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Effect of the use of the microalga *Spirulina maxima* as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry

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Abstract

The present study addresses the use of the microalga *Spirulina maxima* as a protein source in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Animal protein was replaced with algae protein at ratios of 20%, 40%, 60%, 80% and 100%, and the substitution effect was compared with a control diet in which fish meal was the sole protein. An additional 100% spirulina protein diet was supplemented with phosphorous to test for possible mineral deficiency in the plant-protein-based diet. The six treatments were tested in triplicate in a closed-recirculating system where the fish were fed by hand at 6% of their body weight. After a 9-week feeding period, the growth rate and protein utilization of fish fed the diet with 20% and 40% *Spirulina* were elevated and not significantly different ($P > 0.05$) from those fed the control diet. Further increases in the alga protein content significantly decreased the growth and feeding performance. The addition of P to the 100% *Spirulina* diet slightly improved performance in comparison to the same diet without P. None of the treatments produced any clear adverse effects on carcass composition. It is observed that *Spirulina* can replace up to 40% of the fish meal protein in tilapia diets.

Introduction

Financial limitations are depressing the development of aquaculture because of the impact of the increasing prices of fish meal and other traditional protein feedstuffs. In this context, the identification and use of alternative proteins, mainly of plant origin, are considered a priority to support the growth of this activity. Mustafa & Nakagawa (1995) considered algae as a valuable alternative protein source, particularly in tropical aquaculture, where adequate year-round temperatures are advantageous for continuous production. Among microalgae, *Chlorella* spp., *Scenedesmus* spp. and *Spirulina* spp. are the most frequently used in fish feeds, particularly as a dietary supplement or mixed with other protein sources.

Microalgae are essential in aquaculture feeding, where they are used extensively as live feed, particularly for molluscs and marine fish larvae. However, these can also be used in dried form within production diets. For example, *Spirulina* has been used as a low-level feed additive to improve the taste, texture or colour of the whole fish or its flesh, and for its positive effects on growth, feed utilization, physiological condition, and stress and diseases resistance (Hirano & Suyama 1985; Mori, Muranaka, Miki, Yamaguchi, Konosu & Watanabe 1987; Chow & Woo 1990; Liao, Takeuchi,

Watanabe & Yamaguchi 1990; Watanabe, Liao, Takeuchi & Yamamoto 1990; Cysewski 1992; Mustafa, Umino & Nakagawa 1994a; Mustafa, Takeda, Umino, Wakamatsu & Nakagawa 1994b; Okada, Nur-E-Borhan, Watanabe & Yamaguchi 1994; Mustafa & Nakagawa 1995).

Spirulina is one of the most common microalgae in warm saline and alkaline waters. Its growth rate under culture is faster than any other agricultural crop, and close to that of other micro-organisms such as yeasts and bacteria (Richmond 1988). Its short life-cycle results in the possibility of duplicating its biomass in 3–5 days, with a potential production of as much as 25 t ha^{-1} $year^{-1}$, which is equivalent to 15 t of protein ha^{-1} $year^{-1}$ (Richmond 1988; Göhl 1991). The nutritional value of *Spirulina* is higher than that of other single-cell algae because of the high content of protein, vitamin E, B12 and biotin, and it is an important source of essential fatty acids, especially those of the *n*-3 and *n*-6 series (Richmond 1988). The nucleic acid content in this alga is lower than 4%, while its amino acid profile is well balanced, although deficient in sulphur-based amino acids and tryptophan. This microalga is also rich in pigments, mainly phycobiliproteins, c-phycocyanin and allophycocianin, and it is a good source of thiamine, niacin, pyridoxine and cyanocobalamin, with high levels of calcium pantothenate, folic acid, inositol, β-carotene and tocopherol (Santillán 1979; Richmond 1988).

The present study was conducted to evaluate the use of the microalga *Spirulina maxima* as a fish meal replacement in practical diets for tilapia, *Oreochromis mossambicus* (Peters), fry with the objective of evaluating its nutritional quality and determining the best substitution level.

Materials and methods

Diets

Five diets were formulated using the microalga spirulina (*Spirulina maxima*; Sosa Texcoco, Mexico) to substitute for 20% (E20), 40% (E40), 60% (E60), 80% (E80) and 100% (E100) of the dietary animal protein (Chilean brown fish meal). A sixth experimental diet was included containing 100% algae protein, supplemented with 4% calcium phosphate (E100P). Each diet was formulated to provide 35% protein and 10% lipids. These diets were compared with a control diet (CON) in which fish meal was the sole protein source. All the diets were supplemented with 0.5% chromic oxide as a marker for digestibility determination. The diets were prepared following the method described previously by Olvera, Campos, Sabido & Martínez (1990), using a meat mill to form crumbles that were then dried for 24 h at 40 °C in a forced-air stove, and stored in a freezer until use. Table 1 shows the proximal composition and amino acid profiles of the two protein sources, while Table 2 shows the formulation, calculated amino acid contents and proximate composition of the diets (AOAC 1984).

Experimental procedure

The tilapia fry (279 mg mean body weight, 20–30 days old, mixed sexes) were produced in the CINVESTAV-Mérida Aquaculture Laboratory, Mérida, Mexico. Lots made up of 15 fish were randomly distributed among 21 indoor 20-L plastic self-cleaning tanks, arranged in a closed recirculating system as previously described (Olvera *et al.* 1990), which were installed in a laboratory with a 12:12 h light:dark artificial photoperiod. Each tank was supplied with water at a mean rate of 1.5 L min^{-1} and water quality was monitored throughout the 9-week experimental period. The following mean (± SE) values were

Table 1 The proximate composition and essential amino acid content of the protein sources

Content (% wet weight)	*Spirulina maxima*	Brown fish meal
Moisture	10.18	7.82
Crude protein (N × 6.25)	66.86	67.44
Ether extract	0.62	10.52
Crude fibre	1.91	–
Ash	9.54	12.76
NFE	10.89	1.46
Essential amino acid (g 100 g^{-1} sample)		
Arginine	5.22	3.67
Histidine	0.99	1.55
Isoleucine	3.91	3.02
Leucine	5.68	4.84
Lysine	3.48	5.05
Methionine	1.88	1.87
Phenylalanine	3.32	2.69
Threonine	3.68	2.71
Tryptophan	0.98	0.72
Valine	5.10	3.41

Table 2 Formulation, proximate composition and essential amino acid content of experimental diets

		Diet						
Ingredients (%)	**Requirement[3]**	**CON**	**E20**	**E40**	**E60**	**E80**	**E100**	**E100P**
Chilean brown fish meal	–	51.90	41.52	31.14	20.76	10.38	0.00	0.00
Spirulina	–	0.00	10.47	20.94	31.41	41.88	52.35	52.35
Fish oil (cod liver)	–	0.00	0.93	2.03	3.22	4.31	5.50	5.50
Soybean oil	–	5.00	5.24	5.17	5.20	5.14	5.18	5.18
Raw corn starch	–	36.60	35.34	34.22	32.91	31.79	30.47	26.47
Vitamin premix[1]	–	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[2]	–	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Carboximethyl cellulose	–	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Chromic oxide	–	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calcium phosphate	–	0.00	0.00	0.00	0.00	0.00	0.00	4.00
Nutrient contents per analysis (% wet weight)								
Moisture	–	5.81	7.30	7.05	5.36	5.54	6.01	5.81
Crude protein (N × 6.25)	–	35.15	32.06	31.48	34.69	34.95	36.65	31.63
Ether extract	–	9.80	8.75	9.71	9.98	9.52	9.27	9.80
Crude fibre	–	0.67	0.70	0.68	0.79	0.72	0.88	0.67
Ash	–	8.90	8.93	8.85	7.01	7.08	6.18	8.89
N free extract	–	39.67	42.26	42.23	42.17	42.19	41.01	43.20
Chromic oxide	–	0.48	0.49	0.41	0.43	0.54	0.48	0.48
Gross energy (kcal g^{-1})	–	441	441	440	440	439	439	422
Calculated EAA content (g 100 g^{-1} diet as fed)								
Arginine	1.18	1.92	2.08	2.25	2.41	2.57	2.73	2.73
Histidine	0.48	0.81	0.75	0.69	0.64	0.58	0.52	0.52
Isoleucine	0.87	1.58	1.67	1.77	1.86	1.95	2.05	2.05
Leucine	0.95	2.53	2.62	2.71	2.80	2.89	2.97	2.97
Lysine	1.43	2.64	2.48	2.31	2.15	1.99	1.82	1.82
Methionine	0.75	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Phenylalanine	1.05	1.41	1.47	1.54	1.61	1.67	1.74	1.74
Threonine	1.05	1.42	1.52	1.62	1.72	1.82	1.93	1.93
Tryptophan	0.28	0.38	0.40	0.43	0.46	0.49	0.51	0.51
Valine	0.78	1.78	1.96	2.14	2.32	2.49	2.67	2.67

[1]Tacon, Stafford & Edwards (1983).
[2]Tacon, Webster & Martinez (1984).
[3]Santiago & Lovell (1988).

recorded: temperature = 28 °C; pH = 8.31 ± 0.16; oxygen = 5.27 ± 0.10 mg L^{-1}; ammonia = 0.171 ± 0.035 mg L^{-1}; and nitrite = 0.0038 = 0.0007 mg L^{-1}. Temperature and oxygen were measured daily, and pH and N products were recorded weekly.

All fish were fed the control diet during the first 7 days after stocking to adapt them to feeding and handling practices, after which the experimental diets were randomly assigned between tanks, with three replicates per treatment. The fish were fed by hand 7 days a week at a rate of 6% body weight per day divided into three rations during the day. Daily feed consumption was recorded. Every week, the fish in each tank were bulk weighed and counted, and the ration was adjusted accordingly. After 7 days of feeding with the experimental diets, the faeces were collected daily by siphoning these off the bottom before the second feeding. Then the faeces were oven-dried at 105 °C for 24 h and stored under refrigeration for subsequent chromic oxide and total nitrogen analysis.

Analytical methods

The individual protein ingredients, diets and samples of fish taken at the beginning and end of the experiment were analysed for moisture, crude protein (N × 6.25), lipid, crude fibre and ash content

using standard methods (AOAC 1984). The protein content in faeces was determined by the Kjeldhal method. The chromic oxide content in the diets and faeces was determined using the method of Furukawa & Tsukahara (1966). Gross energy in diets was estimated based on the following conversion factors: carbohydrate (as NFE) = 4.1 kcal g^{-1}; protein = 5.65 kcal g^{-1}; and fat = 9.5 kcal g^{-1}. The individual essential amino acid composition of *Spirulina* was provided by the producer (Sosa Texcoco), since those values for fish meal were measured after acid digestion using a Beckman automatic amino acid analyser. Tryptophan was determined separately, as described by Basha & Roberts (1977).

Statistical methods

The results for survival, growth, feed utilization efficiency and body composition were analysed statistically using a one-way analysis of variance (ANOVA). Differences between treatments were tested for significance ($P < 0.05$) using Duncan's multiple range test (Duncan 1955).

Results

The effects of the experimental diets on fish growth and feed utilization throughout the 9-week experimental period are given in Table 3. The diets were well accepted, although a tendency to reject the food as the spirulina content increased was observed. This was attributed to increased particle hardness resulting from the high spirulina content. Survival was variable among treatments and exhibited no diet-related pattern. The highest survival values were obtained with the CON, E100P and E80 diets, with values between 70% and 80%, while the lowest were recorded with the E40 and E100 diets, both with a value of 42%. The survival differences were attributed to handling because no deleterious effect related to the diet spirulina content was observed.

Key variables such as final body weight, weight gain (%), daily weight gain (DWG, mg day^{-1}), specific growth rate (SGR, % day^{-1}), feed intake (FI, mg day^{-1}), PER and apparent nitrogen utilization (ANU, %) showed no significant differences

Table 3 The mean growth and feed utilization of tilapia fed the experimental diets

	Diet							
Mean values[1]	**CON**	**E20**	**E40**	**E60**	**E80**	**E100**	**E100P**	**±SE[2]**
Survival (%)	80.00[a]	68.89[a]	42.22[a]	57.78[a]	71.11[a]	42.22[a]	80.00[a]	1.08
Body weight:								
initial (mg)	294[a]	280[a]	273[a]	276[a]	267[a]	280[a]	280[a]	5.26
final (mg)	5737[a]	5637[a]	4975[a]	2923[b]	1239[c]	577[c]	866[c]	351.53
Weight gain (%)	1857.88[a]	1915.34[a]	1719.58[a]	951.35[b]	362.51[c]	105.33[d]	209.99[c,d]	2.01
DWG (mg day^{-1})[3]	86.408[a]	85.038[a]	74.628[a]	42.014[b]	15.428[c]	4.720[c]	9.298[c]	5.54
SGR (% day^{-1})	4.72[a]	4.76[a]	4.59[ab]	3.65[b]	2.40[c]	1.10[d]	1.78[c]	0.07
FI (mg day^{-1})[4]	89.222[a]	91.158[a]	96.093[a]	58.271[b]	29.218[c]	14.562[c]	17.990[c]	7.73
FCR	1.03[c]	1.07[c]	1.28[b,c]	1.45[b,c]	1.91[b]	3.64[a]	1.96[b]	0.09
N intake (mg day^{-1})	5.018[a]	4.676[a]	4.840[a]	3.234[b]	1.634[c]	0.854[c]	0.910[c]	0.41
PER	2.75[a]	2.93[a]	2.51[a,b]	2.02[b,c]	1.50[c]	0.85[d]	1.62[c]	0.06
CND (mg day^{-1})[5]	2.382[a]	2.035[a,b]	1.864[b]	1.182[c]	0.412[d]	0.139[d]	0.240[d]	0.15
ANU (%)[6]	47.46[a]	43.80[a,b]	39.18[a,b]	35.59[b]	25.05[c]	15.71[d]	26.17[c]	0.25
AOMD (%)	82.55	81.92	69.85	78.17	46.00	45.45	59.66	-
APD (%)	91.95	94.51	90.74	93.97	84.60	80.64	84.83	-

[1]Values followed by the same superscript letters are not significantly different ($P > 0.05$).
[2]SE: standard error, calculated from the mean-square for error of the ANOVA.
[3]WG (mg day^{-1}) = 1000 × [Σ weekly individual WG/time (days)].
[4]FI (mg day^{-1}) = 1000 × [Σ weekly individual food fed/time (days)].
[5]CND (mg day^{-1}) = 1000 × {[(final body weight × % final carcass protein) − (initial weight × % initial carcass protein)]/100/time (days)/6.25}.
[6]ANU (%) = 100 × (CND/N intake).

between the CON and both the E20 and E40 diets ($P > 0.05$). Further increases in the *Spirulina* content in the diet resulted in a significant reduction in fish performance ($P < 0.05$), with the lowest values occurring with complete replacement (E100). Fish fed the diet supplemented with phosphorous (E100P) had a significantly better SGR, PER, and ANU than those fed the E100 diet. Food conversion ratios (FCRs) for fish fed diets with up to 60% *Spirulina* substitution were not significantly different from those fish fed the control diet ($P > 0.05$).

An inverse relationship between plant protein content and apparent organic matter digestibility (AOMD, %) was observed. The lowest values were obtained with the E100 and E80 diets, while the best digestibility was registered for the control diet. This tendency was repeated in the estimations for apparent protein digestibility (APD, %), but the tendency was less marked, since all obtained values were higher than 80%.

Carcass composition was not clearly affected by the inclusion of spirulina (Table 4), and water content was statistically similar among all treatments ($P > 0.05$). Body protein and lipids showed no consistent relation to the diet spirulina content; however, body ash tended to decrease with increasing levels of vegetable protein in the diet. The highest ash values were obtained with the CON diet and the lowest with the E100P diet, each being statistically different from all the other treatments ($P < 0.05$).

Discussion

The present study shows that *Spirulina* algae possess adequate nutritional value for tilapia fry at low inclusion levels, making possible substitution levels up to 40% of the dietary animal protein without adverse effects on growth and feeding efficiency. The results obtained in the present study were superior to those of previous experiments with fish fed this alga. Working with rainbow trout fry and common carp, respectively, Matty & Smith (1978) and Atack, Jauncey & Matty (1979) obtained poor performance when spirulina was the only dietary protein source. However, Chow & Woo (1990) replaced 20% of the eel meal (equivalent to 5% protein) with spirulina meal in diets for *O. mossambicus*, and found no differences in SGR, feed intake or intestine enzymatic activity.

Considering that the calculated amino acid profile in the diets used in the present study was higher than that required for the fish, it is difficult to explain the growth decrease obtained when increasing diet algae content (Table 2). This suggests that the plant proteins in general could have a lower dietary quality than fish meal. Taking into account the positive effect observed with the addition of phosphorous in the diet with 100% algae and the body demineralization observed at increasing levels of spirulina inclusion, another explanation may be related to a deficiency of this mineral in the diet. There are no reported antinutritional factors for spirulina and its nucleic acid content is one of the lowest among micro-organisms (Richmond 1988), and therefore, it is unreasonable to expect adverse effects related to these factors.

Although food avoidance was not observed, feed hardness increases as spirulina content increases, making ingestion more difficult. This may be one reason for feed intake reduction, and as a consequence, lower growth performance in diets with high spirulina content.

Algal protein is of high quality, as shown by the fact that PER values higher than 2 were observed in

Table 4 The carcass composition (per cent wet weight) of tilapia fed *Spirulina* diets[1]

Composition (% wet wt)	Initial	Diet							
		CON	E20	E40	E60	E80	E100	E100P	±SE
Moisture	71.36	73.20^{a}	74.35^{a}	73.64^{a}	71.35^{a}	72.45^{a}	71.33^{a}	73.63^{a}	0.65
Protein	17.77	17.21^{d}	16.06^{g}	16.49^{f}	18.25^{a}	17.42^{c}	17.99^{b}	16.69^{e}	0.03
Lipid	6.18	6.19^{b}	$6.09^{b,c}$	$5.96^{b,c}$	7.28^{a}	6.94^{a}	$5.93^{b,c}$	5.61^{c}	0.16
Ash	3.95	4.17^{a}	3.59^{b}	3.64^{b}	$3.56^{b,c}$	$3.21^{d,e}$	$3.39^{c,d}$	3.13^{e}	0.06

[1]Values followed by the same superscript letters are not significantly different ($P < 0.05$).

diets containing 60% spirulina. However, it is important to note that the better ANU was obtained with plant protein inclusion levels of up to 40%. This efficiency indicator was lower than expected, considering that the Cyanophyta, including *Spirulina* spp. and *Microcystis* sp., represent an important component in the natural diet of *O. mossambicus* and *O. niloticus* (L.). In some African lakes, these algae represent 25% or more of the organic matter consumed by these fish (Getachew 1987; Ekpo & Bender 1989; Northcott, Beveridge & Ross 1991).

The reduction in AOMD with increasing *Spirulina* content in the diet suggests that the tilapia fry in the present experiment were unable to digest the algae efficiently. Nevertheless, the values obtained for the AOMD could be considered as normal for this type of material. Ekpo & Bender (1989) reported a digestibility of 62% for a mixture of Cyanophyta consumed by *O. niloticus* in ponds. In the present study, only the AOMD values calculated for diets E80, E100 and E100P were below the values calculated by those investigators. This drop in digestibility was unexpected considering that cellular walls of the spirulina are made of digestible mucoproteins (Richmond 1988) which have a digestibility of 83–84% (Santillán 1979)

In contrast, protein digestibility was higher than 80%, a value in accordance with Ekpo & Bender's (1989) results for the protein digestibility of *Anabaena* sp. by tilapia. The above authors found that tilapia more efficiently digest blue-green algae than silver carp, *Hypophthalmichtys molitrix* (Cuvier & Valenciennes), which is a strict phytoplankton feeder. The APD in tilapia varied between 90.0% and 94.5% in diets with 20–40% spirulina inclusion levels. These were higher than the values calculated by Hossain, Nahar, Kamal & Islam (1992) for diets with other plant proteins such as linseed, sesame and soybean fed to *O. mossambicus*. This capacity to digest microalgae protein is explained by the low pH in herbivorous fish stomachs, which allows them to leach nutrients from the cell without breaking its wall (Horn & Messer 1992). This process would be very easy for tilapia considering that its stomach has the lowest pH level among the fish, with values close to 1 (Ekpo & Bender 1989).

No regular adverse effect pattern for dietary *Spirulina* was found in the present study. Neither mortality nor carcass composition showed clear trends in relation to algae content. Only ash content was consistently affected, with all of the experimental diets yielding significantly lower values than the control. The use of the *Spirulina* as a feed additive in fish diets is advantageous because it has been reported that algae consumption lowers fish susceptibility to illness or stress, and decreases mortality (Henson 1990; Mustafa & Nakagawa 1995). Additionally, it has been reported that carcass composition for fish fed diets supplemented with spirulina is of a higher quality, with less fat, and a good flavour and texture. Liao *et al.* (1990) and Watanabe *et al.* (1990), observed that 5% spirulina supplementation in the diets of *Pseudocaranx dentex* (Bloch & Schneider) resulted in depression of body lipid levels and better growth rates. *Pagrus major* (Temminck & Schlegel) fed 2% spirulina supplemented diets exhibited a considerable increase in biomass production, and better texture and flesh quality. This was explained by Mustafa *et al.* (1994a,b) as an increase in protein synthesis. According to Henson (1990), the addition of 2.5% spirulina improved growth in cherry salmon, *Onchorhynchus masou* (Breevort), while the inclusion of 0.5% of the alga in the diet promoted growth and reduced mortality in *Seriola quinqueradiata* Temminck & Schlegel. The content of pigments in spirulina also results in a more attractive colouration of the fish. Mori *et al.* (1987) observed that the addition of 3–6% spirulina in diets for *Plecoglossus altivelis* Temminck & Schlegel improved the colour of fish. This effect was also observed by Mustafa *et al.* (1994a,b) and Liao *et al.* (1990.) with *Pseudocaranx dentex* (Bloch & Schneider), although no effect was noticed on the flesh or skin colouration of tilapia in the present study, perhaps because of the low fat content in its flesh or the pink colour of its skin.

The results of the present study show that the inclusion of *Spirulina maxima* in the diet has beneficial effects on the growth of tilapia fry, and can substitute for up to 40% of the animal protein in the diet, although the optimum performance is obtained with 20% substitution. Currently, spirulina production is directed toward its use as a feed additive at low inclusion levels. The present study demonstrates the feasibility of its use as an alternative protein source in complete diets, giving arguments for intensification in *Spirulina* cultivation to provide an increase in the availability of this product for use in substituting animal proteins in fish diets. However, longer-term studies under farm conditions are required in order to confirm these findings and the possible effects of spirulina on flesh quality and pigmentation.

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