Research Article

# Effect of *Arthrospira platensis* (Spirulina) dietary supplementation in the growth performance, digestive enzyme activities and digestibility of the freshwater fish, Common carp (*Cyprinus carpio* L.) exposed to sub-lethal concentration of fluoride.

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***Abstract:*** *Effect of Arthrospira platensis (Spirulina) dietary supplementation against fluoride toxicity induced alteration of growth performance, digestive enzyme activities and digestibility in the freshwater fish, Common Carp (Cyprinus carpio L.) was investigated in the present study. A total of 210 acclimated common carp fingerlings of three-month-old common carp were randomly allocated into 7 experimental groups (T1, T2, T3, T4, T5, T6, and T7) each with 30 fish divided into three replicate (10 fish/ replicate). The T1 was the control group fed on control diet reared in normal water, T2 was the toxic control or negative control group fed on control diet and exposed to 10% of $LC_{50}$ of fluoride, T3 was served as plant control or positive control fed on Spirulina(1%) diet and reared in normal tap water, T4 to T7 were the treatment groups fed on Spirulina dietary supplementation at 0.25%, 0.50%, 0.75% and 1% respectively and exposed to 10% of the $LC_{50}$ of fluoride. The experiment was continued for a total period of 30 days. Study revealed that sub-lethal concentration of fluoride significantly ($p<0.05$) decreased the growth performance, digestibility and digestive enzyme activities in T2 compared to T1 in common carp. However, dietary supplementation by Spirulina significantly ($p<0.05$) increased the growth performance, digestibility and digestive enzyme activities in dietary supplementation groups (T4 to T7) compared to T2. The antioxidant rich Spirulina dietary supplementation improved the fluoride toxicity induced alteration of growth performance by ameliorating the free radical generated due to exposure of fluoride, thus increasing the digestive enzyme activities which in turn improved the digestibility. Spirulina dietary supplementation at 1 % showed the best response hence considered as the optimum dose.*

***Keywords:*** *Growth performance, fish nutrition, fish toxicology, ground water pollution*

## 1. Introduction

Fluoride is the 13$^{th}$ abundant element in the earth contributing 0.06-0.09% of the earth crust [1-5]. Fluoride in drinking water at a concentration of 0.5 to 1 mg/L has beneficial effect in health, preventing dental caries as well as strengthen the teeth and bones [6]. However, fluoride causes dental fluorosis (mottling of teeth) at a concentration of 1.5 to 4.0 mg/L, dental as well as skeletal fluorosis (pain in back and neck bones) at 4.0 to 10 mg/L and crippling skeletal fluorosis above 10 mg/L in drinking water [7-9]. Therefore, the permissible limit of fluoride in drinking water is 1.5 mg/L has been recommended by World Health Organization (WHO), 0.6 to 1.2 mg/L by Bureau of Indian Standard (BIS), 1 mg/L by Indian Council of Medical Research (ICMR) and the Committee on Public Health Engineering Manual and Code of Practice, Government of India [10].

Occurrence of excessive concentration fluoride in ground water is endemic in many parts of the World, including India [11-12]. Ground water fluoride pollution is also a major problem in West Bengal, affecting 42 blocks in 7 districts (Purulia, Bankura, Birbhum, South 24 Parganas, Malda, Uttar Dinajpur, Dakshin Dinajpur) having more than 1.5 mg/L concentration of fluoride [13].

*Arthrospira platensis* (commonly called Spirulina), an ubiquitous organism (blue- green algae), is rich with protein (60-70%) containing a considerable number of essential amino acids, essential fatty acids (γ-linolenic acid), vitamins (Vitamin B complex, Vitamin C, Vitamin E), Minerals (Fe, Mg, Zn, Se) and photosynthetic pigments (β-carotene, phycocyanin) [14-18].

The information about the effect of *A. platensis* dietary supplementation against fluoride toxicity induced alteration of growth performance, digestive enzyme activities and digestability in the fish is very limited in literature. Thus, we investigated the effect of *A. platensis* dietary supplementation in the growth performance, digestive enzyme activities and digestibility of the freshwater fish, *Cyprinus carpio* exposed to sub-lethal concentration of fluoride.

## 2. Materials and Methods

### *2.1. Experimental diets*

The source of Spirulina (*A. platensis*) was the ‘Spirulina capsules’, manufactured by the Surya Herbal Ltd, India. The proximate composition of the Spirulina powder was analyzed before incorporation in the diet [19]. The maximum tolerable limit of Spirulina (*A. platensis*) to common carp (*C. carpio*) fingerlings was determined for a period of 14 days following Organization for Economic Cooperation and Development (OECD) guideline, and the value was 50 g/kg (5%). From this range of tolerance, Spirulina was incorporated at 0, 2.5 g/kg, 5.0 g/kg, 7.5 g/kg and 10 g/kg level to prepare control or basal diet, 0.25%, 0.50%, 0.75%, and 1% Spirulina diet respectively (Table 1) which were analysed by standard methods [19].

**Table 1:** Proximate composition of Spirulina diets (g/kg). Values are in mean ± SE, (n=3 per sample)

| Ingredients (g/Kg) | Control or basal diet (g/kg) | 0.25%Spirulina diet (2.5 g/kg) | 0.50% Spirulina diet (5 g/kg) | 0.75%Spirulina diet (7.5 g /kg) | 1% Spirulina diet (10 g /kg) |
|---|---|---|---|---|---|
| GNO Cake[1] | 600 | 600 | 600 | 600 | 600 |
| Fish Meal[2] | 200 | 200 | 200 | 200 | 200 |
| Rice Bran[3] | 100 | 100 | 100 | 100 | 100 |
| Vit. & Min. Premix | 10 | 10 | 10 | 10 | 10 |
| Wheat Flour | 90 | 87.5 | 85 | 82.5 | 80 |
| Spirulina (*A. platensis*)[4] | - | 2.5 | 5 | 7.5 | 10 |
| Proximate composition | | | | | |
| Crude Protein | 40.673±0.014 | 40.300±0.028 | 40.190±0.037 | 40.546±0.050 | 40.266±0.012 |
| Crude fat | 8.656±0.017 | 8.446±0.014 | 8.456±0.017 | 8.203±0.014 | 8.100±0.017 |
| Fiber | 6.140±0.036 | 6.420±0.017 | 6.206±0.023 | 6.306±0.034 | 5.793±0.021 |
| Ash | 8.093±0.023 | 8.244±0.031 | 8.130±0.011 | 8.243±0.034 | 8.430±0.011 |
| Moisture | 11.546±0.024 | 11.250±0.028 | 11.803±0.014 | 11.506±0.040 | 10.430±0.005 |
| NFE[5] | 24.856±0.014 | 25.123±0.039 | 25.220±0.011 | 25.626±0.040 | 26.970±0.011 |
| GE (kcal/100 g feed)[6] | 372.592 | 369.977 | 369.959 | 370.891 | 374.144 |

*Composition of vitamin & mineral mixture(premix): Each 1 kg contains Vitamin A 8,00,000 IU, Vitamin $D_3$ 80,000 IU, Vitamin E 0.6g, Nicotinamide 1.2 g, Cobalt 2.2g, Copper 4.7g, Iodine 0.6g, Iron 2.2g, Magnesium 6.5g, Manganese 3.3g, Potassium 0.2g, Sodium 0.04g, and Zinc 10g; [1] Ground nut oil (GNO) cake contains 55.43% proteins and 14.45% fat; [2] Contains 51.65% proteins and 7.6% fat.; [3] Contains 9.25% proteins and 8.3% fat; [4] Contains 60% proteins; [5]NFE (Nitrogen Free Extract)=100-(Protein+Fat+Ash+Crude fiber); [6]GE (Gross Energy): Estimated according to NRC (1993)[32] as 4.64, 9.44 and 4.11 Kcal/g for protein, fat and carbohydrate respectively.*

## 2.2. *Experimental Design*

The experimental study was conducted as per the internationally accepted laboratory animal use and care, and guidelines (guiding principles in the use of animals in toxicology, adopted by the Society of Toxicology in 1989), and as per the guidelines of the Institutional Animal Ethics Committee, University of Calcutta, West Bengal, India. In this experiment 10% of the 96 h $LC_{50}$ of fluoride (i.e. 67.5 mg/L of NaF) was used as the sub-lethal dose. Common carp fingerlings were purchased from a local fish market and then brought to the laboratory in plastic bag with sufficient oxygen. The collected fingerling of common carp was stocked in a large glass aquarium and acclimated for a total period of one month. During this period of acclimation, fingerlings of common carp were fed commercial diet at 3% of body weight daily and the water was continuously aerated. A total of 210 acclimated common carp fingerlings of three-month-old common carp were randomly allocated into 7 experimental groups (T1, T2, T3, T4, T5, T6, and T7) each with 30 fish divided into three replicate (10 fish/ replicate). The T1 was the control group fed on control diet reared in normal water, T2 was the toxic control or negative control group fed on control diet and exposed to 10% of $LC_{50}$ of fluoride, T3 was served as plant control or positive control fed on Spirulina(1%) diet and reared in normal tap water, T4 to T7 were the treatment groups fed on Spirulina dietary supplementation at 0.25%, 0.50%, 0.75% and 1% respectively and exposed to 10% of the $LC_{50}$ of fluoride. The experiment or each trial was continued for a total period of 30 days. Water was aerated continuously with the replacement of water in every alternate day. Fishes were provided feed @ 3% of their body weight at 9.00 hrs daily. Standard method [20] was followed to estimate the physico-chemical parameters of water namely dissolve oxygen, free $CO_2$, total alkalinity, total hardness, water temperature, pH, fluoride (Table 2).

**Table 2:** Physico-chemical parameters of the water.Values are means ± SEM, n = 3 per treatment group).

| Water quality parameters | Values |
|---|---|
| Dissolve Oxygen | 4.146±0.260 mg/L |
| Free Carbon dioxide | 4.633±0.120 mg/L |
| Total Alkalinity | 192.333±4.333 mg/L |
| Total Hardness | 125±1.154 mg/L |
| Water Temperature | 30.333±0.440$^{o}$C |
| p*H* | 7.466±0.145 |
| Fluoride | 0.813±0.008 mg/L |

### *2.3. Growth Performance*

Growth Performance of fish: The growth performance of common carp and mosquitofish was calculated as per the following formulae [21].

- *IBW:* Initial body weight
- *FBW:* Final body weight
- *FI:* Feed Intake
- *Weight gain:* Final weight- Initial weight
- *Wt. gain % (Weight gain %)* = (Final body wt. - Initial body wt.) ∕ Initial body wt.) × 100
- *SGR%/day (Specific Growth Rate)* = 100 × (ln final weight-ln initial weight) ∕ days
- *FCR (Feed Conversion Ratio)* = Consumed Feed ∕ Final Weight - Initial weight)
- *PER (Protein Efficiency Ratio)* = Weight Gain ∕ Protein intake

- *HSI (Hepatosomatic Index)* = Liver weight(g)/Body weight(g) x100
- *VSI (Viscerasomatic Index)* =Viscera weight/Body weight x100
- *Survival rate %*= Total number of fish survived/Total number of fish stocked x 100

### 2.4. *Digestibility*

The chromium content of the feces was determined as per the [20]. The fecal matter sample was digested using nitric acid and perchloric acid (2:1), and then made the volume 100 mL. The O.D. value was recorded using atomic absorption spectrophotometer and the chromium content was determined using the standard curve of chromium.

Apparent Dry Matter Digestibility (ADMD) and the Apparent Nutrient Digestibility (ADN) i.e. protein and fat digestibility were calculated following [21].

Apparent Dry Matter Digestibility (ADMD) = 100 - [100(Cr in feed/Cr in feces)]

Apparent Nutrient Digestibility (AND) = 100 - [100(Cr in feed/Cr in feces) × (N in feces/N in feed)]

### 2.5. *Digestive enzyme activities*

The crude digestive enzyme extract was prepared from the intestinal tissues of fishes by homogenization using ice cold distilled water (1:10 w/v) and centrifugation at 15,000 rpm for 30 minutes at 0°C. The supernatant was used for the determination of three digestive enzymes like amylase [22, protease 23, and lipase 24]. Amylase was determined as per the method of [22]. The reaction mixture containing 1 mL of substrate (1% starch), 1 mL of 0.1 M phosphate buffer (pH 7), 1 mL of NaCl and 1 mL of crude enzyme extract in a test tube, and incubate at 37°C for 1 h. After 1 h, the reaction was stopped by adding 0.5 mL of 3,5 dinitrosalicylic acid solution, and absorbance was recorded at 540 nm in a spectrophotometer.  The protein contents of extract were estimated as per Lowry et al. (1951). The specific amylase activity was expressed as mg of maltose released/mg of protein/h. Intestinal protease was determined as per the method of Kunitz (1947). The reaction mixture containing 1 mL of substrate (1% BSA), 1 mL of 0.1 M phosphate buffer (pH 7.6), 1 mL of $CaCl_2$ and 1 mL of crude enzyme extract in a test tube, and incubate at 37°C for 1 h. After 1 h, the reaction was stopped by adding 3 mL of 5% TCA solution. The reaction mixture was then centrifuged at 3000 rpm for 10 min and absorbance was recorded at 660 nm using three blank like buffer only, buffer plus substrate and buffer plus enzyme in a spectrophotometer. The protein content of the extract was estimated as per Lowry et al. (1951). The specific protease activities was expressed as µg of tyrosine/mg of protein/h.

The lipase was estimated as per the method of [24]. Two test tubes marked as 'test' and 'blank' were taken. The reaction mixture of the 'test' contains 2.5 mL water, 10 mL olive oil emulsion and 1 mL buffer (tris aminomethane) and 1 mL of enzyme extract. Whereas, 'blank' contains all the above ingredients except enzyme extract. After the incubation period of 3 hrs, reaction was stopped using in both the test tube by adding 3 mL of 95% ethanol. 1 mL of enzyme extract was also added in 'blank' after adding ethanol. Titration with 0.05 N NaOH was used until the appearance of blue colour. The difference of NaOH (0.050 N) was used to calculate the lipase activity and expressed as unit/mg protein/hr.

### *2.6. Statistical analysis*

All the values were expressed as Mean ± SE. One way Analysis of Variance (ANOVA) followed by Tukey's post hoc test was conducted to compare the means between experimental groups using software's [25, SPSS vesion 20]. The Pearson correlation was performed using Statistical Package for Social Science (SPSS) vesion 20.

## 3. Results

After the end of the feeding trial growth performance, digestibility and digestive enzyme activities of fish were estimated. The selected growth performance were weight gain, feed intake, weight gain percentage, specific growth rate, feed conversion ratio, protein efficiency ratio, hepatosomatic index, viscerosomatic index and survivality. The dry mater, protein and lipid digestibility were also measured. In the digestive enzyme activities, amylase, protease and lipase activities were also estimated.

### *3.1. Growth Performance*

The final body weight was recorded highest in T3(14.8 g) followed significantly ($p<0.05$) in T1(13.3 g) and T7(13.3 g), T6(12.2 g) and others. The lowest final body weight was recorded in T2 (10.2 g) (Table 3).

The highest weight gain was found in T3(5.48 g) followed significantly($p<0.05$) in T1(3.82 g), T7(3.65 g) and other. The lowest value of weight gain was observed in T2(0.9 g) (Table 3).

The highest feed intake was recorded in T3(6.51 g) followed non-significantly by T1(6.16 g) and significantly($p<0.05$) by T7(5.33 g) and others. The lowest value of feed intake was found in T2(2.03 g) (Table 2).

**Table 3:** Effect of Spirulina on growth performance of common carp exposed to sub-lethal concentration of fluoride. Values are in mean ± SE, (n=3 per sample)

| | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
|---|---|---|---|---|---|---|---|
| IBW(g) | 9.48 ± 0.17 | 9.25 ± 0.11 | 9.66 ± 0.07 | 9.37 ± 0.10 | 9.68 ± 0.09 | 9.51 ± 0.28 | 9.65 ± 0.06 |
| FBW(g) | $13.3 \pm 0.26^{b}$ | $10.2 \pm 0.17^{d}$ | $14.8 \pm 0.41^{a}$ | $11 \pm 0.18^{cd}$ | $11.8 \pm 0.16^{c}$ | $12.3 \pm 0.40^{bc}$ | $13.3 \pm 0.12^{b}$ |
| WG(g) | $3.82 \pm 0.09^{b}$ | $0.9 \pm 0.06^{e}$ | $5.48 \pm 0.17^{a}$ | $1.64 \pm 0.07^{d}$ | $2.13 \pm 0.07^{d}$ | $2.79 \pm 0.12^{c}$ | $3.65 \pm 0.05^{b}$ |
| FI(g) | $6.16 \pm 0.18^{a}$ | $2.03 \pm 0.04^{e}$ | $6.51 \pm 0.12^{a}$ | $3.12 \pm 0.01^{d}$ | $3.83 \pm 0.14^{c}$ | $4.3 \pm 0.15^{c}$ | $5.33 \pm 0.04^{b}$ |

*Values in the same row with different superscript letters are significantly different (p < .05).*

Thus, the exposure of sub-lethal concentration of fluoride decreased the weight gain and feed intake in T2 but Spirulina dietary supplementation at 1% increased the weight gain and feed intake in T3. In the treatment groups, the values of weight gain and feed intake were increased from T4 to T7.

### *3.2. Nutritional Efficiency*

The weight gain percentage was found highest in T3 (56.7) followed significantly (p<0.05) by T1(40.3), T7(37.8), T6(29.3) and others. The lowest value of weight gain % was recorded in T2 (9.71) (Table 4).

The specific growth rate was found highest T3(1.42) followed significantly(p<0.05) by T1(1.13), T7(1.07) and others. The lowest value of specific growth rate was recorded in T2(0.39)(Table 4).

The lowest value of feed conversion ratio was observed in T3 (1.19) followed significantly(p<0.05) by T7(1.45), T6(1.54), T1(1.61) and others. The highest value of feed conversion ratio was obtained in T2 (2.27) (Table 4).

The protein efficiency ratio was highest in T3(2.1) followed significantly(p<0.05) by T7(1.71), T6(1.62), T1(1.55) and others. The lowest value of protein efficiency ratio was recorded in T2 (1.1) (Table 4).

**Table 4:** Effect of Spirulina on nutritional efficiencies of common carp exposed to sub-lethal concentration of fluoride. Values are in mean±SE, (n=3 per sample)

| | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
|---|---|---|---|---|---|---|---|
| WG% | $40.3 \pm 0.57^{b}$ | $9.71 \pm 0.63^{f}$ | $56.7 \pm 1.38^{a}$ | $17.5 \pm 0.64^{e}$ | $22 \pm 0.54^{d}$ | $29.3 \pm 0.49^{c}$ | $37.8 \pm 0.35^{b}$ |
| SGR%/day | $1.13 \pm 0.01^{b}$ | $0.387 \pm 0.01^{e}$ | $1.42 \pm 0.06^{a}$ | $0.53 \pm 0.01^{d}$ | $0.65 \pm 0.01^{d}$ | $0.863 \pm 0.02^{c}$ | $1.07 \pm 0.01^{b}$ |
| FCR | $1.61 \pm 0.01^{cd}$ | $2.27 \pm 0.13^{a}$ | $1.19 \pm 0.01^{e}$ | $1.91 \pm 0.08^{b}$ | $1.79 \pm 0.01^{bc}$ | $1.54 \pm 0.01^{cd}$ | $1.45 \pm 0^{de}$ |
| PER | $1.55 \pm 0.01^{bc}$ | $1.1 \pm 0.06^{e}$ | $2.1 \pm 0.02^{a}$ | $1.31 \pm 0.05^{d}$ | $1.39 \pm 0.01^{cd}$ | $1.62 \pm 0.01^{b}$ | $1.71 \pm 0.01^{b}$ |

*Values in the same row with different superscript letters are significantly different (p < .05).*

Thus, the exposure of sub-lethal concentration of fluoride decreased the weight gain percentage, specific growth rate and protein efficiency ratio, and increased the feed conversion ratio in T2. Spirulina dietary supplementation at 1% increased the weight gain percentage, specific growth rate and protein efficiency ratio, and decreased the feed converstion ratio in T3. In dietary supplementation groups, weight gain percentage, specific growth rate and protein efficiency ratio were increased from T4 to T7 with highest value in T7, and feed conversion ratio was decreased from T4 to T7 with lowest value in T7.

### *3.3. Hepatosomatic index and viscerosomatic index:*

There was no significant difference of hepatosomatic index (HSI) and viscerosomatic index (VSI) between the experimental groups. The survivality of fingerlings was significantly ($p<0.05$) improved in all Spirulina dietary supplementation groups compared to the toxic control (T2) (Table 5).

**Table 5:** Effect of Spirulina on growth indices of common carp exposed to sub-lethal concentration of fluoride. Values are in mean±SE, (n=3 per sample)

| | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
|---|---|---|---|---|---|---|---|
| HSI | 0.763±0.0176 | 0.737±0.00882 | 0.787±0.00333 | 0.75 ±0.0252 | 0.76±0.00577 | 0.75±0.00577 | 0.78±0.00577 |
| VSI | 33.9 ± 26.3 | 8.21 ± 0.366 | 7.99 ± 0.0922 | 7.99 ± 0.435 | 7.8 ± 0.373 | 7.86 ± 0.43 | 7.67 ± 0.124 |
| Survivality | 98 ± 1.15$^{a}$ | 81 ± 0.577$^{d}$ | 98.3 ± 0.882$^{a}$ | 82.3±0.882$^{cd}$ | 86.3 ± 0.882$^{c}$ | 91 ± 0.577$^{b}$ | 96.7 ± 0.882$^{a}$ |

*Values in the same row with different superscript letters are significantly different (p < .05).*

### *3.4. Digestibility*

The results of the present study revealed that there was no significant difference of dry matter digestibility between the experimental groups (Table 6).

The protein digestibility was highest in T3(87.9) followed significantly($p<0.05$) by T1(83.8), T7(81.8), T6(79.7) and others. The lowest value of protein digestibility was found in T2(70.3)(Table 6).

The highest value of lipid digestibility was recorded in T3(95.5) followed significantly($p<0.05$) by T1(92.4), T7(88.1) and others. The lowest value of lipid digestibility was observed in T2(77.7)( Table 6).

**Table 6:** Effect of Spirulina on digestibility of common carp exposed to sub-lethal concentration of fluoride. Values are in mean±SE, (n=3 per sample)

| | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
|---|---|---|---|---|---|---|---|
| Dry matter | 58.3 ± 0.333 | 56.3 ± 0.333 | 59.3 ± 0.333 | 58.1 ± 0.41 | 57 ± 1.53 | 57.3 ± 0.586 | 57.1 ± 1.23 |
| Protein | 83.8 ± 0.441$^{b}$ | 70.3 ± 0.333$^{g}$ | 87.9 ± 0.233$^{a}$ | 74.2 ± 0.601$^{f}$ | 77.5 ± 0.289$^{e}$ | 79.7 ± 0.333$^{d}$ | 81.8 ± 0.167$^{c}$ |
| Lipid | 92.4 ± 0.296$^{b}$ | 77.7 ± 0.333$^{f}$ | 95.5 ± 0.5$^{a}$ | 80.8 ± 0.167$^{e}$ | 82.3 ± 0.333$^{e}$ | 85 ± 0.289$^{d}$ | 88.1 ± 0.353$^{c}$ |

*Values in the same row with different superscript letters are significantly different (p < .05).*

Thus, sub-lethal concentration of fluoride reduced the nutrient digestibility in T2, and Spirulina at 1% increased the nutrient digestibility in T3. The nutrient digestibility was increased from T4 to T7 with highest value in T7.

### *3.5. Digestive enzyme activity*

The amylase activity was found highest in T3(0.77 mg maltose/mg protein/hr) followed significantly($p<0.05$) by T1(0.54 mg maltose/mg protein/hr), T7(0.47 mg maltose/mg protein/hr), T6(0.37 mg maltose/mg protein/hr) and others. The lowest activity of amylase was found in T2(0.14 mg maltose/mg protein/hr) (Table 7).

The highest protease activity was recorded in T3 (0.8 µg tyrosine/mg protein/hr) followed significantly ($p<0.05$) by T1(0.58 µg tyrosine/mg protein/hr), T7(0.39 µg tyrosine/mg protein/hr) and others. The lowest protease activity was found in T2(0.03 µg tyrosine/mg protein/hr)(Table 7).

The maximum lipase activity was observed in T3(1.02 unit/mg protein/hr) followed significantly($p<0.05$) by T1(0.83 unit/mg protein/hr), T7(0.69 unit/mg protein/hr). The minimum value of the lipase activity was found in T2(0.12 unit/mg protein/hr)(Table 7).

**Table 7:** Effect of Spirulina on digestive enzyme activities of common carp exposed to sub-lethal concentration of fluoride. Values are in mean±SE, (n=3 per sample)

| | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
|---|---|---|---|---|---|---|---|
| Amylase (mg maltose/mg protein/hr) | 0.54 ± 0$^{b}$ | 0.14 ± 0.01$^{f}$ | 0.77 ± 0.02$^{a}$ | 0.18 ± 0$^{f}$ | 0.28± 0$^{e}$ | 0.36 ± 0$^{d}$ | 0.46 ± 0$^{c}$ |
| Protease (µg tyrosine/mg protein/hr) | 0.58 ± 0.01$^{b}$ | 0.03± 0$^{g}$ | 0.8 ± 0.01$^{a}$ | 0.10 ± 0$^{f}$ | 0.18 ± 0$^{e}$ | 0.26 ± 0$^{d}$ | 0.38 ± 0$^{c}$ |
| Lipase (unit/mg protein/hr) | 0.83 ± 0$^{b}$ | 0.12 ± 0$^{g}$ | 1.02 ± 0.02$^{a}$ | 0.23 ± 0.01$^{f}$ | 0.40 ± 0$^{e}$ | 0.56 ± 0$^{d}$ | 0.68 ± 0$^{c}$ |

*Values in the same row with different superscript letters are significantly different (p < .05).*

The digestive enzyme activities were highest in T3 and lowest in T2. All the digestive enzyme activities were increased from T4 to T7 with highest value in T7.

There was a significant ($p<0.01$) positive correlation between weight gain and feed intake(r=0.964), weight gain and weight gain% (r=0.999), weight gain and SGR (r=0.990), weight gain and PER (r=0.955), weight gain and protein digestibility(r=0.981), weight gain and lipid digestibility(r=0.972), weight gain and amylase activity(r=0.988), weight gain and protease activity(r=0.970), weight gain and lipase activity(r=0.980), protein digestibility and protease activity(r=0.953), and lipid digestibility and lipase activity(r=0.985). However, a significantly ($p<0.01$) negative correlation was found between weight gain and feed conversion ratio (r= - 0.918) (Table 8).

**Table 8:** Correlations between the growth performance, and the digestibility and digestive enzyme activities of common carp fed on Spirulina diet and exposed to sub-lethal concentration of fluoride.

| | weight Gain | Feed Intake | WG% | SGR | FCR | PER | Protein Digestibility | Lipid Digestibility | Amylase | Protease | Lipase |
|---|---|---|---|---|---|---|---|---|---|---|---|
| weight Gain | 1 | | | | | | | | | | |
| Feed Intake | .964** | 1 | | | | | | | | | |
| WG% | .999** | .964** | 1 | | | | | | | | |
| SGR | .990** | .972** | .991** | 1 | | | | | | | |
| FCR | -.918** | -.882** | -.917** | -.911** | 1 | | | | | | |
| PER | .955** | .870** | .953** | .936** | -.969** | 1 | | | | | |
| Protein Digestibility | .981** | .981** | .982** | .980** | -.926** | .928** | 1 | | | | |
| Lipid Digestibility | .972** | .976** | .977** | .972** | -.857** | .880** | .974** | 1 | | | |
| Amylase | .988** | .947** | .989** | .975** | -.871** | .926** | .968** | .977** | 1 | | |
| Protease | .970** | .945** | .975** | .963** | -.831** | .883** | .953** | .986** | .984** | 1 | |
| Lipase | .980** | .981** | .984** | .990** | -.897** | .911** | .988** | .985** | .978** | .972** | 1 |

**. Correlation is significant at the 0.01 level (2-tailed).

Sub-lethal concentration of fluoride significantly ($p<0.05$) decreased the growth performance, digestibility and digestive enzyme activities in T2 compared to T1 in common carp. However, dietary supplementation by Spirulina significantly ($p<0.05$) increased the growth performance, digestibility and digestive enzyme activities in dietary supplementation groups (T4 to T7) compared to T2. Among the treatment groups highest value was achieved in T7.

## 4. Discussion

Fluoride adversely affected the growth and feed utilization parameters in fish by reducing the feed intake, weight gain, weight gain percentage, specific growth rate and protein efficiency ratio, and by increasing the feed conversion ratio in group fed on control diet and exposed to sub-lethal concentration of fluoride, compared to fish fed on control diet and reared in normal surface water. The result of the present study is in consistent with several previous studies in which sub-lethal concentration of fluoride negatively affected the growth performance in fish [26,27,28]. The mechanism through which fluoride inhibits the growth performance in fish are anorexia due to reduction of feed intake [26], higher accumulation in bones [27], and inhibition of enzymatic activity [28, 29].

Dietary supplementation by Spirulina improved the fluoride toxicity induced alteration of growth performance by increasing the weight gain, feed intake, weight gain%, specific growth rate, protein efficiency ratio and decreasing the feed conversion ratio compared to fish fed on control diet and exposed to sub-lethal concentration of fluoride. Fish fed on Spirulina dietary supplementation at 1% and exposed to sub-lethal concentration of fluoride achieved the highest growth among the different doses of Spirulina supplementations. Spirulina dietary supplementation highly enhanced the weight gain and feed intake in common carp fingerlings However, the feed conversion ratio was reduced in common carp fed on 1% Spirulina supplemented diet and exposed to sub-lethal concentration of fluoride compared to fish fed on control diet and exposed to sub-lethal concentration of fluoride. The chlorophyll content of Spirulina (*A. platensis*) has the ability to alleviate different toxic substances [30], thus improved the growth performance of fish.

The result of the present study is in conformity with the study of [31] in which dietary Spirulina improves the copper toxicity induced alteration of growth parameters in carp. Spirulina (*A. platensis*) is rich with protein (60-70%) containing essential amino acids which reduced fluoride bio availability, and thus ameliorates fluoride toxicity [32,33]. The chlorophyll content of Spirulina (*A. platensis*) has the ability to alleviate different toxic substances [30]. It is well established that diet rich with ascorbic acid (vitamin C) and calcium (Ca) can reduce the fluoride absorption [34,35,36,37]. The ascorbic acid as well as calcium rich nature of Spirulina (*A. platensis*) decreased the absorption of fluoride, thus reduced the accumulation of fluoride in the body of fish, results in the amelioration of fluoride toxicity.

The exposure of sub-lethal concentration of fluoride caused the inhibition of all the three digestive enzyme activities in common carp. The result of the present study is in consistent of some previous studies in which fluoride adversely affected the digestive enzyme activities in animals [38,39]. Fluoride inhibits the digestive enzyme activities through the production of free radicals [40, 41].

However, Spirulina (*A. platensis*) dietary supplementation improved the digestive enzyme activities in group fed on dietary Spirulina at 1% and reared in normal water compared to the fish fed on control diet and reared in normal water which in turn might have resulted in the efficient absorption of nutrients into the blood, and improved the growth performance of fish. The result of the present study is in consistent with several previous studies in which Spirulina dietary supplementation stimulates the intestinal digestive enzyme activities in *C. carpio* [42,43] and Oskar fish [44]. Similarly, other plant based dietary supplements like soybean meal improves the digestive enzyme activity in catla [45] and common carp [46, 47]. Similarly, azolla dietary supplementation improves the digestive enzyme activities in *Labeo fimbriatus*, and in common carp [47].

Dietary supplementation by Spirulina highly improved the fluoride toxicity induced alteration of digestive enzyme activities in all dietary supplementation groups, with 1% Spirulina showed the best response. The antioxidant rich Spirulina (phycocyanin, β carotene) dietary supplementation possibly improved the fluoride induced alteration of digestive enzyme activities by ameliorating the free radical generated due to exposure of fluoride, thus increasing the digestive enzyme activities.

In the present study, sub-lehtal concentration of fluoride reduced the protein and lipid digestibility at in common carp. Tao et al. (2005) [48] also reported the inhibitory activity of fluoride on the nutrient digestibility in growing pigs. The fluoride alters the structure and function of intestine through formation of highly damaging hydrogen fluoride [49, 50, 51, 52]. It also reduces the translation in the intestinal tissue by adversely affecting the protein metabolism resulting in the alteration of digestibility in animal [53]. However, dietary supplementation by Spirulina improved the fluoride induced alteration of digestibility in both the fish, with 1% Spirulina supplementation showed the highest value.

Thus, the antioxidant rich Spirulina dietary supplementation improved the fluoride toxicity induced alteration of growth performance by ameliorating the free radical generated due to exposure of fluoride, thus increasing the digestive enzyme activities which in turn improved the digestibility. The increased digestibility was responsible for the increase of feed efficiency results in the reduction of feed conversion ratio and improvement of growth performance of fish. In addition, nutrient rich nature of Spirulina reduced the accumulation of fluoride in the body, and at the same time positively affect the overall metabolism results in the higher growth performance in fish. Spirulina dietary supplementation at 1 % showed the best response hence considered as the optimum dose.

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