Fishery Technology
2003, Vol. 40(1) pp : 5 - 7

Effect of Dietary β-chitosan levels on Survival and Growth of Fingerlings of *Cyprinus carpio, Catla catla* and *Cirrhinus mrigala*

B. Victor, U. Ramesh and M. Maridass
Environmental Carcinogenesis Research Unit, Department of Zoology
St. Xavier's College (Autonomous) Palayamkottai - 627 002
Tamil Nadu, India

A feeding experiment on the fingerlings of *Cyprinus carpio, Catla catla* and *Cirrhinus mrigala* was conducted for 28 day period with four experimental diets added with graded levels of β-chitosan (1,2,5 and 10%) and a control diet which contained 31% crude protein, 7.96% crude fat, 25% crude carbohydrate and 5% crude fibre. Fish fed with the diet containing 1, 2 and 5% β-chitosan had the highest survival, feeding rate and weight gain. The above studies indicate that β-chitosan has growth promoting effect in carps and the inclusion of low levels of β-chitosan (1-2%) in artificial diets is likely to improve carp production.

Key words: β-chitosan, *Cyprinus carpio, Catla catla, Cirrhinus Mrigala*, growth rate, feeding rate, food conversion efficiency

Chitin, N-acetyl glucosamine (NAG) forms 50-80% of organic compounds in crustacean exoskeleton (Muzzarelli, 1977). Deacetylation of chitin with strong alkali yields chitosan, a polymer of b(1-4)-D glucosamine. Both chitin and chitosan are non-starch polysaccharides and are regarded as compounds of dietary fibre. Many fish species are capable of digesting chitin in their digestive tracts by using enzymes like chitinase, chitobiase or N-acetyl-muram-hydrolase (a lysozyme) (Goodrich & Morita, 1977; Danulet & Kausch, 1984; Lindsay, 1987). Experimental feeding studies on cultured fish showed that the growth rate of red seabream, Japanese eel and yellow tail fed with 10% chitin supplemented diet recorded high growth rate compared to control diet, while incorporation of 10% chitosan supplemented diet showed low growth rate (Kono *et al.*, 1987). The effects of graded dietary chitosan levels on fish are not well studied. The study was carried out to determine optimum dietary b-chitosan levels for the fingerlings *Cyprinus carpio, Catla catla* and *Cirrhinus mrigala.*

Materials and Methods

Beta-chitin from squid, *Loligo indica* was prepared from fresh squid pens. They were ground in a blender, dried in air at room temperature and then heated at 40°C. Care was taken to avoid overheating and denaturing. A portion of the ground material, 105 g, was deproteinized in 1300 ml of 1 N NaOH at room temperature for 8 h with mechanical stirring. The suspension was centrifuged at 2000 rpm, and the residue was resuspended in water and centrifuged. The procedure was repeated twice and the suspended material was filtered and washed until neutral. The chitin was air dried at room temperature and then heated at 40°C. It was finally ground to 40 mesh size (Austin *et al.*, 1988).

Deacetylation of chitin was carried out by hydrolyzing with 50% w/v NaOH at the ratio of solid to solvent of 1:20 w/v at 120°C for 1 h. The resulting b-chitosan was washed with filtered tap water until neutral and dried at 60°C for 10 h. (Sumalai *et al.*, 1996).

Fingerlings of *Cyprinus carpio, Catla catla* and *Cirrhinus mrigala* were collected from Tirunelveli, Tamil Nadu and maintained in

the laboratory conditions for two weeks. During the acclimatization period, the fish were fed with compounded and pelletised feed containing 30% protein.

The dry ingredients were pulverized to fine particles and mixed thoroughly in a mixer until homogenous. All the diets were made as dough of good consistency and were steam cooked for 10 min with intermittent stirring. They were then passed through a hand pelletiser to obtain pellets of 3 mm dia. The pellets were air dried for 2 h and then oven dried at 70°C for 12 h to a moisture content of approximately 8%.

Fingerlings of 4-6 g size from the acclimated stock were randomly allocated to experimental troughs (50 L capacity) containing 45 L of well water in triplicate of groups of 20 fishes for 4 test diets (1,2,5 & 10% b-chitosan supplemented diets) and a control. The feeding trial was conducted for 28 days. The fingerlings of *C. carpio*, *C. catla* and *C. mrigala* were fed at 5% of their body weight/day and ration was divided in 2 doses (10 h and 16 h). Unfed diet was removed and weighed to calculate the amount of feed taken. Compressed air was continuously supplied through air stones to all the rearing troughs. Water samples were analysed every day for temperature, DOC, CO_2, pH and ammonia nitrogen (APHA, 1995). Fish were weighed weekly and amount of feed given was readjusted accordingly. Weight gain was measured fortnightly and specific growth rates were calculated (Rangasamy *et al.* 1988). Proximate composition of the diets were estimated by AOAC, (1984). FCR, daily growth and daily feeding rate were statistically analyzed by student's t-test.

Results and Dicsussion

The proximate composition of the feed ingredients and the formulated feed is given in Table 1. The water quality parameters during the feeding trial was found to be temperature 28°C±0.5°C; dissolved oxygen 6-7 mg/l; Carbon dioxide, 5-7 mg/l; ammonia (NH_4-N), 0.1-0.3 mg/l; and pH 7.2-7.6. During the feeding trial, the experimental

Table 1. Ingredient content and proximate analysis of reference diet used in the growth experiment.

Ingredient	Composition %	Feed components	Proximate Composition (%)
Fish Meal (52.03)	30	Dry matter	91.24
Ground nut oil cake (43.59)	29	Crude protein	31.10
Rice bran (13.84)	10	Crude fat	7.96
Wheat flour (12.50)	10	Crude carbohydrate	25.18
Tapioca flour (2.22)	10	Crude fibre	5.55
Sardine oil	10	Ash	10.40
Vitamin and mineral mix	1	Energy kcal/g	2.95

fingerlings readily accepted the diets and no food remained at the bottom of the aquaria. The survival of fingerlings of *Cyprinus carpio*, *Catla catla* and *Cirrhinus mrigala* in all dietary groups was more than 90%. The growth response of the test fishes under various dietary treatments of b-chitosan is presented in Table 2. Fingerlings fed with diet containing 1,2 and 5% b-chitosan gave best growth and survival than 10% b-chitosan supplemented diet. This was evident from the higher weight gain, specific growth rate (SGR), protein efficiency ratio (PER) and survival. The average food conversion ratio (FCR) ranged from 1.13 to 1.96. The highest gross conversion efficiency (GCE) was obtained for the test fishes fed 1,2 and 5% b-chitosan supplemented diet. Better growth of fingerlings receiving 1,2 and 5% b-chitosan incorporated diet appeared to be due to improved food conversion and protein efficiency. The optimum level b-chitosan supplementation was found to be 1-2%. Shi-Yen Shiau & Yi Ping Yu (1999) reported that addition of 2,5 and 10% supplemental chitosan depresses tilapia growth regardless of the supplementation level. However they observed higher weight gain in fish fed 1,2 and 5% chitin diets. Studies with rainbow trout also indicated that chitin was not digested when fed at 10 and 30% of the diet (Lindsay *et al.*, 1984). Apparent chitin digestibility was significantly higher (52.4%) in juvenile *Penaeus duorarum* than in their adult *P. setiferous*

Table 2. Growth response of *C. carpio*, *C. catla* and *C. mrigala* fingerlings fed with test diets containing different doses of chitosan for 28 - day feeding period

Fish species	Feed type	Initial body weight (g)	Final body weight (g)	Weight gain (g)	FCR	SGR	GCE (%)	PER	DFR (g)	DGR (g)	Survival (%)
	Reference diet	5.17±0.025	7.86±0.03	2.69±0.882	1.88±0.14	0.65±0.10	57.60±6.21	8.97±0.20	0.16±0.03	0.08±0.03	90.0±2.7
	1% Chitosan	4.97±0.26	10.89±0.47	5.92±0.20	1.22±0.17*	1.22±0.06*	87.14±4.72	19.34±0.06	0.30±0.06*	0.21±0.02*	100.0±0.00
C. carpio	2% Chitosan	4.90±0.47	10.56±1.62	5.66±0.30	1.34±0.17*	1.19±0.16*	84.60±4.57*	18.20±0.77	0.283±0.04*	0.20±0.01*	96.7±1.60
	5% Chitosan	4.95±0.036	9.38±0.07	5.48±0.667	1.39±0.14*	0.99±0.22*	72.01±6.92*	18.27±0.56	0.26±0.08*	0.16±0.04*	96.7±1.6
	10% Chitosan	5.17±0.21	8.44±0.26	3.27±0.137	1.49±0.08*	0.76±0.03	67.29±4.31	10.90±0.79	0.20±0.02	0.12±0.02	93.3±1.6
	Reference diet	6.25±0.071	8.85±0.071	2.60±0.141	2.23±0.10	0.54±0.12	49.36±2.35	8.67±0.64	0.19±0.03	0.09±0.02	93.3±3.11
	1% Chitosan	6.28±0.21	12.26±1.62	5.98±0.67	1.32±0.16*	0.03±0.17*	79.69±6.92*	19.23±0.79	0.299±0.06*	0.214±0.01*	100.0±0.00
C. catla	2% Chitosan	6.32±0.21	12.05±0.27	5.73±0.047	1.46±0.08*	1.00±0.06*	73.41±4.57*	18.42±0.08	0.287±0.04*	0.205±0.02*	96.7±1.60
	5% Chitosan	6.30±0.47	11.95±0.27	5.65±0.047	1.59±0.12*	0.99±0.15*	62.58±4.57*	18.83±0.82	0.27±0.06*	0.20±0.01*	96.7±2.7
	10% Chitosan	6.30±0.47	10.4±0.14	4.10±0.071	1.96±0.17	0.78±0.06	48.83±3.92	13.67±0.77	0.27±0.04	0.15±0.01	93.3±1.6
	Reference diet	4.48±0.03	8.26±0.53	3.78±0.47	2.25±0.16	0.97±0.17	45.12±3.80	12.6±0.82	0.28±0.05	0.13±0.01	90.0±3.11
	1% Chitosan	4.82±0.04	11.39±1.62	6.57±0.882	1.13±0.08*	1.33±0.16*	92.02±6.21*	21.13±1.00	0.33±0.06*	0.253±0.02*	100.0±0.0
C. mrigala	2% Chitosan	4.86±0.04	11.10±0.26	6.24±0.137	1.24±0.10*	1.27±0.22*	94.26±4.72*	20.06±0.82	0.31±0.01*	0.223±0.01*	96.7±0.10
	5% Chitosan	5.10±1.14	11.25±1.62	6.15±0.30	1.26±0.10*	1.80±0.16*	79.38±4.72*	20.5±0.62	0.42±0.02*	0.22±0.02*	100.0±0.0
	10% Chitosan	4.5±0.42	10.25±0.07	5.75±0.14	1.83±0.05*	1.48±0.23	55.28±1.83	19.17±0.68	0.37±0.06	0.20±0.02	96.7±1.6

Values are mean ± S.D.
Superscript values are significant at 0.05% (t<0.05) level.

32.6%) or juvenile *P. vannamei* 36.2%). The differences in apparent chitin digestion was attributed to food preference differences among *Penaeus subgenera* (Kanazawa, *et al.*, 1970).

Dietary glucosamine has been found to be a growth promoting factor in shrimp providing growth enhancement over other dietary carbohydrate sources (Kanazawa *et al.*, 1970). This growth effect indicated the shrimp's ability to absorb and utilize dietary sources of glucosamine. Moreover substitution of glucosamine by equal amounts of chitin did not produce the same growth promoting response (Kitabayashi *et al.*, 1971) indicating that chitin digestion did not occur at a rate sufficient to replace pure source of glucosamine. The present study indicates that the inclusion of low levels of b-chitosan has growth promoting effect in carps.

The study was supported by University Grants Commission, New Delhi, Grant No. F3-84/ 2001(SRII)/20-03-2001

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