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Effect of Varying Protein and Lipid Levels on the Growth of Rohu, *Labeo rohita*

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Abstract

Rohu (*Labeo rohita*, Cyprinidae) fingerlings (7.5 g) were fed six experimental diets containing two protein levels (25 and 30%) and three lipid levels (4, 6 and 9%) for 98 d. The best growth was obtained with the diet containing 30% protein and 6% lipid. At a given protein/fat level, increase in fat/protein resulted in better weight gain, except in the diet having 30% protein and 9% lipid. Protein efficiency ratio and net protein retention decreased with increase in dietary protein. Muscle RNA/DNA reflected the growth trend. Gut protease and lipase activity had a direct relationship with dietary protein. Dietary fat influenced carcass fat content, but dietary protein had no effect on carcass protein.

Introduction

The metabolic partition of the dietary components between storage and catabolism depends on both dietary composition and energy intake. Energy expenditure associated with the metabolism of the assimilated protein may be reduced in homeothermic and poikilothermic animals by dietary lipid (Shul'man 1972). Further, several studies have shown that providing adequate energy through dietary lipids can minimize the use of more costly protein as an energy source (Watanabe 1977; Reinitz and Hitzel 1980; Clarke et al. 1982; Medland and Beamish 1985; Beamish and Medland 1986; Tabachek 1986; Davies 1989; De Silva et al. 1991; Silver et al. 1991; Bazaz and Keshavanath 1993).

The economically optimum dietary protein for rohu was found to be 30% (Renukaradhya and Varghese 1986). This study was carried out to evaluate the combined effects of different dietary protein and lipid levels on growth, body composition and feed utilization.

Materials and Methods

Diets

Six test diets containing protein levels of 25 and 30%, each with three lipid levels viz., 4, 6 and 9%, were formulated (Table 1). While fishmeal (59.95% protein and 8.5% fat) and tapioca flour (2.1% protein, 0.23% fat and 81.61% nitrogen-free extract) were used as protein and carbohydrate sources, respectively, sugarcane pith served as a filler. The diets were in dry pelleted form and were stable in water for a duration sufficient enough for fish to feed on.

Growth Studies

The growth trial was conducted over a period of 98 d by stocking 25 advanced rohu fingerlings (7.5 g) in 25 m^2 cement cisterns with static water. Evaporation loss was made good at regular intervals. No aeration was provided. All the six test diets were fed to fish in duplicate tanks once daily at 5% body weight. Feed quantity was adjusted based on the weight of fish recorded at fortnightly samplings.

Table 1. Ingredient composition and proximate analyses of experimental diets.

	Diets					
	P25L4	P25L6	P25L9	P30L4	P30L6	P30L9
Ingredients						
Fishmeal	41.66	41.66	41.66	50.00	50.00	50.00
Tapioca	50.00	50.00	50.00	48.00	46.25	43.50
Sunflower oil	0.46	2.45	5.45	-	1.75	4.50
Vitamin mineral mix	2.0	2.0	2.0	2.0	2.0	2.0
Sugarcane pith	5.88	3.89	0.89	-	-	-
Proximate analysis (%)						
Moisture	4.14	4.19	4.63	4.92	4.81	4.16
	(0.04)	(0.03)	(0)	(0.02)	(0.13)	(0.01)
Crude protein	26.00	26.63	25.67	31.21	31.05	30.98
	(0.36)	(0.16)	(0.23)	(0.26)	(0.42)	(0.20)
Crude fat	4.46	6.15	8.86	4.29	6.49	9.33
	(0.32)	(0.27)	(0.48)	(0.13)	(0.11)	(0.18)
Crude fiber	6.01	3.94	1.21	-	-	-
Ash	8.39	8.27	7.88	8.85	8.78	8.30
	(0.02)	(0.10)	(0.04)	(0.02)	(0.10)	(0.06)
NFE	51.00	50.82	51.75	50.73	48.87	47.23
Gross energy ($kJ \cdot g^{-1}$)	15.65	16.39	17.37	16.63	17.12	17.90

Note: Figures in parentheses indicate standard error.

Water Quality

Water quality was monitored fortnightly for temperature, pH, dissolved oxygen, free carbon dioxide and total alkalinity (APHA 1985). Plankton samples were also collected on the sampling days and analyzed quantitatively.

Biochemical Analyses

Fish were killed at the beginning and end of the culture period and analyzed for proximate composition (AOAC 1975). Analysis of muscle DNA (Giles and Myres 1965) and RNA (Ceriotti 1955) and the gut digestive enzymes — protease (Kunitz 1947), amylase (Rick and Stegbaurer 1974) and lipase (Naher 1974) — were carried out on termination of the experiment.

Data Analyses

Specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and net protein retention (NPR) were calculated according to the following equations:

$$\text{SGR} = \frac{\text{Log}_e\text{Final weight} - \text{Log}_e\text{Initial weight}}{\text{Experimental duration (d)}} \times 100$$

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

$$\text{PER} = \frac{\text{Gain in wet weight of fish (g)}}{\text{Dry weight of protein fed (g)}}$$

$$\text{NPR} = \frac{\text{Gain in carcass protein (g)}}{\text{Dry weight of protein fed (g)}} \times 100$$

Data were tested for significance employing one-way analysis of variance (Snedecor and Cochran 1968) and Duncan's (1955) multiple range test.

Results

The water quality parameters (pH 7.10-9.72; DO 5.60-9.07 ppm; free CO_2 0-72 ppm; alkalinity 31.07-66.51 ppm; temperature 27-31°C; dry weight of plankton 0-15 mg$\cdot$40 l^{-1}) were well within the range for fish culture. Dry weight of plankton was very low due to non-fertilization of the cisterns. It is presumed that plankton would have contributed little nitrogen (protein) to the system.

Fish fed P30 L6 showed the highest weight gain, followed by P30 L4; P25 L9; P25 L4; P25 L6 and P30 L9 (Table 2). SGR also followed the same trend. FCR did not show any definite trend with respect to final weight of the fish, rather it increased with dietary protein at a given level of dietary lipid, except for the highest level of 9% (Table 2).

The mean PER and NPR are shown in Table 2. Both PER and NPR were inversely related to dietary protein. Dietary lipid was positively correlated with NPR, except for the P30 L9 diet.

Proximate analysis of fish (Table 2) showed an increase in percentage of carcass protein and fat with increasing dietary lipid. Increase in protein content decreased carcass protein percentage, but increased fat percentage. Ash percentage was higher with higher lipid, except for the P30 L9 diet. RNA:DNA ratio showed a trend similar to SGR, the highest being for diet P30 L6 and the least for diet P30 L9 (Fig. 1)

Gut amylase activity revealed no definite trend with respect to dietary protein and fat (Fig. 2). Protease activity increased with dietary protein. The hepatopancreatic protease activity decreased with increase in dietary lipid. Hepatopancreatic and intestinal lipase activities were directly related to dietary lipid as well as protein levels.

Table 2. Growth indices of *Labeo rohita* fed with experimental diets.

	Experimental diets					
	P25L4	P25L6	P25L9	P30L4	P30L6	P30L9
Initial weight (g)	7.5	7.5	7.5	7.5	7.5	7.5
Final weight (g)	155.71^{ab}	152.37^{a}	162.51^{b}	173.23^{c}	181.24^{d}	149.28^{a}
	(12.46)	(12.21)	(21.68)	(14.72)	(19.83)	(9.58)
Food conversion ratio	2.23^{a}	2.50^{b}	2.88^{d}	2.83^{cd}	2.71^{c}	2.76^{cd}
	(0.04)	(0.03)	(0.04)	(0.05)	(0.01)	(0.09)
Specific growth rate	3.09^{bc}	3.07^{ab}	3.13^{c}	3.20^{d}	3.25^{e}	3.05^{a}
	(0.01)	(0.01)	(0)	(0.01)	(0)	(0.02)
Protein efficiency ratio	1.72^{e}	1.50^{d}	1.35^{c}	1.13^{a}	1.19^{b}	1.16^{ab}
	(0.03)	(0.01)	(0.02)	(0.02)	(0)	(0.04)
Net protein retention	2.96^{c}	3.85^{d}	5.41^{e}	0.65^{a}	2.63^{bc}	1.90^{b}
	(0.19)	(0.12)	(0.11)	(0.18)	(0.14)	(0.14)
Proximate composition of carcass (wet weight basis)						
Moisture	75.68^{b}	74.49^{ab}	71.10^{a}	76.48^{b}	72.05^{a}	73.89^{ab}
	(0.57)	(0.27)	(0.22)	(0.49)	(1.06)	(0.56)
Protein	13.91^{b}	15.08^{c}	16.53^{d}	12.32^{a}	15.13^{c}	13.64^{b}
	(0.12)	(0.08)	(0.09)	(0.19)	(0.16)	(0.12)
Fat	4.10^{a}	4.83^{b}	5.04^{c}	6.16^{de}	6.31^{e}	7.32^{f}
	(0.05)	(0.02)	(0.05)	(0.05)	(0.09)	(0.07)
Ash	2.93^{c}	3.02^{c}	3.71^{d}	2.04^{a}	3.41^{c}	2.63^{b}
	(0.02)	(0)	(0.03)	(0.01)	(0)	(0)

Note: Figures in parentheses indicate standard error.
Figures in the same row having same superscript are not significantly different (P>0.05).

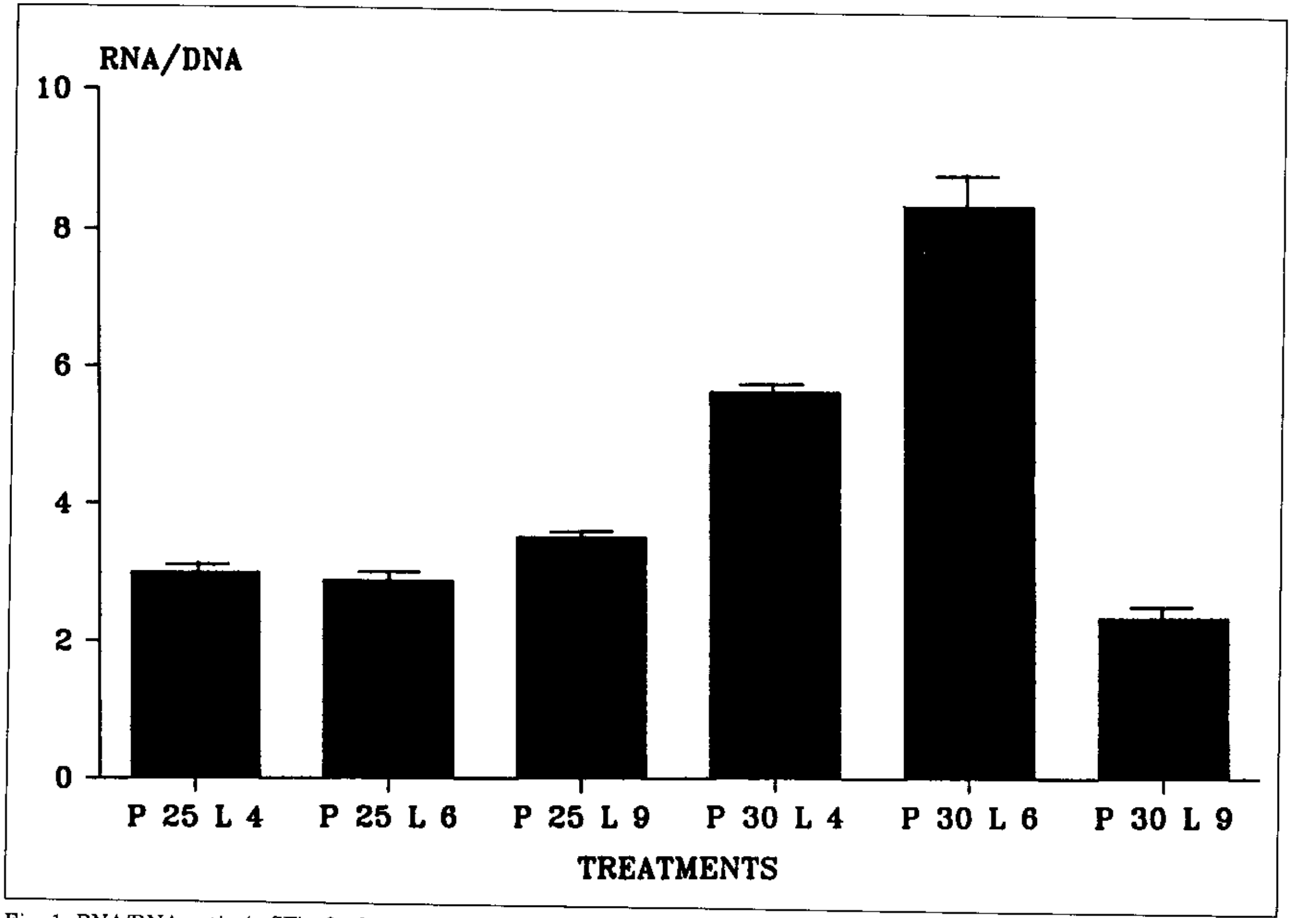

Fig. 1. RNA/DNA ratio (± SE) of rohu (muscle) fed with experimental diets.

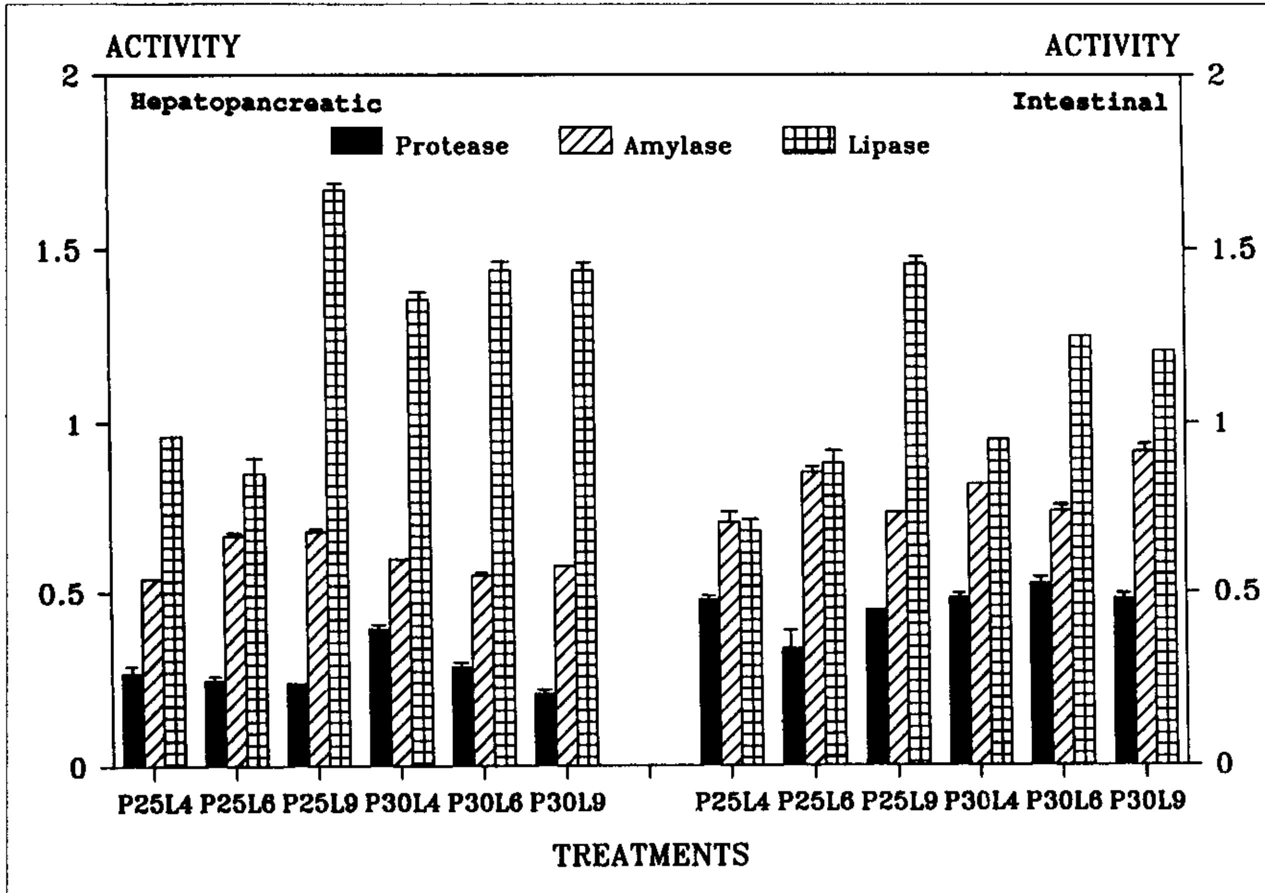

Fig. 2. Specific activity ± SE (m moles of product liberated min·mg^{-1} protein at 28°C) of gut digestive enzymes in common carp.

Discussion

Tacon and Cowey (1985) found a positive correlation between SGR and dietary protein requirement (g kg body weight^{-1}•d^{-1}). In red tilapia (De Silva et al. 1991) and chinook salmon (Silver et al. 1991), growth rate correlated to daily protein consumption, irrespective of dietary lipid content. In the present study, a similar trend was noted, except with the diet having the highest dietary protein and lipid levels (P30 L9). The final weight of fish under this treatment was comparable to that obtained with diets P25 L4 and P25 L6. This might be an indication that 9% lipid is not required at 30% dietary protein for rohu. Growth depression has been recorded when dietary protein exceeded a certain level in rainbow trout (Zeitoun et al. 1973) and striped bass (Millikin 1983). In the present study, growth depression occurred when dietary protein content was increased from 25 to 30% only at the 9% lipid level.

FCR and PER are known to decrease with increasing dietary protein content (Steffens 1981; Jauncey 1982), and the effects vary with species (Dabrowski 1979). Tabachek (1986) obtained a decrease in PER and NPR with each increase in dietary protein level at a constant level of dietary lipid. De Silva et al. (1991) reported the influence of dietary protein and lipid levels on FCR, PER and NPR. They observed that increasing dietary lipid level increased the protein retained, thus enhancing the proportion of dietary protein utilized for growth rather than as an energy source. This type of sparing effect has been observed in rainbow trout (Reinitz and Hitzel 1980; Beamish and Medland 1986), coho salmon (Clarke et al. 1982), Arctic charr (Tabachek 1986), red tilapia (De Silva et al. 1991), chinook salmon (Silver et al. 1991) and mahseer (Bazaz and Keshavanath 1993). Higher dietary protein (30%) did not improve FCR, PER and NPR in rohu. At this protein level, the highest lipid level tested (9%), had a negative effect on growth, FCR, PER and NPR. Thus, for rohu, it is not desirable to increase dietary lipid content to 9% in 30% protein diet.

Increase in carcass lipid with increasing dietary lipid content has been reported for most species investigated (De Silva et al. 1991; Silver et al. 1991; Bazaz and Keshavanath 1993). Changes in the carcass composition of red tilapia (De Silva et al. 1991) in relation to dietary protein level were less consistent than those related to dietary lipid level. Reinitz et al. (1978) obtained no significant influence of dietary protein on either percentage fat or protein deposited in rainbow trout. Silver et al. (1991) reported an inverse relationship of carcass protein percentage with dietary lipid percentage. The increase in carcass protein with increase in dietary lipid obtained in the present study suggests that the additional lipid is not only used for metabolism, but is converted into protein.

RNA:DNA ratio is known to provide dependable indication of growth trend (Sable 1974; Buckley 1980; Mustafa and Mittal 1982; Khan and Jafri 1991). This ratio was the highest in fish receiving diet P30 L6, the treatment that resulted in the best growth of fish. This further indicates that using higher lipid levels is not advisable at the dietary protein level of 30% for rohu. Owing to the constant level of carbohydrate in all the diets, there was no marked variation in amylase activity between different treatments. Nonetheless, the diets affected protease and lipase activity (Fig. 2). Proteolytic activity tends to vary depending on the type of diet and its protein content (Kawai and Ikeda 1972; Scherbina et al. 1976; Hofer 1979). Bazaz and Keshavanath (1993) observed higher induction of lipase and protease activity when the diet of mahseer, *Tor khudree* was supplemented with 6% sardine oil.

In conclusion, it may be stated that the diet formulated to contain 30% protein and 6% lipid maximized weight gain, and feeding rohu with a higher percentage of fat produced fish rich in fat as well as protein.

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