

Growth Performance, Body Composition and Digestive Enzyme Activity of Common carp (*Cyprinus carpio*) Fry Fed on Soybean and Horse Gram Supplemented diets

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Abstract An equal mixture of soybean and horse gram flour was added to the traditional carp diet (a mixture of 1:1 rice bran and oilcake in powder form) and fed to triplicate groups of common carp (*Cyprinus carpio*) fry at 10, 20, 30% inclusion levels. Traditional feed mixture without supplementation served as the control diet. All the diets were provided in pellet form to the fish reared in out door cement tanks for 90 days. The test diets significantly ($P < 0.05$) improved fish growth. Survival of fish ranged from 80 to 87.5%. Net fish production was the highest with the 30% inclusion diet. Carcass protein and lipid levels were significantly higher in fish fed the test diets. Activity of digestive enzymes was also affected by the test diets.

Keywords Common carp; Growth; Soya flour; Horse gram; Carcass composition

1 Introduction

Carp are the mainstay of Indian inland fish production, contributing nearly 85% of the total production. Generally, Indian major carps, catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) are grown together in ponds under extensive or semi-intensive culture systems, along with two or three exotic carps, particularly common carp (*Cyprinus carpio*). In low scale Indian carp culture, fish are fed with the traditional mixture of rice bran and oilcake (1:1) in powder form. This mixture is nutritionally inadequate and a sizable portion of the feed applied goes unutilized as the powdered particles sink to the pond bottom. Production in the former system is based on the natural productivity of the pond, while in the latter, supplemental feed plays an important role. Protein requirement of catla and rohu is around 30% (Renukaradhya and Varghese, 1986). The traditional feed mixture does not meet this protein requirement. Though commercial diets provide higher levels of protein, they are prohibitively expensive, effectively excluding resource poor farmers from using such diets. According to De Silva and Anderson (1995), feed cost in culture fisheries accounts for 30-70% of the production

cost, depending on the type of culture and the intensity of feeding. Therefore, from the economic point of view, it would be advantageous to improve the nutritional quality of the traditional feed mixture and also to provide it in pellet form to improve feed utilization and performance. With this in view, soybean and horse gram flours were selected as additional ingredients to upgrade the traditional feed mixture. They were added to the traditional feed mixture at three different levels and provided to fish in pellet form.

Due to wide availability and consistent quality, soybean (*Glycine max L.*) derived products are being extensively used in fish and prawn feeds in recent years to partially replace the fish meal component (Dias, 2005; Hasanuzzaman et al., 2009; Altan et al., 2010; Antolovic et al., 2012; Azarm and Lee, 2012; Yong et al., 2013; Dawood et al., 2015) which results in reduced feed cost. Horse gram (*Macrotyloma uniflorum*) is also a nutritious feed ingredient which contains more than 22% protein and 58% carbohydrate (Marimuthu and Krishnamoorthi, 2013), with high calcium and iron contents and no known antinutritional factors. It is largely used to feed animals, particularly horse. Ravindran and Bino Sundar (2009) recommended its

use in poultry feed in order to reduce the cost of feed.

The present investigation was carried out with the objective of improving the nutritional quality of the traditional feed mixture used in carp culture in India, through supplementation of plant-based ingredients, and in order to reduce wastage, the diets were provided in pellet form, instead of powder form.

2 Materials and Methods

2.1 Feed ingredients and feed formulation

Rice bran, groundnut cake, soybean, horse gram, tapioca flour and vitamin-mineral mixture were procured locally. Soybean flour and horse gram flour were obtained by grinding soybean and horse gram after soaking in water overnight and shade drying. All the ingredients were sieved using ISI standard mesh No.1. Proximate composition of the ingredients used is given in Table 1.

The control diet (T₀) and the 3 test diets were formulated as shown in Table 2. In the test diets, soybean and

horse gram flours were included at 5% (T₁), 10% (T₂) and 15% (T₃) levels each, replacing equal quantity of rice bran and groundnut oilcake and adjusting the proportion of tapioca flour which was used as a binder. The diets were prepared according to Jayaram and Shetty (1981) to obtain pellets of 2 mm diameter. They were then dried in a thermostatic oven at a temperature of 40°C.

2.2 Experimental set up

The experiment was carried out on triplicate basis in 12 outdoor cement tanks of 25 m³ (5x5x1m) each, without a soil base, over a period of 90 days. Ground water was used to fill the tanks, maintaining a depth of 90±5 cm throughout the experimental period. Common carp fry (avg. wt.=0.87g) were stocked at a density of 40 per tank. They were fed every day once in the morning at 5% body weight. Fish were sampled every fortnight to measure body weight and length. The quantity of feed given was re-adjusted after each sampling, based on the weight recorded.

Table 1 Proximate composition (% ± S.E.) of ingredients used in feed formulation

Parameter	Rice bran	Groundnut cake	Soybean flour	Horse gram flour	Tapioca flour
Moisture	9.79 ± 0.15	7.82 ± 0.81	9.68 ± 0.21	5.40 ± 0.12	9.76 ± 0.13
Crude protein	8.37 ± 0.23	33.26 ± 0.23	34.41 ± 0.40	37.81 ± 0.23	4.01 ± 0.21
Lipid	2.03 ± 0.10	7.12 ± 0.02	12.07 ± 0.20	5.40 ± 0.02	0.30 ± 0.01
Ash	19.76 ± 0.03	6.30 ± 0.39	4.01 ± 0.07	6.67 ± 0.04	1.96 ± 0.03
Crude fibre	30.80 ± 0.12	16.16 ± 0.14	6.40 ± 0.05	4.40 ± 0.03	3.50 ± 0.06
Nitrogen-free extract	29.25	29.34	33.43	40.32	80.47
Gross energy (kJ/g)	7.71	15.33	18.22	17.58	14.86

Table 2 Ingredient proportion and proximate composition (± S.E.) of experimental diets

Ingredient proportion (%)	Diets			
	T ₀	T ₁	T ₂	T ₃
Rice bran	45	40.5	36	31.5
Groundnut cake	45	40.5	36	31.5
Horse gram flour	-	5.0	10	15.0
Soya flour	-	5.0	10	15.0
Tapioca flour	9	8.0	7	6.0
Vitamin-mineral mixture	1	1.0	1	1.0
Proximate Composition (%)				
Moisture	5.23 ± 0.07	5.60 ± 0.12	6.52 ± 0.03	6.94 ± 0.10
Crude protein	18.39 ± 0.07	20.26 ± 0.22	22.59 ± 0.20	23.52 ± 0.32
Lipid	3.70 ± 0.2	4.10 ± 0.3	4.60 ± 0.5	4.90 ± 0.2
Ash	17.55 ± 0.27	17.45 ± 0.05	17.05 ± 0.55	17.47 ± 0.45
Crude fibre	21.60 ± 0.34	18.40 ± 0.22	17.20 ± 0.01	16.50 ± 0.50
Nitrogen-free extract	33.53	34.19	32.04	30.67
Gross energy (kJ/g)	11.36	12.05	12.40	12.50

2.3 Water analyses

Water samples from the experimental tanks were collected fortnightly between 09.00 and 10.00 hr for measuring temperature, dissolved oxygen, pH, free carbon dioxide, total alkalinity and ammonia. Water temperature was recorded using a digital thermometer and pH was measured with a digital pH meter (LI-120, ELICO, India). Dissolved oxygen, free carbon dioxide, total alkalinity and ammonia were determined following standard procedures (APHA, 1992). Plankton samples were also collected on fish sampling days, using a net made of No. 30 bolting silk cloth having 60 µm mesh size, by filtering 100 liters of water from different locations of each experimental tank. Dry weight of plankton was determined by drying the filtrate in a hot-air oven at 80°C, till a constant weight was obtained.

The water quality parameters monitored over the experimental duration ranged as follows. Temperature: 27 to 31°C, pH, 7.4 to 8.4, dissolved oxygen: 8.46 to 12.01 ppm, free carbon dioxide: nil to 0.8 ppm, total alkalinity: 55.74 to 73.8 ppm and ammonia: 0 to 13.78 µg at./L. They were within the acceptable optimum range for common carp, with no drastic variation between treatments, and hence would not be a contributory factor for the difference in fish growth among treatments. The average plankton dry weight in the tanks varied from 2.9 to 39.48 mg/100 L, increasing with the progress of the experiment.

2.4 Proximate composition

Proximate composition of the ingredients, diets and fish carcass was analyzed. Protein, lipid, fiber and ash were determined according to AOAC (1995). NFE was obtained by the difference method (Hastings, 1976). The energy content of the feed ingredients and diets was calculated using values of 22.6 kJ/g for protein, 38.9 kJ/g for lipid and 17.2 kJ/g for carbohydrate as NFE (Mayes, 1990).

2.5 Activity of digestive enzymes

The activity of digestive enzymes; protease, amylase and lipase in the intestine and hepatopancreas of the experimental fish was analyzed at the end of the experiment by the methods of Kunitz (1947), Bernfeld (1955) and Bier (1962), respectively.

2.6 Performance indices and statistical analysis

Fish performance in terms of specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency

ratio (PER), condition factor and yield was calculated using the following formulae:

Specific growth rate (SGR) = $[(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{experimental duration in days}] \times 100$.

Feed conversion ratio (FCR) = $\text{Feed consumed (g)} / \text{Weight gain (g)}$.

Protein efficiency ratio (PER) = $\text{Weight gain (g)} / \text{Protein intake (g)}$.

Condition factor (K) = $\frac{W \times 100}{L^3}$

where W= weight of fish (g), L= length of fish (cm).

Yield (g) = $\text{Mean body weight (g)} \times \text{Total number of viable fish at harvest}$.

Comparison among treatments for various parameters was done by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at $P < 0.05$ (Duncan, 1955; Snedecor and Cochran, 1968).

3 Results

Incorporation of soya and horse gram flours to the traditional feed mixture led to an increase in dietary protein and lipid levels and a decrease in fiber content (Table 2). Final weight and length of fish, along with SGR (%) values are shown in Table 3. The three test diets supplemented with soybean and horse gram flours at 5, 10 or 15% each showed a positive impact on the growth of common carp. Fish fed diet T₁ recorded significantly ($P < 0.05$) higher growth than that of the control, while growth of T₂ and T₃ fish groups was significantly higher than that of the control as well as T₁ group. No significant differences in final weight and SGR values were observed between T₂ and T₃ groups. The condition factor among fish groups varied from 1.10 (T₃) to 1.62 (T₁).

Variation in carcass composition was recorded due to feeds. Protein and lipid levels were significantly ($P < 0.05$) higher in fish fed test diets, compared with fish receiving control diet. Protein showed no significant difference ($P > 0.05$) among fish grown on the three test diets, whereas lipid values varied significantly (Table 3). Ash content was higher in fish fed the control diet. Feeding the test diets had a stimulatory effect on the activity of digestive enzymes. Lipase activity in the treated fish was significantly ($P < 0.05$) higher in both intestine and hepatopancreas, while amylase activity was higher only in the hepatopancreas. Protease activity was higher in T₀ fish group, particularly in the intestine (Table 4).

Table 3 Growth parameters (\pm S.E.) of common carp fed experimental diets

Parameter	Diets			
	T ₀	T ₁	T ₂	T ₃
Final weight (g)	14.0 \pm 0.35 ^a	16.79 \pm 0.29 ^b	17.97 \pm 0.42 ^c	18.22 \pm 0.29 ^c
Final length (cm)	9.88 \pm 0.52 ^a	10.11 \pm 0.26 ^a	10.97 \pm 0.31 ^b	11.79 \pm 0.18 ^c
Specific growth rate (%)	3.08 \pm 0.09 ^a	3.16 \pm 0.03 ^b	3.36 \pm 0.08 ^c	3.38 \pm 0.12 ^c
Food conversion ratio	2.62 \pm 0.26 ^a	2.46 \pm 0.12 ^a	2.35 \pm 0.23 ^a	2.31 \pm 0.38 ^a
Protein efficiency ratio	2.38 \pm 0.27 ^a	2.42 \pm 0.04 ^a	2.18 \pm 0.05 ^a	2.20 \pm 0.22 ^a
Condition factor (K)	1.46	1.62	1.36	1.10
Survival (%)	80.00	82.50	82.50	87.50
Yield (g)	1344.00	1662.21	1779.03	1913.10
Carcass proximate composition (%)				
Moisture	84.02 \pm 0.64 ^a	82.99 \pm 0.31 ^a	83.12 \pm 0.21 ^a	82.14 \pm 0.36 ^a
Protein	11.93 \pm 0.03 ^a	12.75 \pm 0.12 ^b	13.07 \pm 0.04 ^b	13.36 \pm 0.10 ^b
Lipid	1.25 \pm 0.04 ^a	1.38 \pm 0.02 ^b	1.43 \pm 0.03 ^c	1.74 \pm 0.08 ^d
Ash	2.80 \pm 0.02 ^b	1.98 \pm 0.25 ^a	2.18 \pm 0.31 ^a	2.16 \pm 0.27 ^a

Initial average weight of fish was 0.87 g

Values in the same row with the same superscript are not significantly different ($P > 0.05$)

Table 4 Digestive enzyme activity (total activity \pm S.E.)¹ in the gut of common carp fed experimental diets

Diets	Protease		Amylase		Lipase	
	Intestine	Hepato ²	Intestine	Hepato	Intestine	Hepato
T ₀	13.01 \pm 0.29 ^c	12.27 \pm 0.03 ^a	37.27 \pm 0.43 ^c	32.78 \pm 0.57 ^a	1.48 \pm 0.25 ^a	0.89 \pm 0.12 ^a
T ₁	12.18 \pm 0.30 ^b	12.66 \pm 0.14 ^a	35.21 \pm 0.52 ^b	43.24 \pm 0.44 ^c	1.91 \pm 0.21 ^b	1.52 \pm 0.22 ^b
T ₂	11.96 \pm 0.34 ^b	11.67 \pm 0.37 ^a	32.12 \pm 0.35 ^a	40.79 \pm 0.09 ^b	1.87 \pm 0.13 ^b	2.03 \pm 0.12 ^c
T ₃	11.12 \pm 0.06 ^a	12.68 \pm 0.31 ^a	34.15 \pm 0.32 ^b	45.41 \pm 0.94 ^c	1.85 \pm 0.12 ^b	2.78 \pm 0.15 ^d

¹Enzyme activity is expressed as μ moles of product liberated/ g tissue/ min. at 30 °C.

²Hepato. = Hepatopancreas.

Values in the same column with the same superscript are not significantly different ($P > 0.05$).

Survival rate (80 to 87.5%) was not significantly ($P > 0.05$) different among all fish groups. Net fish production was the highest in T₃ and lowest in T₀. The highest production obtained in T₃ treatment is attributed to the obvious increment in growth and survival rate compared with other fish groups.

4 Discussion

The results indicate that soybean and horse gram flours added to the traditional diet fed to common carp, *Cyprinus carpio* fry were well utilized. One of the challenges that fish nutritionists face currently is the need to produce nutritionally well balanced, cost effective and eco-friendly diets. Soybean products have been recognized as one of the most appropriate protein sources in aquafeed because of their consistent nutritional composition, high protein content, comparatively well balanced amino acid profile, easy availability, reasonable price and lower fecal nutrient

output (El-Sayed, 1999; Storebakken et al., 2000; Watanabe, 2002; Castro et al., 2011). Further, the digestibility of the protein fraction of soybean products has consistently been reported to be more than 90% for species such as common carp (Takeuchi et al., 2002), channel catfish (Wilson and Poe, 1985), tilapia (Hanley, 1987) and silver perch (Allan et al., 2000). The protein digestibility coefficient of soybean meal (SBM) can be compared favorably with those of any other high-quality protein feedstuff such as various fish meals (NRC, 1993). Lipid digestibility of SBM also is rather high, with values of 81% and 90% for channel catfish (Cruz, 1975) and hybrid tilapia (Hanley, 1987). Consequently, SBM has been shown to be a valuable protein source for different species of fish.

Experiments conducted with channel catfish in ponds have revealed that diets containing 28 to 32% crude protein primarily from SBM provide growth equivalent

to diets containing some animal protein, such as fish meal and meat and bone meal (Robinson et al., 2000; Li et al., 2000). Excellent results have also been obtained using soybean meal-based diets during fry to fingerling rearing of mirror carp (Cremer et al., 2001). Hasanuzzaman et al. (2009) used SBM to replace fish meal in the diet of freshwater prawn, *Macrobrachium rosenbergii*, and reported that the diet with 80% fish meal protein replacement yielded the best results in terms of weight gain, survival, FCR, PER and SGR. Altan et al. (2010) and Azarm and Lee (2012) observed that SBM could partly replace fish meal in the diets of sea bass (*Dicentrarchus labrax*) and black sea bream (*Acanthopagrus schlegeli*). Castro et al. (2011) reported the possibility of replacing 75% of fish meal in the diet of juvenile rainbow trout (*Oncorhynchus mykiss*) with SBM, coinciding with a decline in fecal P and N output. The positive impacts of using soybean in the diets of juvenile saddled bream (*Oblada melanura*), marble goby (*Oxyeleotris marmoratus*) and amberjack (*Seriola dumerili*) has been reported by Antolovic et al. (2012), Yong et al. (2013) and Dawood et al. (2015), respectively.

Soybean is known to contain antinutrients (Francis et al., 2001), particularly trypsin inhibitor that affects fish growth; however, no negative effect of soybean flour on fish growth was observed in the present study. Soaking of soybean before milling and the heat treatment during feed preparation would have eliminated the antinutritional factors to a great extent.

Horse gram is used as an ingredient in mahseer (*Tor khudree*) broodstock diets (Keshavanath et al., 2006). It is a good source of protein with low crude fiber (Sen et al., 1978) and is suitable for use in fish diets at moderate levels (FAO, 1983). It is also a rich source of essential amino acids such as leucine, lysine, phenylalanine, valine, etc. (Swaminathan, 1974).

Soybean and horse gram flours had low fiber levels, compared to rice bran and groundnut cake (Table 1) and the addition of these two protein sources to the traditional feed mixture resulted in reduced crude fiber content in the test diets (Table 2). Fiber content of the diet affects feed digestibility and food retention in the gut, thereby influencing absorption of nutrients. Hilton et al. (1983) observed that dry matter digestion coefficients of the diets declined as the fiber level

increased, and also Meurer et al. (2003) reported a decrease in food retention time with increased crude fiber.

The test diets had higher protein (20.26 to 23.52 vs. 18.39% of control) and fat (4.1 to 4.9 vs. 3.7% of control) levels, which would have contributed to the superior growth of common carp receiving them. An increase of about 2% protein due to soybean and horse gram supplementation in diet T₁ resulted in significantly higher growth of fish. Diets T₂ and T₃ with 4% and 5% higher protein respectively, induced still superior growth. The quantity of natural food encountered in the different tanks during fortnightly samplings was almost similar, increasing with the progress of the experiment, facilitated by the fertilizing effect of fecal nutrients. Its contribution to fish growth can be considered as similar in all the tanks. Even though the protein requirement of common carp is around 30% (Varghese et al., 1976), since carps are generally raised in manured ponds, natural food satisfies a part of their nutrient requirement (Priyadarshini et al., 2011). In such a system, the diets tested in this study would be nutritionally adequate. The condition factor of fish being above 1.0 in all the treatments (Table 3) is indicative of their well being. Condition factor can be vital to culture system management, because it reflects the specific condition under which organisms grow (Araneda et al., 2008).

Higher nutrient content of the test diets contributed to carcass protein and lipid contents that were significantly ($P < 0.05$) higher in fish fed them. Higher carcass protein indicates protein synthesis and increased tissue production as reported in *Clarias gariepinus* fed soybean-based diets (Fafioye et al., 2005). Dietary lipid level is known to influence carcass lipid positively (Bazaz and Keshavanath, 1993; Manjappa et al., 2009). Enzymes play an important role in the digestion and utilization of feed (Dabrowski and Glogowski, 1977) and dietary nutrients influence digestive enzyme activity (Gangadhara et al., 1997; Manjappa et al., 2009). The test diets employed in this study significantly ($P < 0.05$) influenced amylase and lipase activity. Higher digestive enzyme activity in fish receiving the test diets would have resulted in better utilization of diets, leading to higher growth. Higher protease activity in control fish group indicates better utilization of protein from low protein diets. This is reflected by the PER value also (Table 3).

Keshavanath et al. (2002) reported better utilization of protein from low protein-high carbohydrate diets by common carp grown in manured tanks.

Inclusion of soybean and horse gram at the 3 levels marginally increased the cost which was 10.03, 10.62 and 11.20 Rs./kg for diets T₁, T₂ and T₃ respectively, as against 9.45 Rs./kg for the control (T₀). Cost increase (per kg) compared to control diet works out to 6.14, 12.38 and 18.52% respectively as against the 24, 32 and 42% increase in fish production. The higher yield obtained more than compensated the cost increment, the lowest level of supplementation being more cost-effective.

The results of this study clearly indicate that efficiency of the traditional feed mixture used in low-scale carp culture in India can be significantly improved by the addition of soybean and horse gram flours and by providing the feed in pellet form; this supplementation is economically beneficial.

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