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RESEARCH ARTICLE

NUTRITIONAL ENERGETICS OF AN INDIAN MAJOR CARP *Labeo rohita* (HAM.), FAMILY *CYPRINIDAE*

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ABSTRACT

An eight weeks experimental trial was conducted with variable percentage levels of protein (25, 30, 35, 40, 45 & 50) diet fed to the Indian major carp, *Labeo rohita* to study the satiation time, maximum feed intake in different hours of the day, protein/dietary energy requirement for optimum growth in different size groups, amino acid absorption and quantitative requirement, *in vivo* protein biosynthesis etc. and finally to calculate the requirement of digestible energy for the production of one gram fish to thousand gram in weight. It was observed that the maximum feed intake recorded with smaller size groups (5.0 g) as compared to 20.0 g and 50.0 g, satiated within one hour and highest appetite was recorded at 12.00 hours of the day. Maximum growth were observed with 45.0% protein (5.0 g) followed by 40% (20.0 g) and 35% (50.0 g) in *Labeo rohita,* although growth continued till 50% of dietary protein but not significant at the 0.5% level. Maximum weight increase recorded with 1:14.85 digestible energy / digestible protein ratio. Maximum amino acid absorption takes place in the posterior serosal layer of the intestine. It was also recorded that the same 10 amino acids are essential and their requirement was almost at per with the other fish species, and as in higher vertebrates. However, the importance of cystine and tyrosine for the growth of *Labeo rohita* should not be ignored and should be considered as two additional essential amino acids. The protein biosynthesis *in vivo* is highly correlated with the dietary protein/energy, feed intake and growth performance of fish.

INTRODUCTION

Fish require dietary sources of energy and nutrients for growth, reproduction, immunity and health. Dietary requirements for energy, protein, amino acids, essential lipids, vitamins and minerals have been established for several fish species of commercial importance. Although bioenergetics of the most economic specices (*Catla catla, Labeo rohita and Cirrhinus mrigala*) of Indian waters and much preferred *Labeo rohita* is not yet developed. Assimilation and metabolism of nutrients and energy are similar to those of terrestrial animals, the process of osmoregulation, nitrogen excretion and energy expenditures are unique to fish. To perform optimally, the fish must have all of its nutrients and a supply of energy in optimum balance and quality. In contemporary aquaculture formulated artificial feeds from commercial feedstuffs are the primary source. Thus familiarization of the nutrients and their sources, requirements, and roles in metabolism are necessary for successful aquaculture of Indian major carps in the country. Fate of ingested energy as feed has been estimated by Cho and Kaushik (1990) in rainbow trout (16 to 145g in size) as fasting metabolic heat production, in kcal fish^{-1} day^{-1}, to be $8.85W^{0.82}$, where W is body weight. However, Smith (1989) reported this for the same fish as $4.41W^{0.63}$ in lower size group (4 to 50 g). National Research Council (1993) stated that there are several places where energy is lost between ingestion and weight gain.

A dietary excess or deficiency of useful energy can reduce growth rate. In practice, fish nutritionists have given priority to meeting the requirements for protein (but not the quantity of plant and animal protein and amino acids thereof), major minerals, and the vitamins, and generally have allowed energy to take care of itself but not fat (ratio of plant and animal fat) and carbohydrate (digestible and indigestible ratio). Also, practical feeds (30% protein) for most species made with commonly available ingredients, contains approximately 2.9 kcal g^{-1} which provides a digestible energy (kcal) to digestible protein (gram) ratio of 10:1, but for maximum weight gain for several fish species are ranging from 8.5 to 12.3 kcal g^{-1}. Protein and amino acid allowances as a function of dietary energy plane, that is, as the energy concentration of the diet increases, the protein percentage increases proportionately. The rationale here is that in *ad libitum* feeding, energy intake regulates feed consumption and thus the amount of nutrients the animal ingests daily. Fish, however, are not fed *ad libitum*, and often not to satisfy, and therefore nutrient consumption would be controlled by feed allowance and not energy concentration of the diet.

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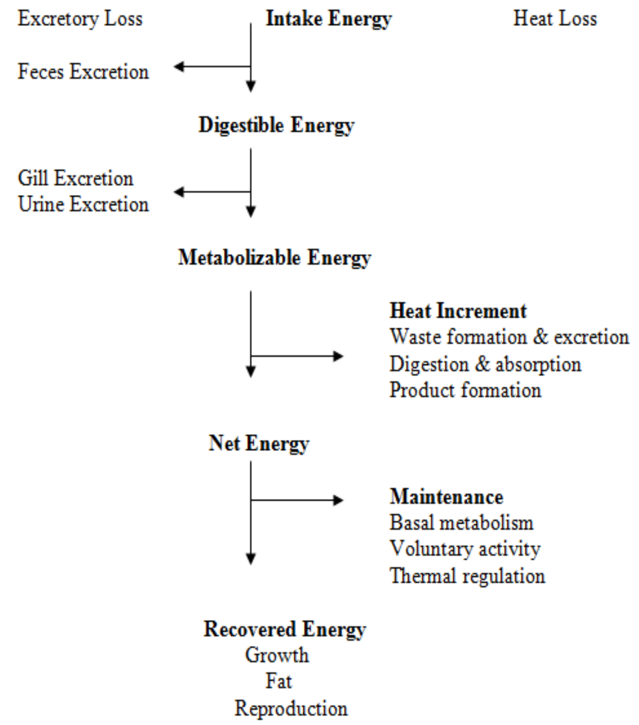

Fishes evolved in an aqueous environment where carbohydrates are scarce, their digestive and metabolic systems to be better adapted to utilization of protein and lipids for energy than carbohydrates. Some fishes, however, such as warm-water herbivore or omnivores, like Indian major carps can digest and metabolize carbohydrates relatively well. Since fish do not regulate body temperature they spend less energy in maintaining position in water than do terrestrial animals. Energy requirements may be calculated empirically, based on energy losses and energy recovery. Cho and Kaushik (1990) constructed a model for calculating the digestible energy required to grow 1 kg of rainbow trout, from 1g to 100g size at 15^0C, based on derived heat and excretory losses and estimated recovery of energy in the fish, and indicated that, 3.56 Mcal of digestible energy was required to produce 1.91 Mcal of recovered energy in 1kg of fish biomass with a recovered energy / digestible energy efficiency ratio of 0.54.

Most monogastric animals, including Indian major carps and other fishes, required the same 10 essential amino acids : arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Amino acid composition of practical feedstuffs is usually presented on a total content basis. Thus, in formulating fish feeds to meet amino acid requirements, the total amino acid contents of the feed ingredients must be corrected for availability. The digestibility of protein may be used in estimating the availability of amino acids in the feedstuff when digestibility of individual amino acids is not known, but digestibility of the protein is. Reports in the technical literature have indicated that the optimum level of protein in feeds for growth of fish has ranged from 25% to 50%. In all of these studies the researchers were probably justified in making their conclusions that a specific percentage of protein was optimum under their experimental conditions because a number of factors influence the growth response of fish to feeds containing different levels of protein, some of these are size of the fish, ambient water temperature, feed allowance, feeding frequencies, amount of non-protein energy, quality of the protein, and natural food availability. Fish has higher protein requirements during early growth than during later phases of growth.

MATERIALS AND METHODS

Experimental diets for evaluating protein requirements prepared from highly purified ingredients (Casein, Gelatin as protein; Dextrin, Alpha-cellulose & Carboxymethyl cellulose as carbohydrate source, Fish oil & Vegetable oil as lipid source; Amino acid mixture, vitamin premix, Mineral premix, etc.) to allow maximum control over the nutrient being tested. All diets were alike in all respect, such as palatability, particle size & texture, water stability, and nutrient and energy content. Fishes were conditioned to the rearing environment for 2 weeks with test diet prior to beginning the experiment. The fish fed *ad libitum* twice daily for enhancing sensitivity to diet differences and maximum growth. Feed allowance was adjusted each week on the basis of weight increment and fed twice daily at 9 and 16 hours of the day. Fish from continuous flow aquarium with water, air, temperature and photoperiodic control facility; chemically analyzed at the beginning and after 56 days i.e. end of the feeding trial for moisture, protein and fat (AOAC, 1984), and rates of *in vivo* protein biosynthesis.

The fishes were starved for 24 hours to allow emptying of intestinal (agastric fish) residues. Thread ligatures was placed at oesophageal (upper and lower), stomach, duodenal, anterior intestinal and posterior intestinal junctions in anaesthetized fish. Thorough and clean washing is essential for correct estimation of absorption. Two small incisions were given at the opposite ends of each ligatured section and the part of the gut was washed with Krebs-Ringer bicarbonate solution or by fish saline at 30°C. Legations at 5-6 cm apart was given in washed lower oesophagus, gastric, duodenum and ileum. One ml. Krebs-Ringer bicarbonate solution / fish saline at 100 mg % protein or amino acid concentration at 30°C was injected into each of the above segment. The vicera and the ligatured segments were kept moist with Krebs-Ringer bicarbonate/ fish saline solution at 30°C in incubator. Absorption was allowed for 30,60 and 90 minutes, following which the segments were separated from the rest of the gut and removed. The post absorption fluid was collected in vials and the wet weight of the separated segments was recorded. An aliquot of the fluid was taken for spectrophotometric determination of protein/ amino acids and was expressed as µg protein / amino acid absorbed $hour^{-1}$sq $cm^{-1.}$ This experiment was repeated for six times. Quantitative requirements of amino acids were determined in fish by feeding a purified diet containing isolated crystalline amino acids as a control diet and feeding test diets similar to the control except that one amino acid at a time has been removed. Test diet that produce no growth or markedly less than the control represented amino acids that were essential to fish. Quantitative requirements for essential amino acids was determined by feeding graded levels of one amino acid at a time in a test diet containing crystalline amino acids.

The amino acid profile of the test diet is usually designed to be similar to that in the fish muscle. In fact, the essential amino acid profile of fish muscle has been found to agree closely with the dietary amino acid profile for optimum growth of the fish. The rates of protein bio-synthesis in the liver and muscle of the experimental fish were measured as incorporation of amino acid (^{14} C-L–leucine) in the liver and muscle. The fish from each of the experimental sets was injected intramuscularly with the isotope ^{14} C-L–leucine (Specific activity 282 M Ci / MMOL ; solution in 0.01 N HCl; obtained from Bhaba Atomic Research Centre, Trombay, Bombay – 400 085) both at the initial stage and at the termination of 60 day experimental period at the rate of 0.05 µ Ci $100g^{-1}$ body weight of the fish. During the commencement of the experiment, the injected fish was sacrificed to measure the steady state of the level of incorporation. The tissues were weighed and proceeds as followed by Patra (1989) and Patra (2002) for counting the radioactivity.

The ratio of digestible energy / digestible protein gradually increased from 8.20 to 16.50 and it highly correlated with the findings of NRC (1993). In the present investigation,

Table1. Percent composition of different ingredients and protein on dry matter basis, and digestible energy content (Kcal g^{-1}) in the prepared diets

Ingredients	Percentage of ingredients					
Casein	20	24	28	32	36	40
Gelatin	05	06	07	08	09	10
Dextrin	40	33	26	19	12	05
α-Cellulose	21	21	21	21	21	21
Carboxy - methyl cellulose	5	7	9	11	13	15
Amino acid mixture	0	0	0	0	0	0
Vitamin premix	1	1	1	1	1	1
Mineral premix	2	2	2	2	2	2
Animal fat	3	3	3	3	3	3
Plant fat	3	3	3	3	3	3
Protein %	25	30	35	40	45	50
Digestible Energy (DE) (KCalg^{-1})	3.05	3.04	3.04	3.04	3.03	3.03
Protein /DE	8.20	9.87	11.51	13.16	14.85	16.50

Table 2. Daily dry matter intake and weight gain of *L. rohita* fed with various levels of protein diet

	Daily dry matter intake (mg 100 g^{-1} fish)					
Percent (%) of protein	25	30	35	40	45	50
Size group of fish	Feed intake					
5±0.37g	4918±10.2	5248±16.0	5862±14.7	6211±19.4	6313±12.1	6320±12.4
20±1.15g	4217±11.1	4432±12.3	4536±10.2	4782±12.4	4799±14.5	4808±16.4
50±2.57g	3729±9.37	3910±11.7	4217±11.2	4298±14.3	4317±16.2	4336±12.5
	Weight gain (g)					
Growth						
5±0.64g	30.38±1.85	40.36±2.10	47.13±1.58	50.31±2.20	54.31±1.37	55.17±2.15
20±1.85g	46.32±2.47	50.78±3.42	58.37±2.18	62.10±1.92	62.70±1.75	62.95±2.05
50±3.20g	78.35±4.16	92.40±2.55	108.1±3.14	109.55±3.1	110.64±3.10	111.42±2.51

RESULTS AND DISCUSSION

Proximate composition of the diets prepared with highly purified ingredients on a dry matter basis, is presented in Table 1. Protein share of the diet varied from 25% to 50% , content of lipid fixed at 6% level and carbohydrate percentage gradually reduced as their digestive and metabolic systems to be better adapted to utilization of protein and lipids for energy than carbohydrates. Although digestible energy content was kept constant within the limit of 3.03 to 3.05 kcal g^{-1}.

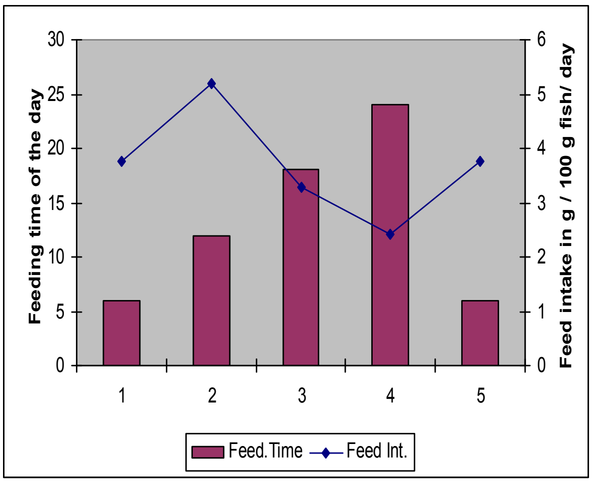

Figure 1. Maximum feed intake of *Labeo rohita* at different hours of the day

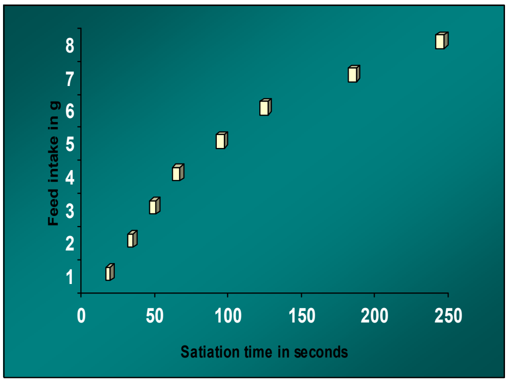

Figure 2. Satiation time of *Labeo rohita*

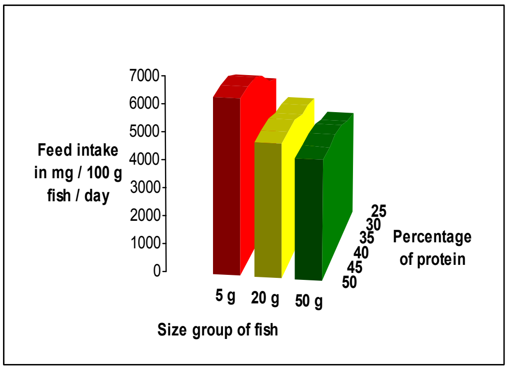

Figure 3. Daily feed intake (mg/100 g $fish^{-1}$) in *Labeo rohita* fed with variable protein diets

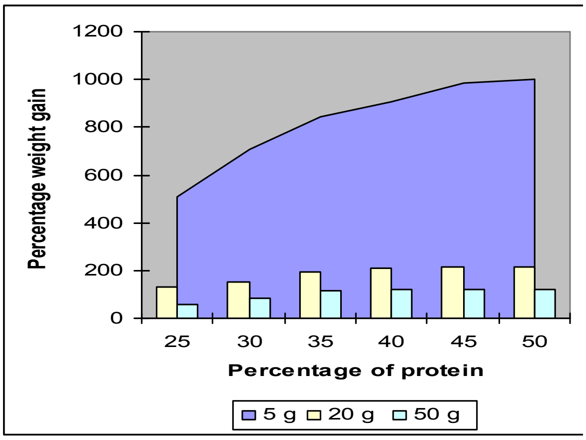

Figure 4. Growth performance of different size groups of *Labeo rohita* fed with variable protein diets

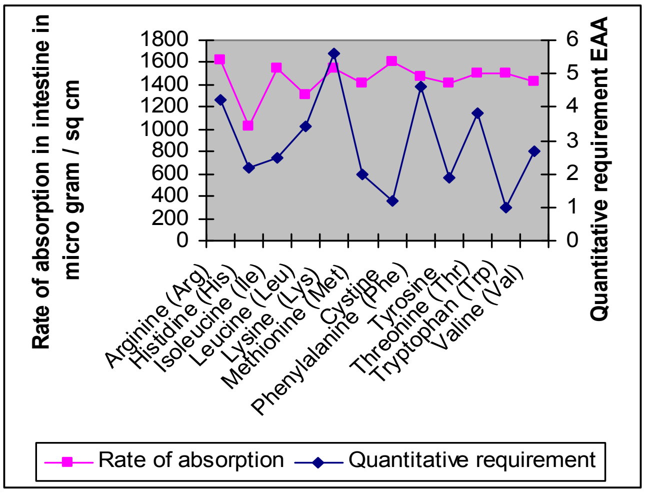

Figure 5. Rate of absorption of essential amino acids (EAA) through the intestinal serosal layer in *L. rohita* (data in the parenthesis indicate the EAA requirements of 5-10 g size group).

the maximum growth was observed at 14.85 for smaller size group (5± 0.64 g) followed by larger size groups of *Labeo*. It was also recorded that satiation time in case of *Labeo* was about 60 minutes (Figure 1) and maximum feed intake was at 12 hours (09.00h – 12.00h), followed by 06.00 hours, 18.00hours and 00.00 hours (midnight)of the day, presented in Figure 2 (Patra, 1993). Highest feed intake recorded in smaller size groups of *Labeo* (5 ± 0.64 g) was 6318 mg $100g^{-1}$ followed by larger size groups 20 ± 1.85 g (4782 mg $100g^{-1}$) and 50 ± 3.20 g (4217 mg $100g^{-1}$) as presented in Figure 3. The protein requirement in terms of growth performance and weight gain was very high in smaller size groups of *Labeo* (5± 0.64 g) followed by larger size groups 20± 1.85 g and 50± 3.20 g respectively as presented in Figure 4. Weight gain is not a measure of true growth. True growth is an increase in muscle both smooth & striated, skeletal and organ tissue whereas weight gain can represent an increase in adipose tissue with relatively less change in the other tissues. Because, muscle is the main marketable product in fish grown for food. Jobling (1994) recorded that, deposition of 1g of fat represents weight increase of 1g, whereas deposition of 1g of protein leads to the gain of about 4g of tissue. Feed conversion is more efficient in smaller size group (5g, 1.32), faster growing fish than in higher size group (20g, 1.81 and 50g, 2.10). In most situations, protein gain is an acceptable measure of growth in fish. In small fish, weight gain correlates well with protein gain but as fish increase in size there is less similarity (Lovell and Li, 1992). It was estimated that, for calculating the digestible energy required to grow 1 kg of *Labeo rohita,* in controlled condition from 1g to 100 g size at 25-30^{0}C water temperature based on estimated recovery of energy in the fish which indicated that 3.71Mcal of digestible energy is required to produce 1kg of fish biomass equivalent to 1.91Mcal recovered energy. Protein retention rarely exceeds 50% in fish fed either purified or practical diet. **A**pparently *Labeo* respond to supplementation of isolated (crystalline) amino acids when the diet is deficient in the amino acid, but little is known about the relative bioavailability of isolated amino acids compared to protein-bound amino acids. Theoretically it should be possible to grow Indian major carps at an acceptable rate on a diet containing 15% essential amino acids, and an additional 10% of non-essential amino acids. Highest rate of EAA absorption recorded through the posterior intestinal segment as compared to anterior and middle segment and presented in Figure 5. (Patra, 2004, 2008a,c). Amino acid requirements also presented in table 3 and it is found that, among the EAA, maximum quantity required in the diet is Lysine (5.6) followed by Phenylalanine (4.6), Arginine (4.2), Threonine (3.8), Leucine (3.4), Valine (2.7), Isoleucine (2.5), Histidine (2.2), Methionine (2.0), Tyrosine (1.9) and Cystine (1.2). Maximum incorporation of U-^{14}C L-leucine takes place at 2 hours in liver (4000 cpm mg^{-1} of protein) and 4 hours in muscle (605 cpm mg^{-1} of protein) in case of *Labeo rohita*. There was no significant differences in assimilation rate either in liver or in muscle with formulated diets and synthetic diets in *Labeo rohita* .It was recorded that maximum assimilation takes place in smaller size groups (4100 cpm mg^{-1} of protein in liver & 604 cpm mg^{-1} of protein in muscle at 10 hours of the day and 637 cpm mg^{-1} of protein in liver & 241 cpm mg^{-1} of protein at 'zero' hours of the day) as compared to larger one which directly correlated with the feed intake, protease enzyme activity, growth performance, fish flesh quality in terms of nutrients and palatability (Patra, 1994, 1995 and 2008b).

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