# Effect of Dietary Lipid Sources on Growth, Enzyme Activities and Immuno-hematological Parameters in *Catla catla* Fingerlings

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**ABSTRACT :** Ninety advanced *Catla catla* fingerlings (av. wt. 16 g) were randomly distributed in six treatment groups with three replicates each for an experimental period of 60 days to study the effect of dietary lipid source on growth, enzyme activities and immuno-hematological parameters. Six isoprotein (40.0-41.9%) and isocaloric (4,260 kcal $kg^{-1}$) semi-purified diets were prepared with varying levels of soybean oil (SBO) and cod liver oil (CLO) within a total of 8% lipid viz., $D_1$ (Control), $D_2$ (8% SBO), $D_3$ (6% SBO and 2% CLO), $D_4$ (4% SBO and 4% CLO), $D_5$ (2% SBO and 6% CLO) and $D_6$ (8% CLO). Highest SGR was noted in $D_5$ (0.73±0.03) group, which was similar with $D_3$ (0.71±0.02) and $D_4$ (0.69±0.01) groups. Activity of intestinal lipase, hepatic glucose-6-phosphate dehydrogenase (G6PDH) and aspartate amino transferase (AST) of the lipid treatment groups were significantly higher ($p<0.05$) than the control group. The respiratory burst activity of the phagocytes (Nitroblue tetrazolium (NBT)) was highest in $D_2$ (1.95±0.21) followed by $D_3$ (1.19±0.15) group, which were significantly ($p<0.05$) higher than the other groups. Globulin level was significantly higher in $D_3$ (1.29±0.08) than in the other groups expect $D_4$. Hemoglobin content and total erythrocyte count did not show any significant difference. From this study, it is concluded that a diet containing 6% soybean oil and 2% cod liver oil ($D_3$) yields higher growth and immune response in *Catla catla* fingerlings and would be cost effective. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1609-1616)***

**Key Words :** *Catla catla*, Cod Liver Oil, Soybean Oil, Enzyme, Immuno-hematological Parameters

## INTRODUCTION

In India, the aquaculture practices mainly revolve around a few species of finfish and shellfish, among which the Indian Major Carps viz. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* contribute substantially to the inland production. Although carp culture is widely practiced, the non- availability of appropriate compounded feed to meet the demands of the species still remains as a major constraint. Amongst the major nutrients in feed, lipid deserves special mention due to its higher calorific value than the proteins and carbohydrates. Lipids are almost completely digestible by fish and seem to be favoured over carbohydrate as an energy source (Cowey and Sargent, 1977; Cho et al., 1985). The protein sparing effect of dietary lipid has also been investigated in several freshwater fish species (Ramachandran and Gopakumar, 1980; Viola and Arieli, 1983; Das et al., 1991). Dietary lipids provide essential fatty acids mainly polyunsaturated fatty acids (PUFAs) that require for the proper functioning of the cells.

A combination of n-3 and n-6 fatty acids has resulted in better growth rate and survival in the fry of *Catla catla* (Mukhopadhyay and Rout, 1996) and *Clarias batrachus* (Mukhopadhyay and Misra, 1998). Lipase activity and the adaptive changes in the activity of digestive enzymes with respect to the quality of dietary lipids have been well documented in carps (Mukhopadhyay and Rout, 1996), *Chanos chanos* (Borlongan, 1990) and *Tor khudree* (Bazaz and Keshavanath, 1993). The effect of diets with different lipid and protein contents on aspartate amino transferase (AST) activity has been observed in rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Rehulka and Parova, 2000), which indicates that the composition of the diet has a strong influence on the enzyme activities of an organism. Experiments conducted in male rats (Torii et al., 1996) proved that diets with different fatty acids caused changes in the lipogenic related enzyme activities in various tissues.

Plant oils are generally rich sources of n-6 fatty acids, while fish oils are rich in n-3 fatty acids. As the global production of plant oils is 100 times higher than that of fish oils (FAOSTAT 1990-98), vegetable oils obviously represent more sustainable sources of lipid for the aquafeed industry. Dietary lipid source has a significant effect on growth and meat quality of terrestrial animal (Jaturasitha et al., 2002; Jung et al., 2003). Many studies have been performed regarding the partial substitution of fish oils with plant oils in the fish diets (Hardy et al., 1987; Thomassen and Rosjo, 1989; Greene and Selivonchick, 1990) and the results indicate that high inclusion of alternative plant lipid sources had no negative impacts on growth of fish.

A particularly recent area of interest in fish is the influence of polyunsaturated fatty acids (PUFAs) on the immune response (Blazer, 1992; Li et al., 1994; Waagbo,

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Received September 22, 2004; Accepted March 14, 2005

**Table 1.** Ingredient and proximate composition of experimental diets (g $kg^{-1}$ )

| Ingredient | Diets | | | | | |
|---|---|---|---|---|---|---|
| | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ |
| Caesin fat and vitamin free (S.D. Fine Chemicals, Mumbai, India) | 350.0 | 350.0 | 350.0 | 350.0 | 350.0 | 350.0 |
| Gelatin (S.D. Fine Chemicals, Mumbai, India) | 95.0 | 95.0 | 95.0 | 95.0 | 95.0 | 95.0 |
| Dextrin (S.D. Fine Chemicals, Mumbai, India) | 180.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Starch (S.D. Fine Chemicals, Mumbai, India) | 330.0 | 250.0 | 250.0 | 250.0 | 250.0 | 250.0 |
| Cellulose (S.D. Fine Chemicals, Mumbai, India) | - | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 |
| Carboxymethyl cellulose | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Soybean oil | - | 80.0 | 60.0 | 40.0 | 20.0 | - |
| Cod liver oil | - | - | 20.0 | 40.0 | 60.0 | 80.0 |
| Vitamin-mineral mix[1] | 26.0 | 26.0 | 26.0 | 26.0 | 26.0 | 26.0 |
| Vitamin B complex[2] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Vitamin C | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Glycine | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Proximate composition | | | | | | |
| Crude protein (g $kg^{-1}$ DM) | 408.1 | 400.0 | 418.2 | 411.2 | 419.0 | 401.3 |
| Ether extract (g $kg^{-1}$ DM) | 0.5 | 78.7 | 77.3 | 80.9 | 79.2 | 79.2 |
| Ash (g $kg^{-1}$ DM) | 32.0 | 32.6 | 31.3 | 32.2 | 32.8 | 33.3 |
| Total carbohydrate (g $kg^{-1}$ DM)[3] | 554.4 | 488.6 | 473.2 | 475.7 | 468.9 | 486.2 |
| Digestible energy (KJ $g^{-1}$ diet) | 16.3 | 17.9 | 17.9 | 17.9 | 17.9 | 17.9 |

BHT (S.D. Fine Chemicals, Mumbai, India) was added at 0.02% of oil. Caesin fat and vitamin free: 86% CP. Gelatin: 96% CP

[1] Composition of vitamin mineral mix (Agrimin) (quantity/kg) Vitamin A, 6.25,000 IU; Vitamin $D_3$, 62.500 IU; Vitamin E, 250 mg; Nicotinamide, 1 g; Cu, 312 mg; Co, 45 mg; Mg, 6 g; Fe, 1.5 g; Zn, 2.13 g; Iodine, 156 mg; Se, 10 mg; Mn, 1.2 g; Ca, 247.34 g; P, 114.68 g; S, 12.2 g; Na, 5.8 mg; K, 48.05 mg.

[2] Composition of vitamin B complex (quantity/g) Thiamine mononitrate, 20 mg; Riboflavin, 20 mg; Pyridoxine hydrochloride, 6 mg; Vitamin $B_{12}$, 30 mcg; Niaciamide, 200 mg; Ca pantothenate, 100 mg; Folic acid, 3 mg; Biotin, 200 mcg.

[3] Total carbohydrate (g $kg^{-1}$): 1,000-(EE−CP−Ash).

1994). Studies conducted on fish show that diet containing different levels of n-3 and n-6 fatty acids from fish and vegetable oils can modify the fatty acid composition of cell phospholipid in turbot and Atlantic salmon (Bell et al., 1991, 1993, 1994), which affect the synthesis of eicosanoid precursors. Fracalossi and Lovell (1994) observed low disease resistance and immune functions such as phagocytic capacity and killing activity in channel catfish fed diets high in n-3 PUFAs. The functional role of n-3 and n-6 PUFA in non specific and specific humoral and cellular immunity has not been studied extensively (Balfry and Higgs, 2001). However, studies conducted on the effect of n-3 and n-6 fatty acids on the immune responses in fish are preliminary and often inconclusive. Studies conducted on the effect of n-3 and n-6 fatty acids on the immune responses in fish are preliminary and often inconclusive. Therefore the objective of the present study was to evaluate the growth, enzyme activities and immuno-hematological changes caused by the dietary incorporation of soyabean oil and codliver oil in *Catla catla* fingerlings

## MATERIALS AND METHODS

### Experimental diets

Six isoprotein (40.0-41.9%) and isocaloric (4,260 kcal $kg^{-1}$) semi-purified diets were prepared, which contained soybean oil, cod liver oil or a combination of both to maintain 8% lipid except in the control diet, which lacked lipids. Ingredients used for diet formulation and the proximate composition of the experimental diets are given in Table 1.

Purified ingredients such as casein, gelatin, dextrin, starch, cellulose, carboxymethyl cellulose, cod liver oil, soybean oil, vitamin and mineral mixture (Agrimin India Ltd), vitamin C (Roche) and vitamin B complex (Glaxo India Ltd.) were used for feed formulation. All the ingredients except vitamin and mineral mixture were mixed well. About 100 ml water was added to 1 kg feed mix to form dough and the required amount of the oils were incorporated in it and mixed well. The dough was conditioned for 1 h and subsequently steam cooked in a pressure cooker for 30 min. Vitamin and mineral premixes and vitamin C were added to the dough after cooling. Pellets were prepared using a hand pelletizer of 2 mm diameter, oven dried at 60°C, packed in air-tight polythene bags and stored at 4°C until use.

### Analysis of tissue and feed

The experimental diets were analysed using standard AOAC (1995) methods for dry matter (dried at 100°C to constant weight), crude protein (2,200 Kjeltec Auto Distillation, Foss Tecator, Sweden), ether extract (solvent extraction with diethyl ether b.p. 40-60°C using a Soxtec system model SD2, 1,045 extraction unit, Tecator) and ash

(muffle furnace incineration at 550°C for 5-6 h). Total carbohydrate was calculated by difference (Hasting. 1969) and approximate digestible energy content was calculated as Halver (1976). Initial and final tissue composition of the fish was analyzed for all the treatment groups as described above.

**Experimental design and feeding trial**

Advanced fingerlings of *Catla catla* were procured from Khopoli Govt. Fish Farm, Maharashtra, India and acclimatized for a period of 2 weeks to the control diet. They were then distributed into six groups. each with three replicates following a completely randomized design (CRD). Five fishes with initial weight ranging from 15 to 16 g were stocked in 75 L plastic tubs with 50 L chlorine free bore well water and round the clock aeration was provided. The experimental tubs were cleaned manually and siphoning was done everyday in the morning at 7:30 in order to remove the excess feed pellets and the remaining faecal matter. About 75% of the water was replaced with fresh chlorine free bore-well water. The experiment was conducted for 60 days.

Water quality parameters. like temperature. pH. dissolved oxygen, free carbon dioxide, carbonate hardness, total ammonia, nitrite-N. and nitrate-N were recorded on every other day. All were found to be within the optimum range. Feeding was done at 40 g $kg^{-1}$ body weight and the feeding rate was adjusted based on weight measurement at every 20 days. The daily ration was divided into two equal parts and was fed at 09.00 and 17.00.

**Enzyme assays**

Lipase activity of intestine tissue and glucose-6-phosphate dehydrogenase (G6PDH). aspartate amino transferase (AST) and alanine amino transferase (ALT) activities in liver tissues were assayed at the end of the experiment. Six specimens were collected randomly from each treatment. The intestines and liver of the fishes were carefully removed and for intestine the contents were squeezed out. The intestinal and the liver tissues were then weighed and homogenised with chilled sucrose solution (0.25 M) in a glass tube using tissue homogeniser. A 5% homogenate was prepared for liver and intestine. which was centrifuged at 5.000 rpm for 10 min at 4°C to collect the supernatant in glass vials and stored at 4°C until use. Quantification of protein in the liver was carried out as Lowry et al. (1951) using bovine serum albumen as the protein standard.

**Analysis of enzymes**

The lipase activity was assayed by the method of Cherry and Crandell (1932). Unit of lipase activity per g tissue was expressed as the volume (ml) of N/20 NaOH solution required for 100 mg intestinal tissue in the experimental tube minus the volume (ml) of N/20 NaOH solution required for the same amount of intestinal tissue in the control tube. The G6PDH activity was assayed by the method of De Moss (1953). The OD was recorded at 340 nm in 15 seconds interval against distilled water. The G6PDH activity was expressed as units/mg protein/minute. One unit was equal to Δ0.01 OD/min/ml at 25°C.

The AST and ALT activities were assayed in tissue homogenate as described by Wooten (1964). The procedure adopted for ALT activity was same as that of AST activity except that the substrate comprised of D. L-alanine instead of aspartic acid. The absorbance was recorded at 540 nm against the blank. One unit of enzyme activity is defined as n moles of product released per mg of protein per minute. The products were oxaloacetate and pyruvate. respectively.

**Immunological parameters**

The respiratory burst activity of the phagocytes was done by Nitroblue Tetrazoleum (NBT) assay following the method of Secombes (1990) as modified by Stasiack and Baumann (1996). The OD of the turquoise blue coloured solution was then read in ELISA reader. Plasma protein was estimated by biuret method (Reinhold. 1953) using the kit. Albumin was estimated by bromocresol green binding method (Doumas et al., 1971). The absorbance of standard and test were measured against blank in a spectrophotometer at 630 nm. Globulin was calculated by subtracting albumin values from total plasma protein. A/G ratio was calculated by dividing albumin values by globulin values.

**Hematological parameters**

The hemoglobin level of blood was analysed following the formation of cyanmethemoglobin method using Drabkin's fluid (Qualigens). About 20 µl of blood was mixed with 5 ml of Drabkin's working solution. The absorbance was measured using a spectrophotometer at wavelength of 540 nm. The final concentration was calculated by comparing with the standard cyanmethemoglobin (Qualligens Diagnostics India Ltd). RBC and WBC diluting fluids were used for taking total erythrocyte count and total leucocyte count. It was done by mixing 20 µl of blood with 3.980 µl of corresponding diluting fluid in a clean test tube and was shaken well to suspend the cells uniformly in the solution. A small drop of this mixture was charged to Neubauer's counting chamber of hemocytometer and counting was done.

No. of cells $mm^{-3}$ = (No. of cells counted×dilution)/ (Area counted×depth of fluid)

**Statistical analyses**

Significance of differences among treatments was

**Table 2.** Growth parameters and survival rate of *Catla catla* fingerlings fed different experimental diets[1]

| Parameters | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ |
|---|---|---|---|---|---|---|
| Initial body wt. | 16.43±0.48 | 16.18±0.51 | 16.42±0.31 | 16.40±0.42 | 16.13±0.48 | 16.18±0.59 |
| Final body wt. | 21.87±0.94 | 24.15±0.54 | 25.11±0.82 | 24.85±0.74 | 24.95±0.38 | 22.00±0.74 |
| Weight gain (%)[2] | $34.58^{a}$±1.28 | $49.37^{b}$±1.53 | $52.90^{b}$±2.14 | $51.53^{b}$±1.45 | $54.85^{b}$±2.30 | $38.70^{a}$±0.94 |
| SGR[3] | $0.49^{a}$±0.01 | $0.67^{b}$±0.01 | $0.71^{bc}$±0.02 | $0.69^{bc}$±0.01 | $0.73^{c}$±0.03 | $0.55^{a}$±0.01 |
| FCR[4] | $5.50^{d}$±0.20 | $4.12^{b}$±0.14 | $3.70^{ab}$±0.16 | $3.84^{ab}$±0.08 | $3.62^{a}$±0.15 | $5.05^{c}$±0.11 |
| PER[5] | $0.43^{a}$±0.02 | $0.61^{b}$±0.01 | $0.65^{b}$±0.03 | $0.63^{b}$±0.02 | $0.66^{b}$±0.01 | $0.49^{a}$±0.01 |
| Survival (%) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

[1] Mean of three replicates±SE. Means in the same row sharing same superscripts are not significantly different ($p>0.05$).
[2] 100 [(final body weight (g)-initial body weight (g))/initial body weight (g)].
[3] 100 [log$_e$ final body weight-log$_e$ initial body weight/experimental duration (60 days)].
[4] Feed intake (g)/weight gain (g).
[5] Weight gain (g)/protein intake (g).

**Table 3.** Whole body composition (g kg$^{-1}$ dry weight basis±SE) of *Catla catla* fingerlings of different experimental groups at the end of the experiment

| Diets | Moisture | Protein | Total carbohydrate | Lipid | Ash |
|---|---|---|---|---|---|
| $D_1$ | 783.7±19.4 | $639.2^{c}$±4.6 | $65.0^{a}$±9.0 | $128.7^{a}$±6.7 | $166.2^{b}$±2.2 |
| $D_2$ | 758.5±1.5 | $600.1^{a}$±11.0 | $71.0^{a}$±10.0 | $165.6^{b}$±2.7 | $163.1^{ab}$±2.8 |
| $D_3$ | 761.6±1.9 | $627.3^{bc}$±9.8 | $50.5^{a}$±5.7 | $168.2^{b}$±7.4 | $153.8^{a}$±2.4 |
| $D_4$ | 757.8±4.5 | $617.9^{abc}$±6.5 | $51.5^{a}$±4.2 | $180.8^{b}$±5.3 | $149.8^{a}$±1.9 |
| $D_5$ | 755.0±0.6 | $620.3^{abc}$±3.6 | $77.1^{a}$±6.4 | $142.5^{a}$±10.7 | $160.0^{ab}$±4.9 |
| $D_6$ | 765.8±6.0 | $608.5^{ab}$±4.6 | $67.9^{a}$±12.4 | $43.9^{a}$±8.9 | $179.6^{c}$±2.1 |

All values are means of three observations.
Means in the same column sharing same superscripts are not significantly different ($p>0.05$).
Total carbohydrate = 1,000-(protein−lipid−ash).

**Table 4.** Enzyme activities of *Catla catla* fingerlings fed different experimental diets

| Parameters | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ |
|---|---|---|---|---|---|---|
| Lipase activity (unit activity/gm of wet tissue) | 16.66±2.92 | $24.33^{b}$±1.21 | $28.66^{b}$±2.42 | $28.00^{b}$±2.32 | $24.67^{b}$±1.77 | $22.00^{b}$±1.16 |
| Glucose-6- phosphate dehydrogenase (units mg$^{-1}$ protein min$^{-1}$) | 11.74±0.83 | $26.71^{c}$±2.25 | $27.70^{c}$±2.46 | $25.32^{c}$±2.15 | $25.98^{c}$±1.53 | $18.23^{b}$±1.51 |
| Aspartate amino transferase activity (n moles of oxaloacetate released mg$^{-1}$ protein min$^{-1}$) | 26.21±1.50 | $48.76^{d}$±3.02 | $45.63^{d}$±2.75 | $42.14^{cd}$±2.15 | $34.64^{b}$±1.74 | $37.71^{bc}$±2.38 |
| Alanine amino transferase activity (n moles of sodium pyruvate released mg$^{-1}$ protein min$^{-1}$) | 25.00±1.13 | $40.81^{c}$±1.13 | $33.88^{b}$±1.07 | $24.46^{a}$±1.54 | $23.98^{a}$±1.14 | $25.34^{a}$±1.20 |

All values are means (±SE) of six observations.
Means in the same row sharing same superscripts are not significantly different ($p>0.05$).

determined by one way analysis of variance (ANOVA) and the differences between mean values were tested using Duncan's multiple range test (DMRT) using the statistical package SPSS version 11.

## RESULTS

### Growth

Different growth parameters of *C. catla* fingerlings fed diets containing soybean oil, codliver oil or a combination of both are given in Table 2. There was significant ($p<0.05$) difference in growth among fish fed the different diets. Weight gain of groups $D_2$, $D_3$, $D_4$ and $D_5$ was significantly ($p<0.05$) higher than the control and $D_6$ group. There were no difference among $D_3$, $D_4$ and $D_5$ groups. FCR showed the same trend as that of SGR. The best FCR was observed in $D_5$ group. but it did not vary significantly ($p>0.05$) from $D_3$ and $D_4$ groups. PER of $D_2$, $D_3$, $D_4$ and $D_5$ was significantly ($p<0.05$) higher than the control and $D_6$ groups. Survival was not affected due to feeding of different lipid source in different treatment groups.

### Tissue composition

The final tissue compositions of all the experimental groups are given in Table 3. There was significant ($p<0.05$) difference in the tissue lipid level among fishes fed the different diets. Lipid level of $D_2$, $D_3$ and $D_4$ were significantly higher ($p<0.05$) when compared to the other groups. However, there was no difference among $D_2$, $D_3$ and $D_4$ groups. The lowest lipid level was found in the

control in which the diets were not supplied with lipids.

**Enzyme activities**

Lipase activity in intestine and G6PDH, AST and ALT activities in the hepatic tissue of fingerlings of *Catla catla* at the end of the experiment are given in Table 4. The activity was found to be significantly higher in treatment groups than the control group. G6PDH activity was significantly ($p<0.05$) higher in the treatment groups than the control groups. Though the highest G6PDH activity was found in $D_3$ group, it did not vary significantly ($p>0.05$) from the other groups except $D_6$. However, the activity of the enzyme in all the treatment groups differed significantly ($p<0.05$) from the control group. AST and ALT activity also showed significant ($p<0.05$) differences among the treatments. AST activity was higher in $D_2$ followed by $D_3$ and $D_4$ groups. Though, there was no significant difference among $D_2$, $D_3$ and $D_4$, it varied significantly with the rest of the treatments and control. The lowest activity could be observed in the control. ALT activity was also higher in $D_2$ and lowest in control group. Though the lowest activity was observed in the control, it did not vary significantly ($p>0.05$) from $D_4$, $D_5$ and $D_6$ groups.

**Immuno-hematological parameters**

Immunological and hematological parameters of *Catla catla* fingerlings fed different experimental diets are given in Table 5. The respiratory burst activity of the phagocytes as measured by the NBT assay showed significant ($p<0.05$) difference among the various treatments. The activity was the highest in $D_2$ followed by $D_3$ and was found to decrease as the amount of the soyabean oil in the diet decreased. The lowest activity was found in $D_6$ group fed only codliver oil as lipid source. Plasma protein, albumin, globulin and A/G values also differed significantly ($p<0.05$) among the treatments. Plasma protein was significantly ($p<0.05$) higher in $D_3$ and $D_4$ groups compared to control. Globulin level was higher in $D_3$ followed by $D_4$ and varied significantly ($p<0.05$) with other groups. Hemoglobin and total erythrocyte count showed no significant difference ($p>0.05$) among the experimental groups. Total leucocyte count was higher in $D_5$ followed by $D_3$ and $D_4$ but did not vary significantly among themselves.

## DISCUSSION

The efficacy of dietary lipids in promoting growth depends mainly upon its composition. Growth depends upon the type and content of fatty acids in the dietary lipid, rather than the total quantity of lipid used in the diet. In the present study, fish fed diets containing a mixture of soybean oil and cod liver oil showed maximum growth rather than only soybean or cod liver oil fed groups. The highest SGR was observed in $D_5$ group, which was also similar with $D_3$ and $D_4$ groups. This might be due to the presence of n-6 fatty acid in soybean oil and n-3 fatty acid in cod liver oil, that are reported as essential for the proper growth of fishes (Mukhopadhyay and Rout, 1996).

Lipids are a concentrated and highly digestible source of energy (Mead et al., 1986) and favoured over carbohydrate as an energy source (Cowey and Sargent, 1977; Cho et al., 1985). The control diet was devoid of lipids and so the proteins might have been used for energy production and not for growth.

Feed conversion ratio was low in groups fed diets containing a combination of soyabean oil and cod liver oil. This indicates that carps prefer a combination of n-6 and n-3 fatty acids in their diet (Mukhopadhyay et al., 1991; Lovell, 1998). Mukhopadhyay and Rout (1996) had reported that best FCR was obtained in the fry of *Catla catla* when fed a combination of sunflower oil and cod liver oil in their diet. The control group without lipids showed the highest feed conversion ratio. This might be due to inefficient utilization of feed. Changde and Paulraj (1997) observed that deletion of lipid from the diet resulted in high feed conversion ratio indicating inefficient utilization of feed by *Macrobrachium rosenbergii*.

The highest PER was recorded in $D_5$ group followed by $D_3$, $D_4$ and $D_2$ groups, which differed significantly ($p<0.05$) from the control and $D_6$ groups. The higher PER in all the

**Table 5.** Immuno-hematological parameters of *Catla catla* fingerlings fed different experimental diets

| Parameters | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ |
|---|---|---|---|---|---|---|
| NBT assay | $0.31^{ab}\pm0.05$ | $1.95^{d}\pm0.21$ | $1.19^{c}\pm0.15$ | $0.65^{b}\pm0.12$ | $0.54^{b}\pm0.06$ | $0.21^{a}\pm0.02$ |
| Plasma protein | $1.49^{ab}\pm0.09$ | $1.34^{a}\pm0.05$ | $1.98^{c}\pm0.11$ | $2.03^{c}\pm0.05$ | $1.37^{a}\pm0.08$ | $1.76^{bc}\pm0.14$ |
| Albumin | $0.78^{ab}\pm0.02$ | $0.67^{a}\pm0.03$ | $0.69^{a}\pm0.03$ | $0.89^{b}\pm0.07$ | $0.62^{a}\pm0.01$ | $0.75^{ab}\pm0.10$ |
| Globulin | $0.70^{a}\pm0.08$ | $0.67^{a}\pm0.03$ | $1.29^{c}\pm0.08$ | $1.13^{bc}\pm0.03$ | $0.75^{a}\pm0.07$ | $1.01^{b}\pm0.06$ |
| A/G (ratio) | $1.14^{d}\pm0.10$ | $1.00^{cd}\pm0.06$ | $0.54^{a}\pm0.01$ | $0.79^{bc}\pm0.08$ | $0.82^{bc}\pm0.05$ | $0.74^{ab}\pm0.08$ |
| Haemoglobin (g (100 ml)$^{-1}$of blood) | 7.53±0.24 | 7.52±0.35 | 8.57±0.51 | 8.52±0.59 | 8.71±0.35 | 9.20±0.24 |
| Total erythrocyte count ($10^6$ cells/mm$^3$) | 1.12±0.06 | 1.14±0.04 | 1.21±0.05 | 1.18±0.04 | 1.24±0.09 | 1.46±0.17 |
| Total leucocyte count ($10^3$ cells/mm$^3$) | $47.00^{a}\pm2.39$ | $52.00^{ab}\pm4.19$ | $58.8^{bc}\pm1.86$ | $55.50^{abc}\pm2.29$ | $62.33^{c}\pm5.04$ | $49.66^{ab}\pm0.88$ |

Each value is the mean (±SE) of three replicates.

Means in the same row sharing same superscripts are not significantly different ($p>0.05$).

treatment groups compared to control is due to the protein sparing effect of dietary lipid as indicated by several studies (Viola and Arieli, 1983; Das et al., 1991). Among the treatment groups, $D_6$ showed a lower PER and reduced growth. In $D_6$, the fishes were not able to utilize the lipid source properly and hence protein utilization was affected and resulted in lower PER and growth. However, no literatures are available in these aspects and needs to be explored. The energy source and energy level of diet having the protein sparing effect, affect the protein utilization (Cowey et al., 1975; Adron et al., 1976; Pieper and Pfeffer, 1980).

Supplementation of oil to the basal diet resulted in significant changes in the tissue lipid content. The results indicate a considerably high tissue deposition of lipids in almost all the treatment groups with comparison to the control group. This is in agreement with Viola and Amidan (1980), who reported that carps respond to fat supplementation by increased growth rates and incorporation of fats and specific fatty acids into the tissue lipids. In the control diet, lipids were not incorporated and hence resulted in a lesser deposition in the tissues. Essential fatty acid deficient fish have lower lipid and high moisture levels in their tissues (Back et al., 1983; Mosconi-Back, 1987). In fish, a deficiency of food lipid results in a low fat deposition (Spangenberg and Schreckenbach, 1984). Koven et al. (1990) also found reduced fat levels and increased moisture content in gilt-head bream fry fed with EFA-deficient diets.

Lipase activity in all the treatment groups were higher than the control. However, there was no significant difference ($p>0.05$) among the treatment groups. Higher enzyme activity could be observed in groups fed a mixture of oils and this observation corroborates the results of Mukhopadhyay and Rout (1996) in fry of *Catla catla*. However, the least activity in the control group may be due to the lack of lipids in the diet that indicates that the enzyme system gets triggered only in the presence of specific substrates.

In the present study, the activity of G6PDH has been studied in liver. The control group given diets devoid of lipids showed lower activity, which indicates that the entry of carbohydrates into the pentose phosphate pathway to activate the enzyme was retarded as it was diverted for energy production in the absence of lipids.

The activity was higher in the groups supplied with lipids with comparison to the control group. This high activity can be correlated with the availability of the substrates like $\alpha$-ketoglutarate for the enzyme action resulting in the production of oxaloacetate, which are the precursors for the synthesis of nonessential amino acids that are involved in protein synthesis. Significant difference in the activity of the enzymes with regard to different sources of dietary lipids could be observed which indicates that the composition of the diet has a strong influence on the enzyme activities of an organism as suggested by Rehulka and Parova (2000). No such trend could be observed in ALT activity.

In the present study, the respiratory burst activity of phagocytes was significantly higher ($p<0.05$) in $D_2$ and $D_3$ groups. A gradual decrease in the activity could be observed when the levels of soybean oil (n-6) was reduced and cod liver oil (n-3) in the diet was increased. The highest respiratory burst activity in $D_2$ group fed with soyabean oil and the lowest activity in $D_6$ group supplied with cod liver oil may be due to the corresponding changes caused by the fatty acids in the phagocyte cell membranes. Changes in the PUFAs composition of phospholipids in the lymphocyte membrane can modify membrane functions including enzyme kinetics, ion transport, receptor expression and signal transmission (Hwang, 1989). Enrichment of the diet with n-3 fatty acids were found to decrease the respiratory burst activity of the phagocytes. This supports the immunosuppressive nature of n-3 fatty acids as reported by Fracalossi and Lovell (1994) and Erdal et al. (1991) in Atlantic salmon. Plasma proteins, albumin, globulin and albumin- globulin ratio were also analyzed. The globulin level was higher in $D_3$ and $D_4$ groups when compared to the control group. Relative and total amounts of plasma protein fractions are affected by infections, inflammation, nutritional and physiological status and are therefore important health indicators in free living animals (Grasman et al., 2000). Further studies are required to substantiate the above result.

## CONCLUSION

Considering all the above factors such as growth performance, enzyme activity, and immunological response it concludes that inclusion of soybean oil and cod liver oil in the diet at 6% and 2%, respectively is utilized optimally by *Catla catla* fingerlings. This ratio of lipids in the diet would yield better growth and immune response in catla fingerlings. In spite of high dietary value of cod liver oil due to its n-3 fatty acids content, the toxicity or hypervitaminosis effect needs to be focused in future research.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the former Director Dr. S. Ayyappan and the present Director, Dr. S. C. Mukherjee, Central Institute of Fisheries Education for providing the facilities for the conduct of this work. We are also thankful to Indian Council of Agricultural Research for financial support for this experiment.

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