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EFFECT OF REPLACEMENT OF FISHMEAL BY *AZOLLA* LEAF MAEL ON GROWTH, FOOD UTILIZATION, PANCREATIC PROTEASE ACTIVITY AND RNA/DNA RATIO IN THE FINGERLINGS OF *LABEO ROHITA* (HAM.)

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ABSTRACT

Dried water fern (*Azolla pinnata*) leaf meal was substituted for fish meal in practical diets of *Labeo rohita* fingerlings. Five isonitrogenous diets (38% crude protein) were formulated in which fish meal (68.9% crude protein) was replaced at varying levels (D_1 = Control, 0% replacement; D_2 = 25%; D_3 = 50%; D_4 = 75% and D_5 = 100%) with protein from *Azolla* meal (25.81% crude protein). Carp fed a control diet had significantly ($p < 0.05$) better growth response and nutrient utilization. Growth depression of fish increased with increasing dietary levels of *Azolla* meal. Diet D_5 containing 100% replacement of *Azolla* meal gave the poorest feed conversion ratio as it contain higher amount of trypsin inhibitor. There was marked reduction in cost the diets incorporated with *Azolla* meal. With the gradual inclusion of *Azolla* meal in the diets, the pancreatic enzyme activity was increased significantly up to 50% replacement and then decreasing trend was noticed during 60 days feeding trial. Growth rate of *Labeo rohita* and RNA/DNA ratio were reduced by insufficiently heated meals but properly heated *Azolla* meal improve nutritional quality as well as improvement of growth rate. Dry processing methods other than sun drying are suggested for the improvement of nutritional quality of *Azolla* leaf meal in carp diets. It was concluded that the antinutritional or limiting factor present in *Azolla* leaf meal are antitryptic and reduces the growth rate, if do not take proper care for the nutritional security.

Keywords: Non-conventional fish feed, *Azolla pinnata*, *Labeo rohita*, antinutritional factor, pancreatic protease, RNA/DNA ratio.

INTRODUCTION

The increasing cost and scarcity of feed ingredients has created an urgent demand for cheaper and more abundant substitutes. There is an increasing research effort taken to assess the nutritive value of different non-conventional resource including terrestrial and aquatic macrophytes (Edwards *et al*., 1985; Wee and Wang, 1987; Patra, 2000). Because of the high cost of fish meal in carp diets, most studies of their nutrition have been concerned with substitution of this component with lower cost protein sources and by-product materials (Hanley, 1991). Most of the studies have included materials of plant origin such as soybean, groundnut, rapeseed, sunflower, cottonseed, alfalfa, *Leucaena* leaves and other macrophytes (Olli and Krogdahl, 1994; Jackson *et al*., 1982; Davies *et al*., 1990; Ray and Das, 1992; Mirnova, 1975; Olvera-Novoa *et al*., 1990; Sadiku and Jauncey, 1995; Hasan *et al*., 1997; Patra and Ray, 1988; Patra, 2000; Patra *et al*., 2002, Patra, 2003) for aquaculture practices.

Most of the feed ingredients used in animal feed manufacture are believed have some potential for inclusion as ingredients for aquaculture feeds. The International Network of Feed formulation has described over 18,000 feed ingredients (Harris, 1980). Martyshev (1983) also gave several combinations of feedstuffs used in Northern Asia for feeding carps. There are several reports on the use of various supplementary feeds for culture of carps in ponds (Lakshmanan *et al*., 1971; Chakraborty *et al*., 1973; Patra *et al*., 1999, 2001; Hajra, 1987; Hajra and Tripathi, 1985).

Recently, formulated and pelleted diets have been used in the experimental culture of carp (Das *et al*., 1994; Sehgal and Sharma, 1991). Edwards *et al*. (1985) tried culture of *Tilapia* sp. in outdoor tank by using compost and dried water hyacinth in pelleted forms. Feed based on Salvinia (Murty and Devraj, 1991) and other aquatic weeds (Chiayvareesajja *et al*., 1989; Patra *et al*., 1999; Patra, 2001) have been used as feed in fish culture. Studies on green plant leaves as dietary sources for fish have focused on the use of leaf protein concentrates such as rye grass and alfalfa leaf protein concentrate (Ogino *et al*., 1978; Olvera-Novoa *et al*., 1990). From a nutritional perspective such protein concentrates have proven beneficial for only a limited number of cultured fish species. However, their potential usefulness may still depend upon the cost of

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their extraction and preparation (Olvera-Novoa *et al*., 1990). To identify economical and locally available feed stuffs, this study was designed to evaluate the use of *Azolla* leaves in formulated diets for carp.

Water fern (*Azolla pinnata*) is a free-floating, tiny aquatic plant which grows as a weed, thereby covering the surface of ponds, especially during the rainy season. It represents a "free food" source, being rapidly propagating (Ayinla and Adindu, 1992). *Azolla* has attracted attention as a nitrogenous fertilizer (Anon, 1985; Peters *et al*., 1982) and as a source of dietary nitrogen for herbivorous fish and livestock (Singh and Subudhi, 1978; Edwards, 1980). Its popularity has stirred a rush among fish farmers for its propagation as livestock-fish-integration culture system (Gavina, 1994; Rav and Shanmugasundaram, 1992).

The Indian major carp, *Labeo rohita* is endemic in Indian subcontinent. The fish enjoys high consumer preference in West Bengal as well as in other Asian countries and its price increases over the years. This is due to the growth and survival which is not so far been satisfactory, one reason possibly is due to the non-availability of suitable artificial feed at a cheaper rate.

In the present study, therefore, aimed to evaluate the nutritive value and nutritional efficiency, feed utilization, growth performance, protease enzyme activity and RNA/DNA ratios of *Labeo rohita* fingerlings fed diets containing varying levels of dehydrated *Azolla* leaf meal and identify the limitations to their use arising from antinutritional factors.

MATERIALS AND METHODS

Collection and Processing of *Azolla*

Fresh colonies of *Azolla pinnata* were harvested from the water bodies of Midnapore district, India and thoroughly washed to remove dirt and mud debris. They were then dried in a hot-air oven at 30-32^{o}C for 72h. The dried *Azolla* was milled and packed in a polythene bag and kept in a freezer at –2^{o}C prior to use. The proximate composition of both fresh and dried *Azolla* were performed following AOAC's (Association of Official Agricultural Chemist) (1990) standard analysis procedure (Table 1).

Table 1. Proximate composition of dried and fresh *Azolla pinnata*.

Constituents	Sundried *Azolla* (% content)	Fresh *Azolla* (% content)
Moisture	13.12	83.17
Crude protein	25.92	3.19
Crude lipid	4.79	2.04

Formulated Experimental diets

Five isonitrogenous (≅ 38% crude protein) diets (D_1-D_5) were formulated, containing increased levels of dried *Azolla* meal as replacement of fish meal at 0% (control), 25%, 50%, 75% and 100% (Table 2) and another four isonitrogenous (CP = 38%) diets (D_6 – D_9) processed with heat treatment (90^{o}C for 10 minutes) using the same ingredients for experimental treatments. All dietary ingredients were hand mixed and **produced pellets in pelletilizer** to form noodle like strands which were mechanically broken into pellets of suitable size (2 mm diameter) for *Labeo rohita* fingerlings. The dry pieces of feed were then stored in a freezer at –20^{o}C in sealed plastic bags until fed.

Experimental Design

The feeding trial was conducted in specially designed glass aquaria of 130 liter capacity in the Aquaculture Research Unit, Dept. of Zoology, Vidyasagar University, Midnapore, India. In order to avoid metabolic accumulation, the aquaria's water was changed daily. Each aquaria was also supplied with air by aerator. Water temperature, dissolve oxygen, alkalinity and pH were monitored everyday alternative day. The photoperiod was set on 12 hour light and dark cycle using fluorescent lamp at the light source.

Labeo rohita fingerlings (mean weight = 10.82 $\pm$ 0.60g) were obtained from rearing pond of Aquaculture Research Unit, Vidyasagar University Campus and acclimatize for two weeks in the laboratory condition with standard diet (30.0% crude protein) containing the mixture of fish meal, mustard oil cake, rice bran. The fingerlings were randomly distributed between the aquaria at a stocking density of 10 fish/aquaria. Fish were fed once daily to satiation (6% body weight) at 15.00 to 16.00 hour daily feeding allowance was adjusted each week on the basis of the average weight of the fish. The study was conducted for 60 days.

Estimation of Protease activity

Protease activity was estimated following the method of Bernfeld (1955) and which was modified by Snell and Snell (1971). 500mg hepatopancreas tissue was taken in a glass homogenizer and the tissue was homogenized in cold temperature (4 - 6°C) with phosphate buffer (0.01M, pH 7) and then centrifuged the homogenate at low speed (13,000 rpm) followed by high speed (20,000 rpm) in 4 - 6°C, continued for 10 minutes. The supernatant was used as enzyme source. 2.4ml phosphate buffer, 1.2ml enzyme extract, 1.8ml distilled water and 0.6ml 0.1% BSA were taken in a test tube and incubate it for 1 hour at 37°C. Then added 6.0ml 10% TCA, shaken thoroughly and centrifuged it at 5000 rpm for 10 minutes in cold condition. 1.0ml supernatant and 1.0 ml ninhydrin were taken in separate test tube and placed it in boiling water bath for 15 minutes. After cooling 8 ml distilled water

was taken in the same test tube and read the OD in UV Spectrophotometer (Hitachi, Japan) at 570nm. The OD compared with standard curve of Glycine for calculation of protease activity. Protein was estimated following the methods of Lowry *et al.* (1951) using BSA (Bovine Serum Albumin) as a standard.

Estimation of DNA and RNA

The DNA and RNA content of fish muscle were estimated according to the method of Munro and Fleck (1969) with some modifications. 200 mg liver (Hepatopancreas) tissue was taken from *L. rohita* and homogenised with 0.25M sucrose solution. 250 µl homogenate and 500 µl 5% TCA (Tri-chloro aceticacid) mixed thoroughly, centrifuge and wait for 15 minutes and then supernatant was discarded. The precipitate was dissolved in 500 µl of 0.6N PCA (Per-chloric acid) and 0.3N PCA each and wait for 15 minutes and then centrifuged for 5 minutes at 5000 rpm at 5°C. The supernatant was discarded and precipitate was again dissolved in 2 ml cold 0.3N PCA and wait for 10 minutes and then centrifuged at 5000 rpm for 5 minutes at 5°C. Now, precipitate was dissolved in 0.3M KOH, and incubated in water bath at 37°C for 2 hours with occasional shaking. Cooled at room temperature. With this solution 1.0ml cold 0.6M PCA and 250 µl cold 0.6N PCA were mixed and wait for 10 minutes. The supernatant was used for RNA estimation (read at 290 nm in UV spectro, Hitachi). The precipitate was dissolved in 1.5ml cold 0.6N PCA and heated at 70°C for 40 – 60 minutes in water bath and cooled at room temperature and then kept at 4°C for 10 minute Then with it 400µl of 0.6N PCA was added and centrifuged for 10 minutes at 5000 rpm in cold condition. The supernatant was read at 270 nm in UV Spectro for DNA estimation. For RNA the blank was prepared by 0.3N PCA and for DNA it was with 0.6N PCA. The calculation was done by the method of Strove and Makaravo, 1989.

Protease inhibitor activity assay

Trypsin inhibitor activity was measured by the methods of Smith *et al.* (1980) and this is the modified method of Kakade *et al.* (1974) using BAPNA (Benzoyl-DL-Arginine-Paranitroanilide) as substrate. A solution containing 100 µl of inhibitor solution, 200 µl (20 µg ml^{-1}) bovine pancreatic trypsin and 100 µl of distilled water was pre-incubated at 37^0C for 20 minutes. Then, 500 µl (0.4 mg ml^{-1}) of BAPNA (pre-warmed at 37^oC) was added and vortex immediately to start the reaction. After incubation for 20 minutes, 100 µl of 30% glacial acetic acid (v/v) was added to terminate the reaction. The reaction mixture was centrifuged at 8000 rpm for 5 minutes. Residual activity of trypsin was measured the absorbance at 410 nm in UV-spectro photometer (Hitachi, Japan) and calculated the protease or trypsin inhibitor activity according to the methods of Hamerstrand *et al.*, 1981.

Analytical Procedure

The chemical analysis of dietary ingredients, diets were performed according to the procedures of the AOAC (1990).

Diet performance was evaluated on experimental fish according to Olvera-Novoa *et al.* (1990) as follows:

$$\text{Weight gain (\%)} = 100 \left[\frac{\text{Final body weight - Initial body weight}}{\text{Initial body weight}} \right]$$

$$\text{Specific Growth Rate (SGR\% day}^{-1}\text{)} = 100 \left[\frac{(\log_e \text{ final body weight} - \log_e \text{ initial body weight}}{\text{days in trial}} \right]$$

$$\text{Feed Conversion Ratio (FCR)} = \left[\frac{\text{Dry weight of feed fed (g)}}{\text{Fish weight gain (g)}} \right]$$

$$\text{Protein Efficiency Ratio (PER)} = \left[\frac{\text{Fish weight gain (g)}}{\text{Protein intake (g, dry weight basis)}} \right]$$

Water Quality Analysis

The water quality was monitored periodically (every alternative day) following the methods of APHA (1989) (American Public Health Association). The water temperature during the experimental period determined by thermometer ranging from 29-30°C. The pH (pH meter, Systronics 302), dissolve oxygen (Winkler's method) content and alkalinity (titrimetric method) of the water ranged between 6.65-7.19, 9.12-9.59 mg $liter^{-1}$ and 102.27 – 105.75 mg $liter^{-1}$ respectively.

Statistical analysis

All calculations and statistical analysis were done on IBM P-III using statistical packages STATISTICA and ASP. The level of significance was chosen at $p < 0.05$, results are means of three separate determinations and presented as means ± SEM (Standard Error of Mean). Analysis of variance was performed and mean comparisons were accomplished using Duncan's multiple range test.

RESULTS

Fish in all the experimental units become accustomed to the general diets within the first week of acclimation. Low mortality (<5%) occurred during this period which may be due to initial stress and they were subsequently replaced with fish of similar size prior to the feeding trial.

However, fish fed on 100% *Azolla* meal (D_5) dietary inclusion has lowest percentage of survivality (Table 3). There was a decreasing rate of survival with an increasing inclusion of *Azolla* meal in the diets during the growth trial. Mortality ranged from 0% in D_1 diet and 65% in D_5 diet.

The summary of growth responses (Table 3 and Fig. 1) showed that *Labeo rohita* fed the control diet (D_1) had significantly ($P<0.05$) better weight gain, specific growth rate as compared to fish fed diets (D_2 - D_5) supplemented at various levels with dried *Azolla* meal. The lowest results were observed for fish fed 100% *Azolla* dietary inclusion. The control diet gave the best FCR (2.08) but diets containing *Azolla* meal at the 100% level (D_5) showed the poorest FCR (7.58) and PER value for control diets (D_1) have best performance (1.18) but poorest D_5 diets (0.33) (Table 3).

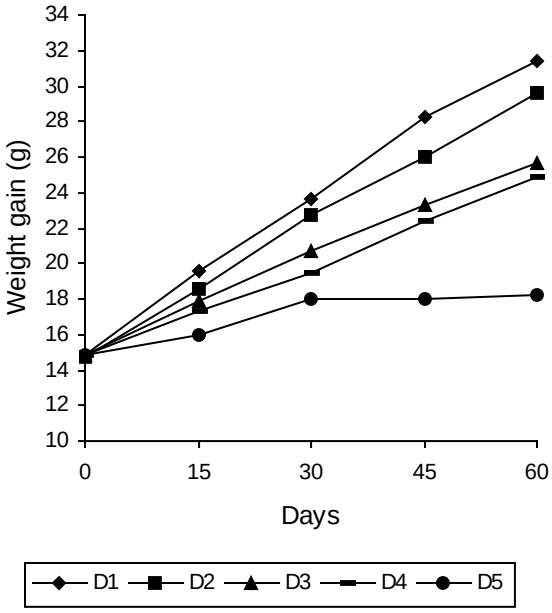

Fig. 1. Growth study of *L. rohita* fingerlings of different experimental diets ($D_1 – D_5$) during 60 days feeding trial.

The response of fingerling *Labeo rohita* fed the different diets (D_2 - D_5) containing *Azolla* meal with varying trypsin inhibitor activity is presented in Table 3. Fish fed the diets containing the unheated *Azolla* meal showed poor growth performance and had a low PER value. Both growth rates and PER values increases in fish fed diets containing less trypsin inhibitor activity. No significant difference ($p < 0.05$) in growth rate or PER was observed in fish fed diets (D_2) containing 11.8 mg TI g^{-1} diet or less. The best growth and PER were observed in fish fed diet (D_6) containing 1.8 mg TI g^{-1} diet prepared with *Azolla* meal in which 90% of the trypsin inhibitor activity has been destroyed (Table 4). *Azolla* meal heated with 90°C for 10 minutes reduces the trypsin inhibitor activity in the diets D_6 - D_9 (Table 4). A comparative growth study was presented in Fig. 2 during 60 days feeding trial, containing control (D_1), unheated *Azolla* meal ($D_2 – D_5$) and heated *Azolla* meal ($D_6 – D_9$).

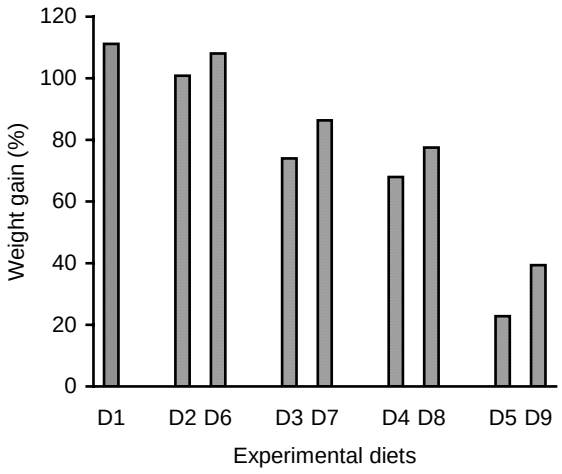

Fig. 2. Comparative study of growth of *L rohita* in control (D_1), unheated (D_2-D_5) and heat treated (D_6-D_9) experimental diets during the experimental period.

The protease activity in hepatopancreas was also recorded during 60 days growth study and presented in Table 5. It was observed that protease activity (µg glycine liberated h^{-1} mg^{-1} tissue protein) in the hepatopancreas gradually increased as compared to control with the inclusion of different level of *Azolla* meal with different T1 activity in the diets (D_2 - D_5) but later decreasing trends was followed with the higher levels of T1 inclusion. During the 60 days feeding trial protease activity in control (D_1) was 92.75 µg glycine liberated h^{-1} mg^{-1} tissue protein and gradually increased in D_2, D_3 (100.34, 102.94 µg glycine liberated h^{-1} mg^{-1} tissue protein respectively but decreased in diets D_4, D_5 (99.32, 94.62 µg glycine liberated h^{-1} mg^{-1} tissue protein respectively). It was also noted that during 60 days feeding trial protease activity increased upto 15 days fed the diet (D_5) and later decreased during 30, 45 and 60 days fed.

Muscle RNA/DNA ratio estimated on 15^{th}, 30^{th}, 45^{th} and 60^{th} day was presented in the Table 6. RNA/DNA ratio gradually decreased with the inclusion of high percentage of unheated *Azolla* meal containing high level of T1 as compared to control. RNA/DNA ratio in control (D_1) was 2.89 but decreased fed diets D_2, D_3, D_4, D_5 (1.91, 1.39, 1.34, 1.27 respectively) containing high inclusion of *Azolla* meal during 60 days feeding trial. This may also be explained and concomittented with the growth and conversion of fingerlings of *L. rohita* during 60 days feeding trial.

Table 2. Ingredients and proximate composition of experimental diets.

Ingredients	Diets (% fishmeal replacement)				
	D_1 (0%, control)	D_2 (25%)	D_3 (50%)	D_4 (75%)	D_5 (100%)
Fish meal	34.86	26.07	22.14	17	-
Azolla powder	-	6.36	13.14	22.07	39.50
α-cellulose	5.00	9.68	9.68	9.62	10.28
Wheat flour	38.14	35.89	33.04	29.31	28.22
Mustard oil cake	12.50	12.50	12.50	12.50	12.50
Rice bran	5.00	5.00	5.00	5.00	5.00
Cod liver oil	1.50	1.50	1.50	1.50	1.50
Vitamin + Mineral	1.00	1.00	1.00	1.00	1.00
Binder (gelatin)	2.00	2.00	2.00	2.00	2.00
Proximate composition (% Dry matter basis)					
Moisture	8.09	8.30	8.20	8.45	8.35
Crude protein	38.82	37.95	39.21	37.75	37.97
Crude lipid	6.43	5.50	5.98	6.02	5.71

Table 3. Cumulative growth performance and feed utilization of fish feed the experimental diets, during 60 days feeding trial.

Parameters	Experimental Diets				
	D_1 (0%, control)	D_2	D_3	D_4	D_5
mg T1 g^{-1} diet	0	11.8	24.4	38.9	48.4
Average initial weight (g)	14.86 ± 0.94	14.78 ± 0.92	14.80 ± 0.62	14.83 ± 0.91	14.80 ± 0.84
Average final weight (g)	31.38 ± 0.38^a	29.65 ± 0.99^a	25.67 ± 0.54^b	24.88 ± 0.75^b	18.18 ± 0.69^c
Weight gain (%)	111.23^a	100.84^b	74.01^c	67.94^d	22.83^e
SGR (% day^{-1})	0.89^a	0.83^a	0.66^b	0.62^b	0.24^c
Feed intake (g day^{-1})	0.41^a	0.39^a	0.37^a	0.29^b	0.28^b
FCR	2.08^a	2.20^a	2.85^a	2.44^a	7.58^b
PER	1.18^a	1.10^a	0.87^a	1.00^a	0.33^b
Survival (%)	100^a	95^a	80^b	75^b	65^c

Results are means of three separate determinations (Mean ± SEM), Values with the same superscript in the same row are not significantly different ($p < 0.05$) from each other.

DISCUSSION

The performance of the fish fed the diets supplemented with different levels of dehydrated *Azolla* meal was inferior ($P<0.05$) to that of the fish fed the control diet. The growth depression of fish tends to be more pronounced with increasing dietary level of *Azolla* meal. This study reveals the inadequacy of total replacment fishmeal with dried *Azolla* meal in diet *L. rohita* fingerlings. Almazan *et al.* (1986) reported similar growth depression and worsening FCR value with increasing dried *Azolla* meal incorporated in the diet for Nile tilapia (*Oreochromis niloticus*). Yousif *et al.* (1994) also supported similar growth depression and decreasing PER value with increasing level dehydrated alfalfa and salt bush (*Atriplex* sp.) leaves in the diets for tilapia (*Oreochromis aureus*). The poor growth responses and feed utilization values of the fish at increasing inclusion levels of dried *Azolla* meal in the diets indicates its nutrient inferiority to fish meal. A possible reason for this may be due to relatively high level of antinutritional factor specially the trypsin inhibitor in 100% *Azolla* dietary inclusion (Yousif *et al*., 1994; Ensminger *et al*., 1990; Olvera Novea *et al*., 1990). High inclusion level of *Azolla* meal in the diets has been resumed results poor digestibility and lowered availability of energy content of plant feeds as well as reduction in fish growth responses (Buddington, 1979; Appler and Jauncey, 1983; Patra *et al*., 1999; Birk, 1989; Maity, 2003).

The growth depression of fish fed diets containing *Azolla* meal incorporation could also be attributed to the

Table 4. Growth performance of *L. rohita* fed with heated diets (D_6 - D_9) *Azolla* meals in the experimental period.

	Experimental diets				
	D_1	D_6	D_7	D_8	D_9
mg T1 g^{-1} diet	0	1.8	8.4	16.5	24.4
%T1 destroyed	100	90	70	60	52
Initial body weight (g)	14.86 ± 0.94	14.80 ± 0.75	14.84 ±0.82	14.42 ± 0.42	14.49 ± 0.39
Final body weight (g)	31.38 ± 0.38^a	30.80 ± 0.31^a	27.65 ± 0.44^b	25.60 ± 0.16^b	20.20 ± 0.69^c
Weight gain (%)	111.23^a	108.10^a	86.32^b	77.53^c	39.40^d
SGR (% day^{-1})	0.89^a	0.79^b	0.56^c	0.48^d	0.41^e
FCR	2.08^a	2.24^a	3.04^b	4.19^c	4.24^c
PER	1.18^a	1.08^a	0.81^b	0.57^c	0.58^c

Results are means of three separate determinations (Mean ± SEM), Values with the same superscript in the same row are not significantly different ($p < 0.05$) from each other.
D_1 = Control : without *Azolla* meal. D_6 - D_9 Heat treated (90^oC for 10 minutes) *Azolla* meal.

Table 5. Protease activity (μg glycine liberated $hour^{-1}$ mg^{-1} of protein) in the hepatopancras of *L. rohita* fingerlings fed with different levels of *Azolla* meal containing different level of TI during 60 days feeding trial.

Experimental diets	Feeding days				
	0	15	30	45	60
D_1 Control	92.51 ± 1.52^a	93.32 ± 0.79	92.15 ± 0.94	93.17 ± 0.39	92.75 ± 0.27^a
D_2	93.01 ± 0.15^a	98.52 ± 0.57	101.32 ± 0.61	102.74 ± 0.50	100.34 ± 0.91^b
D_3	92.98 ± 0.72^a	103.02 ± 0.94	109.17 ± 0.29	108.10 ± 0.97	102.94 ± 0.20^b
D_4	92.21 ± 0.94^a	108.36 ± 0.71	109.27 ± 0.54	104.27 ± 0.72	99.32 ± 0.78^b
D_5	93.27 ± 0.19^a	114.79 ± 0.57	110.38 ± 1.02	103.50 ± 0.74	94.62 ± 0.59^c

Results are means of three separate determinations (Mean ± SEM), Values with the same superscript in the same column are not significantly different ($p < 0.05$) from each other.

Table 6. Muscle RNA/DNA ratio in *L. rohita* fingerlings fed with *Azolla* meal containing different level of T1 during the experimental period.

Experimental diets	Feeding days				
	0	15	30	45	60
D_1 Control	2.02 ± 0.12^a	2.44 ± 0.09	2.69 ± 0.29	2.77 ± 0.51	2.89 ± 0.42^a
D_2	2.15 ± 0.43^a	2.05 ± 0.29	2.02 ± 0.09	1.97 ± 0.12	1.91 ± 0.42^b
D_3	1.99 ± 0.99^a	1.74 ± 0.31	1.68 ± 0.29	1.52 ± 0.14	1.39 ± 0.14^c
D_4	1.95 ± 0.37^a	1.72 ± 0.18	1.62 ± 0.11	1.42 ± 0.19	1.34 ± 0.32^c
D_5	2.10 ± 0.41^a	1.64 ± 0.14	1.58 ± 0.50	1.40 ± 0.09	1.27 ± 0.13^d

Results are means of three separate determinations (Mean ± SEM), Values with the same superscript in the same column are not significantly different ($p < 0.05$) from each other.

presence of possible growth inhibitors such as trypsin inhibitor, which are reported to occur in vegetative tissues of several aquatic plants (Gleen *et al*., 1882; Yousif *et al*., 1994; Fasakin and Balogun, 1998; Soto and Mitchell, 1960; Humphries, 1980; Liener and Kakade, 1980). Trypsin inhibitor impairs the digestion and absorption of protein (Jauncey and Ross, 1982; Maity and Patra, 2003). It is possible from this study that antinutritional factor i.e. trypsin inhibitor might have impaired the absorption of essential components of the diets containing *Azolla* meal and causing depression of growth responses in *L. rohita* fingerlings. The possible effect of these inhibitory factors was more pronounced in 100% *Azolla* dietary substitution for fishmeal.

The present observation demonstrates that high dietary level of trypsin inhibitors may cause negative effect on *L. rohita* either in growth or conversion. In a similar study with rainbow trout (*O. mykiss*) in fresh water, Krogdahl *et al.* (1994) found protein digestibility significantly reduced by increasing dietary inclusion of the same inhibitor. Soybean trypsin inhibitors have been reported to be important when using untreated Soybean products in diets for the carp (Viola *et al.*, 1983), Channel Catfish (Wilson

and Poe, 1985), rainbow trout (Olli and Krogdahl, 1994), Atlantic Salmon (Olli *et al.*, 1994) or in mammals (Krogdahl and Holm, 1983; Kakade *et al*., 1973). Olvera-Novoa *et al.* (1990) evaluated the use of purified alfalfa leaf protein concentrate (LPC) in diets for *O. mossambicus*. They reported reduced performance at higher inclusion levels of LPC (>45%). To explain this reduction in growth, they suggested that at high dietary inclusion levels, and apparent digestibility was adversely affected by the presence of high trypsin inhibitory activity in alfalfa LPC.

It appears that processing of *Azolla* meal heating in 90^{o}C for 10 minutes to enrich the nutritive quality of the feedstuff as a replace of fishmeal in *Labeo rohita* diet. Growth rates and PER values improved as the trypsin inhibitor activity of the *Azolla* meal decreased to tolerable levels. Viola *et al.* (1982, 1983), Maity and Patra (2002) and Sadiku and Jauncey (1998) reported that the growth rates of carp were reduced when fed diets containing insufficiently heated Soybean meal. They observed equal growth rates in carp fed diets containing properly heated and slightly over heated (100% destruction of trypsin inhibitors) Soybean meal. The properly heated and slightly overheated Soybean meals used has 90-100% of the trypsin inhibitor activity destroyed.

During this investigation, the protease activity gradually increased as compared to fish receiving the control diet and later decreased with the higher inclusion of *Azolla* meal containing high level of T1. Similar observations were recorded by Olli *et al.* (1994), Vidal Valverde *et al.* (1997) in the Atlantic Salmon. They suggested that the synthesis of trypsin increases in total trypsin in the pyloric caeca homogenate seemed to be peak and then fall off, suggesting an exhaustion of the pancreatic storage of enzyme and synthesizing capacity. Diets with raw Soybean have been reported to increase pancreatic enzyme secretion in the rat (Crass *et al.*, 1987; Green and Lyman, 1972), the pig (Corring *et al.*, 1985; Zebrowska *et al.*, 1985) and Man (Calam *et al.*, 1987; Holm *et al.*, 1991, 1992).

Again a negative impact has been observed with the incorporation of *Azolla* meal in the diet containing high level of TI on the feed intake, appetite, RNA/DNA level in the muscle. There are several studies in which tissue RNA concentration and RNA/DNA ratios have been used as indicators of recent growth or nutritional status in fish (Bulow *et al*., 1978; Mitra and Mukhopadhyay, 2002, 2003). The usual interpretation is that RNA/DNA ratios are sensitive to nutritional status of fish. The amount of DNA per cell is stable under changing nutritional conditions, whereas cellular RNA, which is directly involved in protein synthesis, fluctuates with nutritional status of the fish (Foster *et al*., 1992). Thus the RNA/DNA ratio indicates metabolic intensity which is deeply influenced by the nutritional status of the diet (Clemmesen, 1987). Similar observations has also been reported previously by Lone and Matty (1980a, b; 1982 a, b; 1983) and Patra *et al.* (2002). In fish, growth can be termed as accretion of protein (Love, 1970, 1980) and it depends on food availability and state of starvation has been shown to effect on the nucleic acid levels in different body tissue (Bulow, 1970, 1971; Bulow *et al.*, 1978, 1981; Haines, 1973; Bouche, *et al.*, 1977). Furthermore, the quality of food has profound effects on cellular growth response in different fishes. So, the present studies definitely establish a direct relationship between the growth and RNA/DNA level.

CONCLUSION

The present investigation indicated that heat treated *Azolla* meal was partially substituted for fish meal resulted a significant growth of *L. rohita*. It may therefore, be incorporated as a non-conventional sources of protein in the diet of IMC on one hand and reduces the cost of feed on the other and gave the nutritional security to improve better aquaculture practices.

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REFERENCES

Almazan, GJ., Pullin, RSV., Angeles, AF., Nanalo, TA., Agbayani, RR. and Trono, MTB. 1986. Assessment of *Azolla pinnata* as dietary component for Nile Tilapia (*O. niloticus*). *In*: Median, JL., Dizon, LB., Hosillos, LV (eds.). First Asian Fisheries Forum, Asian Fisheries Society, Manila, Philippines. 523-528.

Anon, 1985. A nitrogen fix for farmers, *Azolla* and tree legumes. In: Spore, Bi-monthly bulletin of the Technical Centre for Agriculture Rural Cooperation. 56, 6.

AOAC. 1990. Official methods of analysis. Association of Official Agricultural Chemist, Edited by S. Williams (15th Ed.), Arlington, Virginia, USA.

Appler, HN. and Jauncey, K. 1983. The utilization of a filamentous green alga (*C. glomerata* Kutzin) as a protein source in pelleted feeds for *Sarotherodon niloticus* fingerlings. Aquaculture. 30: 21-30.

APHA. 1989. Standard method for the Examination of Water and Wastewater. In: Clesceri, L. S., Greenberg, AE., Trussell, RR. (17th ed.), American Public Health Association, New York, USA.

Ayinla, OA. and Adindu, VE. 1992. Nutritional of *Azolla* and tadpole. In: Nigeria Institute for Oceanography and Marine Research (NIOMR) Annual Reports. pp 93.

Bernfeld, P. 1955. In: *Methods in Enzymology*. Colowick, SP. and Kaplan NO. (eds.). Academic Press, New York. 1: 149.

Birk, Y. 1989. Protein protease inhibitors of plant origin and their significance in nutrition. In : Recent Advances of Research in Antinutritional factors in Legume Seeds. (Huisman, J., Van der Pole, TBF. and Liener, IE. (eds.). Pudoc, Wageningen. 83-94,

Bouche, G., Creach, Y. and Serfaty, A. 1977. Turnover rate of hepatic DNA in carp (*Cyprinus carpio*) after prolonged total starvation. Ann. Biol. Anim. Biochem. Biophys. 17: 21-32.

Buddington , RK. 1979. Digestion of aquatic microphyte *Tilapia zillii* (Gervais). J. Fish. Bull. 15 : 449-455.

Bulow, F. J., 1970. RNA-DNA ratios as indicators of recent growth rates of a fish. J. Fish. Res. Board Can. 27: 2343-2349.

Bulow, FJ. 1971. Selection o suitable tissues for use in the RNA/DNA ratio technique of assessing recent growth rate of a fish. Iowa State. J. Sci. 46(1): 71-78.

Bulow, FJ., Coberun, CB. Jr. and Cobbos, CS. 1978. Comparison of two blue gill populations by means of the RNA-DNA ratios and liver somatic index. Trans. Am. Fish. Soc. 107: 799-803.

Bulow, FJ., Zeman, ME., Winningham, JR. and Hudson, WE. 1981. Seasonal variations in RNA/DNA ratios as indicators of feeding, reproduction, energy storage and condition in a population of blue gill, *Lepomis macrochirus*. J. Fish Biol. 18: 297-303.

Calam, J. Bojarski, J. and Springer, CJ. 1987. Raw soybean flour increases cholecystokinine release in man. Br. J. Nutr. 58: 175-179.

Chakraborty, RD., Sen, PR., Chatterji, DK. and Kawtal GV. 1973. Observations on the relative usefulness of different feed for carp spawn and fry. J. Inland Fish. Soc. India 5: 1982-1988.

Chiayvareesajja, SB., Sirimontrappon, P., Rakkeaw, S., Tansakul, R. and Somprarit, A. 1989. Tilapia cage culture with aquatic weed mixture pellet in Thale Noi, Thailand. 2nd International Symposium on Tilapia in Aquaculture, Bangkok.

Clemmesen CM. 1987. Laboratory studies on RNA/DNA ratios of starved and fed herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. Journal du Conseil International de l'Exploration du mer . 43: 122-128.

Corring, T., Gueugneau, AM. and Chayvialle, JA. 1985. Short term effects of raw soybean diet ingestion upto the exocrine pancreatic secretion in the pig. In: Proc. 3rd. Int. Sem. On Digestive Physiology in the pig (eds. Just, A., Jorgensen, H. and Fernandez, JA), Report from the National Institute of Animal Science, Denmark. p.580.

Crass, R. A., Oates, P. S., Morgan, R. G. H., 1987. The effect of fasting on enzyme levels in the enlarged and involuting rat pancreas. Br. J. Nutr. 58: 427-436.

Das, SK., Manissery, JK. and Verghese, TJ. 1994. Growth response of Indian major carp, *Catla catla* and *Labeo rohita* to formulated diets containing leaf powder as protein source. Fishery Technology. 31(2): 102-107.

Davies, SJ., McConell, S. and Bateson, RL. 1990. Potential of rapeseed meal as an alternative protein source in complete diets for tilapia (*Oreochromis mossambicus* Peters). Aquaculture. 87: 145-154.

Edwards, P. 1980. Food potential of aquatic microphytes. ICLARM Study Rev. International Centre for Living Aquatic Resources Management, Manilla, Philippines. 5**,** p.51.

Edwards, P., Kamal, M. and Wee, KL. 1985. Incorporation of composted and dried water hyacinth in pelleted feed for the tilapia *Oreochromis niloticus* (Peters). Aquaculture and Fisheries Management. 1: 233-248.

Ensminger, ME., Oldfield, JE. and Hein, WW. 1990. Feed and nutrition . Ensminger Publishing Co. CA. p.64.

Fasakin, AE. and Balogun, AM. 1998. Evaluation of dried water fern (*Azolla pinnata*) as a placer for soybean dietary components for *Clarias gariepinus* fingerlings. J. Aqua. Trop. 13(1): 57-64.

Foster, AR., Houlihan, DF., Hall, SJ. and Burren, LJ. 1992. The effect of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod (*Gadus morhua* L.). Canadian Journal of Zoology. 70: 2095-2102.

Gavina, LD. 1994. Pig-Duck-Fish-Azolla integration in La Union. Phillippines. In: NAGA. The ICLARM Quarterly. 17(2) :18.

Gleen, EP., Fontes, MR., Katzen, S. and Colvin, LB. 1982. Nutritional value of holophytes grown on hyper saline sea water. In : San Pietro (editor). Biosaline Research : A Look to the Future. Plenum Press, New York. pp.485- 489.

Green, GM. and Lyman, RL. 1972. Feed back regulation of pancreatic enzyme secretion as a mechanism for trypsin inhibitor induced hyper secretion in rats. Proc. Soc. Exp. Biol. Metd. 140: 6-12.

Haines, TA. 1973. An evaluation of RNA-DNA ratio as a measure of long-term growth in fish. J. Fish. Res. Board Can. 30: 195-199.

Hajra, A. 1987. Biochemical investigations on the protein-caloric availability in grass carp (*Ctenopharyngodon idella* Val.) from an aquatic weed (*Ceratophyllum demersum* Linn.) in the tropics. Aquaculture. 61(2): 113-120.

Hajra, A. and Tripathi, SD. 1985. Nutritive value of aquatic weed, *Spirodela polyrhiza* (Linn.) in grass carp. Indian J. Anim. Sci. 55(8): 702-705.

Hamerstrand, GE., Black, LT. and Glover, JD. 1981. Trypsin inhibitor in soybean products: Modification of standard analysis procedure. Cereal Chem. 58: 42-45.

Hanley, F. 1991. Effect of feeding supplementary diets containing varying levels of lipid on growth, food conversion and body composition of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture. 93: 323-334.

Harris, LF. 1980. Feedstuffs. In : Fish Feed Technology. ADCP/80/11. FAO of the UN, Rome. pp. 111-170.

Hasan, MR., Macintosh, DJ. and Jauncey, K. 1997. Evaluation of some plant ingredients as dietary protein source for Common carp (*Cyprinus carpio* L.) fry. Aquaculture 151: 55-70.

Holm, H., Jorjensen, A. and Hanssen, LE. 1991. Raw soybean and purifies proteinase inhibitor induce the appearance of inhibitor resistant and chymotrypsin activities in Wister rat duodenal juice. J. Nutr. 121: 532-538.

Holm, H., Reseland, JE., Thorsen, LI., Flatmark, A. and Hanssen, LE. 1992. Raw soybean stimulate human pancreatic secretion. J. Nutr. 122: 1407-1416.

Humphries, C. 1980. Trypsin inhibitors in leaf protein concentrate. J. Sci. Food Agric. 31: 1225-1230.

Jackson, AJ., Capper, BS. and Matty, AJ. 1982. Evaluation of some plant proteins in complete diets for the Tilapia, *Sarotherodon mossambicus.* Aquaculture. 27(2): 97-109.

Jauncey, K. and Ross, B. 1982. A guide to tilapia feeds and feeding. Institute of Aquaculture, University of Striling, UK.

Kakade, ML., Hoffa, DE. and Liener, IE. 1973. Contribution of trypsin inhibitors to the deleterious effect of unheated soybean fed to rats. J. Nutr. 103: 1772-1778.

Kakade, ML., Rackis, JJ., McGhee, JE. and Puski, G. 1974. Determination of trypsin inhibitor activity of soybean products: a collaborative analysis of an improved procedure. Cereal Chem. 51: 376-382.

Krogdahl, A. Lea, TB. and Olli, JJ. 1994. Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout. Comp. Biochem. Physiol. 107A: 215-219.

Krogdahl, A. and Holm, H. 1983. Pancreatic proteinase from man, trout, rat, pig, cow, chicken, mink and fox. Enzyme activities and inhibition by soybean and lima bean proteinase inhibitors. Comp. Biochem. Physiol. 74B: 403-409.

Lakshmanan, MAV., Sukumaran, KK., Murty, DS., Chakraborty, D. and Phillioose, MT. 1971. Preliminary observations on intensive fish farming in fresh water pond by the composite culture of Indian and exotic species. J. Inland Fish. Soc. India. 2: 1-21.

Liener, IE. and Kakade, ML. 1980. Protease inhibitors. In: Toxic constituents of plant foodstuffs (eds. Liener, IE), 2nd Edn., American Press, New York. pp 7-71.

Lone, KP. and Matty, AJ. 1980a. The effect of oral administration methyltestosterone on the growth and body composition of common carp (*Cyprinus carpio* L). Gen. Comp. Endocrinol. 40: 409- 427.

Lone, KP. and Matty, AJ. 1980b. The effect of oral administration of trenbolone acetate on the growth and tissue chemistry of common carp, *Cyprinus carpio* L. Pakistan J. Zool. 12: 47-56.

Lone, KP. and Matty, AJ. 1982a. The feeding of 11 ketotestosterone on the food conversion efficiency and tissue protein and nucleic acids contents of juvenile carp, *Cyprinus carpio*. J. Fish Biol. 20: 93-104.

Lone, KP. and Matty, AJ. 1982b. Cellular effects of adrenosterone feeding on juvenile carp, *Cyprinus carpio*. Effect on liver, kidney, brain and muscle protein and nucleic acids. J. Fish Biol. 21: 33-45.

Lone, KP. and Matty, AJ. 1983. The effect of ethylestrenol on the growth, food conversion and tissue chemistry of carp, *Cyprinus carpio*. Aquaculture. 32: 39-55.

Love, RM. 1970. The Chemical Biology of Fishes. Academic Press, London. p. 547.

Love, RM. 1980. The Chemical Biology of Fishes. Academic Press, London. p. 943.

Lowry, OH., Rosebrough, NJ., Farr, AL. and Randall, RJ. 1951. Protein measurement with the folin-phenol reagents. J. Biol. Chem. 193: 265-275.

Maity, J. 2003. Effect of toxic constituents of selected non-conventional fish feed resources, soyabean (*Glycene max*) and water fern (*Azolla pinnata*) on the growth, conversion and physiological changes in Indian major carps. Ph.D. Thesis, Vidyasagar University, Midnapore, West Bengal, India.

Maity, J. and Patra, BC. 2002. Effect of feeding raw or heated soybean meal on the growth and survival of *Cirrhinus mrigala* Ham. fingerlings. Applied Fisheries and Aquaculture, ll (2): 19-24.

Maity, J. and Patra, BC. 2003. Isolation and characterization of trypsin inhibitor from the water fern, *Azolla pinnata*. R. Br. J. Food. Biochem. 27 (4):281-293.

Martyshev, FG. 1983. Pond Fisheries, Russian translation series, A. A. Balkema, Rotterdam. p.454.

Mirnova, NW. 1975. The nutritive value of algae as food for *Tilapia mossambicus*. J. Ichthyol. 15: 510-514.

Mitra, G. and Mukhopadhyay, PK., 2002. Growth, nutrient utilization and tissue biochemical changes in Labeo rohita, fed with natural and prepared diet. Journal of Applied Aquaculture. 12 (3): 65-80.

Mitra, G. and Mukhopadhyay, PK. 2003. Dietary essentiality of ascorbic acid in rohu larvae: Quantification with ascorbic acid enriched zooplankton. Aquaculture International 11: 81-93.

Munro, HN. and Fleck, A. 1969. Analysis of tissue and body fluids for nitrogen constituents in mammalian protein metabolism (Eds. Munro, HN), Academic Press, New York. 3: 433-525.

Murty, HS. and Devraj, KV. 1991. Comparison of growth of carps fed on Salvinia based feed and conventional feed. Fishery Technol. 25(2): 106-110.

Ogino, C., Cowey, C.B. and Chiou, JY. 1978. Leaf protein concentrates as a protein source in diets for carp and rainbow trout. Bull. Jap. Soc. Sci. Fish. 44 (1): 49-52.

Olli, JJ. and Krogdahl, A. 1994. Nutritive value of four soybean products as protein source in diets for rainbow trout (*Oncorhynchus mykiss*, Walbaum) reared in freshwater. Acta Agric. Scand. Sect. A. Animal Sci. 44: 185-192.

Olli, JJ., Hjelmeland, K. and Krogdahl, A. 1994. Soybean trypsin inhibitors in diets for atlantic salmon (*Salmo salar* L): effects on nutrient digestibility and trypsin in pyloric caeca homogenate and intestinal content. Comp. Biochem. Physiol. 109A (4): 923-928.

Olvera-Novoa, MA., Campos, G., Sabido, GM. and Martinez Palacios, CA. 1990. The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oreochromis mossambicus*). Aquaculture. 90:291-302.

Patra, BC. 2000. An inventory of the non-conventional fish feed resource and their nutritional evaluation in the district of Midnapore, West Bengal. V. U. J. Biol. Sci. 6: 41-48.

Patra, BC. 2001. Evaluating the nutritional condition of Indian climbing perch, *Anabas testudineus* (Bloch) fingerlings by the RNA/ DNA, Ca/P ratios and protein biosynthesis. 5th Asian Fisheries Forum, Thailand. Philippine Journal of Science (in press).

Patra, BC., Maity, J., Debnath, J. and Patra, S. 2001. Making aquatic weed useful: as feed for an Indian major carp *Labeo rohita* (Ham.). In: Recent Advances in Animal Science Research (Vol. II), eds. Ghosal, SK. and Ray, D. Orion Press International, West Bengal. pp.293-305.

Patra, BC., Maity, J., Debnath, J. and Patra, S. 2002. Making aquatic weeds useful II : *Nymphoides cristatum* (Roxb.) O. Kuntze as fish feed for an Indian major carp *Labeo rohita* (Ham.). Aquaculture Nutrition. 8(1): 33-42.

Patra, BC., Maity, J., Banerjee, S. and Patra, S. 1999. Making aquatic weeds useful III : Nutritive value of *Nechamandra alternifolia* (Roxb. ex Weight) Thw. meal as feed for the Indian major carps *Labeo rohita* (Hamilton). Vidyasagar University J. Biol. Sci. 5: 35-45.

Patra, BC. and Ray, AK. 1988. A preliminary study on the utilization of the aquatic weed. *Hydrilla verticillata* (L.f.) Rayle as feed by the carp, *Labeo rohita* (Hamilton): Growth and certain biochemical composition of flesh. Ind. Biol. 20(1): 44-50.

Patra, S. 2003. Efficacity of some aquatic weeds on the kinetics of food intake, growth promotion, associated physiological and biochemical changes in Indian major carps. Ph.D. Thesis, Viduasagar University, Midnapore, West Bengal, India.

Peters, GA., Calvet, HE., Kaplan, D. and Toia, RE. Jr. 1982. The Azolla-Anabaena symbiosis : Morphology, Physioloy and use. J. Bot. 31: 305-323.

Rav, K. and Shanmugasundaram, VS. 1992. Rice-Fish-Azolla Integration. In: NAGA. The ICLARM Quarterly. 15(2) :29.

Ray, AK. and Das, I. 1992. Utilization of diets containing composted aquatic weed (*Salvinia cuculata*) by the Indian major carp, Rohu (*Labeo rohita* Ham.), fingerlings. Bioresource Technology. 40: 67-72.

Sadiku, SOE. and Jauncey, K. 1998. Utilization of enrich soybean flour by *Clarious gariepinus*. J. Aqua. Trop. 13(1): 1-10.

Sadiku, SOE. and Jauncey, K. 1995. Soybean flour, poultry meat meal blend as dietary protein source in practical diets of *Oreochromis niloticus* and *Clarias gariepinus*. Asian Fisheries Science. 8: 159-167.

Sehgal, HS. and Sharma, S. 1991. Efficacy of two new supplementary diets for an Indian major carp, *Cirrhina mrigala*: effects on feed composition. J. Aqua. Trop. 6: 25-35.

Smith, RR., Peterson, MC. and Allred, AC. 1980. Effect of leaching on apparent digestion coefficients of feedstuffs for salmonoides. Prog. Fish. Cult. 42: 195-199.

Singh, RK. and Subudhi, BPR. 1978. Utilize *Azolla* in poultry feed. Indian Farming. 27:37 – 39.

Snell, FD. and Snell. CT. 1971. Colorimetric Methods of Analysis. Van Nostrane Reinhold Co., New York, IV AAA: 7- 145.

Soto, J. R. and Mitchell, H. L., 1960. The trypsin inhibitor of alfalfa. J. Agric. Food Chem. 8: 393-395.

Strove, EA. and Makarava, VG. 1989. Nucleic acids. In: Laboratory Manual in Biochemistry, Mir Publishers, Moscow. pp.66-73.

Vidal-Valverde, CV., Frias, J., Pollan, CD., Fernandez, M., Jurado, ML. and Urbano, G. 1997. Influence of processing on trypsin inhibitor activity of Faba beans and its physiological effect. J. Agric. Food Chem. 45: 3559-3564.

Viola, S., Mokady, S., Rappoport, U. and Ariely, Y. 1982. Partial complete replacement of fish meal by Soybean meal in feeds for intensive culture of carp. Aquaculture. 26: 223-236.

Viola, S., Mokady, S. and Arieli, Y. 1983. Effects of soybean processing methods on the growth of carp (*Cyprinus carpio*). Aquaculture. 32: 27-38.

Wee, KL. and Wang, SS. 1987. Nutritive value of *Leucaena* leaf meal in pelleted feed for nile tilapia. Aquaculture. 62: 97-108.

Wilson, RP. and Poe, WE. 1985. Effect of feeding soybean meal with varying trypsin inhibitor activities on growth of feeding channel cat fish. Aquaculture. 46:19-25.

Yousif, OM., Alhadhrami, GA. and Pessarakli, M. 1994. Evaluation of dehydrated alfalfa and salt bush (*Atriplex*) leaves in diets for tilapia (*Oreochromis aureus* L.). Aquaculture. 126: 341-347.

Zebrowska, T., Tanksley, TD. and Kanbe. DA Jr. 1985. The influence of differently processed soybean meals on the exocrine pancreatic secretion of growing pigs. In : Proc. 3rd. Int. Sem. on Digestive Physiology in the pig (eds. Just, A., Jorgensen, H. and Fernandez, J A). pp. 149-151.