Cibtech Journal of Zoology ISSN: 2319–3883 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjz.htm
2014 Vol. 3 (1) January-April, pp.49-54/Rani
Research Article

NUTRITIVE EVALUATION OF SUPPLEMENTED RAW VS HEAT-PROCESSED SOYBEAN TO REPLACE FISHMEAL AS A DIETARY PROTEIN SOURCE FOR *CIRRHINUS MRIGALA*, MRIGAL

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ABSTRACT
Experiment was conducted to study the effect of raw and hydrothermically processed full-fat soybean as a dietary protein source on growth performance and digestibility in *Cirrhinus mrigala* fish fry, (mean BW 0.54 g). Eight isocaloric (gross energy about 18.27 kj g^{-1}) and isonitrogenous (crude protein about 40%) diets (1–4 raw soybean based and 5–8 processed full-fat soybean based) were formulated. A fishmeal-based diet was used as the reference/control diet. Growth performance, nutrient retention and feed conversion ratio (FCR) in fishes correlated well with the trypsin inhibitor activity levels of the diets. The results revealed that the mrigal fry fed on diet contained 25% autoclaved soybean meal and 75% fishmeal showed maximum increase in Growth (%gain in body weight) (461.11±0.12) , FCR (9.24±0.38) and specific growth rate, SGR (2.77±0.002). Minimum growth parameters were observed in fish fed on raw soybean based diets. Carcass phosphorous levels were significantly ($P < 0.05$) higher in fish fed the fishmeal control diet. Significantly ($P < 0.05$) lower levels of total ammonia excretion and reactive phosphate productions (mg kg^{-1} BW d^{-1}) were recorded where the fish were fed processed full-fat soybean diets compared with the fish fed raw soybean diets.

Keywords: *Indian Major Carp, Trypsin Inhibitor, Fishmeal Replacement, Metabolic Waste*

INTRODUCTION
The Indian major carps *Catla catla, Labeo rohita and Cirrhinus mrigala* are the most important commercial fishes in India with a maximum market demand and acceptability as food by the consumers due to their taste and flesh. They contribute about 67% of total freshwater fish production (ICLARM, 2001). Feeding management plays a critical role in the success of fish culture. The current trend in fish culture is towards increased intensification whereby, provision of feeds becomes necessary and success depends significantly on the availability of well balanced nutritionally complete and cost effective compounded feeds. In India, the aquaculture practices mainly revolve around a few species of finfish and shellfish, among which the Indian Major Carp *Cirrhinus mrigala* contribute substantially to the inland production. Although carp culture is widely practiced, the non- availability of appropriate compounded feed to meet the demands of the species still remains as a major constraint. Fish require adequate nutrition in order to grow and survive. Nature offers a great diversity of food to fish including plants and animals. Artificial feed plays an important role in semi intensive fish culture where it is required to maintain a high density of fish than the natural fertility of the water can support (Jhingran, 1991). The role of artificial feed in intensive fish farming cannot be ignored as nutritional requirements of fish depend upon the feed supplied. The quantity and quality of feed consumed have a pronounced effect on growth rate, efficiency of feed conversion and chemical composition of fish (Hassan *et al.,* 1996; Jena *et al.,* 1998; Erfanullah and Jafri, 1998). In this present systematic study aims to assess the growth and digestibility of Indian major carp *Cirrhinus mrigala* fry with different type of feeds ingredients in formulated diets. Fish fry were fed formulated pellet incorporated with different nutritional supplements like groundnut oil cake, raw soybean, autoclaved soybean and fish meal at different concentrations. This study will be very useful to determine the suitable ratio of supplementary nutrition for weight gain and survival rate of the *Cirrhinus mrigala* fry.

MATERIALS AND METHODS

Diet preparation

Before incorporating in diet soybean was hydrothermically processed (for 15 min at 121°C at 15 lb) after then eight diets (1-4 raw soybean based, 5-8 processed soybean based) with 40% protein level were formulated by blending raw and processed soybean at four different inclusion levels viz. 65, 130, 195 and 260g kg¯1 with fish meal. A diet with fish meal as the protein source was used as reference diet. Ground nut oil cake and rice bran were used as base materials. Wheat flour was added as a binder while and 1%chromic oxide used as an external digestible marker for digestibility estimations. All diets were supplemented with a mineral premix (MPA). After then by using a mechanical pelletizer, 0.5mm thicker pellets were obtained which then dried in oven (60-62°C) before using in feeding trials.

Table 1: Ingredient contents (g kg^{-1}) and proximate analysis (%dry weight basis) of experimental diets of experimental diets

	Diet (g kg^{-1}) Reference diet*	1	2	3	4	5	6	7	8
Ingredients									
Groundnut oil cake	650	650	650	650	650	650	650	650	650
Rice bran	26.23	28.35	30.62	32.84	35.00	28.35	30.62	32.84	35.00
Wheat flour	26.23	28.35	30.62	32.84	35.00	28.35	30.62	32.84	35.00
Fish meal	260.00	195.00	130.00	65.00	---	195.00	130.00	65.00	---
Raw soybean	---	65.00	130.00	195.00	260.00	---	---	---	---
Processed fullfat soybean[a]	---	---	---	---	---	65.00	130.00	195.0	260.00
Cod liver oil	17.54	13.30	8.76	4.32	---	13.30	8.76	4.32	---
Mineral premix with amino acids[b] (MPA)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cr_2O_3	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
proximate analysis									
Dry matter	91.67	93.27	92.23	92.99	91.85	91.87	92.04	93.02	92.77
Crude protein	40.12	40.05	40.11	40.23	40.07	40.09	40.12	40.00	40.09
Crude fat	7.19	7.17	7.11	8.33	8.28	7.89	8.03	8.15	8.23
Crude fibre	4.68	4.17	4.33	4.28	4.56	4.23	4.27	4.38	4.34
Ash	11.67	7.00	7.27	7.23	7.70	7.20	7.31	7.25	7.19

[*] Fish meal [a] processed soybean. Soybean was hydrothermically processed in an autoclave at 121^{0}C (15 Lbs) for 15 minutes to remove antinutritional factors (ANFs) [b] Each kg contains : Copper 312 mg, Cobalt 45 mg, Magnesium 2.114 g, Iron 979 mg, Zinc 2.130 g, Iodine 156 mg, DL – Methionine 1.920 g, L – Lysine Mono Hydrochloride 4.4 g, Calcium 30%, Phosphorus 8.25%

Experimental design

The omnivorous fish fry were obtained from Govt. fish seed farm, Jhajjar (India) and maintained in glass aquaria (60×30×30cms) in laboratory where the temperature was maintained at 25±1^{O}C and lighting schedule of LD 12:12. Fry were acclimated for a minimum period of 10 days prior to the commencement of experiment. The aquaria water was renewed daily with water adjusted to the laboratory temperature (25^{O}C). Fry (mean body weight 0.54g) were randomly distributed @ 15 fish per aquarium with two replicates of each dietary treatment. All fish were fed daily twice at 08$^{\mathbf{00}}$ h and in afternoon at 4.00 p.m. The feeding rate being 5% BWd^{-1} for the whole duration of 90 days. Each group of fish were exposed to their respective diet for four hour during each ration, thereafter, the uneaten feed was siphoned out, stored and dried separately for calculating the feed conversion ratio (FCR). The faecal matters voided by the fish were also collected by siphoning separately from each aquarium. The faecal samples were dried in a hot air oven at 60^{O}C and were subsequently analyzed for digestibility estimations. At the termination of experiment, fish from all the treatments were weighed (length was also recorded) individually to nearest

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gram and processed for subsequent analyses. The pH of aquaria water fluctuated between 7.00-7.25.
ANOVA followed by Duncan's multiple range tests and Student's t-test was applied to assess the significance of the differences among treatments.

Analytical techniques

The experimental diets, fish carcass (Initial and final) were analyzed following the procedure of AOAC (1995). pH was monitored using an automatic analyzer.

Growth Parameters: The growth parameters of the *Cirrhinus mrigala* fry were assessed by taking their body weight at 15 days of interval. The growth performance was assessed using the following formulas:

Percentage weight gain

$$\text{Percentage weight gain} = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100$$

Specific growth rate (SGR)

$$\text{Specific growth rate} = \frac{Ln\ (\text{Final weight}) - Ln\ (\text{Initial weight})}{\text{Experimental periods in days}} \times 100$$

Food conversion ratio (FCR)

$$\text{Food conversion ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

RESULTS AND DISCUSSION

The results of the growth performance, nutrient retention and food conversion efficiency of mrigal fry is presented in Table 3. Growth parameters of mrigal fry with different diets clearly showed significant enhancement with 25% autoclaved soybean and 75% fishmeal when compared with other concentrations of seven diets and control. Mrigal fry showed maximum increase in final live weight (3.03g), Growth (%gain in body weight) (461.11±0.12), FCR (9.24±0.38) and SGR (2.77 ±0.002) were observed in 25% autoclaved soybean and 75% fishmeal based diet (Table-3). There are various other benefits of using autoclaved soybean for fish aquaculture including that of carp such as reduction in culture cost of *Cyprinus carpio* (Ghosh *et al.,* 2003) and Indian major carps (Swain *et al,* 1996). Decrease in growth parameters were observed with the increasing inclusion level of raw soybean in the diet. Our results are in agree with, Mazid *et al.,* (1994); Wilson and Poe (1985); Viola *et al.,* (1983); Sadiku and Jauncey (2002); Garg *et al.,* (2002) where Poor growth performance in tilapia, carp, and mrigal fed diets containing soybean has been attributed to the ANFs present in raw soybean. Feeding mrigal with raw soybean diets resulted in significantly lower carcass protein, fat, and energy, and a higher percentage of moisture (Table-6). Increased digestive enzyme activity (protease, amylase, and cellulase) also support high digestibility and nutrient retention in fish fed supplemented processed soybean diets as compared with the other diets (Table-5). The enzyme systems in *C. mrigala* like those of cyprinids that have long guts, are adapted to digest and absorb nutrients from plant feedstuffs (Bairagi *et al*., 2002; Garg *et al*., 2002). In the present studies excretion of wastes (N-NH4 and o-PO4) was significantly ($P < 0.05$) lower in aquaria with fish fed diets containing processed soybean as the protein source (Table-4). Excretion of metabolites was reduced with each increase in the inclusion level of processed soybean in the diet (Kalla and Garg, 2003; Garg *et al.,* 2002; Singh *et al.,* 2003, 2004) also reported a reduction in $N\text{-}NH_4^+$ and $o\text{-}PO_4^-$ levels with the use of processed soybean in fish diets. Since excretory rates of metabolites depend not only on the fish species, but also on the size, temperature, salinity, and other experimental conditions (Porter *et al.,* 1987; Ballestrazzi *et al.,* 1994, 1998), absolute values cannot be compared between different species; however, trends in excretion/production of metabolites and relative magnitude can be compared. These results demonstrate the suitability of hydrothermically processed full-fat soybean as a dietary protein source for *C. mrigala* when growth, digestibility, nutrient retention and excretion of metabolites are taken into consideration.

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Research Article

Table 3: Effect of replacement of fish meal by soybean on growth performance, nutrient retention and food conversion efficiency in *Cirrhinus mrigala* fry under laboratory conditions (LD 12:12 25±1^{0}C)

Parameters	Diets (g kg^{-1})								
	Reference diet*	1[a]	2[a]	3[a]	4[a]	5[b]	6[b]	7[b]	8[b]
Initial live weight (g)	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Final live weight (g)	2.83	2.26	2.09	2.97	2.88	3.03	2.58	2.37	2.36
Growth (%gain in body weight)	424.07 ±0.32	318.52 ±0.42	287.03 ±0.20	264.81 ±0.33	248.15 ±0.43	461.11 ±0.12	377.78 ±0.20	338.89 ±0.22	337.04 ±0.22
Specific growth rate (SGR)	2.54 ±0.001	1.91 ±0.001	1.72 ±0.003	1.59 ±0.002	1.49 ±0.002	2.77 ±0.002	2.04 ±0.002	2.03 ±0.001	2.02 ±0.001
Food conversion ratio (FCR)	9.89 ±0.23	12.38 ±0.27	13.40 ±0.19	14.21 ±0.37	14.89 ±0.32	9.24 ±0.38	10.85 ±0.30	11.85 ±0.40	11.86 ±0.40
Protein efficiency ratio (PER)	0.204 ±0.03	0.154 ±0.03	0.138 ±0.05	0.149 ±0.01	0.120 ±0.04	0.222 ±0.01	0.182 ±0.01	0.163 ±0.02	0.163 ±0.01

*All the vales are mean ±S.E of mean. * Fish meal, [a] Raw soybean base, [b] Processed soybean based*

Table 4: Effect of replacement of fish meal by soybean on Ammonia and reactive phosphate production in *Cirrhinus mrigala* fry under laboratory conditions (LD 12:12 25±1^{0}C)

Parameters	Diets (g kg^{-1})								
	Reference diet*	1[a]	2[a]	3[a]	4[a]	5[b]	6[b]	7[b]	8[b]
Total ammonia (mg kg^{-1} b w d^{-1})	2608.45 ±79.06	2078.65 ±53.10	2169.55 ±27.67	2379.88 ±66.92	2544.93 ±57.80	1267.22 ±27.87	1883.93 ±86.40	1591.63 ±55.19	1394.65 ±69.04
Reactive phosphate production (mg kg^{-1} b w d^{-1})	2500.90 ±55.28	1656.87 ±35.76	1850.30 ±47.68	1999.90 ±35.23	2056.23 ±43.07	899.98 ±58.35	1000.97 ±28.05	1134.04 ±42.02	1200.01 ±66.25

*All the vales are mean ±S.E of mean. * Fish meal, [a] Raw soybean base, [b] Processed soybean based*

Table 5 Effect of replacement of fish meal by soybean on digestive enzyme activities and viscero-somatic index in *Cirrhinus mrigala* fry under laboratory conditions (LD 12:12 25±10C)

Parameters	Diets (g kg^{-1})									
	Initial	Reference diet	1	2	3	4	5	6	7	8
Specific protease activity [A]	0.57 ±0.17	1.49 ±0.11	1.29 ±0.15	1.24 ±0.16	1.03 ±0.19	1.03 ±0.12	1.56 ±0.02	1.47 ±0.13	1.43 ±0.18	1.40 ±0.19
Specific amylase activity[B]	0.11 ±0.03	0.32 ±0.02	0.19 ±0.00	0.19 ±0.03	0.14 ±0.01	0.14 ±0.01	0.47 ±0.00	0.34 ±0.02	0.30 ±0.01	0.27 ±0.01
Specific cellulase activity[C]	0.19 ±0.34	0.54 ±0.12	0.45 ±0.12	0.35 ±0.22	0.32 ±0.23	0.32 ±0.17	0.67 ±0.02	0.59 ±0.01	0.52 ±0.12	0.50 ±0.11
Viscero somatic index (VSI %)	0.11 ±0.04	0.39 ±0.14	.25 ±0.15	0.19 ±0.14	0.19 ±0.14	0.18 ±0.14	0.57 ±0.12	0.40 ±0.14	0.32 ±0.14	0.32 ±0.10

Values are mean ±SE of mean. [A]Mg of tyrosine mg−1 of protein h−1. [B] Mg of maltose mg−1 of protein h−1 .[C]Mg of glycogen mg−1 of protein h−1

Table 6 Effect of replacement of fish meal by soybean on proximate composition of *Cirrhinus mrigala* fry

Parameters	Diets (g kg^{-1})									
	Initial	Reference diet*	1[a]	2[a]	3[a]	4[a]	5[b]	6[b]	7[b]	8[b]
Moisture	71.23 ±0.60	70.02 ±0.09	72.19 ±0.18	74.01 ±0.16	74.89 ±0.17	75.05 ±0.12	69.15 ±0.18	69.40 ±0.08	70.02 ±0.14	70.07 ±0.31
Crude protein	14.00 ±0.13	17.74±0.32	16.91 ±0.10	16.71 ±0.23	16.02 ±0.13	15.93 ±0.24	18.96 ±0.12	18.09 ±0.17	17.49 ±0.08	17.33 ±0.17
Crude fat	3.00 ±0.19	3.84 ±0.11	3.45 ±0.07	3.21 ±0.14	3.06 ±0.22	2.89 ±0.26	4.24 ±0.21	3.89 ±0.15	3.75 ±0.19	3.69 ±0.16
Ash	3.47 ±0.27	3.40 ±0.22	3.72 ±0.19	3.84 ±0.18	3.94 ±0.15	4.06 ±0.16	3.19 ±0.15	3.21 ±0.38	3.24 ±0.11	3.27 ±0.19

All the values are ±S.E. * Fish meal [a] Raw soybean based [b] Processed soybean based

Conclusion

In this present study growth and digestibility of Indian major carp *Cirrhinus mrigala* fry were assessed. The results revealed that the mrigal fry showed maximum increase in growth and digestibility when we partially replace fish meal with processed soybean meal.

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