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Dietary Supplementation of α- and β- Chitosan and Growth and Blood Cell Composition in Common Carp, *Cyprinus carpio L.*

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Fingerlings of *Cyprinus carpio* L. were reared on four supplementary feeds containing 1% each of α-chitin, α-chitosan, β-chitin and β-chitosan for a period of 90 days. Significant differences ($p<0.05$) were observed in weight gain of fish fed with α- and β-chitosan supplemented diets. Diets containing 1% α-chitosan gave food conversion ratio (FCR) 1.16, protein efficiency ratio (PER) 2.11 and protein productive value (PPV) of 14.62. Fish survival ranged from 73–93% in treated fingerlings, while it was only 67% in control. The values of total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were significant in the test groups treated with dietary α-chitosan. An overall improvement in the fish health was observed in the treated groups. α- and β-chitosan can be used as immunomodulatory feed additive in carp nutrition.

Key words: α-chitin, β-chitin, α-chitosan β-chitosan, *Cyprinus carpio*, growth indices, blood cell composition.

Nutrition has an influence on health and immune responses of fish (Blazer, 1992). The immunomodulatory activity of chitin and chitosan in fish has been reported by some authors (Anderson & Siwicki, 1994; Siwicki *et al.*, 1994). Pretreatment of shrimp chitin and chitosan on refrigerated fish fillets has effectively inhibited various bacteria and consequently extended the shelf life. (Tsai *et al.*, 2002). The effective enhancement of nonspecific immunity in rohu (*Labeo rohita)* has been demonstrated after intraperitoneal injection of chitosan (Sahoo & Mukherjee, 1999). This study was carried out to determine the growth promoting and immunomodulatory effects of dietary a-chitin, a-chitosan, b-chitin and b-chitosan supplementation on common carp, *Cyprinus carpio L.* fingerlings.

Materials and Methods

Common carp fingerlings (*Cyprinus carpio* L.) weighing 2.5 – 3.0 g were obtained from the State Fish Seed Farm, Manimuthar, Tamil Nadu, India. The fish were held at 29°C in cement cisterns and fed with a commercial pelleted diet, till the start of the experiment. α-chitin was isolated from shrimp shell waste by sequential treatments with 1N hydrochloric acid for 30 min and 1N sodium hydroxide for one h. The chemically treated chitin was deacetylated with 50% NaOH at 140°C in an oil bath for 3 h. The obtained residue (α-chitosan) was washed thoroughly with deionized water until the effluent became neutral and was then dried in an oven at 70°C for 20 h. (No & Mayers, 1997).

Fresh squid pens of *Loligo indica* were ground in a blender and dried at 40°C. The resulting powder was demineralized by washing repeatedly in excess 0.1N HCl and then treated with 1N NaOH at 50°C for 5 h with continuous stirring to remove proteins, then washed with deionized water until the

washings were neutral. Deacetylation of b-chitin was carried out by hydrolyzing with 50% NaOH at 120°C for 1h. The resulting b-chitosan was washed with deionized water and dried at 60°C for 10h (Austin *et al.*, 1989).

The basal diet used in the present study was prepared by mixing most commonly used ingredients such as fish meal, groundnut oil cake, rice bran and wheat flour. The vitamin and mineral premixes were used as recommended by NRC (1983). Fat and energy levels were kept constant in all diets by cod liver oil supplementation. Four experimental diets were prepared by mixing 1% of each of α-chitin, α-chitosan, β-chitin and β-chitosan.

Feeding trials were carried out for a culture period of 90 d in cement cisterns of 3.0 x 2.0 x 0.5m dimensions without any soil base. Each tank was stocked with 75 common carp fingerlings of 2.5-3.0 g size. Each tank had a filter system and half the water was changed once every week. Also every week, the water was disinfected with 2ppm bleaching powder solution. The water temperature was maintained at 29 ± 0.5°C throughout the experiment. Dissolved oxygen, pH and NH_4 –N were measured once every week by standard methods.

Common carp fingerlings were acclimatized to basal (control) diet for a week. Acclimatization was carried out to ensure better utilization of the experimental diets. The fish were fed with one control and four experimental diets at 10.00 and 16.00 hrs every day. The daily ration was 3.5 – 4.0 % body weight and regulated every week according to a growth rate of 2%. The amount of daily feed intake was recorded. The fish in each cement cistern were weighed once every 10 days. At the end of the experiment, each fish was weighed and its body length was measured and one third of the fish were killed for proximate carcass analysis (AOAC, 1984). Specific growth rate (SGR), weight gain (%), food conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) were calculated after Castell & Tiews, (1980) and the data were statistically analysed by student's t-test.

Packed cell volume (PCV) was determined by microhaematocrit centrifuge. Haemoglobin (Hb) level was determined by the standard photometrical method (Houston, 1990). Total erythrocyte count (TEC) and total leucocyte count (TLC) were performed using the New Improved Neubauer haemocytometer (Hesser, 1960). In both cases, modified Dacie's fluid (Blaxhall & Daisley, 1973) was used as diluant. Blood smears stained with Wright's stain were prepared to determine differential leucocyte counts. Two hundred leucocytes were differentiated morphologically and % occurrence of each type was calculated. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were calculated from the values of PCV, Hb and TEC (Schalm *et al.*, 1975).

Results and Discussion

Dissolved oxygen, pH and NH_4 –N were 5.6–7.2 mg/L, 7.1-7.5 and 0.002 ± 0.68 mg/L respectively during the experiment period.

Basal diet ingredients and the proximate composition of the ingredients are shown in Table 1. During 90-day feeding trials, the fish consumed the feed well and

Table 1. Basal diet ingredients and proximate composition of the ingredients

Ingredients	% of dry diet	Feed components	Diet Composition (%)
Fish meal	30	Dry matter	91.24
Ground nut oil cake	29	Crude protein	31.10
Rice bran	10	Crude fat	7.96
Wheat flour	10	Carbohydrate	25.18
Tapioca flour	10	Crude fibre	5.55
Sardine oil	10	Ash	10.40
Vitamin and mineral mix	1	Energy kcal/g	2.95

the acceptability of all diets was similar. Data for growth, food conversion, protein utilization, survival and carcass composition of common carp fingerlings are presented in Table 2. Significant difference was observed in weight gain of fish fed with a and β-chitosan supplemented diets ($p<0.05$). Diet containing 1% a-chitosan gave the best FCR (1.16) followed by β-chitosan (FCR, 1.38). The highest PER (2.11) and PPV (14.62) were obtained in fish fed with a-chitosan diet. Fish survival ranged from 73-93% in treated fingerlings, while it was only 67% in control. Although the carcass protein, lipid and carbohydrate contents increased after feeding the test diets, significant change in the nutrient composition of the fish was observed only in a-chitosan treatment ($p<0.05$).

Table 2. Growth, food conversion, protein utilization, survival and carcass composition of Common carp fingerlings fed with test diets containing α-chitin, β-chitin, α-chitosan and β-chitosan after 90 - day culture period

Parameter	Diets				
	Basal diet	α- chitin	β- chitin	α- chitosan	β- chitosan
Initial weight (g)	021±0.012	0.26±0.016	0.20 ±0.012	0.23±0.012	0.25±0.022
Final weight (g)	4.26±0.076	5.94±0.071	5.57±0.029	9.71±0.053	8.75±0.148
Weight gain (g)	4.05±0.21	5.68±0.18	5.37±0.12	9.48±0.20*	8.50±.20*
FCR	2.68±0.53	1.85±1.37*	1.68±1.23*	1.16±0.66*	1.38±0.38*
SGR (g/day)	1.19±0.12	1.51±0.37*	1.48±0.42*	1.87±0.36*	1.73±0.10*
GCE (%)	9.01±4.18	23.81±2.90*	23.65±2.36*	42.11±0.11*	40.08±2.99*
PER (%)	1.36±0.51	1.98±0.57*	1.92±0.40*	2.11±1.30*	1.61±0.48
PPV(%)	10.01±1.28	12.84±1.91	10.86±2.31*	14.62±2.62*	13.27±1.75*
Survival (%)	66.67±12.47	76.67 ± 9.43	73.33±12.47	93.33±5.77*	80.00 ± 8.16*
Carcass analysis (%)					
Protein	44.72±0.12	47.14±0.07	46.74±0.08	52.84±0.08*	49.87±0.06
Lipid	7.49±0.07	8.99±0.09	8.93±0.09	10.14±0.06*	9.48±0.08
Carbohydrate	11.76±0.14	12.46±0.13	12.32±0.11	13.56±0.21*	12.81±0.21

Values are mean ± S.D; superscript values are significant at ($P<0.05$) level.

Table 3. Haematological values of Common carp fingerlings fed with test diets containing α-chitin, β-chitin, α-chitosan and β-chitosan after 90 – day culture period

Parameter	Experimental diet				
	Basal diet	α- chitin	β- chitin	α- chitosan	β- chitosan
TEC (x 10^6/mm^3)	2.33±0.14	2.10±0.06*	2.23±0.11*	1.98±1.15*	2.27±0.32
TEC (x 10^4/mm^3)	11.96±0.71	12.42±1.03	12.07±0.85	13.31±1.39*	12.89±1.13
PCV (%)	27.10±0.17	27.05±0.28	27.50±0.25	28.50±0.51	27.60±0.20
Hb (gm%)	9.90±0.15	9.60±0.45	9.60±0.45	10.50±0.91*	10.20±0.27
MCV (mm^3)	116.31±4.44	130.95±8.50*	123.32±6.17*	143.94±12.81*	121.59±11.28
MCH (pg)	42.49±3.06	45.71±2.71	43.05±3.20	53.03±1.60*	44.93±4.71
MCHC (%)	36.53±1.26	34.91±1.76	34.91±1.40*	36.84±1.76	36.96±1.13
DLC (%)					
Lymphocyte	39.00±3.78	40.33±3.05	36.33±2.57	38.00±1.80	43.37±0.96*
Neutrophil	52.67±4.00	52.67±4.04	52.33±3.00	56.43±1.32*	48.33±1.11*
Eosinophil	4.67±1.52	2.67±0.57	6.46±1.14	3.20±0.64*	5.40±1.48
Monocytes	3.70±1.07	3.27±0.51	3.54±1.77	2.90±0.79	2.80±0.27

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

Enzymes capable of digesting chitin occur in the digestive system of many fishes (Fange *et al.*, 1979, Lindsay & Gooday, 1985). Dietary feeding of 4, 10 and 25% chitin over a 12- week period significantly depressed the growth of rainbow trout, *Salmo gairdneri*. On the other hand rainbow trout utilized dietary aminosugars (N-acetyl glucosamine, glucosamine) as energy sources (Lindsay *et al.*, 1984). In another study, Shi-Yen Shian & Yi Ping Yu (1999) reported that addition of 2, 5 and 10% dietary chitosan also depressed the growth of hybrid tilapia, while supplementary feeding of 1, 2 and 5% chitin enhanced the weight gain.

Results of haematological examination of fish from different test diets are presented in Table 3. In general, the values of different blood parameters were significant in the test groups treated with dietary α-chitosan. There was significant ($p<0.05$) difference in the red blood cell indices (MCV, MCH) of fish fed with α-chitosan. No significant difference was found in MCHC levels of α-chitosan treated group. The counts of granulocytic leucocytes increased significantly in the α-chitosan fed groups ($p<0.05$).

It has been suggested that both chitin (N-acetyl glucosamine) and chitosan (N-deacetylated derivative of chitin) has immunomodulatory effects in fishes. Sakai *et al.*, (1992) found rainbow trout injected with 100mg/kg of chitin had increased the activity of the phagocytic cells. A rise in the level of non-specific immunity was observed in the rainbow trout fed with 0.5% chitosan for one week (Siwicki *et al.*, 1994). Treatment of chitosan in brook trout enhanced a higher degree of protection against *Aeromonas salmonicida* infection for a shorter duration (Anderson & Siwicki, 1994). Similarly, intraperitoneal injection of 100mg of chitosan caused effective enhancement of non-specific immunity in *Labeo rohita* (Sahoo & Mukherjee, 1999).

Based on the results of the present investigation, it was observed that the inclusion of a and b-chitosan in diets had significant effect on the fish health and haemostasis. α- and β- chitosan can be used as micronutrients / immunomodulatory feed additive in carp nutrition.

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