

Full Length Research Paper

## Effect of various nutrient combinations on growth and body composition of rohu (*Labeo rohita*)

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A total of 80 *Labeo rohita* fingerlings (mean body weight,  $14.7 \pm 0.08$  g and length,  $11.0 \pm 0.16$  cm) were randomly distributed into four treatments with 20 replicates each, for 60 days, to determine the effect of different feed compositions on the growth and body composition of *L. rohita*. Four isoenergetic ( $17.05 \pm 0.24$  kJ g<sup>-1</sup>) experimental diet viz., control (C), protein rich (PR), fat rich (FR) and carbohydrate rich (CR) were formulated. The proximate composition protein/fat/carbohydrate (P/F/C) of formulated feed were C: P35/F8/C2, PR: P40/F8/C2, FR: P35/F10/C2 and CR: P35/F8/C5. The daily ration size was 5% of fish body weight. The result reveals a highly significant ( $P \leq 0.001$ ) difference in specific growth rate (SGR), weight gain (WG) and protein efficiency (PE) among four feeding groups, while differences were significant for feed conversion ratio (FCR). FR showed maximum growth together with high body fat, CR showed low body fat and high proteins. Results indicate that increasing fat up to 9% in diet showed better growth as compared to increasing dietary protein and carbohydrates

**Key words:** *Labeo rohita*, diet composition, specific growth rate, protein efficiency, body composition.

### INTRODUCTION

All over the world, aquaculture has become the fastest-growing food production sector of the world, with an average annual increase of about 10% since 1984 when compared with 3% increase for livestock meat and 1.6% increase for capture fisheries (FAO, 1997). To sustain such a high rate of increase in aquaculture production, a similar increased level of fish feed production is required. Fish meal covers a major proportion of diet to fulfill the demand of protein (Tacon and Metian, 2008). The major problems confronting the fish farming industry are the increasing cost and inadequate supply of fish meal and the competition of other live stock industries for fish meal (Siddhuraja and Becker, 2003; Ali et al., 2005, 2006a). Over the last decade, carp culture in India and Pakistan has been intensified and there is an increasing demand for cost-effective aqua feeds. Provision of dietary protein contributes significantly to feed cost and it is desirable that it is used for growth rather than for provision of

dietary energy. Adequate levels of non-protein energy sources (lipid and carbohydrate) in the diet may minimize catabolism of protein (Cho and Kaushik, 1990; Iqbal et al., 2006; Ali et al., 2006b).

The significance of quantitative and qualitative protein in aqua feeds is well recognized (Cho et al., 1985; Shiao and Huang, 1989; Umer and Ali, 2009). Dietary protein requirements of a fish species is of fundamental importance in aquaculture, because dietary protein significantly influences growth, survival and yield of fish as well as economics of a farming industry by determining the feed cost which is typically the largest operational cost in aquaculture (Islam and Tanaka, 2004).

Lipid is digested and metabolized with greater relative ease and so serves better as an energy source for protein sparing than carbohydrate. On the other hand, excess lipid results in fat fish. The protein-sparing effect of lipid varies between species (De Silva and Anderson, 1995). Although, fish in general have a limited capacity for carbohydrate utilization (Bergot, 1979), thermal modification of raw dietary carbohydrate, that is, gelatinization, has been found to improve this, resulting in an energy source which is comparable with protein and

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**Table 1.** Feed formulation and proximate composition of diets (1 to 4) applied in the present study.

Ingredient (g.kg <sup>-1</sup> )	International feed number	Diet			
		1 (C)	2 (PR)	3 (FR)	4 (CR)
Rice polish	4-03-943	500	436	600	466
Blood meal	5-00-381	10	241	270	10
Corn gluten (60%)	5-28-242	400	96	24	400
Soybean meal	5-04-604	26	80	22	20
Canola meal	5-06-145	10	50	10	10
Rice bran	4-03-928	10	37	20	20
Canola oil		4	20	14	4
DCP		10	10	10	10
Starch	5-01-162 <sup>a</sup>	20	20	20	50
Mineral <sup>1</sup> and Vitamin <sup>2</sup> Premixes		10	10	10	10
Total		1000	1000	1000	1000
<b>Proximate analysis (percent dry weight)</b>					
DE (kJ g <sup>-1</sup> )		17.3	16.6	17.8	16.2
Crude protein		35.2	40.3	34.6	34.8
Crude fat		7.3	6.8	9.4	7.1
Ash		7.1	6.7	8.2	6.7
Crude fibre		5.8	6.1	6.3	9.2
Cost kg <sup>-1</sup> (\$)		0.14	0.18	0.16	0.14

DCP: Dicalcium-phosphate; DE: dietary energy; C = controlled group; PR = protein rich; FR = fat rich; CR = carbohydrate rich; <sup>1</sup>SB MINERAL MIX(SB Pharma, Rawalpindi, Pakistan) containing (kg<sup>-1</sup>); Copper 5 x 10<sup>3</sup> mg; Iron 5 x 10<sup>4</sup> mg; manganese 6.2 x 10<sup>4</sup> mg; zinc 3 x 10<sup>4</sup> mg; iodine 5 x 10<sup>2</sup> mg and selenium 1 x 10<sup>2</sup> mg. <sup>2</sup>SB VITA-L (SB Pharma) containing (kg<sup>-1</sup>); A 5 x 10<sup>6</sup> IU; D<sub>3</sub> 5 x 10<sup>6</sup> IU; E 7.5 x 10<sup>3</sup> mg; K<sub>3</sub> 5 x 10<sup>2</sup> mg; B<sub>1</sub> 1 x 10<sup>3</sup> mg; B<sub>2</sub> 2.5 x 10<sup>3</sup> mg; B<sub>6</sub> 1.5 x 10<sup>3</sup> mg; B<sub>12</sub> 10 mg; niacin 1.5 x 10<sup>4</sup> mg; biotin 2.5 x 10 mg; pantothenic acid 4 x 10<sup>3</sup> mg; folic acid 5 x 10<sup>2</sup> mg; antioxidant 5 x 10<sup>3</sup> mg and carrier (upto) 1 x 10<sup>3</sup> g.

lipid (Bergot and Breque, 1983). Diet having appropriate amount of carbohydrates affected the growth of herbivore fishes (Abimorad and Carneiro, 2007).

The objective of this study was to determine the effect of nutrient (protein, fat and carbohydrate) combinations in fish diet on the growth of fish to economize the cost of feed and to increase the output of aquaculture by increasing the quality of fish meat.

## MATERIALS AND METHODS

Four almost isocaloric and isonitrogenous experimental diets (17.05 ± 0.24 kJ g<sup>-1</sup>) were prepared at Shabir Fish Feeds, Multan with different levels of crude protein, fat and carbohydrate. Ingredients and proximate composition of the four experimental diets are presented in Table 1. A control group was formulated with proper mixture of all ingredients mentioned earlier. All the diets differ from each other regarding percent protein, fat and carbohydrate contents in their composition. Experimental diets were analyzed using standard AOAC (1995) methods.

### Experimental design and feeding trial

Experiments were conducted at Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. A total of

80 *Labeo rohita* fingerlings were procured from Al-Madina Fish Hatchery Matital Road Multan, Punjab Pakistan and transported in oxygen-filled polythene bags. Fish were acclimated to experimental conditions for two weeks. Then, fishes were randomly housed individually in fiberglass recirculation aerated system (RAS) holding tanks with twenty replicates. Each holding tank had 10 compartments (61 × 61 × 61 cm each). One fish was housed in each compartment. At the start of the experiment, average initial weight and length of fish were recorded. Fish were fed twice per day at the rate of 5% body weight (Khan et al., 2004) in two equal portions for whole experimental period of 60 days. During the experimental period, the water temperature, dissolved oxygen and pH were 26.8 ± 2.5°C, 7.84 ± 0.47 mg L<sup>-1</sup> and 7.16 ± 0.33, respectively (Du et al., 2005).

### Growth performance

Length and weight of all fishes were recorded at the end of the experiments and specific growth rate (SGR), weight gain (WG), feed conversion ratio (FCR) and protein efficiency (PE) were calculated by using the formulae in the foot notes of Table 2.

### Sample collection and data analysis

Fish were sacrificed by immersing in ice water. Moisture and dry weight (oven dry at 60°C to constant weight), ash content (incineration at 550°C for 5 h in a muffle furnace), fat content

**Table 2.** Mean values of standard deviation (parenthesis) of SGR, WG and PE of *L. rohita* for four different feeding groups.

Growth parameter	Feeding group			
	1 (C)	2 (PR)	3 (FR)	4 (CR)
SGR <sup>1</sup> (%day <sup>-1</sup> )	0.48 <sup>a</sup> (0.2)	0.53 <sup>a</sup> (0.1)	0.85 <sup>b</sup> (0.1)	0.78 <sup>b</sup> (0.1)
WG <sup>2</sup> (%)	34.31 <sup>a</sup> (12.3)	37.61 <sup>a</sup> (3.6)	67.12 <sup>b</sup> (11.8)	60.23 <sup>b</sup> (12.1)
FCR <sup>3</sup>	3.503 <sup>b</sup> (2.7)	2.179 <sup>ab</sup> (1.0)	1.258 <sup>a</sup> (0.7)	1.336 <sup>a</sup> (0.4)
PE <sup>4</sup> (g)	15.28 <sup>a</sup> (9.4)	16.19 <sup>a</sup> (6.2)	26.31 <sup>b</sup> (8.2)	20.57 <sup>ab</sup> (6.1)

Values having common superscripts do not differ significantly. C: Controlled group, PR: protein rich, FR: fat rich and CR: carbohydrate rich. <sup>1</sup>Specific growth rate (SGR) = (ln final weight – ln initial weight) × 100/days; <sup>2</sup>weight gain (WG) = (final weight – initial weight) × 100/(initial weight); <sup>3</sup>FCR = [total food intake (g)/total weight gain (g)]; <sup>4</sup>Protein efficiency = final weight – initial weight / protein intake.

**Table 3.** Mean values and standard deviation (parenthesis) of various body constituents of *L. rohita* for four different feeding groups.

Body constituent (%)	Feeding group			
	1 (C)	2 (PR)	3 (FR)	4 (CR)
Water	75.8 (2.04)	75.8 (3.58)	76.3 (2.83)	77 (2.63)
Dry weight	24.2 (2.05)	24.1 (3.52)	23.6 (2.83)	22.9 (2.63)
Ash (wet weight)	2.8 <sup>b</sup> (0.61)	2.9 <sup>b</sup> (0.51)	2.2 <sup>ab</sup> (0.93)	2.0 <sup>a</sup> (0.647)
Organic content (wet weight)	24.15 (2.06)	24.12 (3.59)	23.62 (2.83)	22.95 (2.63)
Fat (wet weight)	8.66 <sup>b</sup> (2.38)	11.31 <sup>c</sup> (2.42)	9.94 <sup>bc</sup> (1.80)	4.56 <sup>a</sup> (2.83)
Protein (wet weight)	15.496 <sup>b</sup> (1.99)	12.805 <sup>a</sup> (1.93)	13.676 <sup>ab</sup> (1.97)	18.388 <sup>c</sup> (3.62)

Values having common/no superscripts do not differ significantly. C: Control group, PR: protein rich, FR: fat rich and CR: carbohydrate rich.

(chloroform-methanol method) and protein content of all feeding groups were calculated based on whole body weight of fish (Salam and Davies, 1994).

All data were expressed as mean and standard deviations. Statistical packages SPSS and Minitab were used for data analysis. One way analysis of variance (ANOVA) was calculated and least significant difference (LSD) was determined by applying Tukey's family error rate test for the comparison of various studied parameters between different treatments.

## RESULTS

Data regarding the feed formulation and proximate composition of diets is shown in Table 1. Mean values of SGR, WG and PE and various body composition parameters of *L. rohita* and their comparison between four different feeding groups are presented in Tables 2 and 3, respectively.

A highly significant ( $P \leq 0.001$ ) difference in SGR, WG and PE among four feeding groups was observed, while differences were significant for feed conversion ratio (FCR). SGR and WG were maximum in FR group and minimum in control (C) group. PE was also maximum in FR but was minimum in PR.

There was a highly significant ( $P \leq 0.001$ ) difference for percent fat and percent protein content among four

treated groups. CR group showed highest protein content and PR group showed highest fat content (Table 3).

## DISCUSSION

The nutritional strategy for protein sparing effect is to increase adequate amount of lipid and carbohydrate in fish diets and reduce protein inclusion without compromising growth and FE in fish. Studies on fish nutrition have shown that fish can use protein, lipid and carbohydrate (PLC) as energy sources (Cho, 1992; Hidalgo et al., 1993; Wilson, 1994; Hardy, 2000), thus, well-balanced ratio of these three dietary components is crucial for fish farming (Umer and Ali, 2009).

The present study indicated that there was highly significant ( $P \leq 0.001$ ) difference among four feeding groups for SGR, WG and PE. SGR, WG and PE were maximum in FR group. But SGR and WG were minimum in C group and PE was lowest in PR group. Bromley (1980) and Watanabe (1982) also agreed that increasing dietary lipid level could improve the growth of fish. Improved feed conversion ratio and protein efficiency with increasing dietary lipid level is in agreement with Nematipour et al. (1992) and Einen and Roem (1997). Sinha and Ray (2001) observed that final weight gain and

SGR both increased proportionately with increasing dietary carbohydrate level up to 40% in *L. rohita* fingerlings. Almost similar results are reported in the present study where CR group showed improved growth after FR group.

Results reveal that fish protein content were higher in CR group than C group and were lowest in PR group. These findings are contradictory to Keshvanath et al. (2002) report that increasing level of carbohydrate in feed increased lipid and decreased protein levels. Protein sparing action of carbohydrate had been reported in various fish species (Cho and Kaushik, 1990; Shiau and Peng, 1993). Erfanullah and Jafri (1995) reported even more pronounced protein sparing by carbohydrate at sub-optimal protein levels than at optimal levels in *L. rohita* fingerling.

We observed a highly significant ( $P \leq 0.001$ ) difference among four feedings groups regarding percent lipid content. Maximum lipid content was observed in PR group and while minimum in CR group. The difference between C and FR groups for the same parameter did not reach statistical significance. These results are also in agreement with the report of Satpathy et al. (2003). William and Robinson (1988) reported an apparent decrease in body protein content when fish were fed on diets with high lipid level. Our finding was also complementary to these results.

## Conclusion

The present study shows that increased level of lipid in feed stimulated the overall growth of *L. rohita*, while increased carbohydrate resulted in improved protein contents in fish meat. FR was found to be a suitable diet for *L. rohita* when growth rates of various treatment groups were compared.

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## REFERENCES

- Ali M, Iqbal F, Salam A, Iram S, Athar M (2005). Comparative study of body composition of different fish species from brackish water pond. *Int. J. Environ. Sci. Technol.* 2: 229-232.
- Ali M, Iqbal R, Rana SA, Athar M, Iqbal F (2006a). Effect of feed cycling on specific growth rate, body composition, condition factor and RNA/DNA ratio of *Labeo rohita*. *Afr. J. Biotechnol.* 5: 155-156.
- Ali M, Iqbal F, Salam A, Sial F, Athar M (2006b). Comparative study of body composition of four fish species from in relation to pond depth. *Int. J. Environ. Sci. Technol.* 2: 359-364.
- Abimorad EG, Carneiro DJ (2007). Digestibility and performance of pacu (*Piaractus mesopotamicus*) juveniles fed diets containing different protein, lipid and carbohydrate levels. *Aquat. Nutr.* 13: 1-9.
- AOAC (Association of Official Analytical Chemists) (1995). Official methods of analysis. AOAC International Arlington, VA.
- Bergot F, Breque J (1983). Digestibility of starch by rainbow trout: effects of the physical state of starch and of the intake level. *Aquaculture*, 34: 203-212.
- Bergot F (1979). Carbohydrates in rainbow trout diets: effects of the level and sucrose of carbohydrates and the number of meals on growth and body composition. *Aquaculture*, 18: 157-167.
- Bromley PJ (1980). Effects of dietary protein, lipid and energy content on the growth turbot (*Scophthalmus maximuma* L.). *Aquaculture*, 19: 359-369.
- Cho CY (1992). Feedings system for rainbow trout and other Salmonides with reference to current estimates of energy and protein requirements. *Aquaculture*, 100: 107-123.
- Cho CY, Cowey CB, Watanabe T (1985). Methodological approaches to research and development. International Development Research Centre, Ottawa, Canada.
- Cho CY, Kaushok SJ (1990). Nutrition energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Rev. Nutr. Diet.* 61: 132-172.
- De Silva SS, Anderson TA (1995). Fish nutrition in aquaculture. Chapman and Hall, London.
- Du ZY, Liu YJ, Tian LX, Wang JT, Wang Y, Liang GY (2005). Effect of dietary lipid level on growth, feed utilization and body composition by juvenile grass carp (*Ctenopharyngodon idella*). *Aquacult. Nutr.* 11: 139-146.
- Einen O, Roem AJ (1997). Dietary protein/energy ratios for Atlantic salmon in relation to fish size: growth, feed utilization and slaughter quality. *Aquacult. Nutr.* 3: 115-126.
- Erfanullah, Jafri AK (1995). Growth response of fingerling Indian major carp, *Labeo rohita* (Ham) to various sources of dietary carbohydrate. *J. Aquacult. Trop.* 10: 287-296.
- FAO (1997). Review of the state of world Aquaculture. FAO Fisheries Circular, Rome, Italy.
- Hardy RW (2003). Advances in the development of low-pollution feeds for Salmonids. *Aquaculture*, 3: 63-67.
- Hidalgo MC, Sanz A, Garcia-Gallego MG, Suarez MD, de la Higuera M (1993). Feeding of the European eel (*Anguilla anguilla*). Influence of dietary carbohydrate level. *Comp. Biochem. Physiol.* 105: 165-169.
- Iqbal F, Ali M, Umer K, Rana SA (2006). Effect of feed cycling on specific growth rate, condition factor, body composition and RNA/DNA ratio of *cirrhinus mrigala*. *J. App. Sci. Environ. Manage.* 10(2): 129-133.
- Islam MS, Tanaka M (2004). Optimization of dietary protein requirement for pond-reared *Tor pititora* Hamilton (Cypriniformes: Cyprinidae). *Aquacult. Res.* 3: 1270-1276.
- Keshvanath P, Manjappa K, Gangadhara B (2002). Evaluation of carbohydrate rich diets through common carp culture in manured tasks. *Aquacult. Nutr.* 8: 169-174.
- Khan MA, Ahmad A, Abid SF (2004). Effect of ration size on growth, conversion efficiency and body composition of fingerling mrigal, *Cirrhinus mrigala* (Hamilton). *Aquacult. Nutr.* 10: 47-53.
- Nematipour GR, Brown ML, Gatlin DM III (1992). Effects of dietary energy: protein ratio on growth characteristics and body composition of hybrid striped bass, *Morone chrysops* x *M. saxatilis*. *Aquaculture*, 107: 359-368.
- Saha AK, Ray AK (2001). Optimum dietary Carbohydrate requirement of Rohu, *Labeo rohita* (Hamilton); Fingerlings. *Acta. Ichthyol. Piscat.* 31: 81-96.
- Salam A, Davies PMC (1994). Body composition of northern pike, *Esox lucius* L. in relation to body size and condition factor. *J. Fish Res.* 19: 193-304.
- Satpathy BB, Mukherjee D, Ray AK (2003). Effects of dietary protein and lipid levels on growth, feed conversion and body composition in rohu, *Labeo rohita* (Hamilton), fingerlings. *Aquacult. Nutr.* 9: 17-24.
- Shiau SY, Huang SL (1989). Optimal dietary protein level for hybrid

- tilapia (*Oreochromis niloticus* x *O. aureus*) reared in seawater. *Aquaculture*, 81: 119-127.
- Shiau SY, Peng CY (1993). Protein sparing effect by carbohydrates in diets for tilapia, *Oreochromis niloticus* x *O. aureus*. *Aquaculture*, 117: 327-334.
- Siddhuraja P, Beacker K (2003). Comparative nutritional evolution of differentially processed mucuna seeds (*Mucuna pruriens* (L.) Dc. Var. *utilis* (Wall ex Weight) Baker ex Bucck) on growth performance, feed utilization and body composition in Nile tilapia (*Oreochromis niloticus* L.). *Aquacult. Res.* 34: 487-500.
- Tacon AGJ, Metian M (2008). Global overview on the use of fish meal and fish oil in industrially compounded aqua feeds: Trends and future prospects. *Aquaculture*, 285: 146–158.
- Umer K, Ali M (2009). Replacement of fishmeal with blend of canola meal and corn gluten meal, and an attempt to find alternate source of milk fat for rohu (*Labeo rohita*). *Pak. J. Zool.* 4: 469-474.
- Watanabe T (1982). Lipid nutrition in fish. *Comp. Biochem. Physiol.* 73: 3-15.
- Williams CD, Robinson EH (1988). Response red drum to various dietary levels of menhaden oil. *Aquaculture*, 70: 107-120.
- Wilson RP (1994). Utilization of dietary carbohydrate by fish. *Aquaculture*, 124: 67-80.