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Effect of Feed with Varying Protein: Energy Ratios on the Growth Performance of Grass Carp *Ctenopharyngodon idella*

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Abstract: Seven diets with varying digestible protein/digestible energy (DP:DE) ratios were tested for growth and nutrient retention in fish body. Six test diets containing DE levels of 310, 3.30 and 360 Kcal/100 g at low (24%) and sub-optimum (28%) DP levels were tested against control having 30% DP and 3100 Kcal/100 g DE. However, the main protein source in control diet was fish meal while in all other six test diets corn gluten meal was provided. Significant differences ($p<0.05$) were observed in the percentage live fish weight gains at different levels of dietary protein and energy. Control fish receiving 30% dietary DP and protein/energy ratio of 81.93 gave the maximum gain in weight of 148.80±5.50%. However, among the six test diets, dietary P/E ratio of 73.75 shared the maximum weight gain (122.29±8.27%). Increase in dietary energy beyond 425.90 kcal/100 g resulted in significantly poor growth. Feed intake by the fish increased significantly with increasing dietary protein while decreased non-significantly with the increased dietary lipid and energy. The results indicate consumption dependent protein and energy retention in fish body as a function of dietary protein level.

Key words: Grass carp, DP:DE ratio, growth, regression

Introduction

The major focus of fish nutrition research in Pakistan must be on making supplementary feeding cost-effective not only by developing diets that are perfect in their nutrient composition but also by improving our knowledge on the digestibility of nutrients in deciding composition and feeding level for a particular fish species. The protein and energy available in the locally raised crops and animal wastes need to be exploited for the supply of correct and cost-effective nutrients to the feeds. The commercial production of grass carp needs rapid growth of fingerlings during the first year of rearing at high stocking densities in earthen ponds. This has caused interest in supplemental feeding of grass carp with formulated feed. In commercial farms the fish must be large enough to feed on aquatic vegetation. Small fish cannot feed effectively on aquatic plants (Shireman *et al.*, 1978). In recent years the yield of grass carp in ponds has reached 7500 kg/ha. One of the major reasons for this increase in production is the use of pelleted feed (Ding, 1991; Javed, 1996). Least-cost diet formulations offer economic advantages in the preparation of a nutritionally complete diet by allowing change in diet formulations when ingredient prices fluctuate. Evaluation of alternate protein sources remains a high priority for fish nutritionists (Hashim *et al.*, 1994a, b; Aoki *et al.*, 1997). However, detailed studies on nutritional energetics for grass carp are needed; particularly in view of the expanding aquaculture interest in herbivorous fish.

Materials and Methods

A 56-day feeding experiment was conducted at the laboratory of Fish Nutrition, Department of Aquatic Biosciences, Tokyo University of Fisheries, Japan. The optimum dietary DP:DE ratio for grass carp (*Crenopharyngodon idella*) fingerlings was determined by using corn gluten meal as a main protein source in fish diets. Six test diets of two dietary DP levels (24 and 28%) were formulated at three DE levels of 300, 330 and 360 kcal/100 g feed for each dietary protein level. Accordingly CP/GE ratios of diets, viz. D1, D2 and D3 were 64.15, 63.08 and 59.12 mg/kcal respectively at 24% DP level while 77,95, 73.75 and 68.75 mg/kcal at 28% DP level in D4, D5 and D6, respectively. However, in control diet, 30% DP and 310 kcal DE was provided by using 32.53% fish meal with CP/GE ratio of 81.93 mg/kcal. However, all six test diets contained corn gluten meal as a main protein source without fish meal (Table 1).

The feeding trial was conducted in 60 litre plastic tanks with a- sloping bottom filled with 45 litre un-chlorinated tap water. Continuous water supply 200-400 ml/min. was maintained throughout the period of this experiment and the dissolved oxygen content of water seldom dropped below 6.40 mg/l. Grass carp fingerlings were acclimated to the experimental diets for one week prior to the beginning of the experiment. The fish were then sorted by weight and introduced into each tank as groups of 20 fish with two replications for each treatment. Fish were fed soft-dry pellets three times a day (at 9:00, 12:00 and 16:30) close to satiation. However, fish were not fed on the day they were weighed. On a bi-weekly basis, all fish were anesthetized in a 0.05% ethy1-4-aminobenzoate solution and their body weights (g) and fork lengths (mm) were recorded. After obtaining the data fish were released back into their respective tanks. Nutrients retention in fish were estimated indirectly through proximate analysis according to the methods described in AOAC (1984). Gross energy of the fish and 'feed were- analyzed by using Shimadzu CA-41Y Bomb calorimeter. One-way analysis of variance and Duncan's multiple-range test were used to analyze experimental data (Steel and Torrie, 1986). Differences for variables within different treatments were considered significant at $p<0.05$. Correlation and regression analyses were performed to findnout relationships/trends among various parameters.

Table 1: Composition of experimental diets for grass carp

Component	DIETS						
	Control Diet	D1	D2	D3	D4	D5	D6
Ingredients (% of total)							
Fish meal 32.53	-	-	-	-	-	-	-
Corn gluten meal	-	21.89	23.11	24.08	26.00	26.20	27.44
Soybean meal[a]	5.00	5.00	5.00	5.00	12.00	12.00	12.00
Wheat flour	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Alfalfa meal	5.00	7.17	7.56	5.31	10.99	7.54	6.48
Rice bran	19.09	19.44	15.00	15.00	-	15.00	10.00
Wheat bran	9.88	20.00	18.00	15.00	24.51	10.00	10.00
Oil[b]	2.00	-	4.83	9.11	-	2.76	7.58
Vitamin mixture[c]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mixture[d]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Chromium Oxid[e]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Proximate analyses of diets							
Moisture (%)	4.03	3.51	3.28	3.68	3.30	3.28	3.41
Protein (%)	35.92	27.66	28.81	28.51	33.20	33.33	32.81
Lipid (%)	11.52	9.28	10.93	16.11	5.04	11.02	14.14
Ash (%)	12.30	9.52	8.52	8.55	8.09	8.93	8.23
Carbohydrate (%)	23.69	31.75	32.64	28.87	29.46	28.32	32.09
Gross energy (Kcal/100g)	438.40	431.20	456.70	482.20	425.90	451.90	477.20
DO[a] (%)	30.00	24.00	24.00	24.00	28.00	28.00	28.00
DE[f] (Kcal/100g)	310.00	300.00	330.00	360.00	300.00	330.00	360.00
DP:DE ratio (mg DP/kcal DE)	96.77	80.00	72.73	66.67	93.33	84.85	77.78
Crude protein:GE ratio (mg/kcal)	81.93	64.15	63.08	59.12	77.95	73.75	68.75
Feed cost per kg (Pak. Rs.)	13.51	7.29	9.50	11.50	7.47	8.88	11.00

[a]Defated product; [b]Soybean oil 3: Pollock liver oil 2; [c]Vitamin mixture provided the following) mg/l of diet); vit. B_1 = 6, vit. B_2 = 10, vit. B_6 = 4, B_{12} = 0.01, vit. C = 500, niacin s = 40, ca-pantothenate = 10, inositol = 200, biotin = 0.6, folic acid = 1.5, p-amino-benzoic acid = 5, vit. K_3 = 5, vit. A = 4000 IU, vit. D3 = 40001U. [d]Ogino salt mixture, [e,f]digestible protein and energy values were calculated from the published data for diet ingredients for carp at 25°C

Table 2: Average weights, fork lengths, weight gain, specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR), protein efficiency ration (PER) and survival rate of fish fed various experimental diets. Values represent Means ± SD of two replications. Means along a column without a letter in common are significantly different at $p<0.05$

Treatment	Average fish weight (g)		Average fish fork length (min)		Weight gain^{-1}	SGR^{-2}	K^{-3}	Survival
	Initial	Final	Initial	Final		1g/day)	(Condition factor)	(%)
Control	28.42 ± 0.63	70.67 ± 0.01[a]	125.53 ± 0.52	163.35 ± 0.70[a]	148.80 ± 5.50[a]	0.7537 ± 0.01[a]	1.60 ± 0.01[a]	100
01	28.05 ± 0.85	41.88 ± 2.84[b]	125.87 ± 0.44	137.05 ± 3.00[de]	49.15 ± 5.62[a]	0.2450 ± 0.01[d]	1.61 ± 0.01[a]	100
02	28.52 ± 0.25	42.37 ± 0.76[a]	125.01 ± 0.29	136.37 ±1.87[a]	48.57 ± 1.38[e]	0.2612 ± 0.02[d]	1.64 ± 0.02[a]	100
03	28.65 ± 0.40	43.50 ± 0.46[de]	125.40 ± 0.65	137.77 ± 0.72[de]	51.88 ± 3.72[a]	0.2650 ± 0.00[d]	1.66 ± 0.03[a]	100
04	28.19 ± 0.34	53.57 ± 1.45[c]	124.95 ± 0.87	149.15 ± 1.60[c]	93.57 ± 2.82[c]	0.4688 ± 0.01[c]	1.65 ± 0.01[a]	100
95	28.52 ± 0.51	63.35 ± 1.22[b]	125.01 ± 0.25	155.70 ± 1.10[b]	122.29 ± 8.27[b]	0.6212 ± 0.01[b]	1.62 ± 0.01[a]	100
D6	28.30 ± 0.59	48.66 ± 0.95[cd]	125.66 ± 0.04	142.42 ± 0.72[d]	71.95 ± 0.23[d]	0.3625 ± 0.01[cd]	1.63 ± 0.00[a]	100

$^{-1}$ = ((Final body weight Initial body weight) + Initial body weight) × 100

$^{-2}$ = (Final weight - Initial weightl + Time interval (days), means were calculated from the data on fortnightly basis

$^{-3}$ = Weight in g (10^5) + fork length^3 (mm)

However, a clear trend was established during the second fortnight (Fig. 1). At 24% DP level, the highest fish growth rate of 51.88 ± 3.72% was obtained at GE level of 482.20 kcal/100g (D3) having PIE ratio of 59.12. However, the difference between D3 and D2 was statistically nonsignificant. At this protein level, increasing the dietary energy beyond 456.70 kcal/100 g resulted in non-significant increase in specific growth rate of fish also. At 28% DP level, 451.90 kcal dietary GE (D5) gave the maximum weight gain of 122.29 ± 8.27% while further increase in dietary GE resulted in significantly poor growth. Among all the treatments condition factor variations were nonsignificant. Control feed was significantly better consumed by the fish (Table 2, 3). Daily feed consumed by the fish was low at high energy diets, viz. D3 and D6. Protein efficiency ratios for both control and D5 were significantly high. However, the difference between these two treatments was statistically non-significant.

Nutrient retention in fish body: The highest protein and energy retention were obtained for the fish fed 28% DP diet at 451.90 kcal GE level (D5). However, the difference between D5 and control treatments was non-significant for protein retention while energy retention in control fish was significantly low (Table 3). The fish fed diet no.4 retained significantly maximum lipid followed by the retention of 77.78 ± 1.87% in fish body under D5. At dietary 28% DP, increasing the GE beyond 451.90 kcal resulted in significantly lowering of energy retention in fish body.

Results and Discussion

Average fish weight in all the treatments increased significantly, which represents a range of 49.15 ± 5.62 148.80 ± 5.50% weight gain. Highest weight gain was observed for the fish fed control diet followed by that for D5. Table 3 depicts protein intake by the fish increased with rising protein level in diets. Fish growth, feed efficiency, protein efficiency ratio, protein and energy retention were directly dependent on *feed* intake which correlated positively ($p<0.01$) with the dietary protein level. Protein retention in fish increased at higher dietary protein level while decreased significantly with increasing GE level beyond 451.90 kcal. When feeding the fish with low-protein diet, the pressure on body protein seems to be high due to the need to meet the demands for tissue repair and metabolic fecal, nitrogen. However, significantly higher protein retention at 73.75 PIE level (D5) would be due to protein sparing effect at high energy level by carbohydrate

Table 3: Nutrient intake and retention by grass carp fed experimental diets^{-1}. Mean are ± SD of two replications for each treatment. Means along a column without a letter in common are significantly different at $p<0.05$

Treatment	Food intake	DFC^{+2}	FE^{-3}	PER^{+4}	Nutrient intake			Nutrient retention in this body^{-3}		
	(g)	(%)			Protein (g)	Lipid (g)	Energy (kcal)	Protein (%)	Lipid (%)	Energy (%)
Control	1129.98 ± 8.57^{a}	2.18 ± 0.04^{a}	0.74 ± 0.01^{a}	2.08 ± 0.01^{a}	405.89 ± 3.08	130.18 ± 0.99	4953.85 ± 37.55	33.15 ± 0.55^{a}	45.89 ± 3.50^{c}	27.68 ± 0.78^{b}
D1	867.40 ± 5.60^{d}	2.17 ± 0.04^{a}	0.31 ± 0.04^{d}	1.15 ± 0.16^{c}	239.92 ± 1.55	80.49 ± 0.52	3740.23 ± 24.15	20.14 ± 2.23^{c}	51.64 ± 0.76^{c}	17.68 ± 1.78^{d}
D2	840.95 ± 11.97^{e}	2.05 ± 0.02^{b}	0.33 ± 0.01^{e}	1.14 ± 0.02^{c}	242.28 ± 3.45	91.92 ± 1.31	3640.61 ± 64.66	19.67 ± 1.33^{c}	71.80 ± 1.91^{b}	24.34 ± 0.58^{c}
D3	823.34 ± 2.58^{e}	1.93 ± 0.04^{e}	0.36 ± 0.02^{d}	1.25 ± 0.07^{c}	234.73 ± 0.73	132.64 ± 0.42	3970.14 ± 12.44	18.44 ± 1.63^{c}	46.97 ± 0.59^{c}	23.52 ± 0.31^{c}
D4	938.91 ± 3.30^{c}	2.00 ± 0.09^{b}	0.56 ± 0.02^{b}	1.69 ± 0.08^{b}	311.72 ± 1.07	47.32 ± 0.16	3998.82 ± 13.63	26.78 ± 0.67^{b}	97.96 ± 0.99^{a}	25.30 ± 0.08^{bc}
D5	1029.10 ± 1.70^{a}	2.04 ± 0.01^{b}	0.67 ± 0.03^{a}	2.02 ± 0.09^{a}	342.99 ± 0.57	113.28 ± 0.06	4650.50 ± 7.68	34.94 ± 1.64^{a}	77.78 ± 1.87^{b}	32.89 ± 1.05^{a}
D6	880.39 ± 4.96^{d}	2.02 ± 0.06^{bc}	0.46 ± 0.01^{c}	1.41 ± 0.03^{bc}	288.86 ± 1.63	124.49 ± 0.71	4201.22 ± 23.67	23.16 ± 0.41^{bc}	55.03 ± 0.18^{c}	24.70 ± 0.32^{c}

*1 = All results are expressed on a dry matter basis

*2 = Daily feed consumption = (Feed intake (g) + (Initial body weight + Final body weight + 2) × days) × 100

*3 = Feed efficiency = Weight gain (g) + Feed intake (g) as dry matter

*4 = Protein efficiency ratio = Body weight gain (g) + Protein intake (g)

*5 = (Final nutrient content-Initial nutrient content + Nutrient intake) × 100

Continued Table 3

Correlation coefficients among different variables	
Dependent Variable (Y)	Independent Variable (x) (Feed Intake by the Fish)
Weight increment	0.9770**
Feed efficiency	0.9410**
Protein efficiency ratio	0.9250**
Protein retention	0.9030**
Energy retention	0.6410**
Dependent Variable (V) (Feed Intake by the Fish)	**Independent Variable (x)**
0.8620**	Dietary protein
-0.2250[5]	Dietary lipids
-0.4440*	Dietary energy

** = Significant at $p<0.01$; * = significant at $p<0.05$

NS = non-significant

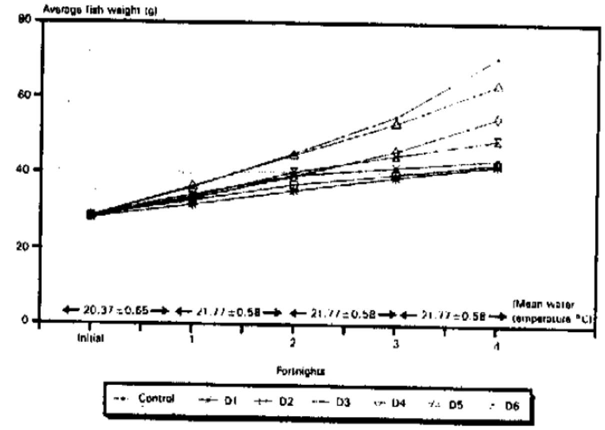

Fig. 1: Growth perofmrance of grass carp under various experimental diets

and lipids (Takeuchi *et al.*, 1979). The results thus indicate consumption dependent protein and energy retention in fish body as a function of dietary protein level. Fish growth and daily feed consumption showed positively significant regression on water temperature. During the last fortnight, a mean water temperature of 24.76 ± 0.49°C gave best growth in fish. Cai and Curtis (1990) provided report about the best growth performance of grass carp between 18.30 and 24.90°C. However, Pfeiffer and Lovell (1990) reported 26 to 30°C as an optimum temperature range for grass carp grown to 175 g in ponds with supplemental feeding. Among six test diets, containing corn gluten meal as major protein source, the best growth was observed in fish fed diet no. 5 containing 26.20% corn gluten meal at a dietary GE level of 451.90 kcal/100 g. However, fish growth was depressed when 27.44% corn gluten meal was added in the diet with increased GE level. Consequently, at this high energy and protein level (D6), feed consumption of fish dropped significantly lowering the intake of necessary amount of nutrients for fish growth and resulted significant decrease in nutrient retention. The high retention of plant origin proteins, in comparison to animal protein based diet (control), may be related to the herbivorous feeding habit of grass carp. This finding strengthen the view that enzyme system in cyprinids, which have a long gut, is better equipped to digest and absorb nutrients from plant feedstuffs (Smith, 1989).

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