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PARTIAL AND COMPLETE REPLACEMENT OF CORN GLUTEN MEAL PROTEIN BY ALGAE PROTEIN IN DIETS FOR NILE TILAPIA, *Oreochromis niloticus* (L.)

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ABSTRACT: *Diets incorporating different levels of corn gluten meal replacement by using biofuel algae or Spirulina protein at 0, 25, 50, 75, and 100% were evaluated for larval/juvenile stage of Nile tilapia (Oreochromis niloticus). Fish averaging 0.02 g were divided into18 groups of 50 fish. There were 3 replicates per every dietary treatment that were fed one of six diets for 11 weeks. Corn gluten protein was replaced with algae on the protein basis. All diets were supplemented with 1.5% lysine and 0.5% methionine. The experimental diets were formulated to contain 34.9 ± 0.1% protein and 12.2 ± 0.1 % lipid in the form of fish oil and soybean lecithin (phospholipids source). The results indicated that algae positively affected feed consumption and fish growth up to the 50% replacement and then performance was depressed. Significant differences in concentration of individual minerals (Al, Fe, Zn, and Cu) in the whole fish body were found. Mineral composition of algae might have affected growth when diets which contained more than 75% of plant protein were replaced with microalgae. These findings suggest that up to 50% of dietary corn gluten meal protein can be replaced with microalgae which significantly enhance fish growth.*

Key words: *Corn gluten, Biofuel algae, Spirulina, Nile tilapia, iron, aluminum*

INTRODUCTION

In the near future, farmed fish will become an even more important source of protein in foods than they are today. Also, the safety of aquaculture products for human consumption is an issue of significance to public health. Quality of fish protein, healthy fat (enhanced level of polyunsaturated fatty acids, PUFA) and low level of contaminants are among the most important. Increased production of aqua feeds has resulted in an increase of global aquaculture, which depends on fish meal with well balanced nutrients as the primary protein source (Naylor *et al*., 2009). The growing demand of fish meal and diminishing fish capture have resulted in the high price of fish meal. Thus, it is essential to evaluate different protein sources as cheaper substitutes to replace fish meal and/or expensive plant protein concentrates as a dietary protein source in aquafeeds (Takeuchi *et al*., 2002). Additionally, it is important to consider economic advantages through cost reduction by using cheaper diets consisting of locally available protein, lipid, and carbohydrate sources (Azaza *et al*., 2008). In this regard, single cell products have a wide spread accessibility as a possible substitute for fish meal and plant protein concentrates in aquafeeds due to their ability to be cultured on a wide variety of substrates and wastes (Nandeesha *et al*., 1998). The effect of algae on growth performance has been previously studied in red sea bream (*Pagrus major)*, Mossambique tilapia (*Oreochromis mossambicus*) and rainbow trout (*Oncorhynchus mykiss*) (Olvera-Novoa *et al*., 1998). Moreover, the high protein content and high production rate of algae in tropical areas, especially in developing countries hold promise as a possible alternative protein source for aquafeeds (Venkataraman *et al.,* 1980). It has been shown that many fish including carnivorous species can ingest algae as a part of diet formulation (Nandeesha *et al.* 1998). Therefore, using algae in fish diets might result in better utilization of artificial diets in cultured fish and enhance fish health (Mustafa & Nagakawa 1995).

Spirulina (*Spirulina platensis*) is one of the most common microalgae in warm saline and alkaline waters. Its growth rate under culture conditions is faster than any other agriculture crop, and close to that of other microorganisms such as yeasts and bacteria. Interestingly, algae have been considered as a valuable substitute as the source of protein, especially in tropical aquaculture where there is continuous production due to adequate temperature throughout the year. Among all algae species Chlorella spp., Scendemus spp., and Spirulina are the most frequently used as a feed additive or mixed with other protein sources (Mustafa & Nakagawa 1995). It was observed that by adding live Spirulina to diets for tilapia enhanced growth rate and improved immune response against common pathogenic bacteria ensued (Abdel-Tawwab & Ahmad 2009). However, there are significant differences in acceptability, digestibility, and bioavailability of various algae species when included in diet formulation for fish (Lu & Takeuchi 2004). Moreover, adding algae as the protein source, feed attractant and source of antibacterial compounds (Li & Tsai 2009) will promote increased aquaculture competitiveness in the production of human food. In addition, algae can be used more widely as a prospective source of biologically active substances for medicine and pharmacy. The algae are able to convert discharged nutrients (wastes) into a valuable biomass. Furthermore, algae are now considered to serve as a source of renewable energy (biofuel production) (Muntean *et al*., 2007). Several studies were conducted to evaluate algae as an alternative source of protein in feeds for food fish and terrestrial animals as part of the human diet. Already a good deal of work has been centered on algae in biodiesel production (Mata *et al*., 2010).

In this study we examined the effect of dietary supplementation with different levels of algae (Algamaxx, Independence Bio-products Development Inc., Dublin, OH) or Spirulina (Ocean Star International, Inc., Snow Ville, UT) as a replacement of the gluten protein concentrate on fish performance during early ontogeny. The main purpose of this study was to evaluate advantages and safety of microalgae produced as biofuels for diet formulations of Nile tilapia.

MATERIALS AND METHODS

Fish, facility and feeding trial

The feeding trial was performed at an indoor installation (Green House) of the Aquaculture Laboratory, School of Environment and Natural Resources (SENR), The Ohio State University (OSU), Columbus, Ohio with larvae/juvenile tilapia averaging 0.02 g initial weight. Tilapia (*Oreochromis niloticus*) were bred for several generations in the OSU laboratory. Larvae were collected from brooding females in September 2009. Fish were maintained without feeding until commencing the feeding trial. Fish were randomly distributed into groups of 50. Each experimental diet was fed to triplicate groups of fish with the feeding rates beginning with 20% of body weight (BW) per day and gradually decreasing to 6% at the end of the feeding trial (NRC 1993). The fish were fed four times a day at 10:00, 12:00, 14:00, and 16:00, 7 days a week, for 11 weeks. All procedures and handling of animals were conducted in compliance with the guidelines of the Institutional Laboratory Animal Care and Use Committee, The Ohio State University. The feeding trials were conducted in glass aquariums (40 L), supplied with city water which was constantly replaced by continuous flow at the rate of 0.25 L min^{-1} and water temperature maintained at 26 ± 2.2°C. Supplemental aeration was also provided to maintain dissolved oxygen levels near saturation. All aquaria were siphoned twice a day to remove fecal materials. Total fish weight in each tank was determined every three weeks to check their growth and to adjust the feeding rate. Feeding was stopped 24 h prior to weighing.

Experimental diets

Six experimental diets were formulated to contain as close as possible levels of crude protein (34.9 ± 0.1%) and crude lipid (12.2 ±

0.1%) (Table 1). Diet formulations were affected by substitution with Algamaxx (composition provided by the Independent Bioproduct Co., Dublin, OH, U.S.A.) and were controlled with wheat flour (only 11% protein) and fish oil and soybean lecithin (53% linoleic acid) to balance protein/lipid ratio. The dietary supplement of 1% lecithin and 2% of cod liver oil covers tilapia requirement for essential fatty acids (Dabrowski & Portella 2005).

Table 1. Ingredient composition and proximate analyses of the six experimental diets fed to juvenile tilapia (*Oreochromis niloticus*) for 11 weeks.

Ingredients (%)	Control	Alga25	Alga50	Alga75	Alga100	Spirulina100
Corn gluten meal*	43.34	32.50	21.67	10.83	-	-
Algamaxx	-	18.75	37.50	56.25	75.00	-
Spirulina	-	-	-	-	-	43.63
Casein (vitamin free)	4.92	5.45	5.97	6.50	7.00	4.00
L-methionine	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine	1.50	1.50	1.50	1.50	1.50	1.50
Wheat flour†	22.70	18.90	15.20	11.40	7.77	29.00
CMC	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin Mix‡	2.00	2.00	2.00	2.00	2.00	2.00
Mineral Mix§	7.50	5.85	4.20	2.60	1.00	4.00
Stay-C 35¶	0.06	0.06	0.06	0.06	0.06	0.06
Choline chloride	0.17	0.17	0.17	0.17	0.17	0.17
Cod liver oil	7.41	6.07	4.71	3.36	2.00	6.14
Lecithin	3.71	3.04	2.36	1.68	1.00	3.07
α-cellulose	4.20	3.21	2.17	1.15	-	3.94
Proximate analyses						
Crude protein	34.9	34.8	34.9	34.9	34.9	35.1
Crude Lipid	12.2	12.1	12.2	12.1	12.1	12.2
Ash	8.60	8.58	8.56	8.59	8.62	8.56

*Corn gluten meal protein was replaced at 25, 50, 75, and 100 with Algamaxx (A) or 100% with *Spirulina* (S) (Ocean Star International, Inc., Snowville, UT).

†Wheat flour is used to balance protein/lipid ratio differences between corn gluten meal and its replacer in the diets.

‡Vitamin mixture (mg kg^{-1} diet) sources were Rovimix series: retinyl acetate, 2.00; cholecalciferol, 0.10; DL-α-tocopheryl acetate, 125.00; menadione niacinamide bisulfite, 5.00; nicotinic acid, 25.00; riboflavin, 20.00; pyridoxine hydrochloride, 15.00; D-calcium pantothenate, 50.00; biotin, 1.00; folic acid, 5.00; cyanocobalamin, 0.05; *myo*-inositol, 500.00; thiamine mononitrate, 10.00 (Aquaculture Research Group, DSM Nutritional Products France, Animal Nutrition & Health Research, Saint-Louis, France).

§Five mg Se in the form of sodium selenite per kg Bernhart Tomarelli salt mixture (MP Biomedicals, LLC, Solon, OH).

¶L-Ascorbic acid monophosphate (35 %).

Diets were supplemented with indispensable amino acids based on already established quantitative methionine and lysine requirements for juvenile tilapia (see review by Dabrowski & Portella 2005). Fish performance was evaluated in a feeding trial where 25, 50, 75, and 100% of the gluten protein concentrate was replaced with algae (Algamaxx, Independence Bio-products Development, Dublin, OH) or Spirulina protein (Ocean Star International, Inc., Snow Ville, UT). The control, as a plant protein-based diet, was made up of corn gluten meal (60% protein, Sigma-Aldrich, St. Louis, MO). All diets were supplemented with 1.5% lysine and 0.5% methionine. The diets were marked as Control, Alga25, Alga50, Alga75, Alga100, and Spirulina100. The composition of the experimental diets for tilapia used in the present study is presented in Table 1. Experimental diets were cold-pelleted into 2.0 mm diameter size, freeze-dried, crushed into a desirable size (0.5-2.0 mm) and stored in sealed plastic bags at -20°C until use. Diet acceptance (daily satiation ration) was examined with juvenile tilapia 9 weeks into the feeding trial. At the end of the feeding trial, samples from the experimental diets were prepared for proximate analysis and minerals (Table 2). Feed conversion ratio (FCR) was calculated for each period and mean weight gain (WG) was calculated at the end of the experiment.

Sample collection and analysis

Analyses of crude protein, moisture, and ash were performed by standard procedures (AOAC 1995). At the end of the feeding trial all fish were counted and weighed to calculate percent weight gain (PWG; [BW – initial BW] × 100/initial BW), feed conversion ratio (FCR; dry feed consumed/WG), protein efficiency ratio (PER; WG/protein intake), specific growth rate (SGR; [ln final BW – ln initial BW] × 100/days), and survival ([no. of fish at the end of the experiment/no. of fish at the beginning of the experiment] × 100). At the end of the feeding trial, six fish from each tank were sampled for biochemical analysis. Approximately 15-20 g of each biomass were freeze-dried, ground into a powder and stored at – 80ºC for proximate chemical analysis at the Ohio State University Research and Extension Analytical Laboratory, Wooster, OH.

Table 2. A mineral composition of the experimental diets fed to juvenile tilapia (*Oreochromis niloticus*) for 11 weeks.

Mineral	Control	Alga25	Alga50	Alga75	Alga100	Spirulina100
P %	1.53^{e}	1.30^{d}	1.12^{c}	0.83^{b}	0.59^{a}	1.30^{d}
K %	0.67^{b}	0.53^{a}	0.57^{a}	0.54^{a}	0.53^{a}	1.10^{c}
Ca %	1.55^{e}	1.21^{d}	1.13^{c}	0.90^{b}	0.68^{a}	0.95^{b}
Na %	0.30^{b}	0.27^{a}	0.29^{ab}	0.27^{a}	0.27^{a}	0.67^{c}
Mg µg g^{-1}	624^{a}	714^{b}	954^{c}	1143^{d}	1337^{e}	1831^{f}
S µg g^{-1}	5353^{d}	5087^{cd}	4998^{c}	4556^{ab}	4401^{a}	4829^{bc}
Al µg g^{-1}	18^{a}	770^{b}	1738^{c}	2816^{d}	3782^{e}	77^{a}
B µg g^{-1}	3.71	<1.523	9.09	5.46	10.04	3.95
Cu µg g^{-1}	15.65^{b}	13.09^{ab}	12.94^{ab}	10.30^{a}	10.27^{a}	10.23^{a}
Fe µg g^{-1}	189^{a}	2300^{c}	4854^{d}	7573^{e}	10075^{f}	525^{b}
Mn µg g^{-1}	238^{b}	285^{c}	336^{e}	300^{cd}	317^{d}	154^{a}
Mo µg g^{-1}	1.09	0.57	<0.225	<0.225	<0.225	0.56
Zn µg g^{-1}	50.0^{b}	64.0^{c}	85.3^{d}	104.0^{e}	123.4^{f}	32.0^{a}

Values are means ± SD of two samples. Superscripts having different letters are significantly different ($P < 0.05$).

Mineral compositions of diets and whole-body were measured by the inductively coupled plasma (ICP) emission spectrophotometric method with the use of an ARI-3560 spectrometer (Applied Research Labs, Valencie, CA, USA), following Watson and Isaac (1990) methods. Minerals were determined in the whole body dry matter. Samples were prepared for analysis using the ashing procedure (AOAC 1995).

Statistical analysis

Each experimental diet was fed to three groups of fish by a completely randomized design. Differences among dietary treatments were tested by one-way ANOVA. The percentage data of weight gain and specific growth rate were arcsine transformed before the ANOVA analysis. Differences were considered significant at the $P < 0.05$. The differences among means were determined using Duncan ´s multiple range test (Duncan 1955).

RESULTS

The results of final BW, SGR, WG, feed intake, FCR, PER and survival rate are presented in Table 3. No significant differences in the survival rate were observed among the different treatments ($P > 0.05$). However, the growth performance significantly differed ($P < 0.05$) in terms of final BW, WG, and SGR among fish groups fed experimental diets for 11 weeks. Fish fed diets Spirulina100, Alga25, and Alga50 grew faster than control group, whereas the fish fed diets either Alga75 or Alga100 showed compromised growth when expressed as the final BW, WG, and SGR (Table 3, and Fig. 3A, $P < 0.05$). The differences in growth were first observed after the 3rd week of the feeding and increased as the feeding period continued (Fig. 1, $P < 0.05$). Moreover, the differences were significant in terms of growth among all treatments during the different periods of the experiment (Fig. 1). There were significant differences ($P < 0.05$) among all treatments in FCR and feeding to satiation (Table 3, and Fig. 2). Fish fed diet Alga25 consumed more feed than the other treatments ($P < 0.05$), while there were no significant differences ($P > 0.05$) between fish fed the Control diet and Spirulina100 diet. Fish fed Spirulina100 diet showed the best FCR (2.31). However, as feeding rate was re-adjusted for weight of fish (the same % of biomass) fish fed Alga100 diet had lower feed intake than other groups (Table 3). The reason for the poor growth of those on the Alga100 diet was the low food utilization efficiency that resulted in the FCR (8.65) and the same trend was observed during the subsequent periods of the experiment (Fig. 2). Fish fed Spirulina100 diet had the highest PER whereas the lowest value was observed in fish fed Alga100 diet.

Fish growth was followed during 11 weeks and "*ad libitum*" feed acceptance was examined at week 9 (Fig. 3B). The results indicated that the growth rate was significantly improved when up to 50% of the plant protein was replaced. This experimental design, however, was based on restricted feeding, that is, where feed rations are adjusted in accordance to body biomass increase. However, feed consumption analysis suggested that when fish were fed to satiation, tilapia may grow at an even faster rate than fish fed control diet (Fig. 3B).

The proximate composition of the experimental fish at the end of the feeding trial is presented in Table 4. No significant difference was found in the moisture content among the different treatments ($P > 0.05$) except for the fish fed Alga100 diet that had the highest value ($P < 0.05$). Fish fed Alga100 diet exhibited higher protein content than that of the control diet ($P < 0.05$). Only fish fed control and Alga100 diets showed a significant difference in the lipid content in their bodies from the other treatments ($P < 0.05$, Table 4). The highest value of ash content was recorded in fish fed Alga100 diet and the lowest was recorded with fish fed Spirulina100 diet. These values in fish body correspond to an increase in ash content in diets containing increased level of algae (Table 2).

Table 3. Final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate of juvenile Nile tilapia *Oreochromis niloticus* (initial wt 0.02 g) fed the experimental diets for 11 weeks. Values are mean ± SD.

Treatments*	Control	Alga25	Alga50	Alga75	Alga100	Spirulina100
FBW (g)	0.97 ± 0.12^{c}	1.52 ± 0.13^{e}	1.22 ± 0.05^{d}	0.77 ± 0.05^{b}	0.26 ± 0.01^{a}	1.82 ± 0.10^{f}
WG (%)†	4767 ± 597^{c}	7517 ± 625^{e}	5983 ± 236^{d}	3733 ± 247^{b}	1200 ± 50^{a}	8983 ± 506^{f}
SGR (%)‡	5.04 ± 0.16^{c}	5.62 ± 0.11^{e}	5.34 ± 0.05^{d}	4.73 ± 0.09^{b}	3.32 ± 0.05^{a}	5.85 ± 0.08^{f}
FI (g)	4.16 ± 0.17^{d}	4.64 ± 0.13^{e}	3.82 ± 0.11^{c}	3.28 ± 0.20^{b}	2.08 ± 0.19^{a}	4.14 ± 0.03^{d}
FCR§	4.39 ± 0.37^{c}	3.10 ± 0.17^{b}	3.19 ± 0.025^{b}	4.40 ± 0.30^{c}	8.65 ± 0.49^{d}	2.31 ± 0.12^{a}
PER¶	0.58 ± 0.05^{b}	0.86 ± 0.05^{d}	0.86 ± 0.01^{d}	0.66 ± 0.05^{c}	0.35 ± 0.02^{a}	1.08 ± 0.06^{e}
Survival (%)‖	81 ± 6^{a}	86 ± 2^{a}	87 ± 1^{a}	87 ± 2^{a}	79 ± 9^{a}	87 ± 1^{a}

*Values in the same row with the same superscript are not significantly different (*P>0.05*).
†WG (%) = 100 × (final body weight – initial body weight)/initial body weight
‡SGR (%/day) = 100 × (Ln final weight – Ln initial weight)/Time (days)
§FCR = Total feed consumed (g fish^{-1})/weight gain (g fish^{-1})
¶Protein efficiency ratio (PER) = weight gain (g fish^{-1})/protein intake (g)
‖Survival rate (%) = [(no. of fish at the end of the experiment / no. of fish at the beginning of the experiment)] ×100.

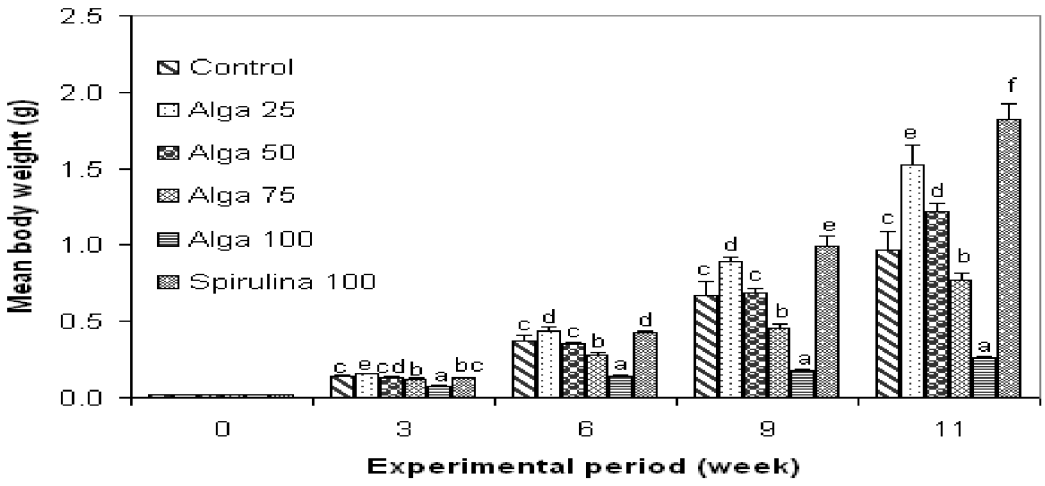

Figure 1. Changes in the mean body weight of Nile tilapia fed the experimental diets for 11 weeks. Values are means ± SD of triplicate groups. Superscripts having different letters are significantly different (*P* < 0.05).

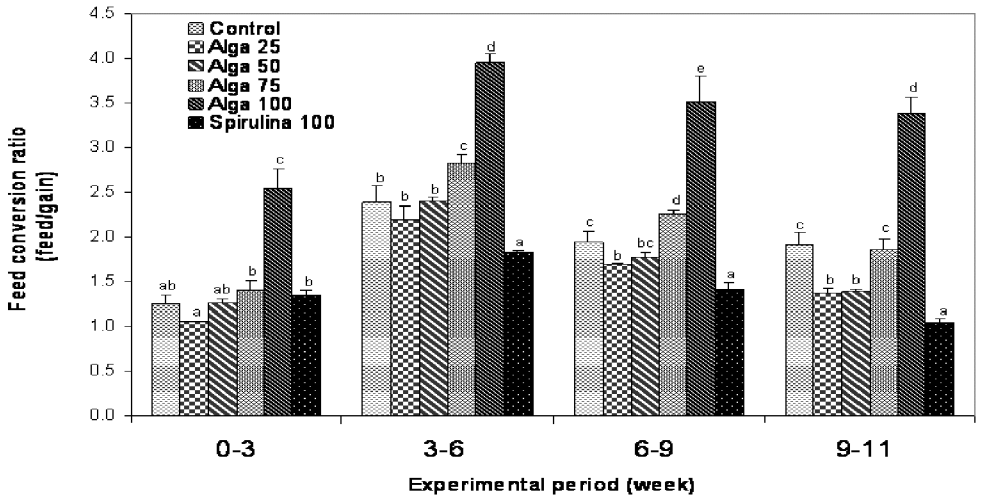

Figure 2. Changes in feed conversion ratio between feeding periods with the experimental diets fed to Nile tilapia for 11 week. Values are means ± SD of triplicate groups. Superscripts having different letters are significantly different (*P* < 0.05).

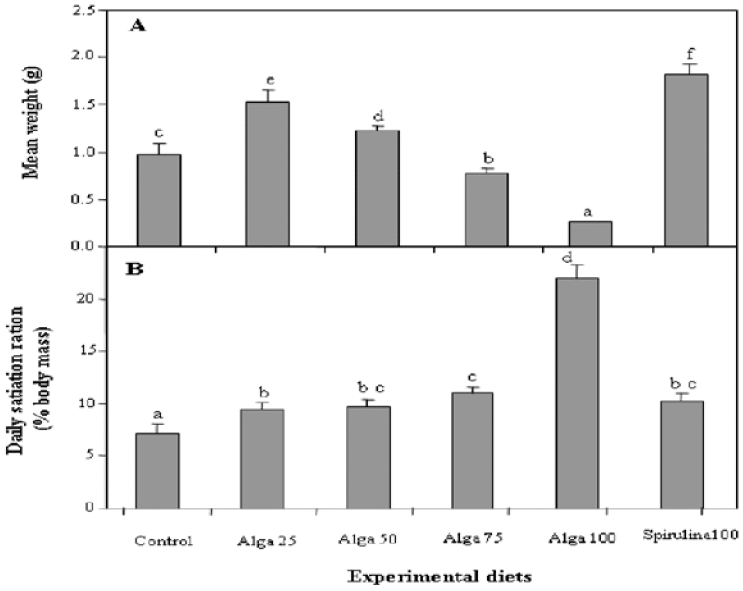

Figure 3. Mean body weight (A), and feed intake (B) of juvenile tilapia fed experimental diets containing 5 different levels of Algamaxx or Spirulina. Final weight was recorded after 11 weeks, whereas feed consumption ad libitum was recorded after 9 weeks. Different superscripts among columns indicate significant differences at *P* < 0.05.

Table 4. Body composition of juvenile tilapia fed the experimental diets containing 5 different levels of Algamaxx or Spirulina for 11 weeks.

Composition (% wet basis)	Control	Alga25	Alga50	Alga75	Alga100	Spirulina100
Moisture	75.5±0.6^{a}	75.3±0.4^{a}	75.4±1^{a}	77.8±1^{a}	81.5±3^{b}	75.4±2^{a}
Protein	49.8±1.7^{a}	53.7±1.6^{b}	56.9±0.5^{c}	59.9±0.4^{d}	65.8±0.8^{e}	56.8±1.2^{c}
Lipid	31.3±1^{c}	23.3±3.3^{b}	26.5±0.6^{b}	22.9±1.8^{b}	14.4±2.4^{a}	24.8±2.7^{b}
Ash	14.3±0.4^{b}	14.0±0.6^{ab}	14.2±0.3^{b}	15.6±0.3^{c}	18.4±0.9^{d}	13.2±0.2^{a}

Values are mean ± SD of triplicate groups. Values followed by the same superscript letters in the same row are not significantly different ($P > 0.05$).

Mineral compositions of the diets used in this study were examined (Table 2). The results showed that algae used in this study contained high levels of Al and Fe which may affect fish growth rates. Several other metals have shown a linear increase of their concentration in fish body (Fig. 4) that paralleled the metal levels in the diets (Table 2). In particular, iron concentrations in the diets which increased up to 1% with 100% algae protein used in replacement might have resulted in some decrease in food utilization and growth depression in fish (FCR; Table 3). The lower growth rate had apparently decreased Fe level in the body of the later group in comparison to fish fed 75% replacement. An exception from a positive correlation between dietary and body minerals in tilapia are Ca and Na (Fig. 5 and Table 2). It is also apparent that the demand for the essential element, phosphorus, increases with an augmentation of algae dietary supplement. Fish fed Spirulina did not conform to the same rule of dietary-body mineral relationship. For instance, high Na levels in Spirulina100 diet did not result in a proportional response in fish body Na level.

DISCUSSION

Live Spirulina as alternative feed source for tilapia larvae and juveniles was previously examined by Lu *et al.*, (2002). The current study was consistent with the latter findings indicating that the major contribution of algae can compromise the growth of tilapia. When live Spirulina was fed *ad libitum* to tilapia larvae as exclusive food for the duration of 84 days, fish weight was severely depressed. However, when algae were offered at a rate of 10% and then tilapia were transferred to Spirulina, no growth differences were observed in comparison to fish fed a commercial diet. The results of the present study show that algae protein can be effectively substituted for up to 50% of corn gluten as plant protein source when larvae/juveniles tilapia were fed Algamaxx. This has been achieved when fish were fed a restricted diet regime. Further improvement of fish growth and feed utilization using Algamaxx or Spirulina may possibly be obtained with feeding to satiation. Spirulina contains several nutrients, especially vitamins and minerals that may enhance fish growth. These results are in agreement with those found by Abdel-Tawwab & Ahmad (2009) who reported that Spirulina has had significant growth and immunity promoting effects in juvenile tilapia (1.8 – 14.0 g) diets when provided at the level of 1%.

Hirahashi *et al.*, (2002) reported that feeding Spirulina to fish and poultry improved survival and growth rates. In this regard, it has been observed that supplemented Spirulina powder in the diet fed to striped jack (*Pseudocaranx dentex*) improved the FCR and growth rates (Watanabe *et al.*, 1990). Also, Olvera-Novoa *et al.*, (1998) suggested that it is possible to replace up to 40% of the animal protein level with Spirulina because it has adequate nutritional value for tilapia juveniles at low inclusion levels without any adverse effects on growth and feeding efficiency. The better feed intake reports of Algamaxx or Spirulina-enriched diets in our studies may have been due to increased fish appetites, resulting in

improved growth. Similar to our results, it has been summarized that many species of fish grow better on algae-enriched diet than on any other fish feeds (Sandbank & Hepher 1978). According to Henson (1990) Spirulina improved growth in a salmonid fish, cherry salmon (*Onchorhynchus masou*), by adding 2.5% of it to the diet. It has been found that Spirulina meal produced a significant enhancement of growth and feed utilization in one year-old red sea bream (*Pagrus major*) when 5% of Spirulina was incorporated into the diet (Mustafa *et al*., 1994). In contrast, poor performance was obtained in rainbow trout and common carp juveniles when Spirulina was the only protein source in the diets (Matty & Smith 1978). Chow & Woo (1990) found no differences in SGR; feed intake or intestine enzymatic activity when replacing 20% of the eel meal (equivalent to 5% protein) with Spirulina meal in the diets for Mossambique tilapia (*O. mossambicus*). Significantly, Nandeesha *et al*. (1998) results suggest that there is no significant difference in SGR, FCR, and PER of common carp ($P > 0.05$) when Spirulina was incorporated into the diet in comparison to control.

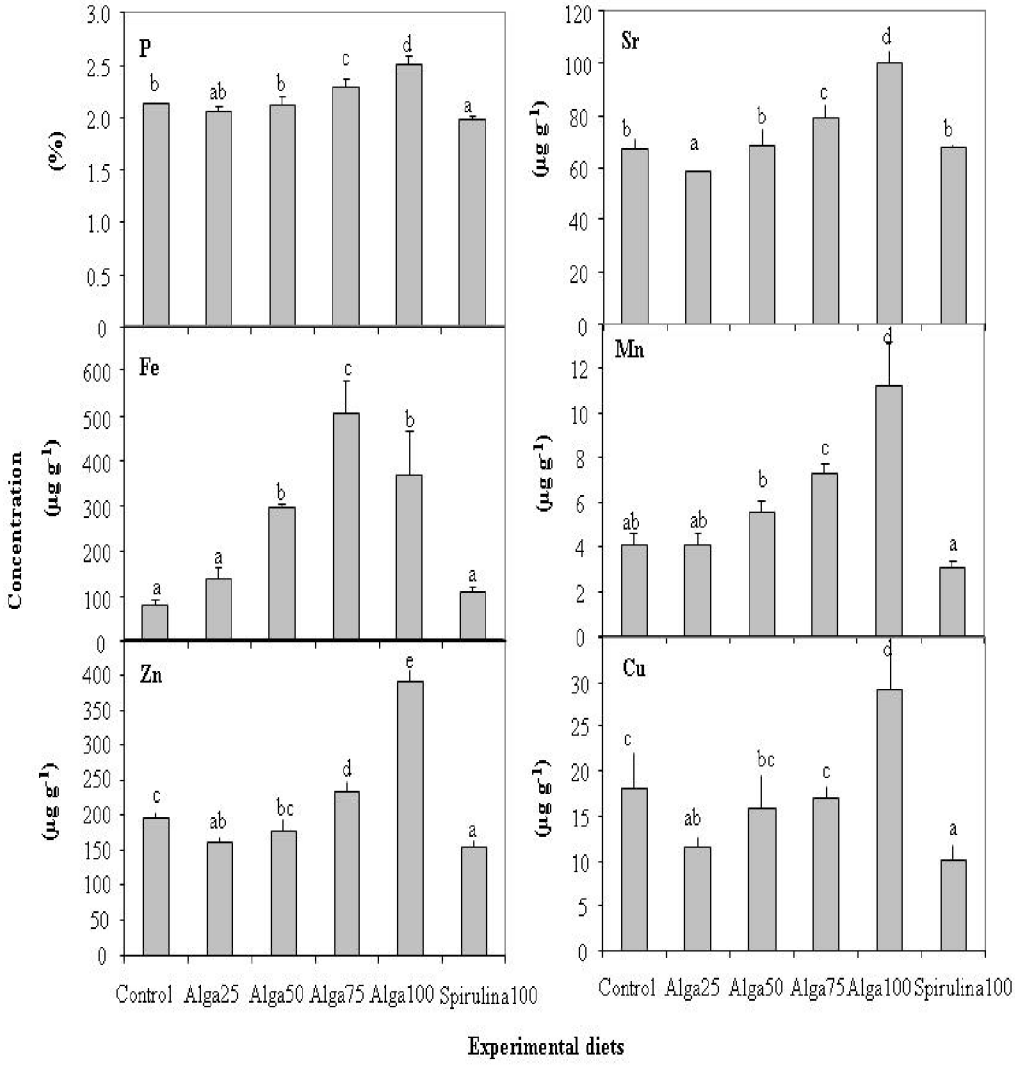

Figure 4. Mineral composition of juvenile tilapia after 11 week feeding experiment with six different diets. Values are means ± SD of triplicate groups. Superscripts having different letters are significantly different ($P < 0.05$).

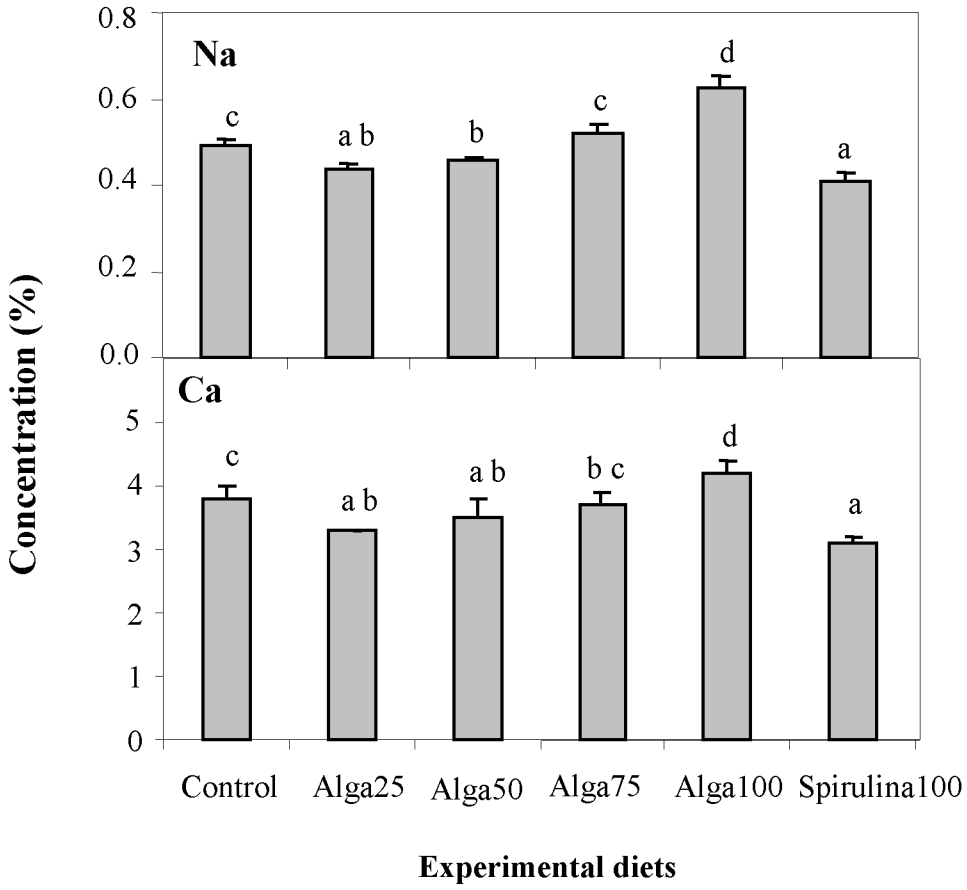

Figure 5. Mineral composition of juvenile tilapia after 11-week feeding experiment with six different diets. Values are means ± SD of triplicate groups. Superscripts having different letters are significantly different ($P < 0.05$).

In the present study, higher amounts of algae (100%) significantly depressed growth and feed utilization in terms of WG, SGR, FI, and FCR of tilapia and these results are in agreement with the results found by Rahnema & Borton (2007) in rainbow trout when fed plant and feather meal based diets (100%). Fish fed on Alga100 diet had slow growth rate and a poor feed-to gain conversion. These results might be related to the high level of Fe and Al contents in the algae (Table 2).

The reduction of voluntary feed intake with plant protein-based diets has been reported in rainbow trout (Gomes & Kaushik 1992) as well as in European sea bass (Dias *et al*., 1997). Also, Silva *et al*., (2010) found low feed intake, poor growth performance and protein accretion when Senegalese sole (*Solea senegalensis*) were fed a plant protein-based (soybean meal, corn, and wheat gluten) diet. The same results were reported by Gomes *et al*., (1995) who showed a depression in feed intake with 100% replacement of fish meal by coextruded rapeseed and pea product which impacted the growth performance of rainbow trout negatively. In contrast, there were no significant differences in growth rate between tilapia fed a plant-based diet (corn gluten meal, soy meal) supplemented with lysine, tryptophan, and threonine and those fed a fish meal-based diet (Wu *et al*., 1997).

The body composition of the experimental fish (Table 4) showed the influence of the dietary composition. Where

fish were fed Alga100 diet, they exhibited the highest ash content and the lowest lipid content. These results are in agreement with Lu *et al*. (2002) who reported that using live Spirulina as the sole diet for tilapia resulted in lipid body content depression although the lipid content in raw Spirulina was almost the same as that in the commercial diets. In the present study the restricted-pair feeding secured the accurate comparison solely on the basis of nutrients intake. We observed significantly higher instantaneous feed intake in fish from groups with lower lipid content (Fig. 3B) that corresponded to 75-100% algae replacement. In fact, diet with 25% replacement (and higher lipid content) was significantly less accepted than diet with 75% of Alagamaxx protein. This clearly indicates that diets with lower lipid levels were not limited in essential fatty acids and growth decrease was related to excessive Al and Fe presence in those two diets (Alga 75 and Alga 100). Furthermore, the general consensus is that tilapia diets are required to have between 5 and 12% of lipids (Lim *et al*., 2011). Therefore, we conclude that the variation in lipid levels in our study was not the confounding factor in diet utilization and fish growth. To the contrary, it has been observed that the inclusion of 5% Spirulina in diets fed to striped jack (*Pseudocaranx dentex*) resulted in depression of body lipid and improved growth rates (Liao *et al*., 1990; Watanabe *et al*. 1990).

Algae used in the current study contained a high level of Al and Fe. Several experiments were carried out to compare the effect of different levels of aluminum or iron supplementation on fish performance, specifically in Nile tilapia (*O. niloticus*) (Santos *et al*., 2011) and African catfish (*Clarias gariepinus*) (Baker *et al*., 1997). In African catfish, the results showed that an increase of ferrous iron to 6,354 mg Fe kg^{-1} diet resulted in liver Fe level of 745 µg g^{-1} in comparison to fish fed a diet unsupplemented with Fe (523 mg kg^{-1}). Our data that relates to the whole body mineral concentrations of tilapia show a proportional increase of Fe corresponding to dietary levels. Lim et al., (2000) demonstrated the effect of ascorbic acid (vitamin C) on Fe metabolism in channel catfish. Supplementation of ascorbic acid to catfish diets containing 300 mg Fe kg^{-1} resulted in hematocrit values that increased from 35.7 to 42.4% and liver iron concentrations rose from 288 to 492 µg Fe g^{-1} that clearly indicated the role of water-soluble vitamins in possible dietary iron toxicity. The toxic effects of dietary aluminum (Al^{+3}) in Nile tilapia were examined by Santos *et al*. (2011). Three different levels of Al $(NO_3)_3 \cdot 9H_2O$ at 50, 75, and 100 mg kg^{-1} were fed to tilapia for 12 months. Some symptoms such as the lack of equilibrium during swimming and the loss of direction were observed. The authors reported that the Al^{+3} taken up by tilapia were not accumulated in the body and subsequently excreted in the feces. The high concentrations of Al in feces indicate low absorption by tilapia organs.

In conclusion, our findings suggest that algae meal protein can replace up to 50% of corn gluten protein in the diets for juvenile Nile tilapia without adverse growth effects. Higher inclusion of algae meal protein resulted in significantly decreased growth performance of juvenile tilapia, probably affected by the increased Fe and/or Al availability. Further research is still necessary to eliminate Al and Fe negative effects on dietary availabilities of phosphorous supplements. Also, to examine other positive aspects of algae supplementation such as attractants being used (increased consumption), enrichment in carotenoids (coloration) and enhanced immunity enhance.

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الإحلال الجزئى والكلى لبروتين مسحوق جلوتين الذرة ببروتين الطحالب فى إعداد علائق أسماك البلطى النيلى

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أجريت هذة الدراسة لتقييم إمكانية إحلال بروتين الطحالب محل بروتين جلوتين الذرة فى إعداد علائق زريعة أسماك البلطى النيلى وذلك بمعدلات إحلال مختلفة وهى صفر (كنترول)، ٢٥، ٥٠ ، ٧٥ ، ١٠٠%. تم عمل عليقة سادسة تحتوى على ١٠٠ % بروتين طحلب اسبرولينا كبديل لبروتين جلوتين الذرة. استخدم فى هذه الدراسة زريعة أسماك البلطى النيلى بمتوسط وزن ابتدائى ٠.٠٢ جم/سمكة و تم تقسيمها الى ١٨ مجموعة كل مجموعة تحتوى على٥٠ سمكة للحوض وكل مجموعة شملت ثلاث مكررات و تم إضافة ١.٥ % حامض أمينى ليسين و ٠.٥% حمض أمينى ميثايونين لكل العلائق التجريبية المستخدمة . تم إعداد العلائق التجريبية لتكون متماثلة فى البروتين والدهن الخام حيث إحتوت على 35 % بروتين خام و 12 % دهن خام فى صورة زيت السمك وزيت فول الصويا كمصدر للفسفوليبيدات . تم تغذية الأسماك بالعلائق التجريبية الستة وذلك لمدة ١١ إسبوع. أوضحت النتائج أن هناك تأثيرات معنوية إيجابية على النمو والأداء و معدل إستهلاك الغذاء لأسماك البلطى النيلى و ذلك حتى معدل إحلال ٥٠ % لبروتين الطحالب بالعلائق ثم انخفض النمو و الأداء فى العلائق المحتوية على أكثر من ٥٠ % بروتين الطحالب. وجد أيضا اختلافات معنوية فى مكونات جسم الأسماك من العناصر المعدنية مثل الألومنيوم و الحديد و الزنك و النحاس . وجد أن محتوى الطحالب من المعادن كان له تأثير معنوي على نمو و أداء الأسماك المغذاة على العلائق المحتوية على أكثر من ٧٥ % نسبة إحلال للطحالب محل بروتين جلوتين الذرة. نستنتج مما سبق أنه يمكن إحلال بروتين الطحالب محل بروتين جلوتين الذرة حتى ٥٠ % في العليقة دون أى تأثيرات ضارة على نمو و أداء زريعة أسماك البلطى النيلى.