

PARTIAL REPLACEMENT OF FISH MEAL WITH DRIED MICROALGA (*CHLORELLA SPP* AND *SCENEDESMUS SPP*) IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) DIETS

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Abstract

This study was designed to investigate the effect of partial replacement of fish meal with dried microalgae (*Chlorella spp* and *Scenedesmus spp*) in Nile tilapia (*Oreochromis niloticus*) diets on fish growth performance, feed efficiency and body composition. Nine isonitrogenous (32% cp), isocaloric (440 kcal/100 g) diets were formulated to contain *Chlorella* and *Scenedesmus* as fish meal replacers at zero (control), 10, 25, 50 and 75% substitution. The diets were fed to triplicate groups of 25 Nile tilapia (*Oreochromis niloticus*) fingerlings (6.5 ± 0.05 g average body weight) in 75 l glass aquaria for 90 days. Results indicated that, growth performance, feed conversion ratio and protein productive value were significantly (P<0.05) higher in fish feed diets containing 50% of both *Chlorella spp* and *Scenedesmus spp*, whereas fish feed diets containing 75% algae had significance lower performance (P<0.05). Carcass analysis showed significantly (P<0.05) higher dry matter and crude protein content, but lower lipid content, in fish received algae at 50% level, compared to other treatments. The present results suggest that dried *Chlorella spp* and *Scenedesmus* can replace fish meal up to 50% substitution level in Nile tilapia diets.

Keywords: algae, fish, growth performance, feed efficiency, body composition

INTRODUCTION

Feed accounts for more than half of the variable operating costs in many aquaculture operations today NRC, (1993). Therefore, the potential use of unconventional feed ingredients such as algae, as feed inputs as a replacement of high cost feed stuffs such as fishmeal has been increasing. Algae have attention as a possible alternative protein source for cultured fish, particularly in tropical and subtropical regions where algae production high and their good protein, vitamins and essential fatty acids contents (El-Hindawy *et al.* 2006). The use of microalgae as fish feed inputs has been studied with encouraging results. (Broun, 1980), reported positive growth performance in fish feed diets containing algae cells. Zeinhom. (2004) found that fish fed diet containing 15% algae increased significant by the digestibility coefficient of dry matter (92.5%), crude protein (87.63%), ether extract (88.45%) and energy (81.41%).

Some studies have been carried out to investigate the chemical composition of microalgae. Tartiel. (2005) found that crude protein content of *Chlorella spp* was 46.7 %, the crude fat content was 14.8 %, total Carbohydrate content was 11.6 % , ash was 17.5 % , crude fiber 9.30 %, nucleic acid content (RNA 2.63 % and DNA 1.72 %), and vitamins group antioxidant B₆, B₁₂, E, C, B-carotene ($\mu\text{g/g}$ dry weight) were found to be 0.05, 0.08, 2.25, 16.0 and 2384.0, respectively. Mean while, *Scenedesmus spp* contained 52.3% crude protein, 12.20% crude fat, 10.06% Carbohydrate, 14.92 % ash, 8.83% crude fiber, 3.16 RNA and 1.43 DNA , 0.27 B₆, 0.78 B₁₂, 0.01 E, 21.8 C and 1890.0 B-carotene ($\mu\text{g/g}$ dry weight). Dawah *et al.* (2002a) reported that, five amino acids (aspartic acid, serine, alanine, leucine and glycine) were collectively responsible for 50% or more of the total dry matter content of *Chlorella* and *Scenedesmus* algae, However, they were deficient in sulfur-amino acids. Soeder (1980) reported that about 67% of fatty acids in *Scenedesmus* are unsaturated. Natural food still remains the major feed for Tilapia rearing so a timely supply of microalgae in sufficient quantity ensures the success of a tilapia hatchery.

The present study was carried out in the Central Laboratory for Aquaculture Research (CLAR), Abbassa, Sharkia Governorate, Egypt, to evaluate the use of *Chlorella* and *Scenedesmus* as fish meal replacers Nile tilapia (*Oreochromis niloticus*) diets under Egyptian conditions.

MATERIALS AND METHODS

Experimental Diets

Nine practical diets were formulated based on the proximate composition of the feed ingredients. Diet 1 (Control diet contained no algae), diets 2, 3, 4 and 5 contained 10, 25, 50 and 75%, respectively of dried *Chlorella spp* while diets 6, 7, 8 and 9 contained 10, 25, 50 and 75%, respectively, of dried *Scenedesmus spp* replacement of fish meal on an equivalent protein basis. The algae used in this study were product according to El-Fouly *et al.* (1998). Composition and proximate analysis of algae and the experimental diets were performed according to (AOAC, 1990) methods (tables 1 and 2.)

Fish and feeding regime

Nile tilapia (*Oreochromis niloticus*) fingerlings with an average weight 6.50 ± 0.05 , were transferred from Abbasa Hatchery to the wet lab of CLAR and acclimatized in fiberglass tanks for three weeks. Fish were randomly stocked in triplicates, in 75 l glass aquaria at a density of 25 fish per aquarium. The fish were fed the test diets at 3% body weight per day. The amount of feed was calculated and readjusted every 15

days according to change in the body weight and distributed in three equal portions for 90 days.

Experimental system

The experimental facility consisted of 27 glass Aquaria (75 liters each). Each aquarium was supplied with aerated and dechlorinated tap water, which was stored in tanks for 24 hours and aerated by air pump (Model-Rina 301) during the experimental period. The water level was maintained to a fixed level by the addition of new well-aerated fresh water.

Data calculation

Body weight gain (g/fish) = Mean of weight (g) at the end of the experimental period – weight (g) at the beginning of the experimental period.

Daily weight gain (DWG) = Gain / experimental period.

Relative weight gain (RWG %) = Gain / initial weight X 100

Specific growth rate (SGR) = $(\ln W_1 - \ln W_0) / T \times 100$

Feed conversion ratio (FCR) = Total feed fed (g/fish) / total wet weight gain (g/fish).

Protein efficiency ratio (PER) = Wet weight gain (g/fish) / amount of protein fed (g/fish).

Protein productive value (PPV) = (Amount of protein retained (g/fish)/ amount of protein fed (g/fish) X 100

Statistical analysis of data

Statistical analysis was performed using the Analysis of variance (ANOVA) and Duncan's multiple Range Test, to determine differences between treatments means at significance rate of $P < 0.05$. The standard errors of treatment means were also estimated. All statistics were carried out using Statistical Analysis program (SAS, 2000).

RESULTS AND DISCUSSION

Growth performance

Growth performance of fingerlings Nile tilapia (*Oreochromis niloticus*) fed on diets with partial replacement of fish meal by algae meal is shown in Table 3. The results indicated that, growth performance (final body weight, BWG, DWG, RWG and SGR) increased significantly ($P < 0.05$) with increasing of both algae species (*Chlorella spp* and *Scenedesmus spp*) replacement from zero to 50% algae. However, the highest values of final body weight (30.7 ± 0.18 & 31.96 ± 0.26), BWG (24.08 ± 0.23 & 25.38 ± 0.21), DWG (0.27 ± 0.003 & 0.28 ± 0.002), RWG (363.9 ± 6.66 & 385.7 ± 2.12) and SGR (1.76 ± 0.01 & 1.70 ± 0.02) were obtained by fish fed 50%

replacement in both algae species (*Chlorella spp* and *Scenedesmus spp*) respectively, while the lower values of final body weight (22.42 ± 0.13 & 23.4 ± 0.22), BWG (15.62 ± 0.09 & 16.47 ± 0.13), DWG (0.17 ± 0.001 & 0.18 ± 0.001), RWG (229.7 ± 1.64 & 237.7 ± 1.58) and SGR (1.33 ± 0.01 & 1.35 ± 0.01) were obtained by fish fed 75% replacement in both algae species (*Chlorella spp* and *Scenedesmus spp*) respectively. These results indicated that the high content of protein and lipid of algae (*Chlorella spp* and *Scenedesmus spp*) are more suitable for fish diets.

These results are in agreement with those obtained by Brune (1980), Ibrahim (2001) and Dawah *et al.* (2002b) who found that the addition of algae in fish diets improved growth performance of Nile tilapia (*O. niloticus*). Also, Zeinhom (2004) found that, Inclusion of algae in fish diets significantly ($P < 0.05$) increased the live body weight (39.69 g), BWG (26.46 g), DWG (0.29g) and SGR (1.22), and Nandeeshha *et al.* (1998) reported that body weight gain of Nile tilapia (*O. niloticus*) increased linearly with increasing the level of algae in fish diet at levels less than 20%. Also these results were in partial agreement with the finding of Nuria and Carmen (1998) who found that the addition of algae, aquatic plant and yeast in fish diets did not differ significantly ($P < 0.05$) less than 20, 25 and 10% respectively, while, Olvera–Novoa, *et al.* (1998) stated that the daily body weight gain did not differ significantly ($P < 0.05$) affecting by different addition of microalgae Spirulina in fish diets for *Tilapia mossambicus* less than 30 and 50% respectively. Similarly Abu Zead (2001), showed that the addition of water hyacinth, water primrose and algae in fish diets improved growth performance of Nile tilapia and common carp.

Feed efficiency

From the presented results in Table 3. the lower significant value of feed intake (48.92 ± 2.28 and 44.69 ± 1.61) was obtained by fish fed diet containing 50% algae in both algae species (*Chlorella spp* and *Scenedesmus spp*) respectively. In addition, the average values of FCR, PER and PPV increased significantly ($P < 0.05$) with increasing of both algae species (*Chlorella spp* and *Scenedesmus spp*) replacement from 0 to 50% algae. However, incorporation 50% algae replacement resulted in the significant greater value of FCR (2.03 ± 0.08 and 1.76 ± 0.05), PER (1.54 ± 0.06 and 1.78 ± 0.05) and PPV (19.98 ± 0.19 and 20.80 ± 0.19) in both algae species (*Chlorella spp* and *Scenedesmus spp*) respectively. These results are agree with those obtained by Dawah, *et al.* (2002b) who found that food conversion ratio and PER were better when the fish were maintained on artificial diets with 10% and 20% dried algae. In addition, Zeinhom (2004) found that, Inclusion of algae in fish diets insignificantly ($P < 0.05$) improved the FCR (2.33), PER (1.34) and PPV (43.10), whereas feed intake was significantly increased. However, these results are good in agreement with those

obtained by Ibrahim (2001) and Abu Zead (2001) who found that the protein efficiency ratio ranged from 1.1 to 1.7 for Nile tilapia and common carp fed on diets containing aquatic plant and algae, while Ibrahim *et al.* (2007) reported that, feed conversion ratio gradually improved with increasing Azolla meal percentage in the diets without significant differences until 31.8% inclusion level after that, significantly lowered, they added that, economical feed efficiency improved as the level of the dietary Azolla meal increased from 10.6 to 31.8% of the diet.

Body composition

Chemical composition of the whole fish body as affected by partial algae replacement at the end of experimental period are illustrated in Table 4. In all groups including algae in the diets, fish body dry matter and protein content was improved except fish fed diet containing 75% algae replacement which are lower body dry matter and protein content than fish fed control diet. Fish fed diet containing 50% algae replacement in both algae species (*Chlorella spp* and *Scenedesmus spp*) showed the highest significant ($P < 0.05$) dry matter (24.16 ± 0.18 and 25.11 ± 0.25), protein body content (65.52 ± 0.64 and 66.00 ± 1.32) and lowest significant ($P < 0.05$) fat (13.83 ± 0.09 and 12.56 ± 0.16) and gross energy body content (510.5 ± 1.05 and 513.9 ± 1.66) respectively. Generally, lower percentage of body fat was observed by increasing the examined algae percentage up to 50% in the diets. Whereas the higher value of ash body contents in the dry matter (20.25 ± 0.12 and 19.50 ± 0.06) was observed in fish fed diet containing 75% algae in both algae species (*Chlorella spp* and *Scenedesmus spp*) respectively. Similar results were observed by Zeinhom (2004) who found that, Inclusion 15% algae resulted the higher value of chemical composition of the whole fish body dry matter(28.11), crude protein (64.12) and ash (19.76), whereas fish fed 25% algae resulted higher value of ether extract(21.4) and gross energy (540.7). In addition, these results are partial agreement with those obtained by Appler and Jauncy (1983) who found that, the moisture content decreased with increased algae level in fish diet, whereas fat content decreased with increasing algae in fish diet. El-Hadidy *et al.* (1993) mentioned that, the fish diet containing algae up to 45% did not cause a significant variation in carcass CP, ash and dry matter comparing with control diet for Nile tilapia (*O. niloticus*). Olevera-Novoa *et al.* (1998) stated that, the body chemical composition of Tilapia mossambica did not clearly affected by the inclusion of Spirulina algae from 20 to 100% in the diet, whereas the highest values of ash content were recorded in the control diet and the lowest values were recorded in fish diet containing 80% Spirulina algae. In addition,

Abu Zead (2001) found that, the dry matter of tilapia and common carp increased with increasing replacement by aquatic plant less than 20% for tilapia and less than 30% for common carp, whereas fat contents were opposite trend by protein contents. On other hand, El-Sayed (1994) found that there is a negative correlation between algae in fish diet and body chemical composition of Nile tilapia and common carp such as CP and EE. This difference body chemical composition my be due to the variation in culture and fish size. Also Ibrahim *et al.* (2007) reported that Body protein and fat content were significantly decreased with increasing Azolla meal percentage in the diets. Nevertheless, moisture and ash content were significantly increased with increasing Azolla meal percentage in the diets.

In conclusion, the present experiment showed that examined algae could be utilized in Nile tilapia (*Oreochromis niloticus*) diets up to 50% instead of the dietary ingredient without any adverse effects on fish growth performances, feed utilization parameters and body composition.

Table 1. The chemical composition of dried examined algae (on DM basis)

	<i>Chlorella spp</i>	<i>Scenedesmus spp</i>
Dry matter (%)	94.01 ± 1.30	95.30 ± 0.33
Crude protein (%)	46.78 ± 0.50	51.17 ± 1.60
Crude fat (%)	14.83 ± 0.90	12.29 ± 0.08
Crude fiber (%)	08.32 ± 0.09	06.91 ± 0.50
Ash (%)	18.75 ± 1.40	19.63 ± 0.62
*NFE (%)	11.32 ± 0.23	10.00 ± 0.05

*Nitrogen free extract = Nitrogen free extract = 100 — (moisture + protein + lipid + fiber + ash)

Table 2. The formulation and chemical composition of the experimental diets

Ingredients	Treatments								
	Control (0.0%)	<i>Chlorella spp</i>				<i>Scenedesmus spp</i>			
		10%	25%	50%	75%	10%	25%	50%	75%
Fish meal (72%CP)	22.23	20.01	16.67	11.12	5.56	20.01	16.67	11.12	5.56
<i>Chlorella spp</i>	---	3.43	8.56	17.11	25.66	---	---	---	---
<i>Scenedesmus spp</i>	---	---	---	---	---	3.13	7.82	15.64	23.46
Soybean meal(44%CP)	35.67	35.6	35.48	35.31	35.13	35.6	35.45	35.21	34.99
Corn starch	31.74	30.24	30.24	28.32	26.32	30.29	30.29	30.06	28.52
Cotton seed oils	3.5	4	3.5	3.5	3	3.95	3.5	3.3	3.23
Vitamin premix ¹	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix ²	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Methionen	0.23	0.27	0.32	0.4	0.48	0.27	0.34	0.44	0.54
Cellulose powder	3.63	3.45	2.23	1.24	0.85	3.75	2.93	1.23	0.7
Total	100	100	100	100	100	100	100	100	100
Chemical composition (on DM basis)									
Dry matter (DM)	90.65	91.1	91.15	91.36	91.59	91	91.06	91.13	91.24
Crude protein (CP)	32.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00
Crude lipid	5.76	6.58	6.56	7.36	7.66	6.41	6.25	6.54	6.97
Crude fiber (CF)	6.23	6.33	6.17	6.16	4.60	6.57	6.06	4.88	4.18
Ash	6.06	6.47	7.07	8.09	9.10	6.10	6.67	7.62	8.89
Nitrogen free extract	49.95	48.62	48.20	46.39	46.64	48.92	49.02	48.96	47.96
Gross energy kcal/100g ³	440.15	442.42	440.51	440.62	444.48	442.05	440.95	443.44	443.39
Digestibility energy kcal/100g ⁴	311.74	316.46	315.44	319.02	322.22	315.53	314.29	316.78	318.65

¹Vitamin premix :- each 2.5Kg contain Vit A 10MIU, D₃ 1MIU, E 10gm, K gm, B₁ gm, B₂ 4gm, B₆ 1.5gm, B₁₂ 10gm, Pantothenic acid 10gm, Nicotinic acid 20gm, Folic acid 1000gm, Biotin 50gm And Coline chloride 500gm.

²Mineral premix. No # 5 (tilapia) source: Jauncey and Ross,1982.

³(kcal/100g diet); based on 5.64 kcal/g protein, 9.44 kcal/g lipid, and 4.11 kcal/g carbohydrate. NRC (1993)

⁴(kcal/100g diet); based on 5.0 kcal/g protein,9.0 kcal/g lipid,2.0 kcal/g carbohydrate (Wee and Shu.1989).

Table 3. Growth performance and food utilization efficiency of Nile tilapia (*Oreochromis niloticus*) fingerlings fed the experimental diets

Items	Treatments								
	Control (0.0%)	<i>Chlorella spp</i>				<i>Scenedesmus spp</i>			
		10%	25%	50%	75%	10%	25%	50%	75%
Initial weight	6.5 ± 0.05 ^a	6.63 ± 0.05 ^a	6.57 ± 0.06 ^a	6.62 ± 0.07 ^a	6.80 ± 0.05 ^a	6.61 ± 0.08 ^a	6.59 ± 0.07 ^a	6.58 ± 0.06 ^a	6.93 ± 0.10 ^a
Final body weight	26.46 ± 0.25 ^d	27.96± 0.14 ^c	28.1 ± 0.21 ^c	30.7 ± 0.18 ^b	22.42 ± 0.13 ^f	28.2 ± 0.22 ^c	28.56 ± 0.32 ^c	31.96 ± 0.26 ^a	23.4 ± 0.22 ^e
PWG (g/fish)	19.94 ± 0.25 ^d	21.33 ± 0.19 ^c	21.53 ± 0.25 ^c	24.08 ± 0.23 ^b	15.62 ± 0.09 ^f	21.59 ± 0.28 ^c	21.97 ± 0.33 ^c	25.38 ± 0.21 ^a	16.47 ± 0.13 ^e
DWG (g)	0.22 ± 0.003 ^d	0.24 ± 0.002 ^c	0.24 ± 0.003 ^c	0.27 ± 0.003 ^b	0.17 ± 0.001 ^f	0.24 ± 0.003 ^c	0.24 ± 0.004 ^c	0.28 ± 0.002 ^a	0.18 ± 0.001 ^e
RWG (%)	305.9 ± 4.68 ^d	321.8 ± 5.12 ^c	327.8 ± 6.21 ^c	363.9 ± 6.66 ^b	229.7 ± 1.64 ^e	326.8 ± 7.78 ^c	333.5 ± 6.47 ^c	385.7 ± 2.12 ^a	237.7 ± 1.58 ^e
SGR	1.56 ± 0.01 ^d	1.60 ± 0.01 ^c	1.61 ± 0.02 ^c	1.70 ± 0.02 ^b	1.33 ± 0.01 ^e	1.61 ± 0.02 ^c	1.63 ± 0.02 ^c	1.76 ± 0.01 ^a	1.35 ± 0.01 ^e
Feed intake	53.47 ± 1.77 ^{ab}	54.62± 1.60 ^{ab}	55.14 ± 1.52 ^a	48.92 ± 2.28 ^{bcd}	50.62 ± 1.29 ^{abc}	55.96 ± 2.94 ^a	56.47 ± 1.58 ^a	44.69 ±1.61 ^d	45.19 ± 0.81 ^{cd}
FCR	2.68 ± 0.06 ^b	2.56 ± 0.05 ^b	2.56 ± 0.04 ^b	2.03 ± 0.08 ^c	3.24 ± 0.06 ^a	2.59 ± 0.11 ^b	2.57± 0.05 ^b	1.76 ± 0.05 ^d	2.74 ± 0.02 ^b
PER	1.17 ± 0.02 ^c	1.22 ± 0.03 ^c	1.22 ± 0.02 ^c	1.54 ± 0.06 ^b	0.97 ± 0.02 ^d	1.21 ± 0.05 ^c	1.22 ± 0.03 ^c	1.78 ± 0.05 ^a	1.14 ± 0.01 ^c
PPV	16.29 ± 0.16 ^e	17.35 ± 0.02 ^d	17.60± 0.17 ^{cd}	19.98 ± 0.19 ^b	13.26 ± 0.09 ^g	17.43 ± 0.13 ^d	17.89 ± 0.09 ^c	20.80 ± 0.19 ^a	14.18 ± 0.12 ^f

Data are represented as mean of three samples replicates ± standard error

Means in the same row with the same letter are not significant difference (P>0.05)

Table 4. Carcass composition (percentage of dry weight) of Nile tilapia (*Oreochromis niloticus*) fed the experimental diets after 90 days growth

Items	Treatments								
	Control (0.0%)	<i>Chlorella spp</i>				<i>Scenedesmus spp</i>			
		10%	25%	50%	75%	10%	25%	50%	75%
Dry matter	21.85 ± 0.05 ^e	22.88 ± 0.05 ^c	23.05 ± 0.12 ^c	24.16 ± 0.18 ^b	22.21 ± 0.12 ^e	23.26 ± 0.09 ^c	23.97 ± 0.09 ^b	25.11 ± 0.25 ^a	23.04 ± 0.14 ^c
Crude protein	62.24 ± 1.13 ^c	62.51 ± 1.08 ^c	63.33 ± 0.95 ^{bc}	65.52 ± 0.64 ^a	60.00 ± 0.82 ^e	62.70 ± 1.22 ^{bc}	63.70 ± 0.96 ^b	66.00 ± 1.32 ^a	61.14 ± 2.12 ^d
Crude Fat	15.92 ± 0.10 ^c	15.69 ± 0.06 ^c	15.92 ± 0.09 ^c	13.83 ± 0.09 ^d	17.42 ± 0.08 ^a	15.63 ± 0.04 ^c	15.77 ± 0.14 ^c	12.56 ± 0.16 ^e	16.80 ± 0.18 ^b
Ash	18.50 ± 0.04 ^e	18.79 ± 0.04 ^d	19.08 ± 0.10 ^c	18.11 ± 0.08 ^f	20.25 ± 0.12 ^a	19.01 ± 0.04 ^c	18.66 ± 0.07 ^{de}	18.24 ± 0.06 ^f	19.50 ± 0.06 ^b
Gross energy	515.0 ± 1.01 ^{ab}	513.0 ± 0.62 ^{abcd}	514.3 ± 0.70 ^{abc}	510.5 ± 1.05 ^d	512.4 ± 0.49 ^{bcd}	512.1 ± 0.59 ^{cd}	515.8 ± 0.15 ^a	504.0 ± 0.69 ^e	513.9 ± 1.66 ^{abc}

Data are represented as mean of three samples replicates ± standard error

Means in the same row with the same letter are not significant difference (P>0.05)

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