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Performance of *Catla catla* (Ham.) fingerlings fed with carbohydrate-rich diets in manured tanks

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Abstract

This study was undertaken to examine the level to which dietary fish meal could be reduced, replacing it with maize, a locally available, cheap carbohydrate source and to study the protein sparing effect of carbohydrate in catla (*Catla catla*), grown in manured tanks. Twelve mud bottomed cement tanks of 18 m^2 (6m x 3m x 1m) each, fertilized initially with poultry manure at 2000 $kg \cdot ha^{-1}$ (3.6 kg per tank) were used. The tanks were subsequently fertilized with poultry manure at 5% of the initial dose at fortnightly intervals. Four diets *viz*. T_0, T_1, T_2 and T_3 containing 0, 10, 20 and 30% fish meal were formulated. Catla fingerlings (av. wt. 1.9 g) stocked at 1 $fish \cdot m^{-2}$ received the diets provided in plastic trays at 5% body weight once daily in the morning hours for 120 days.

The diets contained 18.47, 23.51, 27.78, 31.98% crude protein and 54.48, 46.06, 40.84, 33.89% carbohydrate (NFE), respectively. The highest and lowest growths of fish were obtained with diets T_1 and T_0; fish fed fish meal containing diets exhibited significantly higher growth. FCR in the different treatments was statistically non-significant (P>0.05). PER improved with decreasing dietary protein and increasing carbohydrate. Fish survival ranged from 92.59 to 98.14%. Diets influenced carcass composition and digestive enzyme activity. A significant increase in lipid deposition was recorded with increasing dietary carbohydrate content. A positive relationship was recorded between hepatopancreatic amylase activity and dietary carbohydrate level, while intestinal protease activity was inversely related. Lipase activity was comparatively lower in all the treat-

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ments. The ability of catla to utilize higher levels of carbohydrate, the protein sparing effect of carbohydrate and the economic implications of reducing dietary fish meal content are discussed.

Introduction

Catla catla (Hamilton), a fast growing Indian major carp, is a surface feeder, feeding mainly on zooplankton (Jhingran 1991). It accepts artificial diets and therefore, is a popular species for polyculture in India. Fertilization and artificial feeding are the two important management measures adopted in the country to enhance carrying capacity of ponds. Natural food plays an important role in the growth of carp by directly contributing nutrients and also improving the utilization of artificial diets by supplying certain digestive enzymes (Jhingran 1991).

According to Albrecht and Breitsprecher (1969), the mean protein, carbohydrate and lipid contents of natural food are 51.1, 27.3 and 7.7% respectively, the calorific value ranging from 6.7 to 23.8 $kJ \cdot g^{-1}$. Barash and Schroeder (1984) observed that formulated feeds could be partially replaced by organic manures. A reduction in fish meal, the major protein source of fish diets, is desirable due to high cost and scarcity. According to Pauly et al. (2000), fish meal production is not expected to increase further. Current research is directed at the use of plant ingredients in fish feeds (Mbahinzireki et al. 2001). Inclusion of feedstuff with relatively high level of carbohydrate in formulated fish feeds is preferred in view of its protein sparing action, which makes the diet cost-effective (Hidalgo et al. 1993). Further, carbohydrates improve the pelleting quality and nutrient value of diets (Lovell 1989). Carps and tilapia are known to utilize high levels of carbohydrate (Anderson et al. 1984; Satoh 1991). Hence, incorporation of carbohydrate at higher levels in the diets of these species brings down feed cost substantially.

The purpose of this investigation was to examine the level to which dietary fish meal could be reduced, replacing it with maize, a locally available, cheap carbohydrate source and to study the protein sparing effect of carbohydrate in catla, grown in manured tanks.

Materials and Methods

Diets

The ingredients used in diet formulation (Table 1) were procured from the local market. Locally available commercial fish meal in India is prepared by drying low quality fish on beaches and powdering them. With the result, the quality of the fish meal is poor as it contains high amount of ash (Table 1). Four diets (T_0-T_3) were formulated as per Varghese et al. (1976); fish meal component in them was 0, 10, 20 and 30% respectively. The diets were prepared following the method described by Jayaram and Shetty (1981) to obtain pellets of 3 mm diameter. They were dried overnight soon after pelleting in an electrical oven at a regulated temperature of 40°C.

Experimental set up

The experiment was carried out over a period of 120 days in 12 cement tanks of 18 m^2 each, with a 15 cm thick soil base. The tanks were cleaned and dried, limed at 400 $kg{\cdot}ha^{-1}$ (0.72 $kg{\cdot}tank^{-1}$) and initially fertilized with poultry manure at 2000 $kg{\cdot}ha^{-1}$ (3.6 $kg{\cdot}tank^{-1}$), while subsequent fertilization was done at 5% of the initial dose at fortnightly intervals. The manure contained 2.51% nitrogen, 2.72% phosphorus, 1.95% potassium and 2.30% calcium on dry matter basis. Ground water was used to fill the tanks, maintaining a depth of 90$\pm$5 cm throughout the experimental period. Advanced fry of catla (av.wt. 1.89 g) were stocked at a density of 1 $fish{\cdot}^{m-2}$ (18 per tank). The four diets were fed to triplicate group of fish every day once in the morning at 5% body weight. A minimum of 50% of the stocked fish was collected every fortnight for determining growth. Total length of individual fish was measured using a scale with cm marking. The sampled fish from each tank were weighed together using a field balance to the nearest gram and their average weight was calculated. The quantity of feed given was readjusted after each fish sampling. On termination of the experiment, the surviving fish were weighed, based on which the following parameters were calculated.

$$\text{Specific growth rate (SGR) (\%)} = \frac{Ln_{\text{Final Weight}} - Ln_{\text{Initial Weight}}}{\text{Culture Days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry weight of feed given (g)}}{\text{Wet weight gain (g)}}$$

Table 1. Diet formulation and proximate composition of diets and ingredients

Ingredients (%)	Diets			
	T_0	T_1	T_2	T_3
Fish meal	0	10	20	30
Groundnut oil cake	25	25	25	25
Rice bran	24	24	24	24
Maize	50	40	30	20
Vitamin and mineral mixture[1]	1	1	1	1
Cost (Rs.kg^{-1} diet) (39.60 Rs.=1US$)	6.22	7.62	9.02	10.42
Proximate composition (%) of diets				
Moisture	7.61	8.57	6.70	7.62
	(0.02)	(0.12)	(0.23)	(0.26)
Crude protein	18.47	23.51	27.78	31.98
	(0.0)	(0.30)	(0.13)	(0.10)
Fat	5.98	6.01	6.03	6.12
	(0.18)	(0.07)	(0.13)	(0.21)
Ash	5.15	7.80	10.93	13.00
	(0.12)	(0.16)	(0.23)	(0.12)
Crude fibre	8.31	8.05	7.72	7.39
	(0.91)	(0.34)	(0.28)	(0.84)
NFE	54.48	46.06	40.84	33.89
Energy (kJ·g^{-1})	15.87	15.57	15.65	15.44
P:E ratio	1.16	1.51	1.78	2.07
Proximate composition (%) of ingredients	**Fishmeal**	**Groundnut oilcake**	**Ricebran**	**Maize**
Moisture	8.16	9.46	8.92	8.43
	(0.38)	(0.41)	(0.24)	(0.21)
Crude protein	58.54	39.62	8.17	8.62
	(0.40)	(0.27)	(0.24)	(0.21)
Fat	6.48	8.02	7.26	3.66
	(0.13)	(0.21)	(0.19)	(0.03)
Crude fibre	0	3.42	24.07	3.54
	(0.0)	(0.33)	(0.31)	(0.82)
Ash	18.27	5.74	17.49	1.31
	(0.35)	(0.18)	(1.20)	(1.50)
NFE	8.55	33.74	34.09	74.44
Energy (kJ·g^{-1})	17.22	17.88	10.53	16.17

Figures in parentheses indicate standard error.
[1]Supplevite-M (Sarabhai Company Ltd., India)

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Amount of protein fed (g)}}$$

$$\text{Hepatosomatic index (HIS, \%)} = \frac{\text{Liver weight}}{\text{Somatic weight}} \times 100$$

Water quality

Water samples were collected at fortnightly intervals between 0700 and 0830 hrs for the analysis of temperature, dissolved oxygen, pH, free carbon dioxide and total alkalinity. Water temperature was recorded using a mercury thermometer. pH was measured with a digital pH meter (LI-120, ELICO, India). Dissolved oxygen, total alkalinity and free carbon dioxide were determined following standard procedures (APHA 1992). Simultaneously, plankton samples were collected by filtering 100 L of water from different locations of each experimental tank through a net made of No. 30 bolting silk cloth having 60 µm mesh size. Dry weight of plankton was determined by drying the samples in a hot-air oven at 100°C till a constant weight was obtained. Quantitative estimation of plankton was done by the direct census method using a Sedgewick rafter cell (Jhingran et al. 1969). The planktonic organisms were identified up to the generic level.

Proximate composition

Proximate composition of ingredients, diets and fish carcass was analysed. Protein was determined by Kjeltec (Tecator-1002), lipid by Soxtec (Tecator-1043) and fibre by Fibretec (Tecator-1017) systems. Ash was analysed by incineration (AOAC 1995) and NFE by the difference method (Hastings 1976). The energy content of the feed ingredients and diets was calculated using values of 22.6 $kJ \cdot g^{-1}$ for protein, 38.9 $kJ \cdot g^{-1}$ for lipid and 17.2 $kJ \cdot g^{-1}$ for carbohydrate as NFE (Mayes 1990).

Enzyme assay

The activity of digestive enzymes- amylase, protease and lipase in the intestine and hepatopancreas of the experimental fish was determined on termination of the experiment by the methods of Bernfeld (1955), Kunitz (1947) and Bier (1962), respectively.

Statistical analysis

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

Results

The water quality parameters monitored in the different treatments over the experimental duration ranged as follows: temperature from 19.5 to 22.5^{o}C, pH from 7.64 to 8.65, dissolved oxygen from 4.70 to 8.07mg·L$^{-1,}$ free carbon dioxide from 0 to 2.13 mg·L^{-1} and alkalinity ($CaCO_3$) from 31.68 to 66.93 mg·L^{-1}. These parameters were within tolerable limits for catla (Jhingran 1991). The average plankton dry weight varied from 1.51 to 64.24 mg·100 L^{-1} in T_0, 2.21 to 43.12 mg·100 L^{-1} in T_1, 1.32 to 53.24 mg·100 L^{-1} in T_2 and 1.33 to 68.23 mg·100 L^{-1} in T_3 treatment. Cyanophytes generally dominated the phytoplankton population in all the treatments, followed by chlorophytes and bacillariophytes. Rotifers, copepods, cladocerans, ostracodes and crustacean larvae were the important groups of zooplankton encountered. There was significant variation ($P<0.05$) in planktonic density not only with respect to culture duration, but also different treatments on the same day of sampling (Table 2).

Catla fed with 10% fish meal diet (T_1) attained the highest average individual weight on termination of the experiment. The final average weight recorded under different treatments ranged from 31.48 (T_0) to 48.30 g (T_1). Fish weight in treatments T_2 and T_3 did not differ significantly (Table 3). SGR was also the best in treatment T_1. There was no significant variation in FCR in the different treatments. But PER improved with decrease in dietary fish meal content. The overall survival of fish ranged from 92.59 to 98.14%. HSI in fish receiving the highest level of maize was significantly higher than in the other treatments (Table 3).

Carcass composition revealed a significant decrease in protein and an increase in fat with increasing dietary carbohydrate (maize). No difference ($P>0.05$) in ash level was observed The highest moisture content was recorded in catla from treatment T_1 (Table 3).

Digestive enzyme activity was generally higher in the intestine compared to the hepatopancreas (Table 4). Amylase showed no significant difference ($P>0.05$) in the intestinal tissue, but in the hepatopancreas the highest activity was observed in fish fed diet T_1. There was a progressive increase in the intestinal protease activity with the increase in dietary protein content, but its activity in the hepatopancreas did not vary significantly ($P>0.05$). No significant difference due to diets was noticed in the activity of lipase. Compared to protease and lipase, higher amylase activity was

Table 2. Mean planktonic density (number·L^{-1}) in different treatments

Plankton	Treatment	Days								
		0	15	30	45	60	75	90	105	120
Phytoplankton	T_0	150^{a}	3115^{a}	10737^{a}	26920^{a}	24911^{a}	10920^{a}	6578^{a}	1961^{b}	2563^{ab}
	T_1	158^{a}	1028^{a}	1789^{b}	7880^{c}	2716^{c}	2962^{b}	2945^{a}	7233^{a}	3926^{a}
	T_2	168^{a}	2853^{a}	7305^{a}	11165^{b}	8168^{bc}	12594^{a}	4039^{a}	969^{b}	321^{b}
	T_3	147^{a}	5918^{a}	12491^{a}	22363^{a}	10943^{b}	12672^{a}	2482^{a}	952^{b}	1330^{ab}
Zooplankton	T_0	7^{b}	611^{a}	613^{a}	546^{a}	555^{a}	517^{a}	445^{a}	234^{a}	283^{a}
	T_1	6^{b}	177^{a}	142^{b}	253^{a}	145^{a}	160^{a}	108^{b}	122^{a}	134^{a}
	T_2	6^{b}	391^{a}	621^{a}	365^{a}	248^{a}	178^{a}	91^{b}	194^{a}	81^{a}
	T_3	14^{a}	252^{a}	555^{ab}	206^{a}	407^{a}	287^{a}	413^{a}	133^{a}	207^{a}
Phytoplankton to zooplankton ratio	T_0	21.43	6.00	1.75	49.30	44.08	21.12	14.78	8.38	9.06
	T_1	26.33	5.81	12.60	31.14	18.73	18.51	27.27	59.27	29.30
	T_2	28.00	7.30	11.76	30.59	32.94	70.75	44.38	4.99	3.96
	T_3	10.50	23.48	22.51	108.55	25.78	44.15	6.01	7.16	6.43

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

Table 3. Growth parameters and carcass composition of *Catla catla* in different treatments

Parameter	Treatments			
	T_0	T_1	T_2	T_3
Mean initial individual weight (g)	1.89[a] (0.01)	1.87[a] (0.01)	1.87[a] (0.01)	1.88[a] (0.02)
Mean final individual weight (g)	31.48[c] (0.62)	48.30[a] (0.46)	43.17[b] (0.95)	45.32[b] (0.64)
Mean net weight gain (g)	29.59[c] (0.56)	46.43[a] (0.46)	41.30[b] (0.94)	43.44[b] (0.66)
Daily weight gain (g)	0.25[c] (0.01)	0.39[a] (0.01)	0.34[b] (0.01)	0.36[ab] (0.01)
Specific growth rate (%)	1.01[c] (0.01)	1.16[a] (0.0)	1.14[b] (0.01)	1.15[ab] (0.01)
Food conversion ratio	2.65[a] (0.09)	2.61[a] (0.07)	2.95[a] (0.11)	2.50[a] (0.07)
Protein efficiency ratio (%)	2.03[a] (0.07)	1.55[b] (0.04)	1.21[c] (0.05)	1.24[c] (0.07)
Survival (%)	92.59[a]	98.14[a]	98.14[a]	94.44[a]
Cost of feed·kg fish-^{1} (Rs.)	16.48	19.89	26.61	26.05
Carcass composition (%)				
Moisture	77.66[b] (0.21)	78.37[a] (0.35)	76.48[c] (0.10)	76.61[c] (0.25)
Crude protein	13.95[c] (0.10)	14.72[b] (0.43)	15.15[a] (0.05)	15.16[a] (0.02)
Fat	4.48[a] (0.01)	3.71[b] (0.12)	3.25[c] (0.02)	1.61[d] (0.05)
Ash	3.58[a] (0.01)	3.08[a] (0.02)	3.32[a] (0.18)	3.19[a] (0.10)
Hepato-somatic index	2.04[a] (0.09)	1.64[b] (0.02)	0.94[c] (0.10)	1.68[b] (0.05)

Figures in parentheses indicate standard error. Values with same superscript in each row are not significantly different (P>0.05).

noticed both in the intestine and hepatopancreas of fish from all the treatments.

Discussion

Protein level of the diets used in this study ranged from 18.47 to 31.98% (T_0- T_3). Catla is reported to grow satisfactorily with a fish meal based 30% crude protein diet (Renukaradhya and Varghese 1986). Earlier reports on the protein requirement of carps, indicating values of 30% or more, are based on trials under non-manured condition and the diets used invariably contained higher levels of fish meal (Sen et al. 1978; Singh et al.

Table 4. Digestive enzyme activity in *Catla catla* from different treatments

Treatment	Protease		Amylase		Lipase	
	Intestinal	Hepato[1]	Intestinal	Hepato[1]	Intestinal	Hepato[1]
T_0	0.258[b]	0.090[a]	1.765[a]	0.732[b]	0.109[a]	0.042[a]
	(0.010)	(0.01)	(0.016)	(0.018)	(0.007)	(0.0)
T_1	0.277[ab]	0.088[a]	1.699[a]	1.101[a]	0.084[a]	0.058[a]
	(0.036)	(0.0)	(0.035)	(0.018)	(0.007)	(0.0)
T_2	0.298[ab]	0.116[a]	1.728[a]	0.970[a]	0.119[a]	0.045[a]
	(0.025)	(0.027)	(0.093)	(0.140)	(0.014)	(0.0)
T_3	0.341[b]	0.105[a]	1.604[a]	0.593[c]	0.088[a]	0.041[a]
	(0.014)	(0.013)	(0.019)	(0.016)	(0.022)	(0.0)

[1]Hepatopancreatic

Figures in parentheses indicate standard error. Enzyme activity is expressed in µ moles of product liberated per minute per mg of tissue protein at 28°C. Values with same superscript in each column are not significantly different (P>0.05).

1987; Singh and Bhanot 1988). Even in manured systems, 40% protein requirement has been reported for Indian major carps (Kalla et al. 2004). As against this, the best growth of catla in the present study was obtained with the diet containing 23.51% protein and 46.06% NFE (10% fish meal and 40% maize), having a P/E ratio of 1.51. Therefore, it is clear that natural food has contributed to the nutrient requirement of catla. Nandeesha et al. (1994) reported improvement in the growth of the Indian major carp, rohu due to nutrient contribution by natural food. Further, better growth of catla under T_1 treatment can also be attributed to the protein sparing action of carbohydrate. Mathies et al. (1988) recorded good growth and feed utilization in carp fed a diet containing 33% crude protein, 5% fat and 52% carbohydrate at a temperature of 23°C. Mohapatra et al. (2003) employed six different levels of gelatinized carbohydrate in the diets of rohu (*Labeo rohita*) fry and observed best protein utilization, carbohydrate digestibility and amylase activity with a diet containing 51.7% carbohydrate. Mokoginta et al. (2004) reported that sub-adult gouramy can use dietary carbohydrate as high as 47.5%. Though fish have no true carbohydrate requirement (NAS-NRC 1983), carps tolerate relatively higher levels of carbohydrate in the diet (Shimeno 1982; Viola and Arieli 1983). Protein sparing by carbohydrate has been reported earlier in a few fish species (Jauncey 1982; Rao 1987; Erfanullah and Jafri 1993, 1998; Wilson 1994; Hung et al. 2003). Shiau and Peng (1993) noted that protein sparing effect of carbohydrate in tilapia occurred only when dietary protein was suboptimal. The present results tally with this observation. The significantly lower growth of fish fed diet T_0 reflects that a minimal level of animal protein (fish meal) is necessary in the diet of catla to support good growth.

Apart from supplying essential amino acids, fish meal also supplies some unknown growth factors (Andrews and Page 1974).

There was no significant difference in FCR between treatments, an indication that reduction in protein content of the diet along with an increase in carbohydrate does not affect feed conversion. In contrast, Jauncey (1982) and Erfanullah and Jafri (1998) observed a negative relationship between dietary carbohydrate and FCR. PER was higher with decreasing protein and increasing carbohydrate levels. An inverse relationship between PER and dietary protein content has been reported by Gangadhara et al. (1997) in rohu. The positive relationship observed between dietary carbohydrate and PER in the present study indicates the beneficial effect of carbohydrate inclusion as a non-protein energy source in the diet of catla. Cowey et al. (1972) and Erfanullah and Jafri (1998) recorded an improvement in PER with increasing carbohydrate in rainbow trout and catla respectively.

Increase in dietary carbohydrate resulted in higher carcass fat. Anderson et al. (1984) and Wee and Ng (1986) found fatty carcass in tilapia receiving higher dietary carbohydrate. Wilson (1994) observed that high carbohydrate diets stimulate lipogenic activity. Mokoginta et al. (2004) found significantly higher lipid retention with elevation in dietary carbohydrate level in giant gouramy. According to Erfanullah and Jafri (1998), the absorbed carbohydrate that is not utilized to provide energy gets deposited as lipid in the body. Tacon (1990) suggested that the capacity of a fish to utilize higher amounts of carbohydrate from the diet depends on its ability to convert excess energy to lipid or non-essential amino acids.

Dietary carbohydrate had a stimulatory effect on gut amylase activity. Kawai and Ikeda (1972) and Appleford and Anderson (1996) recorded increased amylase activity in carp fed higher levels of starch. The increase observed in protease activity in the intestine of catla with the increase in dietary protein content is in agreement with the findings that protease activity has a direct relationship with dietary protein level (Kawai and Ikeda 1972; Mukhopadhyay et al. 1978; Gangadhara et al. 1997). However, such an effect was not observed in the hepatopancreas which showed lower protease activity than the intestine. Hidalgo et al. (1999) reported higher proteolytic activity in the digestive tract as compared to that of liver/hepatopancreas in rainbow trout, gilthead sea bream, European eel, gold fish and tench. Proteolytic activity is known to vary depending on the type of diet (Scherbina et al. 1976). Lipase activity in catla from different treatments did not show much variation which could be due to the similar level of fat in the diets.

The costs of the formulated diets are Rs. 6.22 (T_0), 7.62 (T_1), 9.02 (T_2) and 10.42 per kg (T_3), respectively, implying cost reduction with the decrease in fish meal and increase in maize quantity. The significantly higher growth of catla recorded with the diet containing 10% fish meal and 40% maize indicates that replacement of dietary fish meal with maize does not affect the performance of fish in manured tanks, as long as minimal fish meal is present. Effective utilization of carbohydrate by catla, resulting in protein sparing along with the nutrient contribution by natural food led to higher growth of catla. From the results of the present study, it may be concluded that the diet used for catla cultured in manured ponds should have a minimum of 24% protein. The results have great significance in terms of dietary input cost in carp farming, since feed cost works out to 50-60% of the operational cost in semi-intensive carp farming (Veerina et al. 1993).

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