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Effect of Different Levels of Protein, Fat and Carbohydrate on Growth and Body Composition of *Labeo rohita*

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ABSTRACT

Formulation of cheaper diets consisting of locally available ingredients, especially carbohydrate sources, could have a significant impact on carp culture through cost reduction. Protein, fat and carbohydrate are the basic requirement of fish like other vertebrates but the percentage of their requirement is different. Protein is the primary source of energy for fish, then lipid and carbohydrate at the end. Growth of fish is mainly determined by dietary protein input rate whereas dietary lipid and carbohydrate are supplied to satisfy energetic processes that do not require protein specifically, rather than having these fulfilled with dietary protein. There were 200 fingerlings of *L. rohita* distributed into ten treatments, each supplemented with an experimental diet containing 17.1$\pm$0.04kJ DE g^{-1} dietary energy. In experiment I: 35% (35P), 40% (40P) and 45% protein (45P), experiment II: 7% (7F), 12% (12F), 17% fat (17F) and in experiment III: 3% (3C), 5% (5C) and 7% carbohydrate (7C) containing diets were supplemented for 60 days. Growth performance and various parameters of body composition (ash, fat, and carbohydrates) were determined in wet and dry fish weight in order to demonstrate the effect of various diet composition on nutritional value of most economically important carp *Labeo rohita*. Study revealed that diets with increasing carbohydrate levels show better growth among the various dietary treatments of *Labeo rohita* can.

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Introduction

Low input techniques were mostly being practiced in subcontinent in case of carps farming because now a days carps have not a high market price. Formulation of cheaper diets consisting of locally available ingredients, especially carbohydrate sources, could have a significant impact on carp culture through cost reduction (Keshavanath et al. 2002). Protein, fat and carbohydrate are the basic requirement of fish like other vertebrates but the percentage of their requirement is different. Protein is the primary source of energy for fish, then lipid and carbohydrate at the end. Growth of fish is mainly determined by dietary protein input rate whereas dietary lipid and carbohydrate are supplied to satisfy energetic processes that do not require protein specifically, rather than having these fulfilled with dietary protein. The well-known protein sparing action has been demonstrated in many fish species including carps (Erfanullah and Jafri 1995).

Protein has been given priority in nutritional requirement studies because it is the principal diet component for animal growth, and is the highest cost consideration in commercial feeds. The requirement is most commonly understood to mean a minimal percentage of protein needed for optimal growth. However, requirement should rather be termed optimal because a true requirement is a minimal amount of protein needed per animal per day (Guillaume 1997). Investigations into the quantitative dietary crude protein requirements of abalone have been limited to the use of sole protein sources in the experimental diets (Britz 1996), although compound diets have been used by Coote et al. (2000). The protein requirement of fish varies with fish species, fish size, dietary protein quality, dietary non protein energy level and environmental conditions (NRC 1993). Improper protein and energy levels or their ratio will result in an increase in fish production cost and a deterioration in water quality. Insufficient energy in diets causes protein waste due to the increase of dietary protein proportion used for energy, and produced ammonia can reduce the water quality (Hong 1999).

Lipids play an important role in fish nutrition as a source of energy and essential fatty acids (EFA) to maintain biology of cell membranes (Sargent et al. 1999). It was well known that fish utilizes protein preferentially to lipid or carbohydrate as an energy source, but lipid also plays an important role in fish diets, especially for carnivorous fish species. Within certain limits, increasing dietary lipid levels improve diet utilization (Peres and Oliva-Teles 1999). Lipid is one of the major sources of metabolic energy in fish. In terms of energy supplied per kilogram, dietary lipid should have the greatest protein sparing effect (Ellis and Reigh 1991). The main protein sparing effect of lipid is to replace the protein which would otherwise have been catabolized but lipid may also replace protein which would otherwise be used to synthesize lipid (Lee and Putnam 1973). Although the protein sparing effect of dietary lipid has been extensively reported for many species, information on Indian major carps is scarce and the results are not consistent (Watanabe 1982; Satpathy et al. 2003). Some authors have observed no protein sparing effect of lipid in some fish species (Regost et al. 2001) while very little information exists on warm water marine species (Pe´rez et al. 1997), and sea bream in particular (Vergara et al. 1996).

Carbohydrate rich feeds have the advantage of being cheaper and their incorporation also improves pallet quality and nutritive value. Carp have the ability to utilize higher carbohydrate levels, storing energy reserves as glycogen in the liver and muscle. Inclusion of feedstuffs with relatively high levels of carbohydrate in formulated fish feed is preferred in view of its protein-sparing action that may make the diet more cost effective (Hidalgo et al. 1993). Invariably, fish farmers in India employ manure in carp culture as it increases fish yields (Sharma and Olah 1986), mainly through mineralization by microbial degradation (Zhu et al. 1990) and increased natural food production. Barash and Schroeder (1984) observed that formulated feeds could be partially replaced by manures. The feed cost per unit of fish produced can be minimized by optimal use of low-cost energy carriers such as carbohydrate-rich ingredients, ensuring that the use of costly protein is kept as low as possible. Replacing dietary protein energy by lipid or carbohydrate energy may result in a higher production per unit spent of costly protein sources, such as fish meal, and the effluent nitrogen can be reduced per unit of fish produced. The aim of three experiments conducted during this project was to determine the suitable and cost effective combination of protein, fat and carbohydrate in diet of *L. rohita* to get the maximum output in *L. rohita* culturing in Pakistan.

Materials and Methods

Experimental diet

Ten experimental diets having dietary energy (17.1$\pm$0.04kJ DE g^{-1}) were formulated for three experiments at Shabir Fish feeds Multan, Punjab, Pakistan. All diets had different levels of crude protein, fat and carbohydrate (dry weight). Ingredients and proximate composition of all experimental diets are presented in Table1. Experimental diets were analyzed using standard AOAC (1995). All the experiments were approved by the research and ethic committee of Bahauddin Zakariya University Multan, Pakistan.

Experimental design and feeding trials

Experiments were conducted at Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan. A total of 200 *L. rohita* fingerlings were collected from Al-Madina Fish Hatchery Matital Road Multan, Punjab, Pakistan. All the fishes were transferred to experimental lab in oxygen-filled polythene bags where they were acclimatized to experimental condition for 2 weeks.

At the start of experiments, average initial weight and initial length of fishes were measured. All fishes were transferred to twenty fiberglass tanks. Each tank was divided into 10 compartments (1x1x1 ft) and 10 fishes in each compartment with the help of fiberglass partitions each containing a fish. Fish were fed twice a day, with equal portion, (9:00am and 9:00pm) by hand. The feeding rate was 4% of body weight of fish which was recalculated after interval of fifteen days (Khan et al., 2004). The waste material, diet and faeces of fishes were siphoned daily from each tank. During the experimental period, the water temperature, dissolved oxygen and pH were at 22 $\pm$ 2^{o}C, 7.31 $\pm$ 0.46 mg L^{-1}, and 7.02 $\pm$ 0.47 respectively.

Growth performance

At the end of experiment, fishes were weighed and length was taken. Specific Growth Rate (SGR), Weight Gain (WG) and Protein Efficiency (PE) were calculated by using formulae in footnotes in Table 2 Du et al. (2005).

Sample collection and analysis

At the end of the experiment, the fishes were chill-killed by immersing in an ice water. All the chemical analysis were carried out in triplicates. Moisture and dry weight (oven dry at 60°C to constant weight), ash (incinerate at 550°C for 5 hrs in a muffle furnace), fat (chloroform-methanol method: Bligh and Dyer 1959; Cui and Wootton 1988; Salam and Davies 1994) and protein (subtracting fat content from organic content: Salam and Davies 1994) of all the feeding groups based on whole body weight that were carried out following AOAC (1995).

Statistical Analysis

Data were expressed as mean value along with standard deviation and were subjected to one-way analysis of variance (ANOVA) by using statistical software Minitab which is used to calculate the differences in various parameters among all feeding groups.

Results

Effect of dietary protein variations on growth performance and body composition of L. rohita

There were significant ($P<0.01$) differences amongst four feeding groups with respect to SGR & WG and was non-significant ($P>0.05$) difference for PE. SGR and WG were higher in 45P and lower in 40P. PE was slightly high in 45P. While in case of body composition, there were highly significant ($P \leq 0.001$) differences for percent water, dry mass, organic content, fat content and protein content, and significant ($P<0.01$) difference for percent ash. Water and dry weight were high in 45P and control (C) while they were low in C and 45P respectively. Control (C) also showed maximum value for percent ash, organic and fat content but lowest value for percent protein. Percent protein was high in 40P (Table 2, 3).

Table 1: Feed formulation and proximate composition of diets (1 to 10) used in present study

Ingredients (gm/kg)	International Feed Number	Diets									
			Experiment I			Experiment II			Experiment III		
		Control	35P	40P	45P	7F	12F	17F	3C	6C	9C
Fish meal	5-09-835	230	-	-	-	100	100	100	100	100	100
Canola meal	5-06-145	50	50	90	90	84	90	90	90	90	90
Corn gluten (60%)	5-28-242	254	320	460	570	105	358	378	152	160	144
Rice bran	4-03-928	126	80	130	80	80	334	280	180	142	136
Rice polish	4-03-943	210	320	180	110	61	30	20	80	80	80
Soybean meal	5-04-604	50	146	50	50	570	70	50	409	409	400
Animal Fat[1] (milk fat)		10	14	20	30	30	54	114	29	29	30
Starch	5-01-162a	40	40	40	40	40	40	40	30	60	90
Canola oil[2]		10	10	10	10	10	4	8	10	10	10
Mineral[3] & Vitamin[4] Premixes		10	10	10	10	10	10	10	10	10	10
Di-calcium Phosphate		10	10	10	10	10	10	10	10	10	10
Total		1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Proximate analysis (percent) (Dry weight)											
DE (kJ g^{-1})		17.1	17.3	16.8	16.9	16.8	17.5	17.9	16.9	17.0	16.9
Crude protein		38.3	34.8	40.2	44.5	38.1	37.2	37.4	38.1	38.5	38.2
Crude fat		9.1	8.5	8.2	7.9	7.4	12.4	17.5	8.3	8.2	8.2
Ash		9.66	6.69	5.65	4.48	7.94	7.47	6.61	7.79	8.3	8.5
Crude Fiber		4.08	4.89	4.62	3.82	6.82	5.72	4.88	3.23	6.3	9.1
Cost kg^{-1} (US$)		0.126	0.123	0.126	0.139	0.121	0.126	0.131	0.114	0.119	0.127

DE: Dietary energy

35P: 35%Protein; 40P: 40%Protein; 45P: 45%Protein; 7F: 7%Fat; 12F: 12%Fat; 17F: 17%Fat; 3C: 3%Carbohydrate; 6C: 6%Carbohydrate; 9C: 9%Carbohydrate.

Proximate composition, digestible energy and metabolizing energy are taken from "Nutritional Requirements of fish" National Academy of Sciences 1993.

1- BLUE BAND (Unilever Pakistan Ltd) containing skimmed milk, milk fat, salt stabilizer, preservatives, Vit. A, B, D and calcium.

2- SEASON CANOLA OIL (Wali Oil Mills Lahore, Pakistan) contains Fat profile 6%, Saturated fat 62%, Poly saturated fat (linolic acid) 11%.

3- SB MINERAL MIX(SB Pharma, Rawalpindi, Pakistan) containing (kg^{-1}); Copper $5x10^3$mg; Ferrous $5x10^4$mg; Manganese $6.2x10^4$mg; Zinc $3x10^4$mg; Iodine $5x10^2$mg & Selenium 1x102mg.

4- SB VITA-L (SB Pharma) containing (kg^{-1}); A $5x10^6$IU; D_3 $5x10^6$IU; E $7.5x10^3$mg; K_3 $5x10^2$mg; B_1 $1x10^3$mg; B_2 $2.5x10^3$mg; B_6 $1.5x10^3$mg; B_{12} 10mg; Niacin $1.5x10^4$mg; Biotin 2.5x10mg; Pantothenic acid $4x10^3$mg; Folic acid $5x10^2$mg; Anti Oxidant 5 $x10^3$mg & Carrier(upto) 1 $x10^3$gm.

Table 2: Mean values and standard deviation (Parenthesis) of SGR, WG & PE of *Labeo rohita* for ten different feeding groups

Growth Parameters	Feeding Groups	Experiment I			Experiment II			Experiment III		
	Control	**35P**	**40P**	**45P**	**7F**	**12F**	**17F**	**3C**	**6C**	**9C**
SGR[1] (%day^{-1})	2.559^{b} (0.169)	2.566^{b} (0.171)	2.210^{a} (0.432)	2.591^{b} (0.280)	2.797^{b} (0.147)	2.882^{bc} (0.423)	2.670^{b} (0.266)	2.621^{b} (0.386)	2.736^{b} (0.397)	3.160^{c} (0.096)
WG[2] (%)	366.52^{ab} (42.22)	368.57^{ab} (46.28)	287.20^{a} (84.17)	379.89^{b} (80.69)	437.76b^{c} (47.23)	480.70^{cd} (146.52)	402.04^{bc} (75.39)	392.53^{bc} (94.48)	427.87^{bc} (95.84)	566.88^{d} (38.46)
PE[3] (g)	0.090^{a} (0.016)	0.094^{ab} (0.019)	0.088^{a} (0.013)	0.094^{ab} (0.008)	0.144^{d} (0.029)	0.141^{d} (0.008)	0.109^{bc} (0.006)	0.109^{bc} (0.018)	0.120^{c} (0.015)	0.148^{d} (0.023)

All the values are means ± SD, All the vales are verified by homogeneity of variance, Mean sharing the same superscripts do not differ significantly (P>0.05)

35P: 35%Protein; 40P: 40%Protein; 45P: 45%Protein; 7F: 7%Fat; 12F: 12%Fat; 17F: 17%Fat; 3C: 3%Carbohydrate; 6C: 6% Carbohydrate; 9C: 9%Carbohydrate.

1-Specific Growth rate (%day^{-1}) = (ln final weight – ln initial weight) × 100/ days

2-Weight Gain (%) = (final weight – initial weight) × 100/ (initial weight)

3-Protein Efficiency (g) = Final weight – Initial weight / protein intake

Table 3: Mean values and standard deviation (Parenthesis) of various body constituents and condition factor of *Labeo rohita* for ten different feeding groups

Body constituents	**Feeding Groups**									
		Experiment I			**Experiment 2**			**Experiment 3**		
	Control	**35P**	**40P**	**45P**	**7F**	**12F**	**17F**	**3C**	**6C**	**9C**
% Water	82.2^{a} (3.027)	82.43^{ab} (2.199)	83.33^{abc} (2.201)	85.07^{cde} (1.905)	87.30^{ef} (2.468)	87.51^{f} (1.557)	85.70^{df} (1.064)	87.08^{df} (1.560)	87.03^{df} (2.831)	84.82^{bd} (1.630)
% Dry weight	17.767^{f} (3.027)	17.571^{ef} (2.199)	16.674^{def} (2.201)	14.926^{bcd} (1.905)	12.701^{ab} (2.468)	12.488^{a} (1.557)	14.295^{ac} (1.064)	12.922^{ac} (1.560)	12.974^{ac} (2.831)	15.182^{ce} (1.630)
%Ash (wet weight)	3.121^{c} (0.781)	2.013^{ab} (0.315)	2.750^{c} (0.602)	2.120^{b} (0.276)	1.716^{ab} (0.383)	1.679^{ab} (0.223)	1.876^{ab} (0.245)	1.753^{ab} (0.251)	1.614^{a} (0.432)	2.117^{b} (0.336)
% Organic content (wet weight)	17.545^{f} (3.025)	17.413^{ef} (2.225)	16.492^{def} (2.217)	14.780^{bcd} (1.924)	12.588^{ab} (2.493)	12.374^{a} (1.566)	14.155^{ab} (1.068)	12.779^{abc} (1.573)	12.854^{abc} (2.848)	15.067^{cde} (1.644)
% Fat (wet weight)	9.931^{d} (1.980)	8.023^{c} (1.673)	5.245^{ab} (1.533)	4.641^{a} (3.030)	4.290^{a} (1.775)	4.743^{a} (0.891)	4.307^{a} (1.782)	4.766^{a} (1.004)	4.588^{a} (1.421)	7.067^{bc} (1.242)
% Protein (wet weight)	7.614^{a} (1.754)	9.390^{abd} (1.615)	11.246^{d} (1.705)	10.139^{cd} (3.595)	8.298^{abc} (1.660)	7.631^{a} (0.916)	9.848^{bcd} (1.771)	8.013^{ab} (0.915)	8.266^{ac} (1.771)	7.999^{ab} (0.696)

All the values are means ± (SD), All the vales are verified by homogeneity of variance, Mean sharing the same superscripts do not differ significantly (P>0.05)

35P: 35%Protein; 40P: 40%Protein; 45P: 45%Protein; 7F: 7%Fat; 12F: 12%Fat; 17F: 17%Fat; 3C: 3%Carbohydrate; 6C: 6%Carbohydrate; 9C: 9%Carbohydrate.

Effect of dietary fat variations on growth performance and body composition of L. rohita

WG and PE were higher in 12F. No significant (P>0.05) differences were observed in SGR among various feeding treatments. Control (C) had lowest mean values for WG and PE. Highly significant (P<0.001) differences were observed for percent water, dry weight, ash, organic content and fat content but differences among treatments were significant (P<0.01) for percent protein. Percent water and protein were high in 12F and 17F respectively (Table 2, 3).

Effect of dietary carbohydrate variations on growth performance and body composition of L. rohita

There were highly (P<0.001) significant differences among the feeding groups in WG and PE while significant (P<0.01) difference for SGR was observed. Highly significant (P≤0.001) differences were found in percent water, dry weight, ash, organic content and fat. No significant (P>0.05) difference was observed for percent protein.

Overall better growth was observed by increasing carbohydrates with constant protein and fat content (38% protein and fat 8.5%).

Discussion

Several investigators have reported that optimum growth of *Labeo rohita* takes place when it is supplemented with 40–50% protein diets (Sen et al. 1978; Renukaradhya and Varghese 1986; Erfanullah and Jafri 1995). Our experimental results reveled that varying protein levels with constant lipid levels affect fish growth in experiment I. 45P showed maximum growth although that was not significantly better than group C. Similar results were obtained by Satpathy et al. (2003) while working on *L. rohita*. They observed 45% protein with 15% lipid in diets of *L. rohita* was optimal for the growth but Varghese et al. (1976) found opposite results that the optimum protein requirement of common carp to be 310 g kg–1 diet. Similarly Kim et al. (2001) found non-significant differences for 35, 40 and 45% protein in diet of juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf).

Whole-body composition data showed that the protein content increased with the dietary protein levels, while an opposite pattern was recorded for lipid content, which decreased correspondingly. This trend is well supported by the results from channel catfish (Murai et al. 1985). Body moisture increased with the dietary protein levels and showed an inverse relationship with whole-body lipid content. Body ash was unaffected by the dietary protein levels as noted for other fish species such as brown trout (Elliot 1976), tilapia (Jauncey 1982) and catfish (Khan et al. 1993). PE in the present study was directly related to the dietary protein levels linearly; i.e. maximum efficiency occurred at the highest dietary protein level. The trend has also been noted for other species such as grass carp (*Ctenopharyngodon idella* Val.), tilapia (*Sarotherodon mossambicus* Perters) and catfish (*Mystus nemurus* C. and V) (Dabrowski 1977; Jauncey 1982; Khan et al. 1996).

In experiment 2, non-significant differences in growth and body composition were obtained by increasing fat from 7- 17%. Bright et al. (2005) found similar results working on largemouth Bass *Micropterus sulmoide* with 7-16% lipid. Inverse results were obtained with juvenile grass carp (*Ctenopharyngodon idella*) by Du et al. (2005).

Linear increase in growth rate, WG and PE were observed with increase of carbohydrates. The protein sparing action of carbohydrate has been demonstrated in many fish species (Shiau and Peng 1993; Erfanullah and Jafri 1995). Erfanullah and Jafri (1995) reported more pronounced protein sparing by carbohydrate at suboptimal levels of protein than at optimal levels in fingerling *L. rohita*. This was also true for the present study as diets with protein contents of 37% with increase of carbohydrates induced similar growth. The studies of Ufodike and Matty (1983) showed that carp performed well when cassava or rice was included at 450 g kg–1 diet in a 300 g protein kg^{-1} diet as compared with150 g kg–1 diet and 300 g kg–1 diet, rice inducing higher growth than cassava. Use of high energy-low protein diet has the advantage of reduced nitrogen waste in the culture system. Not only did the higher levels of carbohydrate spare protein, but they also altered body composition, particularly the lipid level. There was a significant increase in lipid deposition in the carcass with increasing carbohydrate level in the diet. Interestingly, the protein contents of fish carcasses from different treatments were almost similar as already observed by Keshavanath et al. (2002) while working on common carp. PER also reflected better utilization of diets with lower protein content. PER was known to improve with decreasing dietary protein (Gangadhara et al. 1997).

Based on the present results it may be concluded that increase in protein level (upto 45%) has no growth increasing effect. Similar fashion was also observed in case of increasing lipid level (up to 17%) but increasing carbohydrates showed better growth for *L. rohita*. Therefore, use of carbohydrate rich diets in the carp culture can improve profitability.

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