The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Editor-in-Chief
Dan Mires

Editorial Board

Sheenan Harpaz – Agricultural Research Organization, Beit Dagan, Israel

Zvi Yaron – Dept. of Zoology, Tel Aviv University, Tel Aviv, Israel

Angelo Colorni – National Center for Mariculture, IOLR, Eilat, Israel

Rina Chakrabarti – Aqua Research Lab, Dept. of Zoology, University of Delhi

Ingrid Lupatsch – Swansea University, Singleton Park, Swansea, UK

Jaap van Rijn – The Hebrew University, Faculty of Agriculture, Israel

Spencer Malecha – Dept. of Human Nutrition, Food and Animal Sciences, University of Hawaii

Daniel Golani – The Hebrew University of Jerusalem, Jerusalem, Israel

Emilio Tibaldi – Udine University, Udine, Italy

Copy Editor
Ellen Rosenberg

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB), University of Hawaii at Manoa Library
and
University of Hawaii Aquaculture Program in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

© Israeli Journal of Aquaculture - BAMIGDEH.

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

EFFECTS OF FEEDING RAW SOYBEAN MEAL TO FRY OF INDIAN MAJOR CARP, *CATLA CATLA*, ON GROWTH, SURVIVAL, AND PROTEIN DIGESTIBILITY

Deepak Patnaik, Narottam Prasad Sahu* and Aparna Chaudhari

Division of Fish Nutrition and Biochemistry, Central Institute of Fisheries Education, Versova, Mumbai 400061, India

(Received 3.3.05, Accepted 18.4.05)

Key words: *Catla catla*, growth, soybean meal, trypsin, trypsin inhibitor

Abstract

Effect of feeding raw soybean meal on growth, nutrient utilization, and amylase and protease activity of Indian major carp (*Catla catla*) fry was studied in a 70-day feeding trial. Five isocaloric (1568 kJ/100 g diet) and isonitrogenous (45 g crude protein/100 g diet) semi-purified diets had varying levels of soybean meal (0, 5, 10, 20 or 30%). Ninety *C. catla* fry (0.94±0.02 g) were distributed into the five treatment groups with three replicates of each treatment. The weight gain, SGR, and PER of groups receiving diets with soybean meal were significantly ($p<0.05$) lower than in the control group that contained no soybean meal. Trypsin inhibitor units in the diets negatively correlated with weight gain (r = -0.7557), protein digestibility (r = -0.9541), trypsin (r = -0.9087) and protease activity (r = -0.8596), and positively correlated with carbohydrate digestibility (r = 0.8140) and amylase activity (r = 0.7802). An increase of trypsin inhibitor units resulted in increased hypertrophy of pancreatic, kidney, and liver tissues. Inclusion of as little as 5% soybean meal depressed the growth rate. Hence, although the soybean diets did not result in mortality, inclusion of raw soybean meal in the diet of *C. catla* fry should be discouraged.

Introduction

Fish generally have higher dietary protein requirements than terrestrial animals. Fishmeal is customarily used as a major protein source and constitutes a substantial part of aqua-feed formulations because of its high nutritive value and palatability. However, its rising cost and uncertain availability are forcing aquaculture nutritionists and feed manufacturers to use less expensive and more readily available plant protein sources as substitutes.

Soybeans are universally regarded as the

* Corresponding author. Tel.: +91-22-26361446/7/8, ext 475; fax: +91-22-26361573, e-mail: npsahu1@rediffmail.com

most attractive plant protein source for animal feed including fish (Lovell, 1988; Hughes, 1991). Soybeans are available in many parts of the world at a lower cost than animal protein sources. Among plant proteins, the amino acid profile of soybean best suits the requirements of aquatic species. It is the most promising plant protein source for replacing part or all of the fishmeal protein in diets for the most cultivable teleosts (Tacon, 1993). However, major replacement of fishmeal by soybean meal results in a reduced growth rate and feed efficiency in most fish species (Dabrowski and Kozak, 1979; Balogun and Ologhobo, 1989; Brown et al., 1997).

Although it is a component of pelleted diets for trout, catfish, and other fish, its use is restricted due to the presence of several antimetabolites, especially a trypsin inhibitor. Crude or inadequately heated soybean meal causes growth depression in various animals including fish, with the trypsin inhibitor accounting for about 40% of the total inhibition caused by raw soybean flour (Liener, 1994). There seems to be a varying degree of sensitivity to the inhibitor among the tested species. Rainbow trout seems to be very sensitive, channel catfish more resistant, and carps intermediate in their response (Hendricks and Bailey, 1988). Cyprinids are considered more tolerant to soybean anti-nutritional factors (Viola et al., 1983) and appear to efficiently utilize dietary soybean meal when supplemented with synthetic amino acids to improve the essential amino acid profile of the diet (Murai et al., 1989).

Catla catla is one of the important species cultivated in composite fish culture in India and other tropical countries. There have been several attempts to evaluate the nutritional value of locally available feedstuffs for catla and other Indian major carps (Chakraborty et al., 1973; Singh et al., 1980). Although soybean cultivation has increased over the years throughout the world, it has not gained popularity as an aquafeed ingredient due to the need for pre-treatment to destroy the antimetabolites. Literature pertaining to the use of raw soybean meal in *C. catla* diet is lacking. Hence, this work was conducted to discover the tolerance limit of *C. catla* fry to raw soybean meal by studying its effect on growth, survival, and protein digestibility.

Materials and Methods

Experimental animals. The experiment was conducted during 70 days in the Division of Fish Nutrition and Biochemistry, Central Institute of Fisheries Education, Mumbai. *Catla catla* fry were procured from the Khopoli Fish Seed Farm, Maharashtra. The fry were reared in a fiberglass tank (2.5 x 1 x 1 m) and fed a diet containing 45% crude protein until use in the feeding trial.

Experimental design. There were five experimental groups, each with three replicates, in 15 uniform plastic tanks (50 l capacity) following a completely randomized design. Six *C. catla* fry of 0.94± 0.02 g were stocked into each tank. The fish were acclimatized for two weeks with a control diet containing no raw soybean meal. No attempts were made to control the natural environmental conditions. Forty liters of water were maintained in each tank throughout the experiment and aeration was provided round the clock to all the tanks with a 2 HP air blower. The aeration pipe of each tank was provided with an airstone and a 1/8" diameter plastic regulator to adjust the air pressure. Experimental tanks were cleaned manually by siphoning the water along with the fecal matter and left-over feed daily. Removed water was replaced by fresh chlorine free borewell water.

Diet formulation and preparation. Five isocaloric (375 kcal /100 g feed) and isonitrogenous (45 g crude protein/100 g feed) diets were prepared, each containing a different level (0-30%) of raw soybean meal made by pulverizing raw soybean seeds (Table 1). The ingredients (Table 2) were thoroughly mixed and water was added (40%) to make a dough. Pellets were prepared using a hand pelletizer with a 2 mm-diameter grid. The pellets were dried in a hot air oven at 60°C and kept in airtight polythene until use.

Feeding rate. Feed was given at 2.5% of the total biomass by assessing the weight fortnightly until the end of the experiment. The daily ration was divided into two parts. About

Table 1. Proximate composition of raw soybean meal and experimental diets (% dry matter basis).

	Raw soybean meal	*Diet*				
		0	*5%*	*10%*	*20%*	*30%*
Organic matter	89.80	95.68	95.77	95.82	95.39	94.80
Crude protein	40.09	45.09±0.12	44.92±0.13	45.32±0.20	45.01±0.13	44.95±0.14
Ether extract	15.93	6.09±0.10	6.19±0.20	6.35±0.08	7.09±0.09	8.45±0.10
Crude fiber	4.01	9.13±0.1	8.82±0.19	5.02±0.14	4.59±0.12	3.20±0.08
Nitrogen free extract (NFE)	34.07	35.07±0.13	35.84±0.22	34.13±0.21	32.49±0.17	30.2±0.14
Ash	5.90	4.62±0.2	4.23±0.15	4.18±0.19	4.82±0.21	5.2±0.25
Digestible energy (kJ/100 g)*		1570	1583	1568	1563	1575
P/E (mg/kJ)		28.72	28.38	28.90	28.80	28.54

* Calculated as (% crude protein x 4) + (% NFE x 4) + (% lipid x 9)

two-thirds of the total ration was given at 9:00 and the rest at 18:00.

Physico-chemical water parameters. Water quality parameters including pH, dissolved oxygen, free carbon dioxide, total alkalinity, total hardness, and total ammonia were recorded once a week. Water temperature was recorded twice daily at 19:00 and 21:00 with a mercury thermometer to an accuracy of 0.1°C.

Growth parameters. The body weights of the fish were recorded every 15 days. Fish were starved overnight before measuring body weight. Growth performance was assessed in terms of percent weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER).

Biochemical analysis of tissues and diets. Biochemical analysis of total protein, total carbohydrate, lipid, ash and moisture was done for specimens at the beginning of the experiment to estimate initial values and for experimental animals collected from the tanks at end of the experiment. Proximate composition of diets included moisture, crude protein, crude fat, ash, fiber, and nitrogen free extract (NFE) was determined according to AOAC (1990).

Trypsin assay. Trypsin was assayed as described by Walsh (1970) using TAME (Na-p-tosyl-L-arginine methyl ester) as the specific substrate. Three milliliters of the substrate solution (19.7 mg TAME-HCl dissolved in 50 ml 0.04M Tris/0.01M $CaCl_2$ buffer, pH 8.1) were added to quartz cuvettes kept inside a double beam UV-Vis spectrophotometer for 3 min and balanced for absorbance at 247 nm. Tissue extracts (10% w/v) were prepared by homogenizing entire fry in 0.1 M phosphate

Table 2. Composition of experimental diets (% dry matter basis).

Ingredient	Diet				
	0	5%	10%	20%	30%
Casein, fat free [1]	45	42	40	35	30
Gelatin [2]	6	7	7	7	7
Raw soybean meal	-	5	10	20	30
Dextrin white [3]	28	28	28	28	24
Cellulose [4]	12	9	6	3	0
Sunflower vegetable oil + cod liver oil (2:1)	6	6	6	6	6
Vitamin-mineral mix [5]	2.75	2.75	2.75	2.75	2.75
Vitamin C [6]	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100

[1] 86% crude protein (SD Fine Chemicals Ltd.)
[2] 96% crude protein (Himedia)
[3] from SD Fine Chemicals Ltd.
[4] from Himedia
[5] Composition of vitamin mineral premix (Supplevite M; per kg feed): Vitamin A 2,000,000 IU; Vitamin D_3 400,000 IU; vitamin B_2 0.8 g; vitamin E 300 units; vitamin K 0.4 g; calcium pantothenate 1.0g; nicotinamide 4.0 g; vitamin B_{12} 2.4 g; choline chloride 60.0 g; calcium 300.0 g; manganese 11.0 g; lidine 0.4 g; iron 3.0 g; zinc 6.0 g; copper 0.8 g; cobalt 0.18 g.
[6] from Celin, Glaxo

buffer (pH 7.5) and centrifuging the mixture at 9,000x g for 10 min. In reference cuvettes, 0.1 ml distilled water was added and mixed well. To other cuvettes, 0.1 ml of tissue extract was added and well mixed. The increase in absorbance was read against the reference cuvette at 1 min intervals for 4 min. One unit of TAME activity is the amount of trypsin catalyzing the hydrolysis of 1 mmole of TAME/min. The absorbance accompanying hydrolysis of 1 mmole of TAME/ml assay solution is 0.409/cm/mM. Trypsin, at a concentration of 0.1 mg/ml in the assay cuvette, results in an absorbance change of 0.0101 absorbance units per min per cm. Thus, 1.0 mg of trypsin is equal to 247 TAME units.

Trypsin inhibitor assay. Feeds were assayed for trypsin inhibition as follows. Feed extracts were prepared according to Hammerstrand et al. (1981). One gram of raw soybean flour was extracted in 0.01 N NaOH and the total volume was made up to 50 ml. The extract was diluted to obtain about 60% inhibition of trypsin activity. The reaction mixture containing 800 µl of commercial trypsin solution (1 mg/ml in 1 mM HCl), 10 µl soybean flour extract, and 190 µl of Tris/$CaCl_2$ buffer (pH 8.1) was incubated at room temperature for 5 min. Trypsin activity was then assayed as above. The decrease in trypsin activity (TAME units) caused by the presence of the inhibitor was calculated in TAME trypsin inhibitor units (TAME-TIU) and TAME-TIU/g soybean flour was calculated.

Statistical analysis. Results were subjected to one-way ANOVA to determine significant differences among treatments. Regression analysis of trypsin activity with other related parameters was done. All data were analyzed using software package SPSS (version 11) and differences were considered significant when $p<0.05$.

Results

Proximate composition of experimental diets. The proximate compositions of the soybean meal and experimental diets are given in Table 1. Crude protein in the diets ranged 44.92-45.32%. Crude fiber decreased and ether extract increased as the amount of soybean meal increased. Soluble carbohydrates ranged 30.2-35.84% and the inorganic content (total ash) ranged 4.18-5.2%, with no trend as the soybean component increased. Calculated digestible energy (kJ/g) varied little and was around 1568 kJ/100 g diet. The protein:energy ratio was the same for all diets.

Growth, FCR, PER, and survival. Weight gains at 15 day intervals are given in Fig. 1. Catla fed the experimental diets had significantly lower final weight gains than the control group (Table 3). The FCR of the control was significantly lower and the PER significantly higher than those of the diets containing soybean meal. Survival was 100% in the control and groups fed diets containing only 5 or 10% soybean meal but less in groups fed diets containing a higher soybean level.

Amylase, enzyme, and trypsin activity. The specific amylase activity at the start of the experiment was 0.19±0.02 unit/mg and increased with the percentage of soybean meal in the diet (Table 4). Specific protease activity of the *C. catla* fry at the start of the experiment was 14.42±0.52 unit/mg, whereas the final protease activity varied among treatments but not according to any detectable pattern. Trypsin inhibitor activity was highest

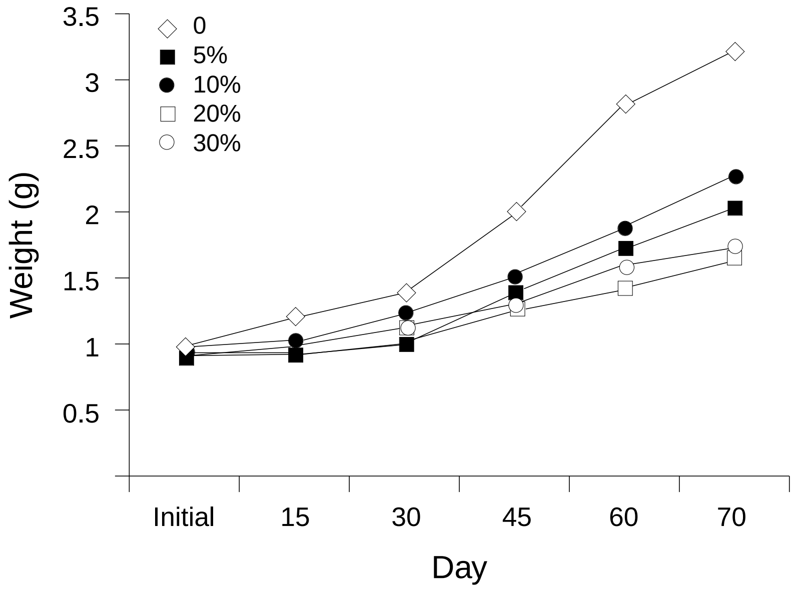

Fig. 1. Growth of *Catla catla fry* fed experimental diets containing different levels of raw soybean meal.

Table 3. Growth and feed efficiency factors (means of 3 replicates±SEM).

Parameter	*Control*	*5%*	*10%*	*20%*	*30%*
Initial body wt (g)	0.98±0.02	0.88±0.02	0.98±0.06	0.91±0.04	0.93±0.01
Final body wt (g)	3.14±0.16	1.97±0.02	2.22±0.05	1.68±0.02	1.70±0.05
Body wt gain (g)	2.16	1.09	1.24	0.77	0.77
Daily wt gain (g)	0.029±0.002	0.015±0.001	0.018±0.001	0.011±0.001	0.011±0.001
Weight gain (%)	220.43±16.62^{a}	124.47±2.86^{c}	126.80±5.03^{c}	84.62±2.70^{b}	82.67±5.88^{b}
Specific growth rate (SGR)	1.67±0.09^{a}	1.15±0.02^{c}	1.17±0.03^{c}	0.88^{b}±0.02^{b}	0.86±0.05^{b}
Food conversion rate (FCR)	1.35±0.11^{a}	1.92±0.08^{c}	1.64±0.05^{c}	2.56±0.13^{b}	2.56±0.21^{b}
Feed efficiency ration (FER)	0.75±0.05^{a}	0.52±0.02^{c}	0.61±0.02^{c}	0.39±0.12^{b}	0.40±0.03^{b}
Protein efficiency ratio (PER)	1.66±0.13^{a}	0.88±0.07^{b}	0.87±0.04^{b}	1.35±0.04^{c}	1.16±0.05^{c}
Survival	100.00	100.00	100.00	94.44±5.56	83.33±9.62

Means in the same row sharing the same superscript do not significantly differ (p>0.05).
SGR = 100($\log_e$ final body wt - $\log_e$ initial body wt)/60 days
FCR = feed intake/weight gain
FER = weight gain/feed intake
PER = weight gain/protein intake

Table 4. Enzyme activity, RNA/DNA ratio, and digestibility the different experimental groups.

Parameter	*Control*	*5%*	*10%*	*20%*	*30%*
Mean specific amylase activity	0.75±0.10^{a}	0.99±0.07^{ad}	1.20±0.18^{bcd}	1.02±0.08^{ac}	1.40±0.99^{b}
Mean specific protease activity	18.22±0.54^{a}	13.72±0.26^{c}	13.99±0.47^{c}	8.93±0.76^{b}	9.37±0.51^{b}
Trypsin inhibitor activity (TAME TIU/g feed)	0	5.35±0.11	18.34±0.09	51.96±0.23	87.10±0.12
Final trypsin assay (TAME units/mg)	0.73±0.01	0.50±0.02	0.62±0.01	0.36±0.01	0.24±0.03
RNA/DNA	2.46	1.60	2.27	1.79	1.44
Carbohydrate digestibility (%)	32.43±2.21	36.78±2.59	35.60±3.55	36.51±1.22	39.60±1.56
Crude protein digestibility (%)	61.23^{a}	60.92^{b}	60.89^{b}	60.67^{c}	60.65^{c}

Means in the same row sharing the same superscript do not significantly differ ($p>0.05$).

in the control and decreased as the soybean component of the feed increased. *In vitro* carbohydrate digestibility was similar in all groups while the control group had significantly higher protein digestibility.

Biochemical composition of body tissue. There were no significant differences in biochemical composition among experimental groups (Table 5).

Discussion

Biochemical composition of animal tissue. There were no differences in final body tissue composition between the control and the groups fed diets containing soybean meal, as found by Pongmaneerat and Watanabe (1992).

Growth parameters. Percent weight gain negatively correlated (r = -0.7557) with TIU. Growth was clearly depressed as the level of raw soybean meal increased due to the presence of trypsin inhibitors. Anti-trypsin activity adversely affects growth and protein utilization in channel catfish (Lovell, 1991), grass carp fry (Dabrowski and Kozak, 1979), hybrid striped bass (Brown et al., 1997), and rainbow trout fingerlings (Murai et al., 1989). The SGR of *Clarias gariepinus* fingerlings decreased from 0.2 to 0.02 as the soybean content increased from 0 to 100% (Balogun and Ologhobo, 1989). Growth response was poor in carp fed insufficiently heated soybean meal (Viola et al., 1983). Low protein utilization is confirmed by the low protein digestibility of the fish fed the diets containing raw soybean meal.

Feed utilization indexes. Poor nutrient utilization was evident as the level of raw soybean meal in the diet increased. Sadiku and Jauncey (1998) found similar results for *C. gariepinus.* In the present study, PER negatively correlated with TIU (r = -0.8173), similar to findings of Shimeno et al. (1997) who reported that PER decreased from 2.06 to 2.03 with an increase in soybean content from 0 to 30% in diets for yellowtail fingerlings. Pongmaneerat and Watanabe (1992) reported significantly reduced feed efficiency (PER and ERV) as the soybean content increased from 0 to 50%. Murai et al. (1989) reported that protein utilization dropped from 30.4% in

Table 5. Biochemical composition of tissue of C. catla fry of different experimental groups (% dry matter basis) at the end of the experiment.

Treatment	*Organic matter*	*Protein*	*Total carbohydrate*	*Lipid*	*Ash*	*Digestible energy (kJ/100 g)*	*Moisture*
Initial	85.95	60.53±0.01	14.17±0.13	11.25±0.21	14.05±0.12	1673.01	79.20
Control	87.98	61.23±0.03	12.53±0.12	12.32±0.09	12.82±0.14	1697.56	78.50
5%	87.27	60.92±0.09	12.65±0.12	13.60±0.14	12.73±0.12	1742.56	78.80
10%	87.31	60.89±0.10	13.40±0.14	13.02±0.12	12.69±0.12	1745.32	78.21
20%	87.28	60.67±0.02	12.91±0.08	13.70±0.17	12.72±0.13	1746.49	77.85
30%	87.19	60.65±0.01	12.34±0.09	14.22±0.15	12.81±0.09	1755.44	77.05

a control feed to 20.1% in feed containing 50.5% raw soybean meal. Brown et al. (1997) observed a reduction in feed efficiency from 0.76 to 0.21 when the inclusion of raw soybean meal in hybrid striped bass diets increased from 20% to 40%.

Sandholm et al. (1976) found a negative correlation between the anti-trypsin content in soybean and protein digestibility in rainbow trout. In the present study, the presence of trypsin inhibitors at 5.3484 TIU/g feed (in the 5% soybean diet) had the same growth depressing effect as 18.3374 TIU/g feed (in the 10% soybean diet).

Survival. The survival rate decreased from 100% to 83.33% as the soybean content rose, in agreement with Shimeno et al. (1997) for yellowtail fingerlings and Brown et al. (1997) for striped bass. However, Murai et al. (1989) concluded that the survival rate was not affected by inclusion of raw soybean meal up to 55.5% in rainbow trout diets. Balogun and Ologhobo (1989) observed 20% mortality when raw soybean meal was included at a level of 50% or above and 10% mortality at an inclusion level of 25% in fingerling *C. gariepinus.* In the present study the differences among treatment groups were not significant, suggesting that *C. catla* fry can tolerate 30% dietary raw soybean meal over a period of 70 days, although the long-term effects of raw soybean meal on survival are unknown.

Nutrient digestibility. Protein digestibility negatively correlated (r = -0.9541) with TIU and positively correlated with protease activity (r = 0.9294). Shimeno et al. (1997) concluded that higher inclusion of full fat soybean meal slightly decreased protein digestibility; they reported 72.9% protein digestibility in yellowtail fingerlings fed a diet without full fat soybean meal and 64.7% in those fed a diet containing 30% raw soybean meal. High TI activity in inadequately heated soybean meal reduced protein digestibility in rainbow trout (Sandholm et al., 1976; Smith et al., 1980).

Hossain et al. (1997) reported that of all the plant proteins, soybean meal was the most digestible with an apparent protein digestion (APD) value of 84.06% for *Labeo rohita* fingerlings. The APD values for soybean in carp (Kim, 1974), jelawat (Law, 1984), rainbow trout (Smith et al., 1980), channel catfish (Brown et al., 1985), and tilapia (Hossain et al., 1992) are 86.8%, 69.5%, 80%, 85%, and 84.67%, respectively. Still higher digestibility was achieved (93%) after eliminating TI by heat treatment (Grabner and Hofer, 1985). Pongmaneerat and Watanabe (1992) reported APD in all cases to be as high as 90-94%, irrespective of whether raw soybean meal or extruded soybean meal with low levels of TI was included.

In the present study, carbohydrate digestibility increased from 32.43 to 39.6% as the soybean content increased from 0 to 30%, although this difference was not significant. There was a positive correlation between carbohydrate digestibility and TIU (r = 0.8140) and a negative correlation between protease and amylase activity (r = -0.6974), although differences were not significant. Amylase activity gradually increased as raw soybean meal increased, possibly compensating for low protease activity. A similar observation was made by Escaffre et al. (1997) in common carp larvae but contradictory findings were reported by Shimeno et al. (1997) in yellowtail fingerlings, where a 38.7-40.5% decrease in carbohydrate digestibility resulted from inclusion of up to 20% raw soybean meal. Similar observations were recorded by Sandholm et al. (1976) for trout and Smith et al. (1980) for salmonids.

Trypsin and trypsin inhibitor activity. The trypsin inhibitor activity was found to be 460.5 TAME TIU/ g raw soybean flour while Sadiku and Jauncey (1998), using n-alpha-DL-arginine-P-nitro-anilide as a trypsin substrate, reported trypsin inhibition in raw soybean meal to be 91.3 TIU/mg.

Trypsin inhibitor activity increased from 0 to 87.10 TIU/g feed as the content of raw soybean meal increased from 0 to 30%. Shimeno et al. (1997) reported that trypsin inhibitor activity increased from 33.5 to 103 TIU/mg as soybean meal content increased from 10 to 30% in experimental diets for yellowtail fingerlings.

Trypsin specific activity dropped as raw soybean content increased, with a negative correlation of r = -0.9087 between TIU and trypsin activity. Robaina et al. (1995) also obtained a significant decrease in trypsin activity (0.15 to 0.02 m units/mg) when the substitution level of raw soybean meal reached 30% in diets fed to gilthead seabream (*Sparus aurata*). Results obtained by Murai et al. (1989) suggest that the effect of soy flour on trypsin activity is influenced by the size of the fingerling in rainbow trout. Addition of soy flour (untreated or treated with methanol) depressed the activity of a gastrointestinal trypsin-like enzyme in small fish (0.7 g), whereas this activity remained unaffected in larger fingerlings (8.9 g).

Enzyme activity. The control, 5%, and 10% treatments registered marginal, though insignificant, increases in protease activity during the experimental period while in the 20% and 30% groups protease activity decreased with time. On the final day of the experiment, the mean protease activity in the control group was higher than in the rest of the groups. A negative correlation (r = -0.8596) was observed between TIU and protease activity, a trend that was also reflected in growth parameters. A similar observation was reported by Dabrowski et al. (1992), who found a reduction in pancreatic enzyme activity in charr (*Salvelinus alphinus* L.) when soybean meal replaced part of the fishmeal-based starter diets. However, Shiau et al. (1987) reported that protease activity in the stomach, liver, and intestine of tilapia fed a fishmeal-based control diet was identical to that of fish receiving hexane extracted soybean meal.

Amylase activity of the experimental groups at the end of the experiment was higher than on the initial day. Interestingly, on the final day of the experiment, amylase activity was lowest in the control group and higher in the treatment groups in relationship to the soybean content. Amylase activity positively correlated (r = 0.7802) with TIU, opposite the trend in growth. Escaffre et al. (1997) observed a slight but insignificant (p>0.05) increase in amylase specific activity in common carp over a period of 20 days.

Histological studies. Hypertrophy in acinar cells of the pancreas and glomerular cells of the kidney increased as the soybean content increased. Lim and Akiyama (1991) observed that fish fed diets containing raw or underheated soybean developed abnormalities in kidney tissues mainly due to the presence of antinutritional factors and poor utilization of the non-denatured protein. However, Robinson and Li (1994) were unable to detect any pancreatic hypertrophy in fingerling channel catfish fed high levels of trypsin inhibitor activity.

Hypotrophy gradually increased in liver cells as the soybean level increased. Sadiku and Jauncey (1998) also observed shrinking hepatocytes (lower vacuolation) in the liver of *C. gariepinus* fed diets containing raw soybean meal. Robaina et al. (1995) reported on increased deposition of lipid and decreased glycogen deposits in the liver of gilthead seabream with increased levels of dietary raw soybean meal.

In summary, the inclusion of raw soybean meal in the diet of *C. catla* fry, even at a level of only 5% (5.35 TAME TIU/g feed), significantly reduced growth and protein digestibility. There was no significant difference in the growth rate between the 5% and 10% inclusion levels. Hypertrophy of pancreas and kidney cells along with hepatic hypotrophy was noticed in groups fed higher levels of soybean meal. Although survival was similar among all the groups during the 70-day period, long-term observations might differ. Thus, the tolerance of catla fry for raw soybean meal is very low and soybean meal should be properly heated whenever its use is recommended.

Acknowledgements

We gratefully acknowledge the Director of the Central Institute of Fisheries Education, Mumbai, for providing facilities and the Indian Council of Agricultural Research for financial support.

References

AOAC, 1990. *Official Methods of Analysis*, 14th ed. Assoc. Official Analytical Chemists, Arlington, VA. 1141 pp.

Balogun A.M. and A.D. Ologhobo, 1989. Growth performance and nutrient utilization for fingerling *Clarias gariepinus* (Burchell) fed raw and cooked soybean diets. *Aquaculture,* 76:119-126.

Brown P.B., Strange R.J. and K.R. Robbins, 1985. Protein digestibility coefficient for yearling channel catfish fed high protein feedstuffs. *Prog. Fish. Cult*., 47:94-97.

Brown P.B., Twibell R., Jonker Y. and K.A. Wilson, 1997. Evaluation of three soybean products in diets fed to juvenile hybrid striped bass *Morone saxatilis x M. chrysops. J. World Aqua. Soc*., 28:215-223.

Chakraborty R.D., Sen P.R., Chatterjee D.K. and G.V. Kowtal, 1973. Observations on the relative usefulness of different feed for carp spawn and fry. *J. Inland Fish Soc. India,* 5:182-188.

Dabrowski K. and B. Kozak, 1979. The use of fishmeal and soybean meal as a protein source in the diet of grass carp fry. *Aquaculture,* 18:107-114.

Dabrowski K., Krumschnabel G., Paukku M. and J. Labanowski, 1992. Cyclic growth and activity of pancreatic enzymes in alevins of Arctic charr *(Salvelinus alpinus* L.). *J. Fish Biol.,* 40:511-521.

Escaffre A.M., Infante J.L.Z., Cahu C.L., Mambrani M., Bergot P. and S.J. Kaushik, 1997. Nutritional value of soy protein concentrate for larvae of common carp *(Cyprinus carpio)* based on growth performance and digestive enzyme activities. *Aquaculture,* 153:63-80.

Grabner M. and R. Hofer, 1985. The digestibility of the proteins of broad beans *(Visia faba)* and soyabean *(Glycine max)* under *in vitro* conditions simulating the elementary tracts of rainbow trout *(Salmo gairdneri)* and carp *(Cyprinus carpio)*. *Aquaculture,* 48:111-122.

Hammerstrand G.E., Black L.T. and J.D. Glover, 1981. Trypsin inhibitor in soy products: Modification of the standard analytical procedure. *Cereal Chem*., 58:42-45.

Hendricks J.D. and G.S. Bailey, 1988. Adventitious toxins. pp. 606-645. In: J.E. Halver (ed.). *Fish Nutrition*, 2nd ed. Academic Press Ltd., London, UK. 798 pp.

Hossain M.A., Nahar N., Kamal M. and M.N. Islam, 1992. Nutrient digestibility coefficients of some plant and animal proteins for tilapia

(Oreochromis mossambicus). J. Aqua. Trop., 7:257-265.

Hossain M.A., Nahar N. and M. Kamal, 1997. Nutrient digestibility coefficients of some plant and animal proteins for rohu *(Labeo rohita)*. *Aquaculture,* 151:37-45.

Hughes S.G., 1991. Use of lupin flour as a replacement for full-fat soy in diets for rainbow trout *(Oncorhynchus mykiss). Aquaculture*, 93:217-277.

Kim Y.K., 1974. Determination of true digestibility of dietary protein with Cr_2O_4 containing diet. *Bull. Jpn. Soc. Sci. Fish.,* 40:651-654.

Law A.T., 1984. Nutritional study of jelawat, *Leptobarbus hoevenii* (Bleeker), fed on pelleted feed. *Aquaculture,* 41:227-233.

Liener I.E., 1994. Implications of antinutritional components in soybean foods. *Critical Rev. Food Sci. Nutr*., 34:31-67.

Lim C. and D.M. Akiyama, 1991. Full fat soybean meal utilization by fish. pp. 188-198. In: M.A. Dean, K.H. Tan Ronnie (eds.). *Proc. Aquaculture Feed Processing and Nutrition Workshop.* American Soybean Association, Singapore.

Lovell R.T., 1991. Use of soybean products in diets for aquaculture species. Revised. pp. 179-187. In: M.A. Dean, K.H. Tan Ronnie (eds.). *Proc. Aquaculture Feed Processing and Nutrition Workshop*. Am. Soybean Assoc*.,* Singapore.

Murai T., Ogata H., Villaneda A. and T. Watanabe, 1989. Utilization of soy flour by fingerling rainbow trout having different body size. *Nippon Suisan Gakkaishi,* 55:1067-1073.

Pongmaneerat J. and T. Watanabe, 1992. Utilization of soybean meal as protein source in diets for rainbow trout. *Nippon Suisan Gakkaishi,* 58:1983-1990.

Robaina L., Izquierdo M.S., Moyano F.J., Socorro J., Vergara J.M., Montero D. and H. Fernandez-Palacios, 1995. Soybean and lupin seed meals as protein sources in diets for gilthead seabream *(Sparus aurata)* nutritional and histological implications. *Aquaculture*, 130:219-233.

Robinson E.H. and M.H. Li, 1994. Use of plant proteins in catfish feeds: Replacement of soybean meal with cottonseed meal and replacement of fish meal with soybean meal and cottonseed meal. *J. World Aqua. Soc*., 25:271-276.

Sadiku S.O.E. and K. Jauncey, 1998. Utilization of enriched soybean flour by *Clarias gariepinus. J. Aqua. Trop.,* 13:1-10.

Sandholm M., Smith R.R., Shih J.C.H. and M.L. Scott, 1976. Determination of antitrypsin activity on agar plates: Relationship between antitrypsin and biological value of soybean for trout *J. Nutr.,* 106:761-766.

Shiau S.Y., Chuang J.I. and C.L. Sun, 1987. Inclusion of soybean meal in tilapia *(Oreochromis niloticus x O. aureus)* diets at two protein levels. *Aquaculture,* 65:251-261.

Shimeno S., Ruchimat T., Matsumoto M. and U. Masaharu, 1997. Inclusion of full-fat soybean meal in diet for fingerling yellowtail. *Nippon Suisan Gakkaishi*, 63:70-76.

Singh B.N., Sinha V.R.P. and D.P. Chakraborty, 1980. Feed intake, absorption, conversion and growth of fry and fingerlings of rohu, *Labeo rohita* (Ham), *Indian J. Fish.*, 27:193-200.

Smith R.R. Peterson M.C. and A.C. Alfred, 1980. Effect of leaching on apparent digestion coefficients of foodstuffs for salmonids. *Prog. Fish Cult.,* 42:195-199.

Tacon A.G.J., 1993. Feed ingredients for warm water fish: Fishmeal with other processed feedstuffs. pp. 64. In: *FAO Fish Circ. No. 856.* FAO, Rome.

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

Walsh K.A., 1970. Trypsinogens and trypsins of various species. pp. 41-42. In: G.E. Perlmann, L. Lorand (eds.). *Methods in Enzymology*, Vol. XIX. Academic Press, New York.