Fishery Technology
2001, Vol. 38(2) pp : 115 - 120

Optimization of Energy to Protein Ratio in Feed for *Labeo rohita* Fingerlings

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Ten semi-purified diets with two levels of dietary protein (32 and 41%) having five energy levels each (324, 341, 364, 384 and 407 kcal.100g^{-1}) were formulated and fed to *Labeo rohita* fingerlings for a period of 45 days. Factorial analysis of variance indicated that although weight gain, food conversion efficiency and protein utilization were significantly affected by dietary energy and protein levels, the energy and protein conversion efficiencies were not influenced by dietary energy and protein interaction. The best growth and food conversion ratio (FCR) were obtained with an average of 379.72 kcal.100g^{-1} at 41% protein diet (P/E 107.87 and E/P 9.29), while maximum protein utilization and conversion occurred with similar energy levels (383 kcal.100g^{-1}) but in low protein (32%) diet. Although dietary energy influenced the body constituents of fish, the influence of protein was not significant. The results indicated that with increased allowance of energy, more protein was utilized for tissue building and enhancement of growth.

Key words: Protein-energy ratio, optimum growth, dietary energy, dietary protein, *Labeo rohita*

To make aquaculture economically viable and environmentally sustainable, it is necessary to reduce the proportion of protein components in the diet, without affecting growth of fishes. Higher levels of protein in the fish diet increase the cost of feed as well as deteriorate the water quality through excessive excretion of nitrogenous (NH_3) wastes. Cowey (1978) observed that unless sufficient energy is provided, the quality and quantity of dietary protein cannot contribute to protein synthesis to the full extent. Excess of dietary protein is wasteful and creates stress in the animal while excess energy means the production of more fatty fish (Das *et al.*, 1991). Attempts to reduce the amount of protein in fish diet was confined mainly to trout, channel catfish and common carp (Lee & Putnam, 1973; Garling & Wilson, 1976; Takeuchi *et al.*, 1979). Better utilization of dietary protein for growth had been observed when sufficient energy from non-protein sources like carbohydrate, lipid, etc. was available (Schwarz *et al.*, 1983; De Silva *et al.*, 1991; El-sayed & Teshima,1992, Hassan, 1993). Information on the effect of the relationship between dietary energy and protein on growth performance of fishes is still not complete.

The protein requirement of *Labeo rohita,* which is an important species in polyculture practices, is reported to be high (40-50%) (Khan, 1991; De Silva & Gunasekara, 1991; Hassan *et al.*, 1995; Hassan & Jafri, 1996). Apart from the above accounts, there is very little information on the dietary energy-protein balance in carps, especially in rohu fingerlings. Therefore, an attempt has been made to investigate dietary energy and protein interaction and their influence on growth, conversion efficiency and body composition with a view to optimize energy to protein ratio in *L. rohita* fingerlings.

Materials and Methods

Using dextrin and casein as major components, 10 types of semi-purified diets were formulated containing 32 and 41% crude protein and each was tested at five different levels of energy (324.34±2.96; 340.87±1.99; 383.85±5.29; 406.48±3.60 $kcal.100g^{-1}$) in 2x5 factorial design (Table 1). α-cellulose and carboxymethyl cellulose were used as fillers and binders. Corn oil and cod liver oil were used as lipid sources. All the ingredients were purchased from Welcome Pharmaceuticals and Sarabhai Chemicals, Baroda, India and were mixed with diet at different concentrations, following the method of Hassan *et al.* (1995). Metabolisable energy equivalents 3.5, 4.5 and 8.5 $kcal.g^{-1}$ for carbohydrates, protein and lipid respectively, were employed for calculating dietary energy levels (Jauncey, 1982).

Rohu fingerlings (3.65±0.26 g) were brought to the laboratory and acclimatized for a period of 15 days in circular cement tanks (200 l capacity). The temperature (28±1°C), pH (7.2 to 7.5), dissolved oxygen (5-7 $mg.l^{-1}$) and photo period (12D:12L) were maintained throughout the experimental period. During the acclimatization period, the fish were fed on commercial carp diets (40% protein).

Fifty fishes of same size were recruited from the stock and divided into ten batches, each consisting of five fish and introduced into circular plastic aquaria (size: 60x75 cm diameter and 75 cm height) connected with PVC tubes with a flow through system having a flow rate of 4 $l.h^{-1}$. Triplicates were maintained for each diet. Fish were fed on the respective feed twice a day (09.00 and 18.00 h) at a rate of 5% of body weight (Haniffa *et al.*, 1987). The feeding trial was conducted for 49 days. Unconsumed feed was collected using a fine sieve after 2 h of feeding and dried at 60°C. Faecal matter was siphoned out every morning before feeding. After the feeding trial, the final weight of the fish was recorded, sacrificed and dried at 80°C and dry weight noted. Samples from each diet were taken and analysed for proximate composition and gross energy. Crude protein was determined by micro kjeldahl method (Nx6.25), while lipid was quantified with petroleum ether using soxhlet extraction technique. Ash content was determined by burning the sample in a muffle furnace at 600°C, for 5 h. Moisture was calculated after fish was dried at 100°C for 24 h.

All data were analysed using one-way ANOVA and the treatment of means were compared using Duncan's new multiple range test (Duncan, 1955). Polynomial regression (2nd order) was employed (Snedecor & Cochran, 1969) to study the relationship between dietary levels of protein and lipid and energy. The variations in the carcass components were calculated through correlation coefficient analyses (Zar, 1984).

The growth performance of fish was evaluated by calculating the relative growth rate (RGR), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), protein productivity value (PPV) and energy retention efficiency (ERE) (Hassan & Jafri, 1996).

Results and Discussion

Both dietary energy and protein significantly affected the growth, FCR and PER (Table 2) in *L. rohita* but the interaction was not found to be significant ($p>0.05$). The maximum growth of rohu fingerlings occurred at 41% protein level, which agrees with the protein requirement value reported by Khan (1991), Hassan *et al.* (1995) and Hassan & Jafri (1996) for *C. mrigala* fingerlings through a dose response study. A lower weight gain was noticed at a low energy diet which may be due to insufficient energy consumption. Fixed ration level (5% of body weight) might have restricted the fish from consuming more food to compensate for insufficient energy supply from the low energy diet and as a result, the dietary protein was presumably catabolised to meet its requirements for energy rather than growth. Increase in the growth with increasing levels of dietary protein, within isocaloric diets suggested that an E/P ratio of 9.29 is sufficient for optimum growth of the fish. A

Table 1. Percentage composition of ingredients and proximate composition of experimental diets

	32% protein level					41% protein level				
	1	2	3	4	5	6	7	8	9	10
Ingredients										
Dextrin	20	18	18	18	18	14	14	12	12	12
Casein (Vitamin free – 92% CP)	32	32	32	32	32	42	42	42	42	42
Gelatin	8	8	8	10	10	10	10	10	10	10
α-cellulose	28.2	26	21.09	16.16	14.19	20.20	18	15.09	12.16	10.19
Cod liver oil	3.33	4.62	5.29	6.47	6.72	3.33	4.62	5.29	6.47	6.72
Corn oil	3.47	4.38	8.62	10.37	12.09	3.47	4.38	8.62	10.37	12.09
Carboxy methyl cellulose	5	5	5	5	5	5	5	5	5	5
Vitamin and mineral mix	2	2	2	2	2	2	2	2	2	2
Proximate composition										
Crude protein (%)	31.82	31.94	32.09	32.18	31.87	40.87	40.92	40.13	40.87	40.93
Crude fat (%)	7.84	9.48	14.74	18.39	21.35	7.9	9.73	15.46	19.82	22.68
Carbohydrate (%)	43.84	41.76	36.35	31.85	26.43	27.49	25.17	21.39	17.84	14.58
Metabolisable energy (Kca/100g^{-1})	326.43	340.5	362.85	386.85	409.09	322.25	341.20	365.67	379.72	403.87
E/P	10.42	10.9	11.31	12.02	12.84	8.11	9.21	9.24	9.29	9.87
P/E	95.95	91.64	88.44	83.18	77.78	123.39	108.53	108.53	107.76	101.34

similar but slightly low value of E/P ratio (8.93) was found to be optimum for *C. mrigala* (Hassan, 1993; Hassan *et al.*, 1995). This result confirms the growth accelerating effect of dietary energy intake up to a certain level with balanced combination of protein and energy intake (Table 1). Analysis of FCR value clearly indicated that both protein and energy of the diet influenced feed conversion of *L. rohita* fingerlings. The increase in weight gain and feeding efficiency up to a certain level of dietary energy has been reported by earlier workers (Takeuchi *et al.*, 1979; Shiau & Huang, 1990; De Silva *et al.*, 1991; Hassan *et al.*, 1995). The low feed conversion and growth rate of rohu fed on high energy diet could be due to poor feed consumption or uptake of high energy diet (Page & Andrews, 1973) or hindrance of digestion and absorption by other dietary nutrients (fat content) present in the diet (Dupree *et al.*, 1979).

The quadratic pattern in relation to SGR to dietary energy revealed that, after a maximum gain at an average of 383 kcal.100g^{-1}, a fall in the growth rate was noticed with increase in dietary energy uptake (Fig.1). However, a better growth was observed with increase in the dietary protein levels of the isocaloric diets. Among the isocaloric diets tested, rohu fingerlings fed on 41% protein diet showed low FCR.

The PER also showed a similar trend in relation to energy content. It decreased with dietary protein when isocaloric diets were compared. The value on PER and PPV clearly indicated the influence of dietary energy and protein content on the protein utilization of the fish. The change in the pattern of PER and PPV in relation of dietary energy levels agrees with the observation of

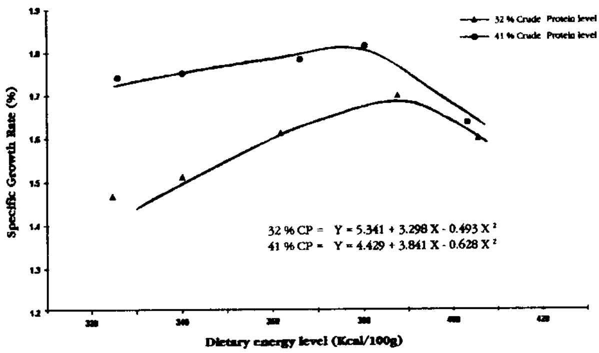

Fig. 1. Effect of dietary energy level on specific growth rate of *L. rohita* fed on diets containing different levels of protein.

Table 2. Effect of two series of diets on the growth performance of *Labeo rohita*

	32% protein level					41% protein level				
	1	2	3	4	5	6	7	8	9	10
Initial weight (g)	3.46±0.06	3.56±0.01	3.58±0.06	3.65±0.08	3.74±0.07	3.66±0.02	3.72±0.02	3.78±0.09	3.86±0.02	3.83±0.05
Final weight (g)	6.33±0.09	6.62±0.02	6.68±0.11	7.30±0.06	6.92±0.02	7.45±0.02	7.57±0.06	7.68±0.12	8.06±0.02	7.40±0.07
Growth (g)	2.88±0.01	3.05±0.02	3.26±0.07	3.61±0.03	3.19±0.01	3.79±0.02	3.85±0.06	3.90±0.11	4.20±0.06	3.57±0.07
RGR (%)	83.3 ± 0.42^{a}	85.75 ± 0.78^{a}	91.16 ± 1.57^{a}	96.83 ± 1.47^{b}	85.28 ± 0.54^{a}	103.75 ± 0.36^{b}	103.63 ± 0.36^{b}	105.64 ± 0.35^{b}	108.71 ± 0.69^{b}	93.10 ± 1.90^{a}
SGR (%)	1.48 ± 0.04^{a}	1.51 ± 0.02^{a}	1.62 ± 0.02^{a}	1.69 ± 0.02^{b}	1.59 ± 0.15^{a}	1.73 ± 0.05^{b}	1.73 ± 0.02^{b}	1.74 ± 0.04^{b}	1.80 ± 0.04^{b}	1.61 ± 0.03^{a}
PCR	3.47 ± 0.51^{b}	3.37 ± 0.25^{b}	3.25 ± 0.07^{b}	3.06 ± 0.08^{a}	3.21 ± 0.06^{b}	3.01 ± 0.11^{a}	2.76 ± 0.6^{a}	2.74 ± 0.08^{a}	2.56 ± 0.04^{a}	2.94 ± 0.05^{a}
PER	0.77 ± 0.09^{a}	0.87 ± 0.09^{b}	0.90 ± 0.09^{b}	1.03 ± 0.07^{b}	0.94 ± 0.4^{b}	0.73 ± 0.04^{b}	0.79 ± 0.21^{a}	0.88 ± 0.01^{b}	0.95 ± 0.02^{b}	0.76 ± 0.03^{a}
PPV	18.68 ± 0.87^{b}	14.70 ± 0.72^{a}	13.52 ± 0.17^{a}	12.59 ± 0.23^{a}	12.35 ± 0.48^{a}	15.49 ± 0.42^{b}	14.73 ± 0.16^{b}	12.62 ± 0.08^{a}	11.66 ± 0.07^{a}	10.61 ± 0.07^{a}
ERE	13.59 ± 0.13^{a}	15.00 ± 0.72^{a}	17.74 ± 0.34^{b}	20.08 ± 0.32^{b}	18.34 ± 0.48^{b}	16.23 ± 0.03^{a}	17.65 ± 0.13^{a}	18.64 ± 0.11^{b}	21.62 ± 0.14^{b}	19.49 ± 0.15^{b}
Survival (%)	96	93	93	95	100	100	100	97	96	94

Figures in each row having the same superscript were not significantly different

El-Sayed & Teshima (1992) in Nile tilapia and Hassan *et al.* (1995) in *C. mrigala*. Increased PER with decreasing level of dietary protein was reported by Daniels & Robinson (1986) and Parazo (1990) in *Sciaenops ocellatus* and *Siganus guttatus* whereas an inverse relationship between PPV and dietary protein was reported by Millikin (1983) on striped bass. The observation on PPV suggests that incorporation of energy in the diet beyond an average of 383 $kcal.100g^{-1}$ is unproductive and wasteful in the case of rohu fingerlings.

In terms of protein utilization and protein retention, the energy contribution from lipid source (36%) to the total average dietary energy of 383 $kcal.100g^{-1}$ revealed a beneficial effect of dietary lipid. The same trend was noticed in *C. carpio* which was efficient in utilizing high levels of dietary lipids (Jauncey, 1982). A sparing effect of 18% dietary lipid was found to influence the growth of tilapia (De Silva *et al.*, 1990). *C. mrigala* was also found to utilize higher dietary energy from dietary lipid source (Hassan *et al.*, 1995). The energy conversion efficiency (ECE) and protein production value (PPV) were influenced by dietary energy and protein and their interaction was found significant ($p<0.05$). ECE was found directly related to protein level in the diets whereas PPV was inversely related to protein and showed a significant difference ($p<0.05$) up to an average of 383 $kcal.100g^{-1}$ of energy

Table 3. Carcass composition of *Labeo rohita* fingerlings fed experimental diets

Feed types	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
32% protein level				
1	77.14 ± 0.75^{a}	57.44 ± 0.75^{c}	16.68 ± 0.10^{a}	12.85 ± 0.14^{b}
2	74.26 ± 0.65^{ad}	53.48 ± 0.94^{a}	17.81 ± 0.03^{a}	11.44 ± 0.06^{a}
3	73.79 ± 0.13^{ad}	53.02 ± 0.54^{a}	18.79 ± 0.13^{b}	10.93 ± 0.09^{a}
4	73.52 ± 0.31^{ad}	52.69 ± 0.04^{a}	21.63 ± 0.14^{b}	10.45 ± 0.43^{a}
5	72.94 ± 0.21^{c}	51.78 ± 0.11^{a}	24.61 ± 0.09^{bc}	9.47 ± 0.10^{a}
41% protein level				
6	77.59 ± 0.25^{a}	57.72 ± 0.19^{c}	18.28 ± 0.04^{b}	13.57 ± 0.12^{b}
7	76.79 ± 0.19^{ab}	55.33 ± 0.12^{b}	19.59 ± 0.16^{b}	12.91 ± 0.04^{b}
8	76.24 ± 0.13^{b}	54.66 ± 0.79^{b}	21.98 ± 0.12^{b}	12.15 ± 0.06^{b}
9	75.69 ± 0.15^{b}	53.92 ± 0.06^{a}	23.56 ± 0.06^{b}	11.51 ± 0.06^{b}
10	74.86 ± 0.11^{b}	52.80 ± 0.45^{a}	28.84 ± 0.11^{c}	10.90 ± 0.05^{a}

Figures in each column having the same superscript were not significantly different at both the protein levels. The interaction of dietary energy and protein appears to influence the energy conversion in *L. rohita*. Increase in energy level beyond 383 kcal. 100 g^{-1} in both the dietary protein levels did not seem to exert a positive influence on energy utilization or growth. So this energy level may be considered to be the optimum for *L. rohita* fingerlings. Earlier works of Lee & Putnam (1973), Cowey (1979) and Hassan *et al.* (1995) support this assumption that surplus protein is used as energy, leading to fat deposition and addition of calorific value of flesh.

The carcass composition (Table 3) showed that the moisture content of the fish was much reduced in animals fed with 32% than 41% protein diets. ANOVA test showed a significant ($p<0.05$) reduction of carcass moisture at both dietary protein levels with increase in energy content. This is similar to the findings in *Ictalurus punctatus* (Garling & Wilson, 1976), hybrid tilapia (*O. niloticus* x *O. aureus*) (Shiau & Huang, 1990), *Cyprinus carpio* (Zeitler *et al.*, 1984) and *C. mrigala* (Hassan *et al.*, 1995). The other body constituents also were significantly affected by dietary energy levels rather than dietary protein.

The whole body protein was negatively correlated to the increase of dietary protein but a positive correlation was reported by De Silva (1991), Hassan *et al.* (1995) Austreng & Refsite (1979) and Millikin (1983). At both the protein levels, the whole body fat showed a significant increase ($p<0.05$) with dietary energy. Positive relationship between whole body lipid with increase of dietary energy was well documented by Watanabe (1982) in rainbow trout. The inverse relationship of body protein with dietary energy might not be due to decreased absolute level of protein but due to the relatively lower level of protein in relation to elevated lipid levels.

One of the authors, T.A. Sethuramalingam, gratefully acknowledges Dr. K. Sampath, Reader, Department of Zoology, V.O. Chidambaram College, Thoothukkudi for his encouragement and timely help.

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