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**Aquaculture**

# EFFECTS OF DIETARY PROTEIN AND CARBOHYDRATE LEVELS ON GROWTH PERFORMANCE, FEED UTILIZATION EFFICIENCY AND NITROGEN METABOLISM IN ROHU, *LABEO ROHITA* (HAMILTON), FINGERLINGS

# WPŁYW ZAWARTOŚCI BIAŁKA I WĘGLOWODANÓW W PASZY NA WZROST, EFEKTYWNOŚĆ WYKORZYSTANIA PASZY I METABOLIZM AZOTU U PALCZAKÓW GRUBOWARGA INDYJSKIEGO, *LABEO ROHITA* (HAMILTON)

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A 60-day feeding experiment was conducted in the laboratory to evaluate the interactive effects of dietary protein and carbohydrate levels on growth, feed utilization efficiency, and nitrogen metabolism in rohu, *Labeo rohita* fingerlings (mean weight 4.06 ±0.08 g). Nine purified diets prepared with 25, 35, and 40% protein level each having 15, 25, and 35% carbohydrate were fed to nine different feeding groups at the rate of 3% of body weight in triplicate treatments. Better performance of fish in terms of percent weight gain, SGR, FCR, and PER was observed with increasing percentage of carbohydrate at a given protein level. Protease and α-amylase activities increased with increase in dietary protein and carbohydrate levels, respectively. Glutamate oxaloacetate transaminase (GOT) activities varied significantly in some groups. No significant difference among different groups regarding glutamate pyruvate transaminase (GPT) activity was observed. Ammonia excretion was found to increase with increased consumption of dietary protein and carbohydrate and was highest in the groups of fish fed 40% protein diet suggesting active nitrogen metabolism in these groups.

## INTRODUCTION

Protein is the premier dietary macronutrient and the most expensive component in a satisfactory fish diet (Cho et al. 1985). The protein is utilized for tissue growth as well as

energy expenditure of fish. A number of studies have been carried out to save protein for growth using fat and / or carbohydrate for energy needs (Artherton and Aitken 1970; Lee and Putnam 1973; Watanabe et al. 1977, 1987; Takeuchi et al. 1979; Pieper and Pfeffer 1980; Kaushik and Oliva-Teles 1985; Beamish and Medland 1986; Tabachek 1986; Walton 1986).

The use of carbohydrate as a protein sparing energy source has received comparatively lesser attention than the use of dietary protein for the same purpose. Native carbohydrates are less suited for carnivorous fish and carbohydrates seem to compete successfully with lipid as an energy source (Dabrowski 1986).

Although there are reports on the protein sparing effects of carbohydrate in the diets for carp fry and fingerlings (Sen et al. 1978 and Seenappa and Devaraj 1995 in *Catla catla* fingerlings; Rao 1987 in fry and fingerlings of *L. rohita*, *Cirrhinus mrigala*, and *Cyprinus carpio*), reports on the effects of varying dietary protein and carbohydrate levels on the metabolic enzymes and ammonia excretion are scarce. In view of this, an attempt has been made during the course of present investigation, to study the interactive effects of different dietary protein and carbohydrate levels on growth performance, body composition, pattern of post-prandial ammonia excretion, and certain enzyme activities in rohu, *Labeo rohita* fingerlings.

## MATERIAL AND METHODS

### Experimental diets

Nine purified diets using casein and gelatin as principal protein source and dextrin as major carbohydrate source, were prepared at 25, 35, and 40% protein levels, each protein level having 15, 25, and 35% carbohydrate. The ingredients and proximate composition of the feeds are shown in Table 1. To each of the formulated diets, 1% chromic oxide was added as an external digestibility marker. All the diets were prepared in pelleted form using carboxymethylcellulose as a binder.

### Experimental design

The feeding trial was conducted in circular 90 $dm^3$ fibre glass aquaria with flow through system. *Labeo rohita* fingerlings (mean weight 4.06 ±0.08 g) were obtained from a local fish seed dealer and were acclimatized in the aquaria for 15 days. The fingerlings were randomly distributed between the aquaria at a stocking density of 20 fish per aquarium. There were three replicates for each experimental diet.

Initial body weight, proximate composition of carcass, activities of different enzymes (viz., α-amylase, protease, glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT)), were determined prior to the commencement of the experiment. The experimental fish were fed with formulated diets twice a day at 0800 hours and 1200 hours

at a fixed feeding rate of 3% wet body weight per day for 60 days. Hardly any uneaten feed remained one hour after feeding. However, any feed remaining after feeding was immediately siphoned out, dried, and weighed to know the exact amount of feed intake. The fish were weighed every $10^{th}$ day and the daily ration allotted was adjusted accordingly.

The digestibility experiment was continued for 20 days in static water system. During this period faecal samples were collected every day in the morning by siphoning, 17 h after removal of the feed following the method described by Spyridakis et al. (1989). Faeces collected from replicate treatments were pooled, dried at 60°C in an oven and kept for subsequent analysis. Before the termination of the experiment, the postprandial (2 h after feeding) ammonia excretion by different groups of fish were determined by estimating the ammonia content in the ambient water. This part of the experiment was also conducted in the static water system. At the termination of the experiment the carcass composition, activities of aforementioned enzymes in fish from each treatment were determined.

Throughout the experimental period the water temperature, pH, and dissolved oxygen assumed the following values: 30–33°C, 7.0–7.6 and 6.5–8.8 $mg \cdot dm^{-3}$, respectively.

## Chemical analyses

The proximate composition (on dry matter basis) of experimental diets, faecal samples, and whole body of the fish (on wet weight basis) was determined according to AOAC (Helrich 1990) procedures as follows: moisture was determined by ovendrying at 105°C for 24 h; crude protein (N × 6.25) by the semi-micro Kjeldahl digestion and distillation after acid digestion; crude lipid by Soxhlet ether extraction; ash by combustion at 550°C in a Muffle furnace to a constant weight; crude fibre by acid/alkali digestion. Nitrogen-free extract (NFE) was computed by taking the sum values for crude protein, lipid, ash, crude fibre, and moisture and substracting this from 100 (Maynard et al. 1979). Chromic oxide in the diets and faeces was estimated spectrophotometrically following the method of Bolin et al. (1952). Water analyses followed the methods outlined by the APHA (Anonymous 1980). Ammonia in ambient water was estimated by indophenol method. At 0 h after feeding, 50 ml of water from each tank was taken in a conical flask. To the collected water, 2 ml of phenol solution, 2 ml of sodium nitroprusside, and 5 ml of oxidizing solution were added. After a thorough mixing, the flask was allowed to stand at room temperature for 1–2 hours. The top of the flask was covered with aluminium foil at this stage to lessen the contamination by atmospheric ammonia. The extinction was read at 640 nm in a spectrophotometer against a distilled water blank. The same procedure was followed to estimate the ammonia content in water after 2 hours of feeding.

**Table 1**

Ingredient composition (% dry weight) and proximate composition of the experimental diets (on dry matter basis)

| Ingredients | Diets (dietary codes) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | D1 (P25/C15) | D2 (P25/C25) | D3 (P25/C35) | D4 (P35/C15) | D5 (P35/C25) | D6 (P35/C35) | D7 (P40/C15) | D8 (P40/C25) | D9 (P40/C35) |
| Casein | 22.50 | 22.50 | 22.50 | 31.50 | 31.50 | 31.50 | 36.00 | 36.00 | 36.00 |
| Gelatin | 4.50 | 7.50 | 7.50 | 10.50 | 10.50 | 10.50 | 12.00 | 12.00 | 12.00 |
| Dextrin | 15.00 | 25.00 | 35.00 | 15.00 | 25.00 | 35.00 | 15.00 | 25.00 | 35.00 |
| Sunflower oil | 6.00 | 6.00 | 6.00 | 4.00 | 4.00 | 4.00 | 2.00 | 2.00 | 2.00 |
| Cod liver oil | 6.00 | 6.00 | 6.00 | 4.00 | 4.00 | 4.00 | 2.00 | 2.00 | 2.00 |
| Cellulose powder | 40.00 | 30.00 | 20.00 | 32.00 | 22.00 | 12.00 | 30.00 | 20.00 | 10.00 |
| Vit. Min. Premix* | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Chromic oxide | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Parameter | Proximate composition (% dry matter) | | | | | | | | |
| Moisture | 5.55 | 5.99 | 6.07 | 6.61 | 6.10 | 5.78 | 6.02 | 5.52 | 5.87 |
| Protein | 26.00 | 25.61 | 25.60 | 35.68 | 35.46 | 36.33 | 40.93 | 40.27 | 40.49 |
| Lipid | 11.51 | 11.39 | 10.99 | 7.89 | 7.51 | 7.02 | 3.41 | 3.58 | 3.75 |
| Ash | 4.98 | 4.95 | 4.95 | 4.47 | 4.97 | 5.00 | 4.47 | 4.50 | 3.94 |
| Crude fibre | 36.80 | 26.40 | 17.01 | 29.66 | 21.09 | 11.01 | 29.91 | 20.16 | 10.01 |
| NFE* | 15.16 | 25.66 | 35.58 | 15.69 | 24.87 | 34.86 | 15.26 | 25.97 | 34.94 |
| Gross energy (kcal/100 g) | 317.82 | 357.53 | 393.50 | 340.48 | 373.28 | 414.52 | 326.04 | 367.83 | 407.46 |

* Vitamin and mineral mixture (Vitaminetes forte, Roche Products Ltd., Mumbai-400 034, India).

** Nitrogen-free extract.

Activities of α-amylase (EC.3.2.1.1) and protease (EC.3.5.1.5) were determined following the methods of Bernfeld (1955) and Moore and Stein (1948), respectively. Activities of glutamate oxaloacetate transaminase (GOT, EC.2.6.1.1) and glutamate pyruvate transaminase (GPT, EC.2.6.1.2) were determined following the methods of Reitman and Frankel (Bergmeyer and Bernt 1974).

### Data processing and statistical analyses

Fish performance in terms of weight gain (%), specific growth rate, feed conversion ratio, protein efficiency ratio and apparent nutrient digestibility was determined using the following formulae:

$$\text{Weight gain (\%)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{Specific growth rate } (SGR;\ \%\cdot\text{day}^{-1}) = \frac{\ln W_t - \ln W_i}{T} \times 100$$

where $W_t$ is the weight of fish at time $t$, $W_i$ is the weight of fish at time 0, and $T$ is the culture period in days.

$$\text{Feed conversion ratio } (FCR) = \frac{\text{Weight consumed (g)}}{\text{Weight gain (g)}}$$

$$\text{Protein efficiency ratio } (PER) = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

Apparent nutrient digestibility was calculated according to De Silva and Anderson (1995) as follows:

$$\text{Apparent nutrient digestibility (\%)} = 10^2 - \left\{10^2 \times \frac{I_d}{I_f} \times \frac{N_f}{N_d}\right\}$$

where $I_d$ represents chromic oxide in diet and $I_f$, chromic oxide in faeces, $N_d$ is nutrient in diet, and $N_f$ nutrient in faeces.

The data were analysed by one-way analysis of variance (ANOVA). Mean differences between treatments were tested for significance at $P < 0.05$ and comparison was made by Duncan's multiple range test (Duncan 1955).

## RESULTS

The ingredient composition and proximate composition of the experimental diets are presented in the Table 1.

The data regarding growth performance and nutrient utilization by rohu fingerlings fed experimental diets are presented in Table 2. The average final weight of the fish increased considerably from the initial value in all dietary treatments. Rohu fingerlings fed

diet D9 containing 40% protein and 35% carbohydrate had the highest weight gain which was significantly higher ($P < 0.05$) than those fed other diets: At any given dietary protein level, increase in carbohydrate level from 15% to 25, or 35% produced well marked and significant improvement in percent live weight gain, SGR, PER, and FCR. Best PER was recorded in fish fed diet D6 which was significantly different ($P < 0.05$) from all diets. PER was lowest with diet D1. SGR was highest with diet D9. The FCR was recorded best for diet D9.

Results regarding nutrient digestibility are depicted in Table 3. No significant difference regarding protein and lipid digestibility was observed among different dietary treatments. Dry matter digestibility was found highest for diet D8 and lowest for diet D5.

The proximate carcass composition of fish receiving various experimental diets is presented in Table 4. The moisture, lipid and ash contents in the carcass decreased in comparison to respective initial values in all the dietary treatments. The whole body protein, on the other hand, was found to increase over the initial value in all dietary treatments. No significant difference ($P < 0.05$) in carcass moisture content was noticed in the groups of fish fed experimental diets except diet D1. The carcass protein content was highest in the fish fed diet D9 which was significantly higher from that in the dietary groups D1, D2, D3, D4, D5, and D7. The carcass protein was recorded lowest in the fish fed diet D1. The carcass lipid was recorded highest in the fish fed diet D3 and lowest in dietary group D1.

The profiles of the digestive enzymes (α-amylase and protease) in the intestine of fish fed experimental diets are presented in Fig. 1. In all dietary groups activities of protease and α-amylase were found to increase from the initial group. Protease and α-amylase activities increased with increase in dietary protein and carbohydrate levels, respectively. The activities of both protease and α-amylase were found maximum in fish fed diet D9 which differed significantly ($P < 0.05$) from the rest of the groups.

Fig. 2. depicts the changes in glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in the liver of rohu fingerlings fed experimental diets. Except diet D1 the activities of both GOT and GPT decreased form the initial values. No significant difference regarding GPT activity among different dietary groups was observed.

Post-prandial ammonia excretion by different groups of fish is presented in Fig. 3. After 2 hours of feeding the ammonia excretion was found to increase with increasing dietary protein level. The maximum ammonia excretion was recorded in dietary group D9 (P40/C35) which was significantly higher than that in the remaining groups.

**Table 2**

Growth performance and feed utilization efficiency of *Labeo rohita* fingerlings fed formulated diets for 60 days

| Parameters | Diets | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 |
| Avg. initial body weight | 4.10 ±0.11$^{a}$ | 4.10 ±0.15$^{a}$ | 4.10 ±0.12$^{a}$ | 4.10 ±0.13$^{a}$ | 4.10 ±0.13$^{a}$ | 4.10 ±0.13$^{a}$ | 4.10 ±0.13$^{a}$ | 4.10 ±0.14$^{a}$ | 4.10 ±0.14$^{a}$ |
| Weight gain (%) | 33.97 ±1.2$^{a}$ | 47.88 ±1.1$^{b}$ | 70.13 ±1.9$^{d}$ | 61.07 ±1.8$^{c}$ | 89.11 ±2.1$^{e}$ | 118.87 ±2.2$^{g}$ | 109.19 ±2.0$^{f}$ | 126.93 ±1.9$^{h}$ | 144.63 ±2.1$^{i}$ |
| SGR (%·day$^{-1}$) | 0.48 ±0.01$^{a}$ | 0.64 ±0.02$^{b}$ | 0.87 ±0.03$^{d}$ | 0.78 ±0.02$^{c}$ | 1.05 ±0.04$^{e}$ | 1.30 ±0.05$^{g}$ | 1.22 ±0.05$^{f}$ | 1.36 ±0.05$^{g}$ | 1.48 ±0.05$^{h}$ |
| FCR | 5.97 ±0.21$^{g}$ | 4.45 ±0.19$^{f}$ | 3.37 ±0.13$^{d}$ | 3.69 ±0.13$^{e}$ | 2.77 ±0.10$^{c}$ | 2.33 ±0.10$^{b}$ | 2.45 ±0.10$^{b}$ | 2.26 ±0.09$^{ab}$ | 2.04 ±0.09$^{a}$ |
| PER | 0.64 ±0.02$^{a}$ | 0.87 ±0.03$^{c}$ | 1.15 ±0.04$^{e}$ | 0.75 ±0.03$^{b}$ | 1.10 ±0.04$^{e}$ | 1.78 ±0.06$^{f}$ | 1.00 ±0.03$^{d}$ | 1.09 ±0.04$^{e}$ | 1.18 ±0.04$^{e}$ |

**Table 3**

Apparent digestibility of nutrients in *Labeo rohita* fingerlings fed experimental diets

| Parameters | Diets | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 |
| Dry matter | 73.58 ±1.5$^{a}$ | 73.34 ±1.2$^{a}$ | 69.60 ±1.7$^{a}$ | 68.80 ±2.0$^{a}$ | 66.81 ±1.9$^{a}$ | 68.80 ±1.6$^{a}$ | 73.82 ±1.4$^{a}$ | 74.55 ±1.3$^{a}$ | 71.80 ±2.0$^{a}$ |
| Protein | 91.02 ±1.1$^{a}$ | 85.27 ±1.0$^{a}$ | 89.03 ±1.9$^{a}$ | 92.47 ±1.4$^{a}$ | 86.88 ±1.6$^{a}$ | 87.05 ±1.8$^{a}$ | 88.33 ±1.9$^{a}$ | 87.89 ±1.8$^{a}$ | 87.55 ±1.8$^{a}$ |
| Lipid | 90.01 ±1.6$^{a}$ | 89.96 ±1.3$^{a}$ | 88.21 ±1.4$^{a}$ | 87.03 ±1.5$^{a}$ | 85.84 ±1.8$^{a}$ | 86.43 ±1.7$^{a}$ | 85.71 ±1.6$^{a}$ | 87.01 ±1.3$^{a}$ | 85.22 ±1.8$^{a}$ |

**Table 4**

Carcass composition (% wet weight) of *Labeo rohita* fingerlings fed experimental diets for 60 days

| Parameters | Initial | Diets | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 |
| Moisture | 80,42 | 79,00 ±1,10$^{b}$ | 74,50 ±1,20$^{a}$ | 72,00 ±1,50$^{a}$ | 75,00 ±1,50$^{a}$ | 75,50 ±1,40$^{a}$ | 75,00 ±1,20$^{a}$ | 72,92 ±1,10$^{a}$ | 74,38 ±1,30$^{a}$ | 72,21 ±1,10$^{a}$ |
| Lipid | 6,03 | 2,70 ±0,10$^{a}$ | 3,20 ±0,13$^{b}$ | 4,90 ±0,19$^{d}$ | 3,20 ±0,13$^{b}$ | 3,20 ±0,13$^{b}$ | 3,20 ±0,13$^{b}$ | 4,20 ±0,15$^{c}$ | 3,20 ±0,13$^{b}$ | 4,20 ±0,15$^{c}$ |
| Protein | 11,30 | 12,89 ±0,50$^{a}$ | 13,69 ±0,60$^{a}$ | 14,32 ±0,71$^{b}$ | 15,42 ±0,71$^{b}$ | 15,76 ±0,70$^{c}$ | 17,51 ±0,75$^{d}$ | 16,16 ±0,72$^{c}$ | 17,51 ±0,73$^{d}$ | 17,79 ±0,75$^{d}$ |
| Ash | 5,22 | 5,00 ±0,10$^{c}$ | 5,00 ±0,10$^{c}$ | 5,00 ±0,10$^{c}$ | 4,47 ±0,10$^{b}$ | 4,00 ±0,10$^{b}$ | 3,47 ±0,09$^{a}$ | 4,47 ±0,10$^{b}$ | 3,47 ±0,09$^{a}$ | 3,98 ±0,10$^{b}$ |

Figures with same superscripts in the same row were not significantly different ($P < 0.05$).

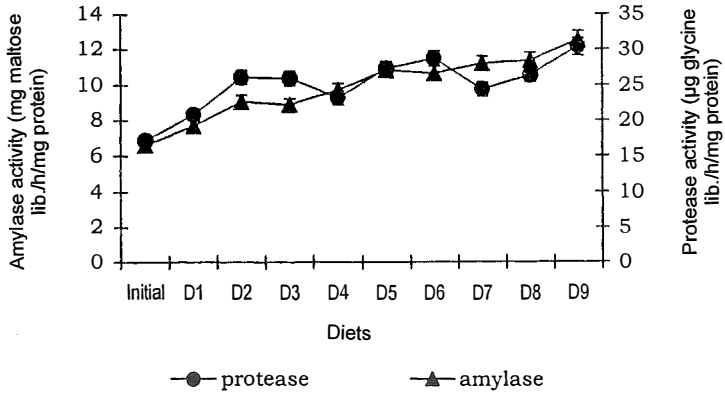


Fig. 1. Intestinal α-amylase and protease activities in *L. rohita* fingerlings fed experimental diets for 60 days

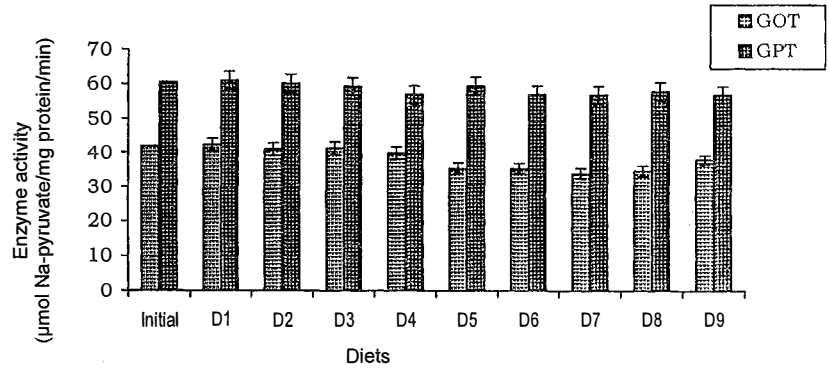


Fig. 2. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities of *L. rohita* fed experimental diets for 60 days

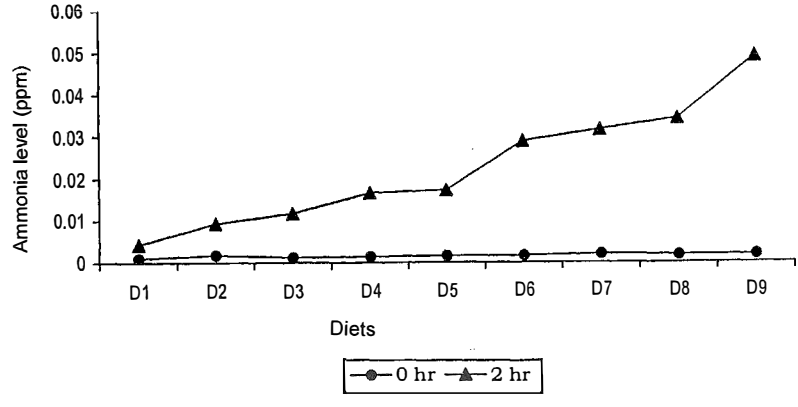


Fig. 3. Post prandial dissolved ammonia in ambient water

## DISCUSSION

The results of the present study indicated that diets containing 40% protein and 35% carbohydrate supported maximum growth and feed conversion in rohu fingerlings under restricted feeding regime. The corresponding gross energy of the diet was 407.46 kcal·100 $g^{-1}$. Mohanty et al. (1990) and Das et al. (1991) reported the protein requirement of rohu fingerlings to be 40 to 50% (gross energy 400 kcal·100 $g^{-1}$). Seenappa and Devaraj (1995) observed that catla, *Catla catla* fingerlings attained maximum growth with diets containing 30% protein and 35% carbohydrate. Kaushik and Oliva-Teles (1985) and Kaushik et al. (1989) clearly demonstrated that incorporation of high levels of carbohydrate in diets did not adversely affect growth performance or retention of major nutrients in rainbow trout, *Oncorhynchus mykiss*. In the present investigation comparison between diets P40/C15 and P40/C35 showed that increase in carbohydrate level in the diets exerted a beneficial effect on weight gain (%), SGR, FCR, and PER, indicating the possibility of raising the carbohydrate level from 15 to 35% in formulated diets. The results corroborated with the observations of Buhler and Halver (1961) showing that chinook salmon, *Oncorhynchus tshawytscha* tolerated 48% dextrin or 20% digestible sugar in their diets. The restricted feeding regime used in the present study which did not allow feed intake to the choice of the fish, helped in bringing out the favourable effects of carbohydrate on growth more clearly (Bergot 1979). Feeding an excess of high carbohydrate diets might cause growth depression in fish (Satpathy 1997). Sen et al. (1978) observed no difference in weight gain in *Catla catla* when fed diets containing 40 : 30 and 45 : 25 protein/carbohydrate levels and reported sparing of protein by 5 percentage points corresponding to an increase of 5 percentage points carbohydrate level in diets. Rao (1987) demonstrated protein sparing by carbohydrate in fiy and fingerlings of *L. rohita*, *Cirrhinus mrigala*, and *Cyprinus carpio* through feeding diets containing higher carbohydrate and lower protein levels. At a given dietary protein level, raising 15 to 25 or 35% produced well marked and significant improvement in weight gain (%), SGR, PER, and FCR; such improvements being more pronounced at optimum protein diets (40% protein). In the present study, the observed effect of protein sparing by carbohydrate was more pronounced at 40% dietary protein level. This is in agreement with the findings of Ahmed and Matty (1989) who reported that at 40% protein level some of the protein that is normally used up for energy was spared for growth by antibiotics in carp.

The results indicated that protein and lipid digestibility in experimental diets were high (above 85%) despite the variation in carbohydrate content. It was also evident that increasing the level of dietary carbohydrate did not affect the digestibility of protein and lipid

in rohu fingerlings. Similar observations have also been reported by Hemre et al. (1989) for cod, *Gadus morhua*.

In the present experiment, raising available energy supply (gross energy) up to 407.46 kcal·100 $g^{-1}$ diet using carbohydrate did not increase body lipid deposits, nor there was a depression in protein accretion. The findings are in agreement with the observation by Beamish et al. (1986) who reported that when energy was supplied by carbohydrate rather than lipid, fish contained lower levels of lipid and higher levels of protein. Bergot (1979), however, found no differences in body composition attributed to feeding graded levels of carbohydrate in the diet. The carcass moisture and ash contents also showed decreasing trend with increase in dietary carbohydrate level at a given dietary protein level. These results could be cited as an evidence of proper utilization of dietary protein for protein synthesis and less for energy needs.

In the present study, diet-related changes of digestive enzyme activities (protease and α-amylase) were noted which led to diet-related growth differences: high dietary protein and carbohydrate levels resulted in high protease and α -amylase activities, respectively and corresponding good growth rates. Kawai and Ikeda (1973) also noted a positive linear correlation between dietary protein content, fish growth and activities of protease, amylase and maltase in young rainbow trout. Likewise, in *Salmo salar* fry, a significant growth depression was induced by that diet which evoked reduced protease activities (Holm and Torrissen 1987). On the other hand, Hofer and Nassir Uddin (1985) described an inverse relationship between dietary protein content, trypsin activities and growth in larval roach, *Rutilus rutilus*. Baragi and Lovell (1986) and Segner et al. (1989) however, did not find any relationship between enzyme response and growth response in larval striped bass, *Morone* sp. and larval *Coregonus lavaretus*, respectively.

In all vertebrates, dietary proteins are broken down into amino acids during digestion, absorbed in the intestine and mixed in the body pools of free amino acids. There also exists another source of amino acids, that is catabolism of tissue proteins, which supplies 70 to 80% of the free amino acids in the precursor pools in rates. This source is quite limited in fish, and is less than 50% (Walton and Cowey 1977), compelling fishes to be more dependent on dietary sources for their body pools. Basically, amino acids are required for protein synthesis and in fish the major amount of amino acids are catabolised after protein synthesis requirement is met, for production of energy. Just after feeding, the supply of amino acids to the tissue often exceeds their capacity to synthesize protein and the excess is catabolised immediately by removal of the amino group and forming an α-keto acid. The amino group in teleost fishes is mainly excreted as ammonia. Thus the rate of post-prandial ammonia excretion is related to rate of amino acid catabolism. The major effects of high dietary protein intakes compared to low protein intakes are increases in body amino acid

concentration, excretion of ammonia, protein synthesis, activities of gluconeogenic enzymes and decreases in activities of glycolytic enzymes (De Silva and Anderson 1995). There is little effect on the activities of amino acid catabolising enzymes. Rates of ammonia excretion are directly related to the amount of protein consumed (Rychly 1980; Beamish and Thomas 1984). In trout, nitrogen consumption was regarded as being the most important variable influencing ammonia excretion. Dabrowski et al. (1987) reported that a rapid increase in post-prandial ammonia nitrogen excretion followed by a precipitus decline to pre-feeding levels was inimical for efficient protein utilization. In contrast, a gradual post-prandial rise in excretion reflected efficient use of protein for fish growth. Because amino nitrogen cannot be stored in large quantities, a sudden surge in amino acids in the blood led to increased deamination and nitrogen loss (Cowey 1980). In the present study, post-prandial ammonia excretion after 2 hours of feeding was found to increase with increasing dietary protein and carbohydrate levels. Paul et al. (1997) also observed highest ammonia excretion rate within 1–2 h after feeding in *L. rohita* fingerlings fed five isocaloric and isonitrogenous diets containing certain unconventional animal protein sources. Brett and Zala (1975) found that ammonia excretion increased within four hours after feeding in sockeye salmon, *Oncorhynchus nerka* and showed that ammonia excretion was related to protein catabolism and dietary amino acids are catabolised rapidly after feeding. The results of post-prandial ammonia excretion in the present experiment also clearly indicate the increasing rate of amino acid catabolism and active nitrogen metabolism with increasing dietary protein and carbohydrate levels.

For each amino acid there is a specific enzyme responsible for initiating its catabolism. Generally, the enzymes responsible for catabolising amino acids are not increased by increasing dietary amino acid concentrations. The transdeamination is considered to be the major contributor to ammonia production in fish (Walton and Cowey 1977). Aminotransferase enzymes also play an important role in transdeamination and the two aminotransferases considered to be the most important in fish are glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate tansaminase (GPT). As stated earlier the adaptation to dietary carbohydrate resulted in a sparing effect of protein. Kheyyali et al. (1989) reported significant decrease in the activity of GPT in the hepatopancreas of carp, *Cyprinus carpio* when protein energy was partially replaced by carbohydrate and only a slight decrease when this energy was principally replaced by lipid. They, however, observed no significant change in the activity of GOT. In the present investigation activities of both GOT and GPT in the liver of *L. rohita* fingerlings were found to decrease with increase in dietary carbohydrate level at any given dietary protein level. The response of GOT and GPT in our experiment is consistent with the findings of Kheyyali et al. (1989) in that carbohydrate, which was more metabolizable in carp, depressed the GPT more efficiently.

The results of the present study established the possibility of incorporating up to 35% carbohydrate in compounded diets with 40% dietary protein content to support optimal growth rate in rohu fmgerlings. The protein sparing effect of carbohydrate was confirmed from the highly significant positive effect on growth, nutrient utilization and nitrogen metabolism which was best evident with the diet containing 40% protein and 35% carbohydrate.

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## REFERENCES

**Ahmed T.S., A.J. Matty,** 1989: The effect of feeding antibiotics on growth and body composition of carp (*Cyprinus carpio*). Aquaculture, **77**: 211–220.

**Anonymous,** 1980: Standard methods for the examination of water and waste water. 15th edn. American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington DC.

**Artherton W.D., A. Aitken,** 1970: Growth, nitrogen metabolism and fat metabolism in *Salmo gairdneri* Rich. Comp. Biochem. Physiol., **36**: 719–747.

**Baragi V., R.T. Lovell,** 1986: Digestive enzyme activities in striped bass from first feeding through larval development. Trans. Am. Fish. Soc., **115**: 478–484.

**Beamish F.W.H., J.W. Hilton, E. Niimi, S.J. Slinger,** 1986: Dietary carbohydrate and growth, body composition, and heat increment in rainbow trout (*Salmo gairdneri*). Fish. Physiol. Biochem., **2**: 85–91.

**Beamish F.W.H., T.E. Medland,** 1986: Protein sparing effects in large rainbow trout, *Salmo gairdneri*. Aquaculture, **55**: 35–42.

**Beamish F.W.H., E. Thomas,** 1984: Effects of dietary protein and lipid on nitrogen losses in rainbow trout *Salmo gairdneri*. Aquaculture, **41**: 359–371.

**Bergmeyer H.U., E. Bernt,** 1974: Colorimetric assay of Reitman and Frankel. In: [Bergmeyer H.U. (ed.)], Methods of Enzymatic Analysis. Vol. II. Academic Press, Inc., New York: 735–739, 760–764.

**Bergot F.,** 1979: Carbohydrate in rainbow trout diets: Effect of level and source of carbohydrate and the number of meals on growth and body composition. Aquaculture, **18**: 157–161.

**Bernfeld P.,** 1955: Methods in Enzymology. vol. I [Colowick S.P., N.O. Kaplan (eds.)]. Academic Press, New York.

**Bolin D.W., R.P. King, F.W. Klosterman,** 1952: A simplified method for the determination of chromic oxide ($Cr_2O_3$) when used as a index substance. Science, **116**: 634–635.

**Brett J.R., C.A. Zala,** 1975: Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled condition. J. Fish. Res. Bd. Canada, **32**: 2479–2486.

**Buhler D.R. J.E. Halver,** 1961: Nutrition of salmonoid fishes IX. Carbohydrate requirements of chinook salmon. J. Nutr., **74**: 307–318.

**Cho C.Y., C.B. Cowey, T. Watanabe,** 1985: Finfish nutrition in Asia: methodological approaches to research and development. IDRC, Ottawa, Ontario, Canada.

**Cowey C.B.,** 1980: Protein metabolism in fish. In: [Buttery P.J., D.B. Lindsay (eds.)], Protein Deposition in Animals. Butterworth, London: 271–288.

**Dabrowski K.,** 1986: Mini-review on ontological aspects of nutritional requirements in fish. Comp. Biochem. Physiol., **85**A: 639–655.

**Dabrowski K., S.J. Kaushik, B. Fauconneau,** 1987: Rearing of Baer's sturgeon (*Acipenser baeri* Brandt). Larvae III. Nitrogen and energy metabolism and amino acid absorption. Aquaculture, **65**: 31–41.

**Das K.M., S. Mohanty, S. Sarkar,** 1991: Optimum dietary protein to energy ratio for *Labeo rohita* fingerlings. In: [De Silva S.S. (ed.)], Fish Nutrition Research in Asia, Proceedings of Fourth Asian Fish Nutrition Workshop. Asian Fish. Soc. Spec. Publ., **5**: 69–74.

**De Silva S.S., T.A. Anderson,** 1995: Fish nutrition in Aquaculture, Chapman & Hall, London.

**Duncan D.B.,** 1955: Multiple range and multiple F-tests: Biometrics, **11**: 1–42.

**Helrich K. (ed.),** 1990: Official Methods of Analysis of AOAC. vol. 1, 15th edn. Association of Analytical Chemists, Inc., Arlington, VA.

**Hemre G.L, O. Lie, E. Lied, G. Lambertson,** 1989: Starch as an energy source in feed for cod (*Gadus morhua*): digestibility and retention. Aquaculture, **80**: 261–270.

**Hofer, R., A. Nasir Uddin,** 1985: Digestive processes during the development of the roach, *Rutilus rutilus* L. J. Fish Biol., **26**: 683–689.

**Holm J.C., K.R. Torrissen,** 1987: Growth depression and acclimatization of protease activity in Atlantic salmon first-feeding fry responding to a diet supplemented with zooplankton. Aquaculture, **65**: 171–174.

**Kaushik S.J., A. Oliwa-Teles,** 1985: Effect of digestible energy on nitrogen and energy balance in rainbow trout. Aquaculture, **50**: 89–101.

**Kaushik S.J., F. Medale, B. Fauconneau, D. Blank,** 1989: Effect of digestible carbohydrate on protein/energy utilization and glucose metabolism in rainbow trout (*Salmo gairdneri*). Aquaculture, **79**: 63–74.

**Kawai S., S. Ikeda,** 1973: Studies on digestive enzymes of fishes: III. Development of digestive enzymes of rainbow trout after hatching and effect of dietary change on the activities of digestive enzymes in the juvenile stage. Bull. Jap. Soc. Sci. Fish., **39**: 817–823.

**Kheyyali D., S. Shimeno, M. Takeda,** 1989: Effect of dietary carbohydrate and lipid levels on hepatopancreatic enzymes and body composition in carp. In: [Takeda M., T. Watanabe (eds.)], The Current Status of Fish Nutrition in Aquaculture. Toba, Japan: 451–460

**Lee D.J., G.B. Putnam,** 1973: The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr., **103**: 916–922.

**Maynard L., J. Loosil, H. Hintz, R. Warner,** 1979: Animal Nutrition. 7th edn. [Zappa C.R. (ed.)]. Mc Graw-Hill, New York.

**Mohanty S.N., D. N. Swamy, S.D. Tripathi,** 1990: Growth, nutritional indices and carcass composition of Indian major carp fry *Catla catla* , *Labeo rohita* and *Cirrhinus mrigala* fed four dietary protein levels. Aquaculture Hungarica, **6**: 211–217.

**Moore S., W.W. Stein,** 1948: Photometric ninhydrin method for use in the chromatography of amino acids. J. Biol. Chem., **176**: 367–388.

**Paul B.N., S. Nandi, S. Sarkar, P.K. Mukhopadhyay,** 1997: Effects of feeding unconventional animal protein sources on nitrogen metabolism in rohu *Labeo rohita* (Hamilton). The Israeli Journal of Aquaculture; Bamidegh, **49**: 183–192.

**Pieper A., E. Pfeffer,** 1980: Studies on the comparative efficiency of utilization of gross energy from some carbohydrates, proteins and fats by rainbow trout (*Salmo gairdneri* Rich.). Aquaculture, **20**: 323–332.

**Rao N.G.S.,** 1987: Studies on the growth of fry and fingerlings of selected carps fed on formulated fish feeds. PhD Thesis. Bangalore University, Bangalore, India.

**Rychly J.,** 1980: Nitrogen balance in trout II. Nitrogen excretion and retention after feeding diets with varying protein and carbohydrate levels. Aquaculture, **20**: 343–350.

**Satpathy B.B.,** 1997: Nutritional evaluation of protein level and sources in formulated diets of rohu, *Labeo rohita* (Hamilton). PhD Thesis. Kalyani University, Kalyani, India.

**Seenappa D., K.V. Devaraj,** 1995: Effect of different levels of protein, fat and carbohydrate on growth, feed utilization and body carcass composition of fingerlings in *Catla catla* (Ham.). Aquaculture, **129**: 243–249.

**Segner H., R. Rosch, H. Schmidt, K.J. Poeppinghausen von,** 1989: Digestive enzymes in larval *Coregonus lavaretus* L. J. Fish Biol., **35**: 249–263.

**Sen P.R., N.G.S. Rao, S.R. Ghosh, M. Rout,** 1978: Observations on protein, fat and carbohydrate requirements of carp. Aquaculture, **13**: 245-255.

**Spyridakis P., R. Metailler, J. Gabaudan, A. Riaza,** 1989: Studies on nutrient digestibility in European seabass (*Dicentrarchus labrax*). I. Methodological aspects concerning faeces collection. Aquaculture, **77**: 61–70.

**Tabachek J.L.,** 1986: Influence of dietary protein and lipid levels on growth, body composition and utilization efficiencies of artic charr, *Salvelinus alpinus* L. J. Fish Biol., **32**: 139–151.

**Takeuchi T., T. Watanabe, C. Ogino,** 1979: Availability of carbohydrate and lipid as dietary energy sources for carps. Bull. Jan. Soc. Sci. Fish., **45**: 977–982.

**Walton M.J.,** 1986: Metabolic effects of feeding a high protein / low carbohydrate diet as compared to a low protein/high carbohydrate diet to rainbow trout (*Salmo gairdneri*). Fish Physiol. Biochem., **1**: 7–15.

**Walton M.J., C.B. Cowey,** 1977: Aspects of intermediary metabolism in salmonoid fish. Comp. Biochem. Physiol., **73**B: 59–79.

**Watanabe T., T. Takeuchi, C. Ogino,** 1977: Studies on the sparing effect of lipids on dietary protein in rainbow trout (*Salmo gairdneri*). In: [Halver J.E., K. Tiews (eds.)], Proceedings of World Symposium on Finfish Nutrition and Fishfeed Technol. vol. 1, Humburg, Berlin:113–125.

**Watanabe T., T. Takeuchi, S. Satoh, T. Ida, M. Yaguchi,** 1987: Development of low protein-high energy diets for practical carp culture with special reference to reduction of total nitrogen excretion. Nippon Suisan Gakkashi, **53**: 1413–1423.

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## WPŁYW ZAWARTOŚCI BIAŁKA I WĘGLOWODANÓW W PASZY NA WZROST, EFEKTYWNOŚĆ WYKORZYSTANIA PASZY I METABOLIZM AZOTU U PALCZAKÓW GRUBOWARGA INDYJSKIEGO, *LABEO ROHITA* (HAMILTON)

### STRESZCZENIE

W warunkach laboratoryjnych przeprowadzono 60-dniowe doświadczenie żywieniowe dla określenia wpływu zawartosci białka i węglowodanów na wzrost, efektywność wykorzystania paszy i metabolizm azotu przez palczaki grubowarga indyjskiego, *Labeo rohita* (średnia masa 4,06 +0,08 g). Przygotowano dziewięć receptur paszowych zawierających 25, 35 i 40% białka. Dla każdego poziomu białka przygotowano trzy zawartości węglowodanów: 15, 25 i 35%. Pasze były podawane dziewięciu grupom ryb i były dozowane w ilości 3% ich masy ciała. Doświadczenie przeprowadzono w trzech powtórzeniach. Dla każdego poziomu białka, wzrastającej zawartości węglowodanów towarzyszył zwiększony wzrost procentowy masy ciała oraz polepszenie wartości SGR, FCR i PER. Aktywność proteazy i α-amylazy zwiększały się odpowiednio wraz ze wzrostem poziomu białek i węglowodanów. Aktywność transaminazy glutaminianowo-szczawiowo-octowej (GOT) różniła się istotnie w poszczególnych grupach. Nie zaobserwowano natomiast istotnych różnic w odniesieniu do aktywności transaminazy glutaminianowo-pirogronowej (GPT). Wydalanie amoniaku wzrastało wraz ze wzrostem spożycia białek i węglowodanów i było najwyższe w grupach ryb żywionych paszami zawierającymi 40% białek, co może sugerować aktywny metabolizm azotu w tych grupach.

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