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Effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio* L.

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Abstract

The effect of feeding *Spirulina platensis* on the growth, carcass composition, organoleptic quality, digestive enzyme activity and digestibility of common carp, *Cyprinus carpio* L., was studied through a culture trial lasting 120 days. Four experimental diets were employed by replacing fish meal protein from the standard diet at 25%, 50%, 75% and 100% through the incorporation of *Spirulina*. Another diet with *Spirulina* as the sole source of protein was also used. The final weight gain, specific growth rate, food conversion ratio and protein efficiency ratio of common carp were not affected by *Spirulina* supplementation. However, the diet with *Spirulina* as the sole source of protein resulted in better net protein retention. The muscle RNA:DNA ratio of fish fed *Spirulina* diets was higher than that of fish fed control diet. There was no significant difference in carcass moisture and protein content in the fish fed *Spirulina* diets as compared to fish-meal-based control diet. The carcass ash and fat contents were positively and negatively correlated with dietary *Spirulina* level, respectively. Organoleptic evaluation revealed no effect of *Spirulina* feeding on the quality of both raw and cooked fish. The gut digestive enzyme activity did not show any definite trend with respect to *Spirulina* supplementation. *Spirulina* improved the protein digestibility of the diets.

Introduction

Although fishmeal represents an ideal nutritional source of dietary protein and lipid for fish, there is an urgent need to reduce the current total dependence of the feed industry upon this expensive and finite commodity of uncertain supply and cost. Thus, if intensive farming systems are to become sustainable, it is essential that alternative protein sources are to be found for use in compounded aquafeeds. In this regard, single-cell proteins hold promise as possible substitutes for fish meal by virtue of their ability to be produced on a wide variety of substrates and wastes, and because of the possibility of genetically modifying their nutrient profile, within limits, to approximate the dietary needs of the cultured species. A good deal of work has been centred around yeast single-cell protein (Spinelli, Mahnken & Steinberg 1979; Tiews, Gropp, Beck & Koops 1979; Mahnken, Spinelli & Waknitz 1980; Wee 1988) and bacterial single-cell protein (Spinelli *et al.* 1979; Tacon 1979; Tacon & Jackson 1985; Kiessling & Askbrandt 1993). Algae are receiving increasing attention as a possible protein source for fish diets, particularly in tropical developing countries, because of their high protein content and production rate (Venkataraman, Nigam & Ramanatham 1980). Earlier studies have indicated that algae contribute to increased growth (protein assimilation), feed utilization, physiological activity, stress response, starvation tolerance, disease resistance and carcass quality of cultured fish. Feeding behaviour studies have shown that many fish, including carnivorous fish, ingest algae as a food source. Thus, the use of algae as a feed additive might help in effective utilization of artificial diets in cultured fish (Mustafa & Nagakawa 1995). Growth studies with a cyanobacterium, *Spirulina*, have confirmed that it improves carcass quality (Liao, Takeuchi, Watanabe & Yamaguchi 1990), encourages growth (Mustafa, Umino & Nakagawa 1994b; M. C. Nandeesha, unpublished results) and induces early maturity, resulting in shorter cultivation and a shorter breeding cycle (Braun 1988). In the present study, *Spirulina platensis* was evaluated for its efficacy as a protein source replacing fish meal in the diet of common carp, *Cyprinus carpio* L., in terms of growth, carcass proximate composition, organoleptic property, digestibility and digestive enzyme activity.

Materials and methods

Experimental diets

The alga *S. platensis* was produced in clean water with commercial fertilizers under standardized conditions on a gravity filter and dried in a cross-flow drier at the Central Food Technological Research Institute, Mysore, India (Venkataraman 1983). *Spirulina* had 54.5% crude protein content and 1.1% fat. Fish meal was procured from a Government fish meal production plant situated in Karnataka, India. Fish meal had 67% crude protein content and 2% fat content. A standard fish-meal-based diet developed by Varghese, Devaraj, Shantharam & Shetty (1976) was used as the control diet (SP1), and from this, fish meal protein was replaced at 25% (SP2), 50% (SP3), 75% (SP4) and 100% (SP5) through incorporation of *Spirulina* at nearly isonitrogenous levels (Table 1). Another diet was also prepared with *Spirulina* as the sole source of protein (SP6). The ingredients were mixed with water and cooked at 105°C for 30 min. Cooked dough was cooled and extruded through a pelletizing machine to obtain pellets of 2.5 mm in diameter. These were dried to less than 10% moisture at 70°C and stored in polythene bags.

Experimental procedure

The experiment was conducted in 10 cement cisterns, each 25 m^2 in area. Common carp fry were procured from a Government fish seed farm, B.R. Project, and reared in the College of Fisheries, University of Agricultural Sciences, Mangalore, India, for a week with a 30% protein diet in fertilized ponds. Duplicate cisterns stocked with advanced fry (0.56 g) of common carp (25 per cistern) were used for each treatment. The fish were fed once daily in the morning at 5% body weight. Water qualities such as temperature, dissolved oxygen, pH and total alkalinity were measured every fortnight (APHA 1985). On all sampling days, plankton samples were also collected by filtering 100 L of water through 60-μm bolting silk cloth, and the dry weight of plankton was determined. Fish were also sampled at fortnightly intervals and growth recorded.

The digestibility of the experimental diets was determined by a short-term trial conducted in 77 × 38 × 38 cm glass aquaria. In each aquarium, 10 fish (average weight 20 g) were stocked and acclimatized to the respective diet over a period of 10 days. Fish in each aquarium were fed daily with one of the experimental diets at 5% body weight at 1000 h; leftover feed was removed at 1600 h. The faecal samples were collected through siphoning the next morning at 0900 h. Water in the tank was completely changed after collection of faecal matter. Faecal samples collected over a period of 20 days from each treatment were pooled together and the digestibility was determined by employing crude fibre as a marker using the following formula:

$$\text{Apparent digestibility of nutrient} = 100\text{–}100 \times \frac{\%\ \text{marker in diet}}{\%\ \text{marker in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in diet}}$$

Analytical methods

Proximate composition of the ingredients, experimental diets, faecal matter, and the initial and final fish carcass was determined following AOAC (1975) procedures. Hastings' (1976) difference method was used for obtaining nitrogen-free extract (NFE) values. On termination of the experiment, the digestive enzymes [amylase (Bernfeld 1955), protease (Kunitz 1974) and lipase (Bier 1962)] and the nucleic acid content of the muscle (Ceriotti 1955; Giles & Myres 1965) were estimated. The organoleptic evaluation was done by 12 expert panellists. The food conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio and net protein retention (NPR) were computed as indicated in a previous paper (Nandeesha, De Silva, Krishnamurthy & Datharthi 1994). Duncan's (1955) multiple range test was applied to rank the treatment means tested for significance ($P < 0.05$) with analysis of variance (ANOVA) for different parameters.

Table 1 Percentage composition of ingredients and proximate composition of formulated diets. Figures in parenthesis are standard deviations

	Diet					
	SP1	SP2	SP3	SP4	SP5	SP6
Ingredients (%)						
Spirulina powder	—	7.44	15.48	23.21	30.95	55.00
Fishmeal	25.00	18.75	12.50	6.25	—	—
Rice bran	40.00	38.00	37.00	35.50	35.50	—
Groundnut oil cake	24.00	24.00	24.00	24.00	24.00	24.00
Tapioca flour	10.00	10.81	10.00	10.00	9.55	29.50
Code liver oil	—	—	—	—	—	6.50
Sugarcane pith powder[1]	—	—	—	—	—	8
Vitamin and mineral mixture[2]	1	1	1	1	1	1
Proximate composition (%)						
Moisture	4.01	4.30	4.49	4.21	5.19	4.81
	(0.08)	(0.04)	(0.03)	(0.03)	(0)	(0.16)
Crude protein	30.93	31.72	31.18	30.52	29.78	28.02
	(0.37)	(0.24)	(0.32)	(0.42)	(0.14)	(0.42)
Crude fat	5.86	5.54	5.03	4.16	3.61	5.94
	(0.05)	(0.19)	(0.04)	(0.12)	(0.39)	(0.09)
Crude fibre	13.61	12.07	11.87	11.59	10.75	5.89
	(0.30)	(0.10)	(0.05)	(0.27)	(0.22)	(0.12)
Ash	16.62	15.98	15.76	15.25	14.82	8.34
	(0.07)	(0.02)	(0.21)	(0.04)	(0.07)	(0.19)
Nitrogen free extract	28.93	30.37	31.66	34.26	35.85	46.39
Energy content (kJ g^{-1})	14.59	14.90	14.79	14.74	14.61	17.01

[1]Used only as a filler.
[2] Supplevite-M, each 250 g provides: vitamin A, 500 000 IU; vitamin D_3, 100 000 IU; vitamin B_2, 0.2 g; vitamin E, 75 U; vitamin K, 0.1 g; calcium pantothenate, 0.25 g; nicotinamide, 1.0 g; vitamin B_{12}, 0.6 mg; choline chloride, 15 g; calcium, 75 g; manganese, 2.75 g; iodine, 0.1 g; iron, 0.75 g; zinc, 1.5 g; copper, 0.2 g; and cobalt, 0.0045 g.

Results and discussion

Water qualities such as temperature (26–29°C), dissolved oxygen (5.70–8.35 ppm), pH (6.80–9.43) and total alkalinity (33.20–87.50 ppm) were within the range optimal for carp culture. Dry weight of plankton (0–12.5 mg 100 L^{-1}) was insignificant as compared to that encountered in fertilized ponds (Wohlfarth & Schroeder 1979) and this probably did not influence the growth of fish.

All the diets except SP6 were nearly isocaloric. However, with the increasing level of *Spirulina* in the diet, fat percentage declined because of the low fat content of *Spirulina* (except in the SP6 diet where oil was added; Table 1). The growth of common carp fed the experimental diets did not differ significantly ($P > 0.05$) in terms of weight and length (Table 2). *Spirulina* incorporation in the diet did not significantly affect the SGR, FCR and protein efficiency ratio of common carp ($P > 0.05$). However, NPR of fish fed *Spirulina* as the sole source of protein was significantly better.

Because of its high protein content, and the presence of essential amino acids, gamma linolenic acid, beta carotene and phycocyanin pigments, and vitamins and minerals in adequate quantities, *S. platensis* has been identified as a potential protein source for animal feed (Braun 1988). A 5% supplementation of *Spirulina* resulted in a higher body weight gain in the nibbler, *Girella punctata* (Grey), (Nakazoe, Kimura, Yokoyama & Iida 1986). Keshavanath, Varghese, Shetty, Murthy & Gogoi (1986) recorded good growth of Deccan mahseer, *Tor khudree* (Sykes), when fed with diets containing *S. platensis* in place of fish meal. M. C. Nandeesha (unpublished results) reported that fish meal protein can be effectively replaced by *S. platensis* without

Table 2 Growth and its indicators in fish fed with the experimental diets. Figures in parenthesis are standard deviations. For each parameter, mean values with different superscript are significantly different ($P < 0.05$)

	Diet					
Parameter	**SP1**	**SP2**	**SP3**	**SP4**	**SP5**	**SP6**
Final mean weight (g)[1]	38.25	41.52	41.02	41.03	41.13	42.33
	(4.48)	(6.72)	(5.36)	(5.77)	(6.71)	(7.29)
Final mean length (cm)	11.88	12.23	12.29	12.44	12.65	13.24
	(0.76)	(0.72)	(0.60)	(0.87)	(0.65)	(1.10)
Average specific growth rate (%)	4.69	4.78	4.76	4.76	4.76	4.81
	(0.04)	(0.01)	(0.05)	(0.01)	(0.10)	(0.01)
Food conversion ratio	1.94	1.78	1.81	1.81	1.82	1.74
	(0.07)	(0.01)	(0.08)	(0.01)	(0.17)	(0.01)
Protein efficiency ratio	1.66	1.75	1.76	1.80	1.90	1.95
	(0.06)	(0.01)	(0.08)	(0.01)	(0.18)	(0.01)
Net protein retention (%)	21.13[a]	20.30[a]	21.25[a]	21.25[a]	21.34[a]	28.83[b]
	(0.49)	(0.82)	(0.55)	(0.12)	(0.55)	(0.34)
Overall survival (%)	94	84	94	84	84	84
Carcass composition (%)						
	75.14	75.53	76.70	76.84	77.40	76.59
Moisture	(0.52)	(0.30)	(0.53)	(0.02)	(0.96)	(0.68)
	15.40	15.35	15.33	15.46	15.26	15.39
Protein	(0.04)	(0.19)	(0.12)	(0.02)	(0.12)	(0.24)
	5.36[e]	5.10[d]	3.27[c]	3.22[c]	2.61[a]	2.98[b]
Fat	(0)	(0.10)	(0.04)	(0.39)	(0.04)	(0.07)
	2.49[a]	2.54[a]	2.60[a,b]	2.67[b]	3.09[c]	3.10[c]
Ash	(0.07)	(0.07)	(0.03)	(0.03)	(0)	(0.04)
Nitrogen-free extract	1.61	1.48	2.10	1.81	1.64	1.94

[1]The initial weight and length of fry were 0.56 ± 0.05 g and 3.53 ± 0.14 cm, respectively.

sacrificing the growth of *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton). In fact, the growth rate and protein efficiency ratio improved in rohu when these fish were fed with *Spirulina* incorporated diet, while in catla, these factors did not differ significantly from the fish-meal-based control diet. At 5% incorporation in the diet, *Spirulina* meal produced a significant enhancement of growth and feed utilization in one-year-old red sea bream, *Pagrus major* (Günther), (Mustafa, Takeda, Umino, Wakamatsu & Nakagawa 1994c). A 3% supplementation of *Spirulina* meal in moist pellets reconfirmed the efficacy of *Spirulina* in improving growth performance and feed utilization in fish (Mustafa *et al.* 1994b). In contrast to these results, Atack & Matty (1979) and Atack, Jauncey & Matty (1979) found poor growth with *Spirulina maxima* when it was used as sole protein source in the diet of rainbow trout, *Oncorhynchus mykiss* (Walbaum), and common carp respectively. This might be a result of variation in the nutritive value of different species of the alga. With *Spirulina* as the sole source of protein, the improved growth of common carp observed in the present study indicated its suitability as a good protein source. Studies on the utilization of microalgae as a feed for fish have revealed that fish grow better on an algae-enriched diet than on any conventional fish feeds (Sandbank & Hepher 1978).

The RNA:DNA ratio of *Spirulina*-fed fish was significantly higher than in those fed the control diet (Fig. 1). Since growth is accomplished primarily by protein synthesis, the RNA:DNA ratio is considered to be an effective index for monitoring growth in fish (Khan & Jafri 1991; Mathers, Houlihan, McCarthy & Burren 1993). Red sea bream fed the *Spirulina* diet also showed elevated RNA:DNA concomitant with improved growth (Mustafa *et al.* 1994b).

The data on carcass proximate composition are given in Table 2. *Spirulina* incorporation did not

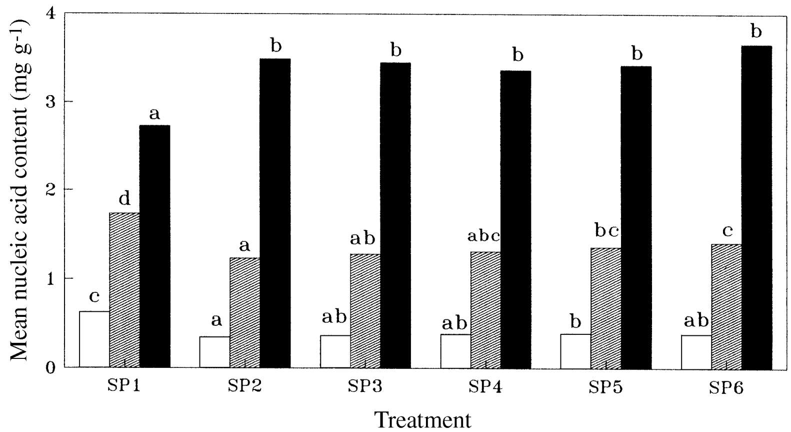

Figure 1 Mean nucleic acid contents (DNA, □; RNA, ▨) and RNA:DNA ratios (■) in the muscle of common carp fed with experimental diets. Different superscripts for the same parameter indicate a significant difference ($P < 0.05$).

affect the moisture and protein content. However, the fat content decreased concomitant with the decrease in dietary fat level. An increase in carcass ash percentage was observed with an increased *Spirulina* supplementation level. Mustafa *et al.* (1994b) recorded no difference in the muscle protein content of red sea bream fed a moist diet with 2% *Spirulina*. The feeding of algae is reported to have elevated body lipids in yellow tail, *Seriola quinqueradiata* (Richardson), and young red sea bream (Nakagawa, Kumai, Nakamura & Kasahara 1982; Yone, Furuichi & Urano 1986; Mustafa, Takeda, Umino, Wakamatsu & Nakagawa 1995). *Spirulina* is known to increase fat deposition (Atack *et al.* 1979; Watanabe, Liao & Takeuchi 1990). In contrast to these observations, feeding 2% *Chlorella* extract reduced lipid levels in the muscle and liver of ayu (Amano & Noda 1985). *Spirulina* in a moist diet suppressed excessive lipid accumulation in the muscle of 2-year-old red sea bream (Mustafa, Umino, Miyake & Nakagawa 1994a). Thus, the effect of *Spirulina* on lipid level appears to vary from species to species.

Carcass quality is a matter of great importance from the perspective of consumer acceptance. The organoleptic evaluation revealed no effect ($P > 0.05$) of *Spirulina* addition on the quality of both raw and cooked fish (Table 3). The carcass quality of red sea bream and striped jack, *Caranx delicatissimus*, was reported to have improved by feeding algae (Nakagawa & Kasahara 1986; Liao *et al.* 1990; Watanabe *et al.* 1990). Dietary algae were also effective in improving the flavour and texture of cultured ayu (Amano & Noda 1985; Hirano & Suyama 1985). Feeding *Spirulina* improved the muscle firmness, texture and taste of red sea bream and striped jack (Liao *et al.* 1990; Mustafa *et al.* 1994a,b). Furthermore, the algal carotenoids contributed to improved colouration in the skin and flesh of the cultured fish (Katsuyma, Komori & Matsuno 1987; Mori, Marunaka, Miki, Yamaguchi, Konosu & Watanabe 1987; Sommer, D'Souza & Morissy 1992).

Table 4 shows the gut enzyme activity of common carp. A reduction in intestinal protease and lipase activity was observed at higher levels of *Spirulina* supplementation. The hepatopancreatic protease activity of all the *Spirulina* fed fish was poorer in comparison with the control. While the hepatopancreatic amylase activity of *Spirulina*-fed fish was better, the intestinal amylase and hepatopancreatic lipase activity remained unaffected. The protein digestibility data support the growth trend (Table 4). While the protein digestibility of *Spirulina*-incorporated diets did not differ between the treatments, it was significantly better than the control diet ($P < 0.05$). The fat digestibility was the highest with treatments SP2 and SP6. The lower values recorded with other diets can be attributed to the low level of fat in the diets. Atack & Matty (1979) and Atack *et al.* (1979) have also observed good digestibility of protein from *S. maxima* in trout and common carp, respectively. Digestibility evaluation studies with common carp using *S. platensis* have shown that maximum protein digestibility can be obtained at an incorporation level of 50% in a 28% protein diet (Umesh, Dathathri, Nandeesha, Gangadhara & Varghese 1994). Studies conducted with other species have revealed an improvement in the protein digestibility of catla and rohu fed diets with *S. platensis* replacing protein from fishmeal at 25%, 50%, 75% and 100%. (M. C.

Table 3 Mean panel scores of the organoleptic evaluation of fish treated with different *Spirulina* diets

Treatment	Overall quality		Odour of flesh		Texture of flesh		Flavour of flesh: cooked	Appearance of fish
	Raw	Cooked	Raw	Cooked	Raw	Cooked		
SP1	3.36	3.23	3.20	2.93	3.40	3.40	3.21	3.43
SP2	3.43	3.21	3.28	3.33	3.60	3.26	3.21	3.35
SP3	3.57	3.23	3.43	3.00	3.60	3.13	3.21	3.67
SP4	3.57	3.18	3.64	3.13	3.24	3.46	3.00	3.43
SP5	3.75	3.26	3.64	3.20	3.73	3.20	3.14	3.50
SP6	3.67	3.16	3.64	3.06	3.53	3.20	3.14	3.57

Table 4 Gut digestive enzyme activity (μmol product liberated min^{-1} mg $protein^{-1}$ at 28°C) and apparent digestibility of diets. Figures in parenthesis are standard deviations. Figures in the same column with the same superscript are not significantly different ($P > 0.05$)

	Protease		Amylase		Lipase		Apparent digestibility (%)	
	Intestine	Hepatopancreas	Intestine	Hepatopancreas	Intestine	Hepatopancreas	Protein	Fat
SP1	0.214^{d}	1.087^{b}	0.995	2.565^{a}	0.010^{d}	0.012	81.73^{a}	84.89^{d}
	(0.009)	(0.043)	(0)	(0)	(0)	(0)	(1.20)	(0.02)
SP2	0.215^{d}	0.169^{a}	1.082	3.040^{c}	0.011^{d}	0.013	88.93^{b}	88.82^{e}
	(0.001)	(0.016)	(0.004)	(0.014)	(0)	(0)	(0.02)	(0.03)
SP3	$0.199^{c,d}$	0.145^{a}	1.192	3.059^{c}	0.010^{d}	0.011	87.23^{b}	80.50^{c}
	(0.008)	(0.016)	(0.014)	(0.014)	(0)	(0)	(0.28)	(0.40)
SP4	0.187^{c}	0.131^{a}	1.212	2.879^{b}	0.006^{c}	0.012	87.70^{b}	77.12^{a}
	(0.003)	(0.002)	(0.012)	(0.008)	(0)	(0)	(0.50)	(0.06)
SP5	0.162^{b}	0.128^{a}	1.183	2.781^{b}	0.005^{b}	0.012	88.94^{b}	77.81^{b}
	(0.001)	(0.013)	(0)	(0.002)	(0)	(0.001)	(0.30)	(0.08)
SP6	0.139^{a}	0.117^{a}	1.103	2.766^{b}	0.004^{a}	0.013	88.39^{b}	87.79^{e}
	(0)	(0.004)	(0)	(0.009)	(0)	(0)	(0)	(0)

Nandeesha, unpublished results). This author also observed a reduction in fat digestibility at higher *Spirulina* incorporation levels. *Spirulina platensis* has no cellulosic cell wall which helps in better digestion and absorption (Becker & Venkataraman 1984). The better digestibility of protein and fat observed in the *Spirulina*-incoporated diets must be a result of better absorption since the protease and lipase activity were not enhanced by *Spirulina*. Alga contributes to an increase in protein assimilation and feed utilization (Mustafa & Nakagawa 1995). *Undaria pinnatifida* and *Ascophyllum nodosum* contributed to the absorption of dietary carbohydrate and protein as an energy source, and stimulated assimilation of nutrients into body constituents (Yone *et al*. 1986).

The present study suggests that *S. platensis* could be used as a sole source of dietary protein in common carp. Innovation of cost-effective technologies for cultivation of *S. platensis* (Venkataraman, Somasekarappa, Somasekaran & Lalitha 1982; Saxena, Ahmad, Shyam & Amla 1983) would help exploitation of this alga as a fish feed ingredient.

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