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## Use of Freshwater Aquatic Plants as a Substitute of Fishmeal in the Diet of *Labeo rohita* Fry

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**Abstract:** Azolla Protein Concentrate (APC) mixed with dry Spirogyra Powder (SP) at 4:1 ratio was evaluated in the diet of *Labeo rohita* fry as a substitute of fish meal. One hundred and sixty fry (avg. wt.  $1.3 \pm 0.2$  g) were distributed in five experimental groups with each of four replicates and fed for 60 days. Five isonitrogenous and isocaloric diets were prepared by replacing fish meal with APC-SP mixture on equal protein basis viz., T<sub>1</sub> (0%); T<sub>2</sub> (25%); T<sub>3</sub> (50%); T<sub>4</sub> (75%) and T<sub>5</sub> (100%). Crude protein content of the APC increased to 44.96% than the dry azolla containing 24.06% CP. Growth and nutrient utilization of the *Labeo rohita* fry were evaluated in terms of percent weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, net protein utilization, protease activity, *in vitro* protein and carbohydrate digestibility. Highest percent weight gain (135.57), SGR (1.45%), protease activity (18.56 units mg protein<sup>-1</sup>), protein digestibility (93.16%) carbohydrate digestibility (35.08%), FCE (0.75), PER (1.9), NPU (28.90) was recorded in control (0% APC-SP), which was similar with T<sub>2</sub> and T<sub>3</sub> groups but decreased significantly ( $p < 0.05$ ) as the APC-SP content increased in the diet. There were no histological changes except the vacuolation of hepatic cells with increased concentration of APC-SP in the diet. No mortality was registered in any of the experimental groups signifying no adverse effect of feeding APC-SP. It concludes that APC is a good source of protein and can be used to the maximum extent of 16.25% by replacing 10% fish meal in the diet of *Labeo rohita* fry.

**Key words:** Azolla protein concentrate, spirogyra, fish meal, growth, digestibility, *Labeo rohita*

### Introduction

Aquaculture has been considered as answer to the shortfall from the global capture fisheries production. The intensification of aquaculture has led to a dependence on artificial feed ingredients especially on fishmeal due to its high quality protein and presence of some unidentified growth factors. Increased demand of this noble ingredient concomitant with its static production has put extra pressure on the animal or fish nutritionist to search for the alternate protein sources. Hardy (2000) predicted that the fishmeal requirement during the year 2010 would be 2.83 mmt, which is 44% of the total global fishmeal production. This is somewhat 716000 mt over estimate of fishmeal use in 2000. If fishmeal production remains same as of today, the demand in aquaculture sector may go upto 63% of the total global fishmeal production. Hence, alternate protein sources need to be explored to overcome this problem.

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Lot of works have been done to include many of the unconventional protein sources to be used in animal or fish feed, but use of aquatic plant has given little emphasis. In this connection aquatic weeds or plant fodder, rich in protein may be considered as the alternative protein sources in the aqua-feed as suggested by Chiayvareesajja *et al.* (1989) Ray and Das (1995). Aquatic weeds have been used as complete or partial replacement of the fishmeal. These include *lemna*, *eicchornia*, *azolla*, *najas* etc. in different forms such as meal or protein concentrate.

*Azolla* is a heterosporous free-floating aquatic fern that lives symbiotically with the nitrogen fixing algae, *Anabena azollae* (Peters *et al.*, 1979). It has attracted the attention of many livestock, poultry and fish farmers due to its high protein content (19-31%) and a favourable essential amino acid composition for animal nutrition (Buu, 1971; Antoine *et al.*, 1987; Santiago *et al.*, 1988). The growth rate of azolla is very rapid and it becomes double the weight in 2-3 days. It has been used as fresh feed in cage culture (Cagaun and Nerona, 1986) in integrated rice-azolla-fish (FAO, 1988).

Algae have been identified as an alternate protein source in the fish diet, particularly in the tropical developing countries, where algal production rates are high (Venkataraman *et al.*, 1980). The most commonly mass cultured algae evaluated, as protein sources in the fish feed, are unicellular micro algae *chlorella*, *Scenedesmus* and *Spirulina* (Terao, 1960; Stanley and Jones, 1976; Sandbank and Hopher, 1978). However, from practical point of view, these are costly to produce and harvest. Hence, it appears logical to evaluate other types of algae, which grows extensively under natural conditions and incurs little or no production cost.

The filamentous green alga, *Spirogyra maxima* grow extensively in freshwater ponds, shallow ditches and in inundated areas. It is frequently a problem in nursery ponds trapping the fries, impediment to boating, when in bloom and on decay it produces offensive odour together with dissolved oxygen depletion resulting in fish kills.

Though azolla has been used in various forms such as fresh (Almazan *et al.*, 1986), sun dried (Almazan *et al.*, 1986; Santiago *et al.*, 1988; Joseph *et al.*, 1994), either singly or in combination (Antoine *et al.*, 1987; El-Sayeed, 1992; Micha *et al.*, 1988), its use as a protein concentrate has not been reported till date.

In the present study two aquatic plants were selected with specific purposes. Though the protein content of both the aquatic plant is similar, azolla was selected due to its better protein quality and spirogyra due to its abundant availability. Azolla Protein Concentrate (APC) was made for more addition of protein from azolla so as to replace fishmeal. Hence, in the present study a mixture was prepared with APC and dry spirogyra with a ratio of 4:1 and added in the feed at a graded level as a substitute of fishmeal in the diet of *Labeo rohita* fry.

## **Materials and Methods**

### *Experimental Design*

The experiment was conducted from 21st December, 1998 to 18th March, 1999 at Digestive Physiology, Nutrition and Feed laboratory of Central Institute of Fisheries Education, Versova, Mumbai. *Labeo rohita* fry were procured from Khapoli fish seed farm, Maharashtra, India and acclimatized for 15 days in a FRP circular tank (1000 L). One hundred and eighty fry of uniform size (1.3±0.2 g) were distributed in 5 treatments with each of 4 replicates. Nine fry were stocked in each tub containing 40 L water. Round the clock aeration was provided to all the tubs to maintain the dissolved oxygen level. Experimental tubs were cleaned manually by siphoning at least 50% of the water daily along with the faecal matter and left over feeds. The siphoned water was replaced by equal

volume of fresh water. Feed was given twice daily at 09.00 and 16.00 h at 5% of their body weight. Feeding rate was adjusted by daily observation of the refusal.

#### *Diet Preparation*

##### *Preparation of Azolla Protein Concentrate (APC)*

APC was prepared by modifying the method of Harendranath and Singh (1984). *Azolla caroliniana* was collected from azolla culture unit of Central Institute of Fresh water Aquaculture, Bhubaneswar, Orissa, India. It was then pulped in a domestic mixer with little addition of water to facilitate better maceration. The pulp was then hand pressed in a cloth of 5  $\mu$  mesh size to collect the juice and acid precipitated by using 2% HCl with a juice to acid ratio of 9:1. Complete precipitation of protein was done within 90 min. The supernatant was filtered off and the precipitate was dried in a hot air oven at 50°C until complete drying and used in the feed.

*Spirogyra maxima* was collected locally, washed thoroughly to remove the adhering dirt followed by drying in a hot air oven at 60°C. It was pulverized to fine powder and mixed in the feed.

#### *Experimental Diets*

Before feed formulation the proximate composition of the feed ingredients especially APC, fishmeal, spirogyra were determined (Table 1). Five semi-purified diets were prepared by gradual replacement of fishmeal with a mixture of Azolla Protein Concentrate (APC): Spirogyra Powder (SP) at a ratio of 4:1. Equal amount of protein from the replaced fishmeal was substituted with same amount of protein from APC-SP viz., T<sub>1</sub> (0% substitution, control), T<sub>2</sub> (25%), T<sub>3</sub> (50%), T<sub>4</sub> (75%), T<sub>5</sub> (100%) (Table 2). The percent inclusions of different ingredients were adjusted to make all the diets iso-proteinous (39-40%) and iso-caloric (347-387 Kcal DE/100 g). All the ingredients except the vitamin, mineral mixture were mixed thoroughly in a mix blender and the required amount of water and oil were added. Mixed ingredients were kept in an airtight polyethylene packet for one hour for proper conditioning followed by steam cooking for 20 min in a pressure cooker. Vitamin and minerals were added to the feed mix after cooling and passed through an extruder with a 2 mm die (Twin Screw Extruder, Basic Technology Private Ltd., Calcutta-12) at barrel screw speed of 430 rpm, feeding rate of 90 rpm, barrel temperature of 90°C and cutter speed of 1100 rpm. Pellets thus obtained were dried at 60°C in an oven and put in airtight polyethylene bag until further use.

#### *Growth Parameters*

Fishes were weighed at every 15 days to assess the growth performance in terms of percent weight gain, Specific Growth Rate (SGR), Feed Conversion Efficiency (FCE), Protein Efficiency Ratio (PER) and protein gain. Fishes were counted at the end of the experiment and compared with the initial stock to calculate survival.

#### *Water Quality Parameters*

Temperature, pH, dissolved oxygen, ammonia, free carbon dioxide and total alkalinity were measured once in every week according to standard methodology (APHA, 1985). Water temperature of different experimental groups ranged from 22.1-26.8°C. pH value in all the experimental groups varied within a range of 7.2-7.7. Dissolved oxygen of all the experimental tubs recorded was within the range of 6.15-6.97. Free carbon dioxide was not detected in any of the experimental container. Range of total alkalinity and hardness were 183-210 and 153-167 ppm, respectively. Ammonia and nitrate concentration of different container were within the range of 0.62-0.92 and 0.53-0.85 ppm, respectively.

Table 1: Proximate composition (% DM basis) of the ingredients

Ingredients	Crude protein	Total lipids	Crude fibre	NFE	Ash
Fish meal	65.10±2.31	7.30±0.42	2.50±0.07	2.90±0.05	22.20±1.20
<i>Azolla</i> protein concentrate	44.96±3.82	6.92±0.38	1.75±0.05	41.45±3.66	4.92±0.29
<i>Spirogyra</i>	24.44±1.86	14.83±0.92	5.34±0.38	32.88±2.20	22.51±1.98
Sun dried					
<i>Azolla caroliniana</i>	24.06±1.11	4.03±0.13	10.18±0.98	39.52±2.08	22.22±1.36

Table 2: Composition of the ingredients for 100 g experimental diets of the different groups

Ingredients (g)	Diet code				
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>
Fish meal	20	15	10	5	0
Caesin	22	22	22	22	22
Gelatin	8	8	8	8	8
Dextrin	25	25	25	25	25
Cellulose	15	12	8.85	5.63	2.5
Veg. oil	4	4	4	4	4
Cod liver oil	4	4	4	4	4
APC + <i>spirogyra</i> powder	0	8.0	16.25	24.37	32.5
Vit-mix*	1.75	1.75	1.75	1.75	1.75
Vit. C	0.25	0.25	0.25	0.25	0.25
<b>Proximate composition</b>					
Dry matter	92.92	93.04	93.08	92.28	92.82
Crude protein	39.11	40.37	39.77	40.82	40.28
Lipid	9.40	9.60	9.77	9.66	10.15
Crude fibre	15.5	12.9	10.3	7.2	4.3
Ash	9.82	9.94	10.13	10.23	10.35
NFE	26.17	27.19	30.03	31.79	34.92
Digestible energy (kcal/100 g)	347	358	371	377	387
P/E ratio					
(g protein/kcal DE)	11.27	11.28	10.72	10.83	10.40

\*Composition of vitamin mineral mixture of 1 kg: Vitamin A - 50,00,000 IU; Vitamin D<sub>3</sub> - 10,00,000 IU; Vitamin B<sub>2</sub> - 2.0 g; Vitamin E - 750 units; Vitamin K - 1.0 g; Calcium pantothenate 2.5 g; Nicotinamide - 10.0 g; Vitamin B<sub>12</sub> - 6.0 g; Choline Chloride - 150.0 g; Calcium - 750.0 g; Manganese - 27.5 g; Iodine - 1.0 g; Iron - 7.5 g; Zinc - 15.0 g; Copper - 2.0 g; Cobalt - 0.45 g

#### *Protease Assay*

The reaction mixture containing 100 µL Hammerstein casein (1 mg mL<sup>-1</sup>), 780 L buffer (50 mM Tris-HCL, pH 8.1 containing 2 mM CaCl<sub>2</sub>) and 20 µL of extract was incubated at 37°C.

#### *Bio-chemical Analysis of Feed and Tissues*

Feed and feed ingredients were analysed for the proximate contents viz., crude protein, ether extract, ash, crude fibre and nitrogen-free-extract content as per standard methods of AOAC (1990). Similarly, tissues were analysed at the end of the experiment using the similar methodology.

#### *Statistical Analysis*

Data were statistically processed for one-way analysis of variance (ANOVA) and significance differences between two groups were compared by Duncan's multiple range tests at 5% level of significance. All the analysis was done by SPSS software (version 11).

**Results**

*Proximate Composition of Feed, Feed Ingredients and Tissues*

Proximate composition of feed ingredients and feed are given in Table 1 and 2, respectively. Protein content of APC increased to 87% than the protein content of dry azolla. Fibre and ash content of APC was reduced to the extent of 82.33 and 77.86%, whereas, the lipid contents increased to 71%. Crude protein and lipid content of the experimental diets was within the range of 39.11-40.82 and 9.40 to 10.15%, respectively. Crude fibre content widely varied between 4.3-15.5%. Ash content was recorded in the range of 9.82%-10.35%. Soluble carbohydrate (NFE) ranged from 26.17-34.92%.

Crude protein content of different experimental groups ranged from 81.69 to 83.56%. Lipid content varied from 6.23 to 7.17%. Moisture and total ash content varied within a range of 81.87-83.78 and 8.83-10.91%, respectively (Table 3). However, difference in mean values among different groups were not significantly different ( $p > 0.05$ ).

*Growth Parameters*

Growth parameters of different experimental groups are given in Table 4. Weight of fish at 15 days interval are given in Table 5. Highest body weight gain% was recorded in control group, which were similar with T<sub>2</sub> and T<sub>3</sub> groups and lowest weight gain% in T<sub>5</sub> group. Weight gain % of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups was significantly higher ( $p < 0.05$ ) than the T<sub>4</sub> and T<sub>5</sub> groups. Similar trend was also recorded for SGR and FCR. Protein efficiency ratio of T<sub>4</sub> and T<sub>5</sub> were similar and significantly lower ( $p < 0.05$ ) than the T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups. But NPU was lowest in T<sub>5</sub> group and less than ( $p < 0.05$ ) T<sub>4</sub> group. There was no significant variation in NPU of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups.

Table 3: Biochemical composition of the tissue of fingerlings of *Labeo rohita* of different experimental groups (%DM basis)

Treatments	Moisture	Protein	Lipid	Ash
Initial	82.03	79.91	6.73	6.29
T <sub>1</sub>	81.87	83.56	7.17	8.83
T <sub>2</sub>	81.85	83.55	7.04	9.00
T <sub>3</sub>	82.09	83.53	6.76	9.38
T <sub>4</sub>	83.26	82.74	6.63	10.55
T <sub>5</sub>	83.78	81.69	6.23	10.91

Table 4: Growth and nutrient digestibility of different experimental groups<sup>1</sup>

Parameters	Experimental groups				
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>
Ratio of FM: APC-SP dietary protein	33.2:0	24.2:8.1	16.4:16.7	8.0:24.4	0:33
Initial body weight (g)	1.31±0.14	1.31±0.27	1.30±0.15	1.31±0.17	1.31±0.21
Final body wt (g)	3.13±0.24	3.10±0.51	3.06±0.33	2.67±0.23	2.44±0.27
% Weight gain <sup>m</sup>	138.57 <sup>a</sup> ±0.592	136.97 <sup>a</sup> ±1.5557	136.97 <sup>a</sup> ±1.0364	104.55 <sup>b</sup> ±0.3028	86.11 <sup>c</sup> ±2.7704
SGR <sup>a</sup>	1.45 <sup>a</sup> ±0.0041	1.44 <sup>a</sup> ±0.0109	1.44 <sup>a</sup> ±0.0073	1.23 <sup>b</sup> ±0.0345	1.04 <sup>c</sup> ±0.0244
FCR <sup>p</sup>	1.34 <sup>a</sup> ±0.0110	1.36 <sup>a</sup> ±0.0288	1.38 <sup>a</sup> ±0.0200	1.51 <sup>b</sup> ±0.0146	1.83 <sup>c</sup> ±0.0513
PER <sup>q</sup>	1.91 <sup>a</sup> ±0.0156	1.88 <sup>a</sup> ±0.0385	1.80 <sup>a</sup> ±0.0262	1.41 <sup>b</sup> ±0.0362	1.15 <sup>b</sup> ±0.0317
NPU <sup>r</sup>	28.90 <sup>a</sup> ±0.2365	27.49 <sup>a</sup> ±0.5813	27.31 <sup>a</sup> ±0.3988	22.88 <sup>b</sup> ±0.5855	18.01 <sup>c</sup> ±0.4984
Survival	100	100	100	100	100
Mean specific protease activity	18.56 <sup>a</sup> ±0.249	18.16 <sup>a</sup> ±0.322	17.40 <sup>a</sup> ±0.277	15.03 <sup>b</sup> ±0.193	11.99 <sup>c</sup> ±0.299
Protein digestibility (%)	93.16 <sup>a</sup> ±0.3873	93.13 <sup>a</sup> ±0.0886	92.82 <sup>a</sup> ±0.2199	86.99 <sup>b</sup> ±0.1897	85.15 <sup>b</sup> ±0.2264
Carbohydrate digestibility (%)	35.08 <sup>a</sup> ±0.099	34.85 <sup>a</sup> ±0.205	34.76 <sup>a</sup> ±0.285	32.95 <sup>b</sup> ±0.207	31.98 <sup>c</sup> ±0.114

<sup>1</sup>-Means of 3 replicates ±SEM. Means in the same row sharing same superscripts are not significantly different ( $p > 0.05$ )  
<sup>m</sup> = 100(Final body wt (g) - Initial body wt (g))/Initial body wt (g), n = 100(Log, Final body wt - log, initial body wt)/Experimental duration (60 days), p = Feed intake (g)/Weight gain (g), q = Weight gain (g)/Protein intake (g), r = protein retention/ protein fed

Table 5: Body weight (g) of different experimental groups at 15 days interval during experimental period

Groups	Initial	15 days	30 days	45 days	60 day
T <sub>1</sub>	1.31±0.14	2.12±0.14	2.21±0.24	2.64±0.23	3.13±0.24
T <sub>2</sub>	1.31±0.27	2.48±0.30	2.59±0.39	2.75±0.38	3.10±0.51
T <sub>3</sub>	1.30±0.15	2.41±0.18	2.54±0.17	2.74±0.19	3.06±0.33
T <sub>4</sub>	1.31±0.17	2.06±0.16	2.16±0.24	2.55±0.28	2.67±0.23
T <sub>5</sub>	1.31±0.21	2.11±0.50	2.15±0.47	2.28±0.28	2.44±0.27

#### *Enzyme Activity and In vitro Digestibility*

Specific protease activity of the different experimental groups are given in Table 4. Higher protease activity was recorded in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups, while the lowest in T<sub>5</sub> group. Protease activity of T<sub>4</sub> and T<sub>5</sub> group was significantly lower ( $p < 0.05$ ) than the other groups. However, no significant difference was found among T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups.

Carbohydrate digestibility of T<sub>4</sub> and T<sub>5</sub> groups were significantly lower ( $p < 0.05$ ) than the T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups. However, there was no difference between T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups. Protein digestibility of T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> groups were similar ( $p > 0.05$ ) except T<sub>5</sub> groups. However, there was no significant difference between the protein digestibility of T<sub>4</sub> and T<sub>5</sub> groups.

#### *Histology of Hepatic Tissue*

There were absolutely no histological changes in the hepatic cells of the control and T<sub>2</sub> groups. Cells were moderately hypertrophy in the T<sub>3</sub> and T<sub>4</sub> groups, whereas maximum hypertrophy was seen in T<sub>5</sub> groups.

### **Discussion**

#### *Proximate Composition of APC*

Increase in protein content of APC as compared to sun-dried azolla is because of extraction of protein along with removal of fibre. Acid precipitation using HCl followed by thermal coagulation was found to be effective than the only heat coagulation. Borhami and El-shazly (1984) had also reported HCl (pH 2) was more efficient for coagulating of about 30% more protein than heating, while extracting protein from water hyacinth. Kositsup *et al.* (1992) observed a higher extraction of 23% from water hyacinth using acid and thermal coagulation.

#### *Growth Parameters*

There was significant decrease in weight gain % and SGR of T<sub>4</sub> and T<sub>5</sub> groups when the level of APC-SP mixture increased in the diet. However, there was no significant difference ( $p > 0.05$ ) within T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups. This indicates that 16.25% APC-SP mixture can replace 10% of fish meal in the diet of *L. rohita* fry. Although literature is lacking but in a similar type experiment Ogino *et al.* (1978) had reported that LPC from rye grass could replace upto 43 and 40% of the total protein in diets for carp and rainbow trout, respectively. Similarly cowpea protein concentrate could replace upto 30% fishmeal in tilapia (Olvera *et al.*, 1997). Feeding fresh or dried azolla to fish resulted in poor growth (Almazan *et al.*, 1986; Antoine *et al.*, 1987; Micha *et al.*, 1988). El-Sayeed (1992) had reported that azolla could be used only by replacing 25% of fishmeal in the diet of tilapia. However, APC-SP could replace upto 50% protein from the fishmeal of control group in the present experiment. It appears that APC could be better utilized by the *Labeo rohita* fry. Growth rate was favourable for the *L. rohita* fry when 50% of total protein from fish meal was replaced by APC-SP, after which it reduced significantly implying low protein utilization at higher inclusion of APC-SP. This may be due to imbalanced amino

acid make-up as it is evident from low NPU from T<sub>4</sub> and T<sub>5</sub> groups. Protein utilization was reduced when essential amino acid in required proportion and amount are not available (De Silva, 1995). Almazan *et al.* (1986) had reported that *Azolla pinnata* strain was limiting in tryptophan and slightly deficient in threonine. Hence, increased concentration of azolla in the diet increases the demand for the deficient amino acid.

High FCR was registered at high inclusion level of APC-SP, especially when protein from APC-SP increased more than 50% of fishmeal protein in T<sub>4</sub> and T<sub>5</sub> groups. This is due to low nutrient utilization from APC-SP at high inclusion level (>24.37%). Increased FCR due to higher feeding of azolla had also reported by many workers (Joseph *et al.*, 1994 in *Etrophus suratensis*; Fasakin and Balogun, 1998 in *Clarias gariepinus*; Almazan *et al.*, 1986 in *Oreochromis niloticus*; Appler and Jauncey, 1983).

Similar trend was also observed for PER as with FCR. The PER of T<sub>4</sub> and T<sub>5</sub> groups were similar and were significantly lower ( $p < 0.05$ ) than T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups. Increased level of APC-SP with concomitant decrease of fishmeal might have resulted essential amino acid deficiency and thus low utilization of protein. Most of the workers were also found the same results when they used alfalfa and *Azolla pinnata* in the diet of tilapia (Santiago *et al.*, 1988; Olvera-Novoa *et al.*, 1990). Fasakin and Balogun (1998) had reported reduced PER and NPU with increasing level of azolla in the diet of *Clarias gariepinus*.

#### *Enzyme Activity and In Vitro Digestibility*

Protein digestibility of T<sub>5</sub> group containing only APC and no fishmeal was significantly lower ( $p < 0.05$ ) than the other groups except T<sub>4</sub>. Ogino *et al.* (1978) also reported a decreasing trend of protein digestibility when LPC of the rye grass was used at higher concentration. Similarly Appler and Jauncey (1983) reported at higher levels of algae in the diet, the decrease in APD with increasing level of algal meal might be due to an actual reduction in the availability of the protein or a decrease in gastrointestinal passage time. Increasing fibre contents result in more rapid passage of food through the gut of higher animals (Bender, 1967), thus decreasing the time available for digestion and absorption of the diet. A negative correlation was found out between protease activity and APC content of the feed ( $r^2 = 0.87$ , Fig. 1). Kawai and Ikeda (1972) and Shcherbina *et al.* (1976) had reported adaptive changes in the activity of the proteolytic enzymes in *Cyprinus carpio* in relation to the type of diet. Fishmeal based diet in T<sub>1</sub> group may be more adaptive for proteolytic enzyme, which gradually decrease due to the presence of APC-SP. Moreover, the proteolytic activity might have decreased probably due to the presence of trypsin inhibitors, which are reported to occur in vegetative tissues of several aquatic plants (Gleen *et al.*, 1982; Yousif *et al.*, 1994). The trypsin inhibitors impairs the digestion and absorption of protein (Olvera-Novoa *et al.*, 1990).

Carbohydrate digestibility was recorded in decreasing mode as NFE content increased in the diet. Fish have a genetic inheritance to low carbohydrate utilization (Palmer and Ryan, 1972). Moreover, NFE which normally represent available carbohydrate in cereals and pulses may be of limited value for weeds due to physico-chemical characteristics of gel polysaccharides and water soluble nature of fibre in the diet.

#### *Histological Study*

However, results show no mortality was registered in any of the experimental groups, which explain no adverse effect of APC-SP for short term feeding. However, it's effect in long term feeding needs confirmation.



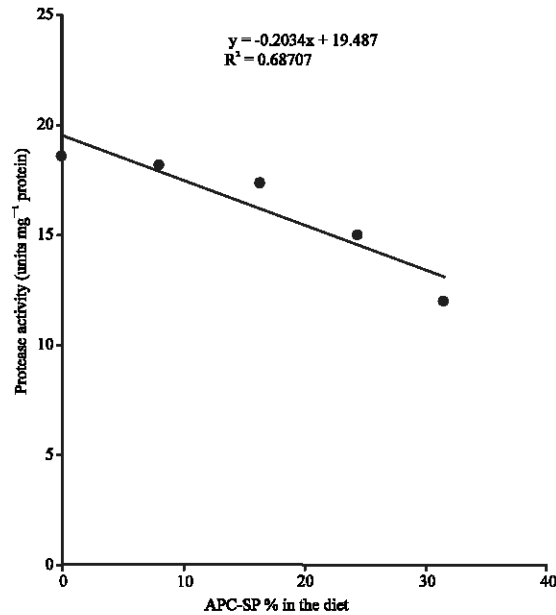


Fig. 1: Relationship between APC-SP content of feed and protease activity

### Conclusions

It concludes that fresh water aquatic plants can be used in the diet of *L. rohita* fry as protein source. Preparation of azolla protein concentrates enhanced the protein percent nearly 1.87 times than its normal protein content. Azolla protein concentrate and spirogyra mixture (4:1) can be used maximum upto 16.25% by replacing 10% fish meal in the diet of *L. rohita* fry. However, detailed studies on its amino acid make-up of these ingredients may help for its higher inclusion level by supplementing the deficient amino acids. Short term feeding had no adverse effect on *L. rohita* but long term effect needs further research.

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