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Energy Budget of Indian Major Carp, *Labeo rohita* Fingerlings Fed on Diets with Different Protein Levels

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Abstract

Energy partitioning of Indian major carp, *Labeo rohita* Ham. fingerlings of 3.3 ± 0.25 g size fed on different protein diets containing indigenous ingredients of pellets with 23, 28, 33 and 38% crude protein were investigated in respirometers for 40 days. Ingested energy (C), energy lost in metabolism (R), faecal matter (F), nitrogenous excretion (U) and growth (P) were measured separately and simultaneously. The outcomes were compiled to construct energy budgets. The percent energy balance from the observed growth were 77.97, 77.58, 88.40 and 92.85% in fish fed on diets having 23, 28, 33 and 38% crude protein respectively. The percent loss of daily ingested energy through metabolism were 31.9, 33.49, 37.5 and 41.0% in fish fed on diets containing 23, 28, 33 and 38% crude protein respectively, whereas, faecal energy were 28.9, 26.1, 25.1 and 25.2% and 5.8, 6.1, 6.9 and 7.6% nitrogenous excretion in fish fed on 23, 28, 33 and 38% dietary protein respectively. Growth was found optimum in fishes fed on diet having 33% crude protein.

Introduction

Bioenergetics involves the examination of energy gains, losses, and transfers within the whole organism, leading to the partitioning of ingested energy into the major physiological components of the energy budget equation. The digestible energy of a feed is the total energy consumed 'C' of the feed less the energy in the faeces 'F' excreted. The energy available for the 'building blocks' of growth 'P' is what remains after the energy for metabolism 'R' and excretion 'U'. The energy budget can be expressed in simplest form as C = R + F+ U+ P (Petrusewicz and MacFadyen 1970), where, C=The energy content of food ingested, R=The respired heat energy due to total metabolism, F=The energy lost in faeces, U=The energy lost in nitrogenous excretion, and P=The production or energy in growth materials. An accurate and simultaneous measure of C and F in a long term experiment gives the energy absorbed and

retained by the fish (Brafield 1985) while measurement of R and U separately and simultaneously helps in constructing the total energy budget among fish in both laboratory studies (Cui and Wootton 1988). Numerous attempts have been made to compile a complete energy budget for fish species (Carter and Brafield 1991, Chakraborty et al. 1995). A number of studies have been carried out on the relationship between dietary energy and protein content on growth of carp (Schwarz et al. 1983; Gongnet et al. 1987). In an intensive culture, this energy budget is of particular interest in making rapid prediction of effects on growth of a certain diet formulation on feed regime or in an acute culture stress.

One of the Indian major carps, commonly known as rohu (*Labeo rohita* Ham.), is an important culture fish throughout the Indian sub-Continent. The appropriate feeding and nutritional requirements of Indian major carp fingerlings are not yet fully established. If energy budget models of the different energy components for carp fingerlings is developed on the basis of feeding and nutritional requirement, the problem of carp production from fingerlings could be helped. Until now, there have been no studies investigating energy partitioning of the consumed food to describe a complete energy budget for Indian major carp (*Labeo rohita*).

The present work attempts not only to study the energy expenditure of ingested pelleted diets of different protein contents by simultaneously quantifying respiration, faecal loss, nitrogenous excretion, and growth but also to construct complete energy budgets.

Materials and Methods

The experiments for energy budgeting were conducted in a flow-through water recirculatory system in the laboratory of Fisheries Technology Department, Bangladesh Agricultural University, Mymensingh. For simultaneous measurement of respiratory rate (R), faecal loss (F), nitrogenous excretion (U) and growth (P), a water recirculatory system was constructed comprising five metabolism chambers connected with a cuvette to serve as ammonia and oxygen measuring chambers through faecal column and a flow meter as devised by Chakraborty et al. (1992b). In this system five, "slope partitioned" rectangular metabolism chambers (38 x 26 x 27cm) each connected with a faecal column were used. The third chamber was used as the reference chamber and contained no fish. The water recirculatory system comprised of one sump tank, one header tank and two filtration tanks connected by one inch diameter PVC pipe. Water from the sump tank was pumped by an immersion pump to the header tank to distribute the water through a half inch diameter pipe into each metabolism chamber. The flow of the water was regulated by the ball valves. The metabolism chambers were partitioned internally by setting a glass sheet sloping diagonally which made water tight so that no water could transfer between the two sections. At the top corner, a small gap was provided to allow the lower chamber to be filled with water. The capacity of the active upper chamber was 10 l of water. The outlet of each metabolism chamber was

set at the lowest point leading to a faecal column. The upper outlet of the faecal column was connected by a 5 mm diameter rubber pipe to a three-way valve which allowed the effluent water to be directed either via a flow-meter into a cuvette which receives an oxygen probe through a hole for determination of oxygen in the cuvette's water and then into the outlet leading to the filtration tank, or directly into the filtration tank. The cuvette had another small hole at the top wherein water was made tight by a rubber stopper. Through this rubber stopper, a needle of a 5 ml hypodermic syringe was introduced to collect water sample to determine ammonia in the water passing through it. The continuous and constant water supply was controlled by valves. Natural photoperiod of about 12h light and 12h dark was maintained during the experimental period.

Four experimental pelleted diets having 23, 28, 33 and 38% crude protein named I, II, III and IV respectively were prepared (Table 1) using a peletting machine (Hobart). The proximate analysis of the diets was done according to the method outlined by the AOAC (1990). Gross energy content of dried faeces, feeds and fish carcass - both at the initial stage and at the end of the experiment were determined using a bomb calorimetry (Adiabatic Bomb Calorimeter, Gallankamp).

Fingerlings of rohu fish, Labeo rohita of 3.3 $\pm$0.25g size were collected from the Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh. The fingerlings were acclimatized in a plastic water pool with continuous aeration for 3 weeks in natural dark-light regime during which they were fed a diet containing 30% crude protein at a rate of about 10g kg^{-1} body weight as a maintenance ration.

Among the five metabolism chambers A, B, C, D and E, chamber "C" was used as reference and contained no fish. The other four chambers A, B, D and E contained eight fish each with a total mean weight of 25.82, 26.54, 25.55 and 25.64g respectively. For this, fingerlings were netted, anaesthetized with 1 ppm benzocaine (Ross and Geddes 1979) in water, gently blotted on soft tissue paper before individual weighing. The water temperature in the system water was 27 $\pm$1.0 °C. Fishes in the metabolism chambers were kept unfed for the first 24 hours followed by two days unfed condition during which the resting metabolic rate and endogenous nitrogenous excretion were measured. Each of the two replicate's experiment continued for a total of 40 days and the feeding in the metabolism chamber continued for 36 days. Water flow rate of 30L/h through the flow-meter was maintained in all metabolism chambers during the experiment. The air-saturated (by Daivo pump, NS 8200) water was fed through the metabolism chambers of the system from the header tank. From the third day, either of the diets I, II, III or IV containing 23, 28, 33 or 38% protein level respectively were fed to the fishes in the metabolism chambers A, B, D and E respectively at their satiation levels from 9 a.m. to 4 p.m. Fishes in each chamber were fed every two hours by giving three to four pellets at a time until the fish responded positively to the pellets. The uneaten pellets (if any) that entered into the faecal column were carefully collected and recorded to be excluded from the daily consumption 'C'. Hourly monitoring of oxygen consumption (measured by an oxygen probe, Check mate;90, Metler Toledo)

Table 1. Formulation and proximate composition of the experimental diets (dry weight basis).

	Diets			
	I	II	III	IV
Ingredients (per 100g dry diet)				
Fishmeal	15.00	20.00	35.00	50.00
Mustard oil-cake	11.50	21.50	17.50	18.50
Duck-weed	15.00	21.00	22.00	16.00
Rice-bran	36.00	25.00	18.00	8.00
Wheat flour	20.00	10.00	5.00	5.00
Chromic Oxide	0.50	0.50	0.50	0.50
Vitamin & Mineral Mix*	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00
Composition (%)				
Moisture	6.12	4.84	6.52	6.23
Crude protein	23.77	28.20	33.09	37.29
Lipid	9.76	8.27	7.83	10.66
Ash	16.12	16.11	18.70	21.91
Crude fibre	10.56	9.32	8.12	6.45
**Energy value	20.68 kJ g-1	21.15 kJ g-1	21.31 kJg-1	21.74 kJ g-1

** Calculated by bomb calorimetry.
*Vitamins: Active Ingredients (I.U. or mg gm^{-1}): Vit A, 3,750 I.U.;Vit D3, 375 I.U.; Vit E, 37.5 mg; Vit K3, 3.0 mg; Vit C, 87.5 mg; Vit B1, 3.75 mg; Vit B2, 6.00 mg; Vit B6, 3.00 mg; Niacin (Nicotinic acid), 30.00 mg; Ca-pantothenate, 12.50 mg; Folic acid, 1.00 mg; d-Biotin, 0.125 mg; Vitamin B12, 0.075 mg; Choline chloride, 150.00 mg; Inositol, 37.5 mg.
Minerals: Active Ingredients (mg gm^{-1} diet): Magnesium, 5.00; Zinc, 7.50; Iron, 12.50; Copper, 0.75; Manganese, 5.00; Iodine, 0.25; Cobalt, 0.0025; Selenium, 0.025.
Amino acids: Methionine, 50.00; Lysine, 30.00; Ethoxyquine (Anti-oxidant) 31.25
Ref. Rhone-Poulenc, Dhaka, Bangladesh

and ammonia excretion (Stirling 1985) of the fed fish groups in chambers over 24h were measured on days 3, 5, 8, 11, 13, 16, 20, 24, 28, 32, 36, 38 and 40.

The respired energy 'R' was measured in terms of oxygen consumption (Brafield 1985) expressed as $mgO_2\ kg^{-1}h^{-1}$. This value was then multiplied by appropriate oxy-calorific co-efficient (Q_{ox}) value in order to get the estimate of energy expenditure in terms of kJ. A computed mixed Q_{ox} of 14.20, 14.12, 14.01 and 13.87 J mgO_2^{-1} was used for the 230, 280, 330 and 380g kg^{-1} protein diets respectively and Q_{ox} used for unfed fish was 13.56JmgO_2^{-1} (Brafield and Llewellyn 1982). It was assumed that the respiratory substrates were respired in proportions in which they were ingested. The nitrogenous excretion was measured as $mgNH_3\ kg^{-1}h^{-1}$ using phenolnitroprusside method (Stirling 1985) and an energy equivalent of 24.83 J $mgNH_3^{-1}$ was used to calculate the total energy loss in excretion (Elliott and Davison 1975).

Faeces from metabolism chambers settled at the bottom of the faecal column were collected together with little amount of water, centrifuged, dried at 70 °C overnight and pooled together by chambers in vials for further analysis.

A group of 25 fishes from the stock fish sample were removed at the start of the experiment, sacrificed, weighed, dried, weighed again, macerated, and analysed to give the initial carcass composition of fish in the experiment.

Feed conversion ratio (FCR) is expressed as the ratio of food consumed (kg) and increase in fish weight (kg); feed conversion efficiency (FCE) or gross

efficiency (K_1) is represented by the energy content of the body weight increase as a proportion of the energy content of the food intake, protein utilization in terms of protein efficiency ratio (PER) is expressed as the ratio between increase in body weight (g) and protein consumption, apparent protein digestibility (APD) % (Moynard & Loosli 1969) using the marker (Cr_2O_3) method of Furukawa and Tsukahara, (1966). Energy budgets were constructed based on the formula $C = R + F + U + P$

In the energy budget compilation, 'P' was considered in two ways-observed growth as 'P_1' and predicted growth as 'P_2'. Predicted growth was calculated by the method of difference from the energy budget equation. The observed percent balance of the energy of the budgets in this experiment was calculated using the formula $((P_1 + R + F + U) \times 100)/C$. Simple analysis of variance (Zar 1984) was used to test the significance of variation of values. For this purpose the results as percent-data were converted into arcsine value. Regression models were developed by using a statistical software-'Minitab' (Ryan et al. 1985) on a microcomputer.

Results

The energy ingested (kJ day^{-1}) from different dietary protein sources in each treatment are shown in Table 2. The mean routine metabolic rate of unfed fishes in the chambers did not vary significantly ($p>0.05$) over a 24 hour period and was calculated as 159.8, 161.3, 161.1, and 157.8 mgO_2 $kg^{-1}h^{-1}$ in A, B, D and E treatments respectively (Table 2). Post-feeding oxygen consumption over routine rate by the fish fed on different protein diets increased significantly ($p<0.05$) depending on the protein content of the diets (Fig. 1). The effect of feeding 380g kg^{-1} protein diet showed the maximum increase in peak value (Table 2) of 820.4mgO_2 $kg^{-1}h^{-1}$ with highest duration value showing a direct relationship with dietary protein content.

Post-feeding percent increase of respiration over routine rate ranged from 319.3 to 520.0% and the daily mean metabolic rate was recorded as 401.7, and 487.5 mgO_2 $kg^{-1}h^{-1}$ with fish fed on 230 and 380 g kg^{-1} crude protein diet respectively (Table 2). Energy lost in routine metabolism in four

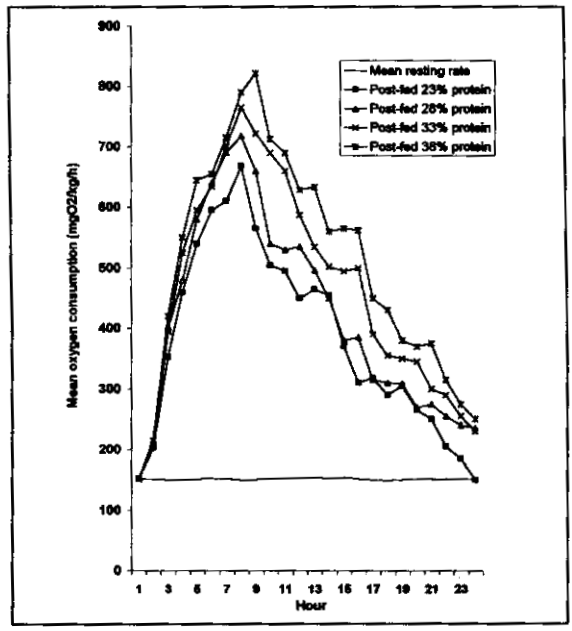

Fig. 1. Changes of mean oxygen consumption over mean resting rate in *Labeo rohita* on 23, 28, 33 and 38% protein diet. Feeding started at 9:00 am which is shown as 1 hour on the X axis.

treatments A, B, D and E were 52.02 (±3.56), 52.49 (±2.42), 52.44 (±2.65) and 51.34 kJ $kg^{-1}day^{-1}$ respectively. Energy lost as heat of metabolism 'R' (kJ) were 31.92, 33.48, 37.38 and 40.95% of ingested energy 'C' on fish fed with 230, 280, 330 and 380g kg^{-1} protein diet respectively (Table 2). The relationship between respiratory energy loss and dietary protein level in the experiment is expressed in the following regression equation:

$R = 17.0 + 0.620\ P$

$p<0.05$ ($r^2 = 0.9788$, N = 13 = no. of samples, observing dates)

where, R = respiratory energy loss expressed as % of 'C'; P = dietary protein level (within range between 230 and 380g kg^{-1}).

Daily mean ingestion (C) of 14.47, 14.81, 15.34 and 14.13 kJ from diets I, II, III and IV produced a faecal energy loss (F) of 4.18, 3.87, 3.85 and 3.56 kJ respectively showing no significantly different ($p>0.05$) relationship between faecal energy and dietary protein level (Table 3). In this case, 'F' as percent of 'C' ranged from 25.19 to 28.87%. The energy lost as 'U' were 0.84, 0.91, 1.06 and 1.08 kJ $chamber^{-1}day^{-1}$ in fish fed on 230, 280, 330 & 380g kg^{-1} dietary protein respectively (Table 3) and 'U' as percent of 'C' ranged from 5.80 to 7.64% in different treatments and increased significantly different ($p<0.05$) with an increase in crude protein in the diets.

The values for assimilation efficiency (AE, expressed as %) were significantly different, and ranged from 71.11% on fish fed with 230 g kg^{-1} protein diet to 75.80% with 330 g kg^{-1} protein diet respectively (Table 4). Apparent protein digestibility (APD%) of feeds ranged between 70.68 and 78.12 (Table 3). No significant variation ($p>0.05$) was found in APD in treatments D and E containing 330 and 380 g kg^{-1} dietary protein but the rest were found to be significantly different ($p<0.05$).

The growth performances of fish fed with different protein diets are summarized in Table 4. Changes in wet weight were considered as 'growth' in the experiments. Growth (P) increased as the dietary protein level increased. Significantly higher ($p<0.05$) growth was observed in fish fed with 330 g kg^{-1} dietary protein (Table 4). Total mean initial body energy of fish fed on diets I, II, III and IV were 103.97, 106.75, 102.69 and 103.33 kJ which increased to 161.68, 167.90, 204.65 and 197.31 kJ respectively at the end of the experiment. The observed growth energy (P_1) as percentage of ingested energy (C) were 11.40, 11.82, 18.98 in 19.03 with fish fed with 230, 280, 330 and 380g kg^{-1} protein diet respectively (Table 4).

The food conversion ratio (FCR) i.e. feed : gain ratio values of the diets I, II, III and IV were 1.76, 1.99, 1.40 and 1.30 respectively and they were found to be significantly different. The protein efficiency ratio (PER) was found as 1.39, 1.80, 2.11 and 2.07 for diets I, II, III and IV containing 230, 280, 330 and 380g kg^{-1} crude protein respectively. The daily energy budgets of different components are shown in Table 5. In this case growth (P) have been shown as predicted growth P_2 (using the method of subtraction from 100) and observed growth as P_1.

Table 2. Oxygen consumption during unfed and post-feeding of *Labeo rohita* when fed on 23, 28, 33 and 38% protein diets. (values are arithmetic means (±SD), n = 13)

Dietary protein g kg^{-1}	Fish wt. (g/tank)	Mean routine rate(mgO_2 /kg/h	Equivalent energy (kJ/kg/day)	Mean peak respiratory rate (mgO_2/kg/h)	% increase over routine rate	Time to reach at peak (h)	Duration above routine rate(h)	Daily mean metabolic rate (R) mgO2/kg/h	Ingested energy (kJ/day)	'R' as % of 'C'
230	25.82	159.84 (±10.94)	52.02 (±3.56)	670.15 (±41.33)	319.26	6	18	401.68 (±145.94)	14.47	31.91
280	26.54	161.28 (±7.45)	52.49 (±2.42)	741.44 (±20.12)	359.72	6	22	434.68 (±151.07)	14.81	33.49
330	25.55	161.14 (±8.13)	52.44 (±2.65)	774.06 (±16.56)	380.36	6	22+	469.26 (±167.94)	15.34	37.41
380	25.64	157.77 (±6.46)	51.34 (±2.10)	820.40 (±16.33)	520.00	6	22+	487.50 (±185.03)	14.13	40.98

Table 3. Energy losses in faeces (F) and nitrogenous excretion (U).

Dietary protein (g kg^{-1})	Mean total wt. of fish (g)	Consumption (C) $kJday^{-1}$	Energy value of faeces kJ g^{-1}	Energy in faeces kJ day^{-1}	'F'as % of 'C' $mgNH_3$ kg^{-1} day^{-1}	Nitrogenous excretion (U) kJ /day	Energy of 'U'/chamber	'U' as % of 'C'
Unfed	25.89	—	—	—	—	115.9	0.074	—
230	25.82	14.41	17.44	4.18	28.87	1310.2	0.84	5.80
280	26.54	14.81	19.38	3.87	26.10	1380.9	0.91	6.14
330	25.55	15.34	19.19	3.85	25.09	1670.8	1.06	6.91
38	25.65	14.13	19.23	3.56	25.19	1676.4	1.08	7.64

Absorption efficiency in energy ranged from 71 to 78% which increased with dietary protein content (Table 4). A comparatively higher value of 79.20% for absorption efficiency was recorded in gold fish, Carrasius auratus by Spillett (1978) (quoted from Chakraborty 1992). The small variation of mean AE in this study may be due to better quality and higher protein content of the diets.

The daily energy gain in growth (kJ) showed a best gain in fish fed with 33% dietary protein. The obtained FCR values of fish fed with different protein diets suggest an optimum growth of Labeo rohita with fish fed with 330 g kg-1 dietary protein than with 380g kg-1 protein diet. The PER values are also found within a good range (Table 4) and found to be better utilized in fish fed on diets C and D. This study showed optimum growth with 33% dietary protein (Table 5). From other studies with common carp, optimum dietary protein levels have been found between 35 (Jauncey 1981) and 39 (Sin 1973; Ogino and Saito 1970).

The pattern of energy allocation in the daily budget in this study with balance of energy was found less than 100% in all fed fishes. Overall energy balance in these experiments varied from 78 to 93% (Table 5). In this study of energy budgets, the balance of energy is never more than 100% which was similar with most of the experiments with grass carp *Ctenopharyngodon idella* (Carter and Brafield 1991), common carp, *Cyprinus carpio* (Chakraborty et al. 1995), and tilapia, *Sarotherodon mossambicus* (Musisi 1984). Thus, Musisi, obtained an energy balance of 102.24% and 66.01-107.12% with tubifex and protein diets respectively. The energy balance in grass carp. *Ctenopharyngodon idella* was found between 81.1 to 93.4% with high protein diet and 61 to 74% with *Lemna* but a higher value of 103% was obtained with high lipid diet (Carter and Brafield 1991).

The energy balance in the present study is clearly below 100% implying that some of the energy lost, not measured, or probably not accounted for was due to some stress effect of confinement of fish in the metabolism chambers. One source of error may be the consumption of energy 'C', some or very little portion may in fact have been lost due to particles escaping through the opercula or unobserved, uneaten, or regurgitated. A portion of the energy expended for respiration (R) in fish fed on diets II, III and IV could not be accounted for during the latter period of each data-collection day (which may be about 3-6% roughly, of total 'R') when post-feeding effects extended beyond 24 hr. A small amount of faecal energy escaped due to unavoidable leaching (about 4% in case of natural food) of total 'F' as determined by Elliott 1976, in brown trout) into water. Moreover, the unmeasured 'U' such as urea as was found between 2.77 and 19.90% of the total nitrogen in *Ctenopharyngodon idella* by Carter and Brafield (1991) and other nitrogenous wastes may also be an energy source which was not counted in these experiments.

The regression models developed on the basis of the data in this experiment could be helpful to serve as a basic and important information relating to the growth and other components of the energy budgets for one of the Indian major carps, *Labeo rohita*. Moreover, applications of the relationship between energy components developed in this short-term trial may be applied in field situation in long time growth trial to verify its limitation.

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