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# Effect of Various Concentrations of Protein, Fat and Carbohydrate in Diet on Growth and Body Composition of Most Economically Important Fish in Pakistan, *Labeo rohita*

K. UMER, M. ALI, R. IQBAL, N. T. NAREJO[++]* A. R. ABBASI*

Institute of Pure and Applied Biology, Zoology Division. Bahauddin Zakariya University Multan, Pakistan.

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**Abstract:** 200 *L. rohita* fingerlings were distributed into ten treatments, each supplemented with experimental diet containing 17.1±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, 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 shows better growth among the various dietary treatments of *Labeo rohita* can.

**Keywords**: Diet composition, Body composition, *Labeo rohita*, Specific growth rate.

## 1. INTRODUCTION

Low input techniques are mostly being practiced in subcontinent in case of carps farming because carps have not a high market price. Carp culture could be more beneficial if the fish feed is manufactured from locally available sources, especially carbohydrate as it will significantly reduce the feed cost (Keshavanath *et al*., 2002; Umer *et al*., 2011). 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. Protein in fish diet is the main determinant of fish growth while lipid and carbohydrate are added to diet to maintain the non protein dependant metabolic processes (Tacon & Cowey ,1985). This protein sparing mechanism is well known and documented in several fish species including carps (Erfanullah & Jafri, 1995).

When ever studies are conducted for diet formulation in animals, proteins are considered as primary component as it is ultimate requirement for growth and also it has highest share in feed cost. A minimal percentage of protein is always required in diet for optimal growth (Guillaume, 1997; Ali *et al*., 2005). Fish protein requirements varies with fish size, species, dietary non protein energy level, dietary protein quality and environmental conditions (NRC, 1993). Generally in feed formulation studies, the crude dietary protein required by fish is obtained by few traditional sources (Britz, 1996) and rarely compound diets are used (Coote *et al*., 2000). Dietary protein levels are important as improper protein levels will lead to wastage of energy or their disturbed ratio will raise the fish production cost and will also deteriorate the quality of fresh water by over production of ammonia (Hong, 1999).

Fish prefer to utilize protein as primary energy source rather than using lipid or carbohydrate but lipid is an important part of fish diets, especially for carnivorous fish species (Ali *et al*., 2006). Lipids are important component in fish diet as they are rich source of essential fatty acids (EFA) and hence energy and also required to maintain the cell and organelle membranes (Sargent *et al*., 1999). Diet utilization by fish can be improved increasing lipid proportion to certain extant under specific conditions (Peres & Oliva-Teles, 1999). Lipid in diet should have the greatest protein sparing effect as they replace those proteins which would have otherwise been catabolized (Ellis & Reigh, 1991). Information on Indian major carps is limited regarding the protein sparing effect of dietary lipid although this topic has been extensively investigated for several fish species (Pe´rez *et al*., 1997; Satpathy *et al*., 2003) and even it is observed in certain species that dietry lipid has no protein sparing effect (Vergara *et al*., 1996; Regost *et al*, 2001).

Fish feed rich in carbohydrate is cheapest among all the commercially available diets, it has high protein sparing efficiency and presence of carbohydrates in feed also improves the nutritive value and pallet quality (Zhu *et al*., 1990). Carp are capable of utilizing carbohydrate rich diets, can store the reserve energy as glycogen in their liver and muscle (Hidalgo *et al*., 1993) By optimizing the use of low cost energy carriers (e.g. carbohydrate-rich ingredients) and decreasing the ratio of costly proteins in diet to minimum, feed cost per fish

[++]Correspondence ,author: dr_ntnarejo46@yahoo.com
*Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro.

produced can be significantly minimized. This would be more beneficial as by replacing the energy provided by dietary protein with that available from lipid or carbohydrate may result in a higher production per unit spent and the excreted nitrogen levels 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 *Labeo rohita* culturing in Pakistan.

## 2. MATERIALS AND METHODS

**Experimental diet**

Ten experimental diets having dietary energy (17.1$\pm$0.04kJ DE $g^{-1}$) were formulated at Shabir Fish Feeds Multan, Punjab, Pakistan. These formulated diets varied regarding ratio of crude protein, fat and carbohydrate in their composition (Table1) Experimental diets were analyzed using standard AOAC (1995). All the experimental protocols and fish handling procedure were approved by the research and ethic committee of Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan.

**Experimental design and feeding trial**

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 length of fishes were measured. All fishes were transferred to twenty fiberglass tanks. Each tank was divided into 10 compartments (1x1x1 ft) 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 with was recalculated after interval of fifteen days (Khan *et al*., 2004). Waste, diet and faeces were siphoned daily from each tank. During the experimental period, the average water temperature, dissolved oxygen and pH were 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, fish 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 following Du *et al*. (2005).

**Sample collection and analysis**

At the end of the experiment, fish were chill-killed by immersing in ice water. All the chemical analyses were carried out in triplicate. 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: Cui and Wootton, 1988; Salam and Davies, 1994) and protein (subtracting fat content from organic content: Salam and Davies (1994) of all feeding groups based on whole body weight were carried out following AOAC (1995).

**Statistical Analysis**

Data was expressed as mean along with standard deviation and was subject to one-way analysis of variance (ANOVA) by statistical software Minitab (Version 11, USA) to calculate the differences in various studied parameters among all feeding groups.

## 3. 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 ($P\leq0.001$) 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. 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 1, 2, 3).**

**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. 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. 9C showed maximum and C showed minimum mean values for all growth parameters. Highly significant ($P\leq0.001$) 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%).

## 4. 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 & Varghese, 1986; Erfanullah & 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 C group. 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 Kim *et al*. (2001), following their experiments on juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf), observed non significant differences in fish growth following 35 %, 40 % and 45 % dietry protein supplementation.

Proximate-body composition data analysis indicated that the protein content in fish body increased while lipid contents decreased following an increase in dietary protein levels. These results are in agreement with Murai *et al*. (1985) who had similar observations during their experiments with channel catfish. Results indicated that water contents of *L. rohita* increased following a rise in dietary protein levels while an inverse relationship of water content was observed with body lipid content. Inorganic contants of *L. rohita* remained unaffected by changing the dietary protein levels. Similar trend was also reported in tilapia by Jauncey (1982) and in cat fish by Khan *et al.* (1993).

Dietary protein levels and PE were directly related in the present study; i.e. maximum efficiency occurred at the highest dietary protein level. This corelation has also been previously reported in other fish species like grass 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 where 7-17% fat was used in fish diet, non significant differences in growth and body composition were observed. Bright *et al*. (2005) had reported similar results while feeding 7-16 % lipid to large mouth Bass (*Micropterus sulmoide*). While contrdictory results were observed by Du *et al*. (2005) with working with juvenile grass carp (*Ctenopharyngodon idella*).

Our results indicated a linear increase in growth rate, WG and PE with increasing carbohydrate levels in diet. Erfanullah and Jafri (1995) had reported more pronounced that sub optimal levels of carbohydrate are more effective than optimal levels for protein sparing in fingerling *Labeo rohita*. Our results are also in agreement with Erfanullah and Jafri (1995) as diets having 37 % protein contents and those with increasing levels of carbohydrates observed similar growth. Ufodike and Matty (1983) had demonstrated that carp showed better growth when cassava or rice was included at the rate of 450 g kg–1 diet to a 300 g protein $kg^{-1}$ diet. Higher levels of carbohydrate not only spare proteins but they also changes the fish body composition, especially they affects the lipid levels. We observed a significant increase in lipid deposition in the carcass with increasing carbohydrate level in the diet during present study. 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 and this observation is in agreement with Gangadhara *et al*. (1997) who had also reported an improvement in PER following decreasing dietary protein in *L. rohita*.

Based on the present results it may be concluded that increase in protein level (up to 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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**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 Parameter | 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 different 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 different 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.

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