analysis (%) on DM basis						
Dry matter (DM)	92.09	93.01	94.08	94.19	90.87	94.74
Crude protein (CP)	31.14	31.02	31.60	31.70	30.30	30.54
Ether extract (EE)	7.22675	7.49	8.73	8.67375	10.51	9.16
Crude fiber (CF)	8.84	8.92	9.17	9.11	9.12	9.99
Ash	4.9265	4.48	3.3805	3.505	2.36	2.66
Nitrogen free extract (NFE)[†]	47.8668	48.09	47.1195	47.0113	47.71	47.65
Gross energy (GE; KJ 1 g^{-1} DM)[‡]	18.43	18.54	19.00	18.98	19.50	19.01
P/E ratio (mg CP: kJ)[§]	16.90	16.73	16.63	16.70	15.54	16.06

[1]Composition of vitamin mineral mixture of 1 kg: Vitamin A - 50,00,000 IU; Vitamin D_3 - 10,00,000 IU; Vitamin B_2 - 2.0 g; Vitamin E - 750 units; Vitamin K - 1.0 g; Calcium pantothenate 2.5 g; Nicotinamide - 10.0 g; Vitamin B_{12} - 6.0 g; Choline Chloride - 150.0 g; Calcium - 750.0 g; Manganese - 27.5 g; Iodine - 1.0 g; Ion - 7.5 g; Zinc - 15.0 g; Copper - 2.0 g; Cobalt - 0.45 g
[†]NFE: Nitrogen free extract calculated using the following equation: NFE = 100- (crude protein + ether extract + crude fiber + ash).
[‡]GE: Gross energy calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy g^{-1} protein, ether extract and NFE, respectively [71].
[§]P/E ratio: Protein energy ratio (mg crude protein kJ^{-1} gross energy) = CP/GE × 1000.

Table 1: Feed ingredients and proximate chemical composition (% of dry weight) of the experimental diets.

Histological investigation of hepatopancreas and intestine

The histological investigation of hepatopancreas showed a normal hepatic architecture and pancreatic islets histology in most studied treatments (Figures 1a-1f). Meanwhile, the hepatic glycogen content showed a moderate level in fish fed SBM based diet, and low level with moderate hepatic fatty changes in CGM and DDG based diet. Spirulina supplementation improved liver histopathological featuring and glycogen content (Figures 1b, 1d and 1f).

The intestinal sections showed a significant increase of both intestinal villi length and area of absorption values in fish fed SBM based diet (Figures 2a-2f and Figure 3). Moreover, fish fed spirulina supplemented diet showed the highest intestinal villi length and area of absorption values. The interaction of both protein source and spirulina showed the highest area of absorption in fish fed SBM and supplemented with spirulina.

Discussion

Selection of feed ingredients is one of the most important factors for best formulation and high-quality commercial feed production for any aquatic species [29]. Fish meal is the widely used feed ingredients as animal protein source, and it is accepted for its higher protein composition, and essential amino acids. Nevertheless, it is rather more expensive than the available plant protein sources [30].

In the present study, the used ingredients were selected considering their nutritional quality (high protein content required to reach the target dietary protein level), levels of anti-nutritional factors as well as their cost effectiveness as recommended by Francis et al. [31]. The experimental diets were formulated to be iso-nitrogenous and iso-caloric. Accordingly, any difference in the performance of fish received such diets could be attributed to the quality and nutritive value of the tested materials.

Growth performance (FW, WG, ADG and SGR) and feed utilization (FCR, PER, PPV and EU) increased significantly in fish fed diet containing SBM compared to other used plant protein sources. These results may possibly be due to the better utilization of SBM by Nile tilapia and it's a nutritional quality, as compared to other used plant protein sources, as SBM is highly digestible by fish and the digestion coefficients are comparable or even higher than fish meal protein [13]. In addition, the obvious improvement of growth performance and feed utilization in the current study may be due to the improvements of liver histology (Figure 1), and glycogen contents with increasing intestinal absorption area as presented in Table 5 and Figure 2 in fish fed on SBM based diets. Accordingly, better absorption and metabolism is detected [32-34]. Fish fed on diet containing CGM and DDGs showed lower growth performance and survival rate (%) than fish fed SBM based diets. These results may occur due to the lower amounts of lysine and methionine in CGM and DDGs, which present the most limited amino acids in protein [35,36]. Moreover, the higher inclusion rate of CGM (34.5%) and DDGs (45%) in the present study may adversely affect the growth performance of treated fish, whereas Shimeno et al., [37] reported that the proper inclusion level of CGM was 13-26% in yellow tail fish diet. Therefore, Wu et al. [14] concluded that proper amino acid balance must be maintained with using CGM in tilapia fish meal-free diet for better growth.

Furthermore, juvenile hybrid tilapia (*O. niloticus* × *O. aureus*) fed on diet contained 30% DDG reported lower WG, SGR and feed efficiency ratio (FER) [15]. Also, El-Ebiary [38] showed that Nile tilapia fingerlings fed diet content 25% of CGM showed considerably lower growth and feed utilization performance. Shelby et al., [39] found that WG, FCR of Nile tilapia fed diet containing 60% DDG without lysine supplementation were lower compared with SBM-based diet and diets containing lesser amount of DDGS (0, 30 and 30% + lysine). However, the relevance of dietary inclusion of both CGM and DDGs is due to the relative availability, low cost, and nutrient composition to provide lower-cost diet formulations, particularly for omnivorous species [15].

It is worth mentioning that the acceptability of feed by fish could be affected by increasing levels of plant material since the texture and taste of diets are bound to differ. The low feed intake of CGM and DDGs in the present study could have been due to the high fiber content, low level of lysine, methionine and threonine, and higher levels of anti-nutritional factors of the diets [40]. Concerning proximate whole-body composition, CP and EE contents of Nile tilapia were not influenced by dietary protein source. Similarly, El-Saidy & Gaber [41] did not find any effects of different plant protein on the whole-body protein and ash contents in Nile tilapia.

Regarding blood metabolites, the serum protein profile did not show any significant differences in total protein and albumin. Meanwhile, globulin and A/G ratio levels were significantly increased in fish fed SBM and CGM based diet compared to those fed DDG based diet. The Haematological evaluations are gradually becoming a routine practice for determining health status in fish [42]. As blood composition is usually altered during diseases or malnutrition conditions [43] using SBM and CGM is preferable than DDGs as a plant protein source in Nile tilapia diet in the current study.

The supplementation of spirulina, *A. platensis,* to different plant protein sources based diet significantly increased growth performance, feed utilization and survival (%) than in non-supplemented groups. In accordance, Abu-Zead [44] and Ibrahim [45] found that algae and aquatic plant supplementation significantly improved growth and protein efficiency in Nile tilapia and common carp fingerlings. Supportively, Jaime et al., [46] reported that *L. Schmitti* larvae fed with *A. platensis* supplemented diet showed higher growth performance and body proximate composition. The inclusion of algae (*Scenedesmus Bijuga* (Chlorophyceae)) at 10% and 20% in artificial diets improved FCR and PER of in Nile tilapia fry diet [47].

Moreover, Win [48] reported that addition of 5% *A. platensis* in the diet showed the best growth performance and survival rate in *Anabas testudineus*. Similarly Su [49] also observed that 10% of *A. platensis* incorporated into formulate diet of *Ctenopharyngodon idellus* fingerlings resulted in good health, better growth performance and brighter body color. Dernekbasi et al., [50] reported that FCR increased with increasing dietary Spirulina meal level and ranged from 2.31- 1.09 in dose dependent manner. These results agree with Sudaporn et al., [51] who reported that *Spirulina* could improve growth, reduction of mortality; overall elements of fish quality, firmness of flesh, and brightness of skin color as well as improving the cost performance ratio of the fish feed.

The better growth, feed utilization and higher feed intake which recorded with spirulina supplemented diet in the present study might be due to the high protein (50 to 70%) content of spirulina with a good amino acid profile [52], besides several nutrients content especially vitamins, minerals, pigments and carbohydrates complex, such as glucans [53]. Moreover, Wahbeh [54] and Lin et al., [55] reported that *A. platensis* appears to be a potential source of essential amino acid and fatty acids (n-3 and n-6 series) for fish. Let also not forget to mention that Spirulina is a rich source of carotenes and other pigments that have antioxidants activity [55].

Items	Body weight: Final body weight(g/fish)	Weight gain (g/fish)	Average daily gain(g/fish/day)	Specific growth rate (%/day)	Survival (%)
Protein sources (A)					
SBM	17.60 ± 0.62[a]	13.82 ± 0.62[a]	0.17 ± 0.01[a]	1.83 ± 0.04[a]	97.92 ± 2.08
CGM	15.48 ± 0.76[b]	11.70 ± 0.76[b]	0.14 ± 0.01[b]	1.68 ± 0.06[b]	91.67 ± 8.33
DDG	15.56 ± 0.38[b]	11.79 ± 0.38[b]	0.14 ± 0.01[b]	1.69 ± 0.03[b]	91.67 ± 5.89
Spirulina, *Arthrospira platensis* (B)					
With Sp	17.12 ± 0.52[a]	13.34 ± 0.52[a]	0.16 ± 0.01[a]	1.8 ± 0.04[a]	100.0 ± 0.00
Without Sp	15.30 ± 0.48[b]	11.53 ± 0.47[b]	0.14 ± 0.01[b]	1.7 ± 0.03[b]	87.50 ± 5.59
Interaction A × B					
SBM	16.75 ± 0.75	12.98 ± 0.75	0.16 ± 0.01	1.77 ± 0.05	95.83 ± 4.17
SBM Sp	18.45 ± 0.55	14.67 ± 0.56	0.18 ± 0.01	1.89 ± 0.03	100.0 ± 0.00
CGM	14.20 ± 0.30	10.43 ± 0.31	0.13 ± 0.01	1.58 ± 0.03	83.33 ± 16.67
CGM Sp	16.75 ± 0.35	12.98 ± 0.36	0.16 ± 0.01	1.78 ± 0.03	100.0 ± 0.00
DDG	14.96 ± 0.16	11.19 ± 0.15	0.14 ± 0.01	1.64 ± 0.01	83.33 ± 8.33
DDG Sp	16.15 ± 0.35	12.39 ± 0.35	0.15 ± 0.01	1.74 ± 0.03	100.0 ± 0.00

Means in the same column bearing different superscript are significantly different ($P \leq 0.05$).
SBM: Soybean Meal, CGM: Corn Gluten Meal, DDG: Distillers Dried Grains, Sp: Spirulina, *Arthrospira platensis.*

Table 2: Effect of different plant protein sources with or without spirulina, *Arthrospira platensis,* supplementation on growth performance parameters and the survival (%) of Nile tilapia, *Oreochromis niloticus*, fingerlings.

Items	Feed intake (gm/fish)	Food conversion ratio	Protein utilization: Protein efficiency ratio	Protein utilization: Protein productive Value (%)	Energy Utilization (%)
Protein sources (A)					
SBM	21.49 ± 0.76	1.56 ± 0.03[b]	2.09 ± 0.04[a]	31.58 ± 1.42[a]	21.40 ± 0.88[a]
CGM	19.86 ± 0.49	1.71 ± 0.09[a]	1.89 ± 0.10[a]	25.83 ± 1.73[a]	18.90 ± 0.97[a]
DDG	20.00 ± 0.24	1.70 ± 0.05[a]	1.89 ± 0.06[b]	25.03 ± 1.08[b]	19.30 ± 1.00[b]
Spirulina, *Arthrospira platensis* (B)					
With Sp	21.00 ± 0.28	1.57 ± 0.06[b]	2.06 ± 0.07[a]	29.47 ± 1.65[a]	21.36 ± 0.72[a]
Without Sp	19.90 ± 0.61	1.73 ± 0.02[a]	1.86 ± 0.03[b]	25.49 ± 1.26[b]	18.37 ± 0.40[b]
Interaction A × B					
SBM	20.32 ± 0.34	1.57 ± 0.06[bc]	2.06 ± 0.09	30.10 ± 2.79	20.26 ± 1.41
SBM Sp	22.66 ± 0.78	1.55 ± 0.01[c]	2.12 ± 0.05	33.06 ± 0.15	22.54 ± 0.12
CGM	19.43 ± 0.57	1.86 ± 0.01[a]	1.73 ± 0.01	22.84 ± 0.16	17.25 ± 0.33
CGM Sp	20.29 ± 0.86	1.57 ± 0.03[bc]	2.06 ± 0.03	28.82 ± 0.04	20.56 ± 0.10
DDG	19.96 ± 0.59	1.78 ± 0.03[ab]	1.80 ± 0.05	23.53 ± 0.88	17.62 ± 0.47
DDG Sp	20.04 ± 0.05	1.62 ± 0.04[b]	1.99 ± 0.05	26.54 ± 1.32	20.97 ± 0.41

Means in the same column bearing different superscript are significantly different ($P \leq 0.05$).
SBM: Soybean Meal, CGM: Corn Gluten Meal, DDG: Distillers Dried Grains, Sp: Spirulina, *Arthrospira platensis*

Table 3: Effect of different plant protein sources with or without spirulina, *Arthrospira platensis,* supplementation on nutrient utilization in Nile tilapia, *Oreochromis niloticus*, fingrlings.

Furthermore, Spirulina supplementation did not affect the whole-body protein and EE contents in the present study. Similarly, Olvera-Novoa et al., [56] stated that, the body chemical composition of tilapia *Mossambica* did not clearly get affected by the inclusion of Spirulina algae with different doses in the diet.

The serum protein profile did not show any significant difference in total protein and albumin. Meanwhile, globulin and A/G ratio levels were significantly increased in fish fed spirulina supplemented diet compared to fish fed non-supplemented diet. These results proved the improvement of fish health when fed Spirulina-supplemented diets. Thus, the measurement of albumin, globulin, and total protein in serum or plasma is of considerable diagnostic value in fish, as it affects the general nutritional status as well as the integrity of the vascular system and liver function [57].

In accordance, the feeding of *S. platensis* to fish and poultry results in increased disease resistance, improved immune functions and antioxidant properties [58,59]. Abdel-Tawwab et al., [60]. also proved that dietary supplementation of Spirulina enhanced fish growth and immunity of Nile tilapia. Bermejo et al., [61] reported that most antioxidant capacities of Spirulina protein extract are attributable to the biliproteins contained in this microalga, such as phycocyanin, so we can use Spirulina to improve the immune capacity of the animals which consume it. Consumption of Spirulina algae also increases the ability to absorb nutrients. Moreover, the nutritional elements that influence the immunological and haemolytical systems in modern intensive fish farming are necessary to appreciate the many complex relationship and interaction between diet, stress and susceptibility to disease in fish [62].

Items	Whole-body chemical composition			
	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)
Protein sources (A)				
SBM	22.47 ± 0.44[c]	61.78 ± 1.50	20.35 ± 0.75	17.87 ± 0.92[a]
CGM	24.57 ± 1.29[a]	64.67 ± 0.65	18.43 ± 0.70	16.91 ± 0.10[ab]
DDG	24.20 ± 0.29[b]	62.75 ± 2.09	20.93 ± 1.75	16.32 ± 0.48[b]
Spirulina, *Arthrospira platensis* (B)				
With Sp	24.58 ± 0.58[a]	63.35 ± 1.15	19.77 ± 0.60	16.89 ± 0.75
Without Sp	22.92 ± 0.71[b]	62.79 ± 1.43	20.04 ± 1.32	17.18 ± 0.18
Interaction A × B				
SBM	21.71 ± 0.02[f]	59.23 ± 0.69	21.60 ± 0.55	19.17 ± 1.25[a]
SBM Sp	23.24 ± 0.08[d]	64.33 ± 0.27	19.11 ± 0.04	16.56 ± 0.31[c]
CGM	22.34 ± 0.09[e]	64.62 ± 0.34	18.58 ± 0.50	16.79 ± 0.16[c]
CGM Sp	26.80 ± 0.02[a]	64.71 ± 1.55	18.27 ± 1.61	17.02 ± 0.07[b]
DDG	24.70 ± 0.09[b]	64.50 ± 0.48	19.94 ± 0.55	15.56 ± 0.07[c]
DDG Sp	23.70 ± 0.01[c]	61.01 ± 4.47	21.92 ± 4.01	17.08 ± 0.46[b]

Means in the same column bearing different superscript are significantly different ($P \le 0.05$).
SBM: Soybean Meal, CGM: Corn Gluten Meal, DDG: Distillers Dried Grains, Sp: Spirulina, *Arthrospira platensis*

Table 4: Effect of different plant protein sources with or without spirulina, *Arthrospira platensis,* supplementation on whole-body chemical composition in Nile tilapia, *Oreochromis niloticus*, fingrtlings

Items	Protein profile			
	Total protein	Albumin	Glob ulin	A/G ratio
Protein sources (A)				
SBM	4.04 ± 0.28	1.98 ± 0.22	2.05 ± 0.07[a]	0.97 ± 0.08[c]
CGM	4.53 ± 0.16	2.61 ± 0.39	1.93 ± 0.34[a]	1.45 ± 0.25[b]
DDG	4.19 ± 0.33	2.77 ± 0.35	1.43 ± 0.07[b]	1.94 ± 0.10[a]
Spirulina, *Arthrospira platensis* (B)				
With Sp	4.09 ± 0.16	2.10 ± 0.21	1.99 ± 0.17[a]	1.06 ± 0.11[b]
Without Sp	4.41 ± 0.25	2.80 ± 0.28	1.61 ± 0.20[b]	1.74 ± 0.14[a]
Interaction A × B				
SBM	4.36 ± 0.49	2.26 ± 0.36	2.10 ± 0.14[ab]	1.08 ± 0.09[b]
SBM Sp	3.71 ± 0.08	1.71 ± 0.02	2.01 ± 0.06[b]	0.85 ± 0.04[c]
CGM	4.65 ± 0.20	3.29 ± 0.06	1.37 ± 0.14[c]	2.40 ± 0.04[a]
CGM Sp	4.42 ± 0.29	1.93 ± 0.04	2.49 ± 0.24[a]	0.78 ± 0.10[c]
DDG	4.23 ± 0.06	2.86 ± 0.11	1.38 ± 0.17[c]	2.07 ± 0.08[a]
DDG Sp	4.15 ± 0.80	2.68 ± 0.84	1.48 ± 0.04[c]	1.81 ± 0.20[a]

Means in the same column bearing different superscript are significantly different ($P \le 0.05$).
SBM: Soybean Meal, CGM: Corn Gluten Meal, DDG: Distillers Dried Grains, Sp: Spirulina, *Arthrospira platensis*.

Table 5: Effect of different plant protein sources with or without spirulina, *Arthrospira platensis,* and supplementation on serum protein profile in Nile tilapia, *Oreochromis niloticus*, fingerlings.

Furthermore, there are improvements of liver and intestine histological observation in group fed SBM based diet than groups fed on CGM and DDG based diets. The supplementation of *A. platensis* especially with SBM diet expressed the highest improvement in both liver and intestine. In accordance, *S. platensis* succeed to normalize the cytotoxicity effect of monosodium glutamate in liver of treated mice [63] and Torres-Duran et al., [64] also supported the potential hepatoprotective role of Spirulina. The improvement of liver histology may be attributed to its content of phytopigments such as phycobilins, phycocyanin, allophycocyanin, and xanthophylls, which seem to be related to its antioxidant activity [61]. Moreover, Wu et al., [65] and Hu et al., [66] reported that the algal carotenoid extract had significant antioxidant activity. The usefulness of antioxidants is to protect cellular components against oxidative stress [67].

Regarding the intestinal villous length and width parameters, the increase in villous length and width may cause nutrient absorption improvement due to the increase of the absorption surface. The obtained result pointed to the role of spirulina in intestinal villi length improvement in all groups especially when added to soybean meal. These results proved the improvement of fish health and better feed utilization when fed Spirulina-supplemented diets in the current study. This also may be due to its valuable content of selenium, chlorophyll, carotene, γ-linolenic acid, and vitamins E and C. Moreover, some strains of Spirulina may produce bioactive substances that may inhibit or promote intestinal microbial growth, with consequent potential for proliferation of beneficial intestinal bacteria [68].

Finally, the nutritive value of mixed ratios depends on the nutrient composition of the individual feed components, and the ability of fish to digest and absorb the nutrients [69].

Protein quality depends on the amino acid composition and their digestibility. Deficiency of an essential amino acid leads to poor utilization of the dietary protein, and consequently reduces growth and decreases feed efficiency [70]. Therefore, spirulina supplementation

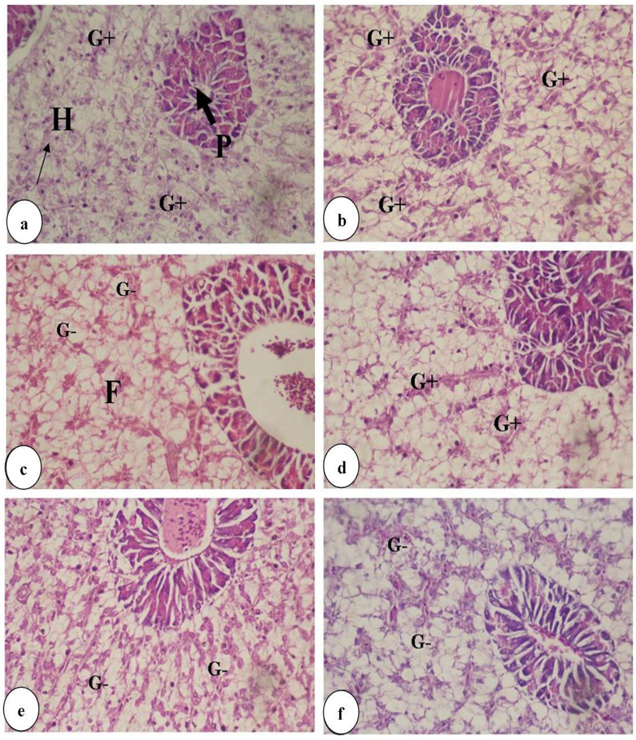

Figure 1: Effect of different plant protein sources with or without spirulina, *Arthrospira platensis,* supplementation on hepatopancreas histology (Hematoxylin and eosin stain) of Nile tilapia, *Oreochromis niloticus*, fingerlings. 1a) 160x and inset, 400x; scale bar 200 µm. Figures order are a: SBM, 1b) SBM+SP, 1c) CGM, 1d) CGM+SP, 1e) DDG, 1f) DDG+SP. H: hepatocyte, P: pancreatic islets, G+: high glycogen content, G-: low glycogen content.

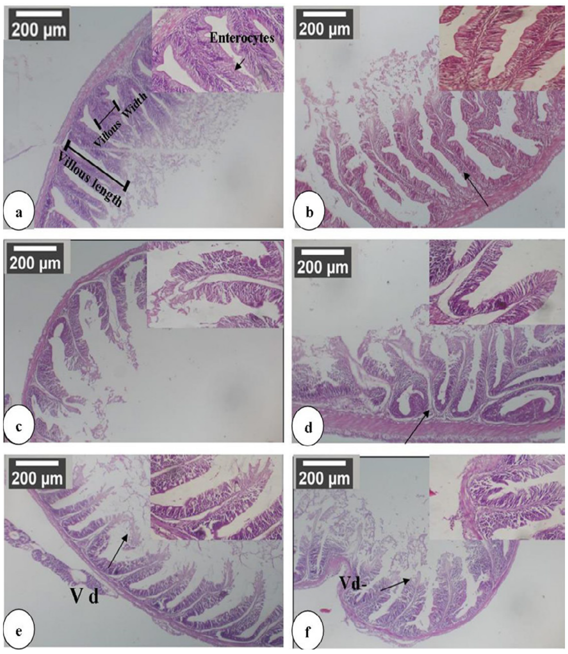

Figure 2: Effect of different plant protein sources with or without spirulina, *Arthrospira platensis,* supplementation on intestine histology (Hematoxylin and eosin stain) of Nile tilapia, *Oreochromis niloticus*, fingerlings. A, 160x and inset, 400x; scale bar 200 µm). Figures order are 2a) SBM, 2b) SBM+SP, 2c) CGM, 2d) CGM+SP, 2e) DDG, 2f) DDG+SP., Vd: Villi desquamation.

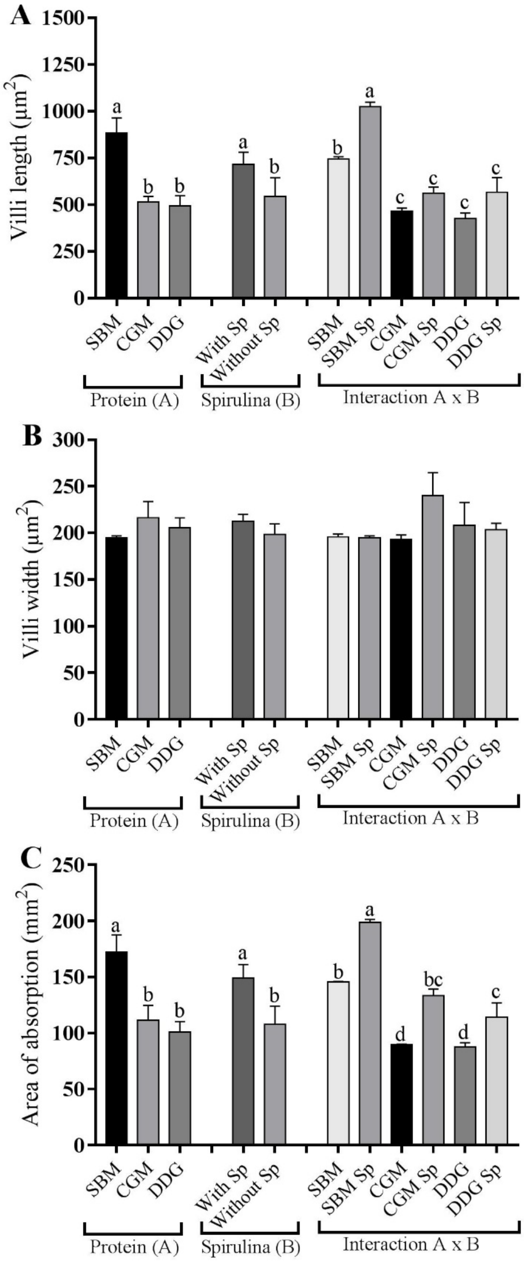

Figure 3: Effect of different plant protein sources with or without spirulina, *Arthrospira platensis*, supplementation on intestine histological changes of Nile tilapia, *Oreochromis niloticus*, fingerlings. Means in the same column bearing different superscript are significantly different ($p \leq 0.05$). SBM: Soybean Meal, CGM: Corn Gluten Meal, DDG: Distillers Dried Grains, Sp: *Spirulina, Arthrospira platensi*.

Area of absorption (mm) = (Villi length × villi width/1000).

is one of the recommended feed additives in plant protein-based diet for better growth performance, feed utilization and physiological status [71].

Conclusion

Our findings indicated that alternative plant protein sources like soybean meal supplemented with spirulina have a positive effect on growth parameters, feed utilization, carcass composition and the health status of the fish which offers it as a suitable protein source in practical diets. Moreover, global spirulina production should continue to increase as to provide adequate amounts needed for aquaculture because of its economic advantage for the commercial diets.

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