

**THE USE OF AQUATIC-PLANT-PROTEINS IN COMPLETE DIETS
FOR THE FLORIDA RED TILAPIA
(*Oreochromis urolepia* hornorum X *O. mossambicus* hybrid)**

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ABSTRACT

Florida red tilapia (*Oreochromis urolepia* hornorum X *O. mossambicus* hybrid) fingerlings were fed on three levels of aquatic plant proteins (10, 20 and 30% of seaweeds, macro algae and azolla) in complete diets. The results showed that replacing plant proteins (Soybean meal and Wheat milling by products) with aquatic plant proteins increased growth performance and feed utilization of Florida red tilapia at the level of 10% Seaweeds or Algae and 20% of Azolla respectively. Productive protein value and energy utilization by Florida red tilapia decreased by increasing the level of replacement with Seaweeds or Algae to more than 10% and level of Azolla more than 20%. It is therefore, recommended to use 10% of Seaweeds or Algae and 20% of Azolla to diets containing mixture of fish meal and soybean meal for feeding Florida red tilapia fingerlings.

INTRODUCTION

Intensification of tilapia culture has made it essential to develop suitable feeds to be used either as supplementary diet in ponds or as a complete diet in tanks. For economic and practical reasons; these diets must use locally available protein sources, preferably those unsuitable for direct human consumption. Fishmeal remains an important but very expensive ingredient and its presence even in small amounts greatly improves the nutritional value of the entire diet

(FAO, 1983). The present study was therefore undertaken to determine the value of some widely available plant protein sources used to feed red tilapia. The plant proteins chosen were Seaweeds, Macro algae and *Azolla pinnata* in practical diets for red tilapia fingerlings. Seaweeds have been used for many years in human nutrition and largely as a vitamin additive and feeding stuff for ruminants (Hegazy, 1974), Poultry (Assar *et al.*, 1972) and fish (Venkatesh and Shetty, 1978). El-Shazly (1955) studied the chemical composition of some species of the seaweeds spread over the shores of Alexandria, and reported that, their chemical composition seems to be comparable to Berseem hay (*Trifolium alexandrinum*).

Algae received attention as a possible alternative protein source for farmed fish, because of their high protein content and high production rate. The most common algae containing high protein and used as protein sources for several fishes are the micro-algae *Chlorella* sp., *Scenedesmus* sp. and *Spirulina* sp. (Nose, 1960; Stanley and Jones, 1976; Matty and Smith, 1978; Tsai, 1979 and Harroun, 1995). However, using algae as the sole protein source in fish feed sometimes results in malformation and impaired growth (Meske and Pfeffer, 1978). The addition of a small amount of algal meal to the fish diet can produce considerable effects on the growth, feed utilization, physiological condition, stress response, disease resistance, body constituents and carcass quality of cultured fish (Mustafa and Nakagawa, 1995).

Azolla pinnata is used in many areas as a feed for livestock for its reasonable protein content (24 - 30%) and good amino acid profile (El-Sayed, 1992). Few studies have been conducted on the use of *Azolla* as a supplemental or complete feed ingredient for fish. The results of these studies, however are inconsistent and ambiguous. For example, Al-Mazan *et al.* (1986) and Micha *et al.* (1988) reported that, the inclusion of different strains and levels of *Azolla* in Nile tilapia diets has resulted in poor growth and feed utilization. Conversely, Santiago *et al.* (1988) found that up to 42% of dietary fish meal was replaced with *Azolla pinnata* in diets fed to *Oreochromis niloticus* (L.) fry without adversely affecting fish performance. Moreover, a diet supplemented with *Azolla pinnata* produced better growth and feed conversion than a fishmeal based diet. Therefore, more research is needed to settle the existing dispute on the effect of quantity of *Azolla* on growth and survival of different fish species, if it used in complete diets.

The main objectives of the present study are to determine the maximum inclusion rates for the aquatic plant sea weeds, macro algae and azolla in the diets and their effects on growth performance and feed utilization of Florida red tilapia reared in seawater.

MATERIAL AND METHODS

The present study was conducted at the Rearing Laboratory of the National Institute of Oceanography and Fisheries (N.I.O.F.), Alexandria, to investigate the effect of inclusion of three levels (10, 20 and 30%) of aquatic plant proteins [seaweeds, macro algae (*Spirulina* sp.) and *Azolla pinnata*] in complete diets, on growth performance and feed utilization of Florida red tilapia (*Oreochromis urolepia* hornorum x *O. mossambicus* hybrid) fingerlings.

Fish were obtained from Mariut Fish Farm, Alexandria, Egypt. Fresh marine seaweeds were collected on March 1996 from Anfoushy "Alexandria seacoast"; macro algae (*Spirulina* sp.) and Azolla were collected from the Experimental Fish Farm, at "Abbis", Faculty of Agriculture, Alexandria University. The aquatic plants were dried at 70°C in the oven for 48 hours, then dried and ground, to pass through 1.0 mm sieve. Then the meals were kept in plastic bags until mixed with the rest of the constituents of the diet. The mixture was processed by California Pellet Meal (CPM) machine. The pellets were soft with particle size of 0.6 mm diameter and 2 mm length.

Twenty 105l glass aquariums each containing salt water (32 ppt) were arranged to perform 10 feeding treatments. Each aquarium was stocked with 10 red tilapia fingerlings. The replacement levels of dried seaweed; macro algae (*Spirulina* sp.) and Azolla were 0, 10, 20 and 30% respectively in the diets. Formulation of the tested diets is presented in Table (1).

Fish were fed two times daily at 9.00 and at 16.00 hr at a rate of 4% of fish body weight (Omar *et al.*, 1994) and were weighed alive bi-weekly. The daily feed allowance were increased on the basis of body weight. After weighing, each tank was cleaned to prevent accumulation of faeces and to reduce algal

growth. Tanks were refilled with the same source of salt water that was stocked in fiberglass tank and aerated by electrical air pumps. Water in each tank was partially changed once every day and completely changed every three days. Supplemental aeration was provided using electrical air pump for each aquarium. The experiments began in August 14, 1996 and ended after 84 days. At the end of the experiment all fish in the different treatment were weighed, sacrificed and frozen at -20°C. Proximate composition for the experimental aquatic plants, diets and fish has been done according to A.O.A.C (1984). A completely randomized design for the analysis of variance, the Duncan Multiple Range test and the least significant difference test were made according to Snedecor and Cochran (1967).

Table (1): Proximate composition (%) of the tested diets.

Ingredients	1*	2	3	4	5*	6	7	8	9*	10	11	12
Fish meal	20	20	20	20	20	20	20	20	20	20	20	20
Soybean meal	20	17	15	13	20	20	19	19	20	16	12	8
Sea weed	--	10	20	30	--	--	--	--	--	--	--	--
Algae "Spirulina"	--	--	--	--	--	10	20	30	--	--	--	--
Azolla	--	--	--	--	--	--	--	--	--	10	20	30
Wheat milling by-product	56	49	41	33	56	46	37	27	56	50	44	38
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral Mex	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin Mex	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

* Control diets (1, 5 and 9).

RESULTS AND DISCUSSION

- Growth performance:

Table (2) shows the chemical analysis of the experimental diets. The control diet (contained neither seaweeds, algae nor Azolla) contained less crude fiber and ash and more nitrogen free extract and energy contents.

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Table (2): Chemical analysis of the tested diets.

Aquatic plant replacement	% on DM basis					Gross energy* kcal/kg
	CP	EE	CF	Ash	NFE	
<u>Sea weed</u>						
0	30.51	11.22	8.70	10.16	39.41	4411.1
10	30.17	11.73	9.72	11.54	36.84	4323.0
20	30.08	12.37	10.61	13.17	33.77	4252.2
30	29.95	12.93	11.26	14.48	31.38	4199.5
<u>Algae "Spirulina"</u>						
0	30.51	11.22	8.70	10.16	39.41	4411.1
10	30.11	11.31	12.47	11.51	34.60	4187.9
20	30.06	11.43	17.24	12.83	28.44	3943.3
30	29.88	11.56	22.89	13.76	21.91	3677.0
<u>Azolla</u>						
0	30.51	11.22	8.70	10.16	39.41	4411.1
10	30.46	10.08	9.91	11.29	38.26	4242.0
20	30.31	9.36	10.18	12.58	37.57	4137.2
30	30.16	8.02	11.31	13.77	36.74	3968.1

* Calculated as based on 5.64, 9.44 and 4.11 (kcal/g) of protein, lipid and carbohydrate respectively (NRC, 1993).

Table (3): Chemical analysis of Seaweed, Algae "*Spirulina* sp." and Azolla.

Aquatic plant	% on DM basis				
	CP	EE	CF	Ash	NFE
Sea weed	19.87	6.14	14.07	15.05	45.11
Algae " <i>Spirulina</i> "	11.72	0.88	47.72	13.83	25.85
Azolla	25.00	3.89	14.06	12.00	45.05

CP: Crude Protein. CF: Crude fiber.
NFE: Nitrogen free extract. EE: Ether extract.

The use of some of aquatic plants as partial replacement for the protein source (Soybean meal and Wheat milling by-products) in the diets showed better performance than the control diet (Table 4). While the use of 20% Azolla in the diet showed better body weight gain than control diet and another aquatic plants (seaweed and algae). Also the results of replacement 10% from Azolla, Macro algae and Seaweeds instead of plant protein gave better performance than control diet. These results may be due to that, the chemical analysis of Azolla contained higher crude protein (25%) than seaweed (19.87%) and macro algae (11.72%); and Azolla contained less crude fiber and ash see table (3). Santiago *et al.* (1988) found that, up to 42% of dietary fish meal was replaced with *Azolla pinnata* in diets fed to *Oreochromis niloticus* (L.) fry without adversely affecting fish performance. Moreover, diet supplemented with *Azolla pinnata* produced better growth and feed conversion than a fishmeal based diet.

Erdem (1983) found that, no significant differences in live body weight gain between the groups of mirror carp fed 3, 6 and 9% Seaweeds. The present results showed that, it could be replaced Seaweeds in red tilapia diet up to 10% without any significant decreasing in growth performance.

Mustafa and Nakagawa (1995) recorded that; the addition of a small amount of algae to meal produced a significant increase in the growth and feed utilization of fish. 5% supplementation of *Chlorella*, *Spirulina* and *Ulva* meal resulted in a higher body weight gain in the nibbler, *Girella punctata* (Nakazoe *et al.*, 1986). A nearly significant effect on growth was observed in rainbow trout, *Oncorhynchus mykiss*, when fed meal prepared from the green algae, *Haematococcus pluvialis* (Sommer *et al.*, 1992).

Table (4): Growth performance of Florida red tilapia fed on different levels of aquatic plants.

Aquatic plant (%)	Live weight/fish g		Gain/fish.(g)*		SGR** (%)
	Initial	Final	Total	mg/day	
<u>Sea weed</u>					
0	2.79	10.19 ^d	7.40 ^d	88.10 ^c	1.54 ^d
10	2.80	11.44 ^c	8.64 ^c	102.80 ^{bc}	1.68 ^c
20	2.80	9.18 ^c	6.38 ^c	85.96 ^c	1.42 ^e
30	2.80	6.78 ^f	3.98 ^f	47.38 ^d	1.06 ^f
<u>Algae "Spirulina"</u>					
0	2.79	10.19 ^d	7.40 ^d	88.10 ^c	1.54 ^d
10	2.80	11.48 ^c	8.68 ^c	103.30 ^{bc}	1.68 ^c
20	2.80	8.97 ^e	6.17 ^c	73.46 ^{cd}	1.39 ^e
30	2.80	6.70 ^f	3.90 ^f	46.37 ^d	1.04 ^f
<u>Azolla</u>					
0	2.79	10.19 ^d	7.40 ^d	88.10 ^c	1.54 ^d
10	2.80	13.39 ^b	10.60 ^b	126.10 ^{ab}	1.87 ^b
20	2.80	14.68 ^a	11.90 ^a	141.50 ^a	1.98 ^a
30	2.80	8.80 ^e	6.00 ^e	71.43 ^{cd}	1.37 ^e
L.S.D (p<0.05)		0.5016	0.4358	32.83	0.0689

*Average in the same column having different superscripts are significantly different (p<0.05).

**SGR, specific growth rate % = 100 (Ln final weight - Ln initial weight)/days.

- Carcass composition:

The effect of different levels of aquatic plants inclusion within test diets on body chemical composition are presented in Table (5). Comparing the chemical composition at the start and at the end of experiment indicated that, as the fishes grow, their protein and lipid contents increased. Also there was a decrease in their ash content.

Table (5): Chemical composition of Florida red tilapia fed at different levels of aquatic plants, on dry matter basis.

Aquatic plant (%)	DM (%)	% on DM basis			Gross energy* kcal/kg
		CP	EE	Ash	
<u>At start</u>	24.40	54.01	22.73	23.25	5190
<u>Sea weed</u>					
0	25.70	58.90 ^a	25.87 ^a	15.23 ^g	5770 ^a
10	24.62	58.62 ^{ab}	25.42 ^{ab}	16.03 ^f	5700 ^{ab}
20	24.37	58.09 ^{bc}	25.03 ^{bc}	16.88 ^e	5640 ^{bc}
30	24.18	57.92 ^c	24.60 ^c	16.48 ^d	5590 ^{cd}
<u>Algae "Spirulina"</u>					
0	25.70	58.90 ^a	25.87 ^a	15.23 ^g	5770 ^a
10	25.55	58.80 ^a	25.47 ^{ab}	15.70 ^{fg}	5720 ^a
20	24.95	57.92 ^c	25.08 ^{bc}	17.01 ^{dc}	5640 ^{bc}
30	24.08	56.60 ^d	24.80 ^c	18.60 ^c	5540 ^d
<u>Azolla</u>					
0	25.70	58.90 ^a	25.87 ^a	15.23 ^g	5770 ^a
10	25.28	58.68 ^{ab}	25.52 ^c	16.80 ^e	5620 ^c
20	25.02	55.12 ^e	25.58 ^d	21.29 ^b	5340 ^e
30	23.90	54.83 ^e	22.73 ^e	22.44 ^a	5240 ^f
L.S.D (p<0.05)		0.6573	0.5682	0.4920	68.9

Average in the same column having different superscripts are different (p<0.05).

DM : Dry matter.

* Calculated based on 5.64, 9.44 and 4.11 (kcal/g) of protein, lipid and carbohydrate respectively.

The highest dry matter, protein, lipid and gross energy are observed for the fish fed control diets (zero level of aquatic plants), which contained the lowest ash content with a significant differences (p<0.05). While the highest ash content was that when the fish fed 30% of aquatic plants (Azolla, Algae and Sea

weed respectively). These results are in agreement with those of Nour *et al.* (1989), who found that, the lowest ash content in carp was obtained with the zero level of water hyacinth and the highest was with the 30% level. Similar results have been reported also with Azolla by Micha *et al.* (1988).

- Feed and nutrient utilization:

Table (6) shows feed and nutrients (protein and energy) utilization for Florida red tilapia fed at different levels of aquatic plants (Seaweeds, Macro algae and Azolla) instead of plant protein. The highest feed intake was observed with fish fed 20% and 10% Azolla respectively followed by the fish that fed control diet. The same treatments showed a good response in feed protein and energy utilization, especially the diet which contained 20% Azolla. Santiago *et al.* (1988) found that up to 42% of dietary fish meal was replaced with Azolla in diets fed to *O. niloticus* (L.) fry produced better growth and feed conversion, than a fish meal based diet. The diets, which contained 30% aquatic, plant significantly ($p < 0.05$) reduced feed intake, feed utilization and nutrient (protein & energy) utilization. The results showed also that, the use of 30% of aquatic plants (Seaweed, Macro algae and Azolla) increased feed conversion ratio (FCR) respectively, (which means decreasing in utilization efficiency).

Erdem (1983) reported that, the use of seaweed up to 10% in the diet had no effect on feed efficiency. Reduced efficiency of feed utilization associated with diets containing higher levels of sea weed was expected due to the presence of antinutritional factors known to influence the digestion and utilization of many nutrients (Zaki *et al.*, 1994). The lower growth rate was noted for fish fed diets supplemented with a higher level of dehydrated plant leaves could be attributed to the presence of growth inhibitors such as high ash content in the aquatic plants and antinutritional factors. Essa (1994) found that, the low nutritive values of some aquatic plants were probably associated with high ash content (20 - 50%).

Table (6): Feed utilization of Florida red tilapia fed at different levels of aquatic plants.

Aquatic plant (%)	Feed intake (g/fish)	FCR*	Protein utilization*		EU (%)
			PER	PPV(%)	
Sea weed					
0	17.38 ^c	2.35 ^b	1.40 ^f	22.15 ^{cd}	15.04 ^{cd}
10	16.74 ^{cd}	1.94 ^{de}	1.71 ^{cd}	25.32 ^b	17.20 ^b
20	13.41 ^e	2.11 ^{cd}	1.58 ^e	23.05 ^c	15.81 ^c
30	10.84 ^f	2.73 ^a	1.23 ^g	17.87 ^f	12.24 ^e
Algae "Spirulina"					
0	17.38 ^c	2.35 ^b	1.40 ^f	22.15 ^{cd}	15.04 ^{cd}
10	16.23 ^d	1.87 ^{ef}	1.78 ^{bc}	27.74 ^a	19.34 ^a
20	12.89 ^e	2.09 ^{cd}	1.60 ^{de}	23.90 ^{bc}	17.72 ^b
30	9.58 ^g	2.46 ^b	1.36 ^f	18.85 ^{cf}	15.01 ^{cd}
Azolla					
0	17.38 ^c	2.35 ^b	1.40 ^f	22.15 ^{cd}	15.04 ^{cd}
10	18.69 ^b	1.77 ^{fg}	1.86 ^{ab}	28.39 ^a	19.47 ^a
20	19.86 ^a	1.68 ^g	1.98 ^a	27.50 ^a	19.51 ^a
30	12.97 ^e	2.17 ^c	1.54 ^e	20.05 ^{de}	14.45 ^d
L.S.D (p<0.05)	0.9913	0.1688	0.1193	2.149	1.207

Different superscripts in the same column indicate that the differences are significant (p<0.05).

* FCR, Feed conversion ratio ; PER, Protein efficiency ratio ; PPV, Productive protein value and EU, energy utilization (Retention).

In the present study, inclusion of a 10% algae in the diet has raising growth performance and feed utilization for Florida red tilapia than that of the control diet (zero algae). The advantageous effects of algae are assumed to derive from dietary fiber, carotenoids, vitamins and minerals (Mustafa & Nakagawa, 1995).

CONCLUSION

From the obtained results, it could be concluded that, growth performance and feed utilization of Florida red tilapia is improved when fed on different levels of aquatic plants.

Therefore, it could be recommended to add Azolla, Seaweeds and Macro algae (*Spirulina* sp.) at a level of 20%, or 10% as a dietary component in the diets for feeding Florida red tilapia fingerlings.

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