

Effects Of Dietary Energy, Protein Levels And Their Interactions On Growth, Utilization Efficiencies And Body Composition Of A Catfish, *Clarias Gariepinus* (Burchell 1822).

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ABSTRACT

Fingerlings of *Clarias gariepinus* (Burchell, 1822) were fed with semi-purified diets containing three levels of crude protein (25, 40 and 45%) and two levels of metabolizable energy (360.8 kcal/100g and 406.7 kcal/100 g) at each protein level, over a period of three weeks feeding trial which was conducted in flow-through troughs at a temperature of $26 \pm 1^\circ \text{C}$. This experiment was conducted for evaluating the effect of dietary energy and protein on growth, utilization efficiencies and proximate composition of fish. Growth of fish in terms of live weight gain (%), increased with increasing dietary protein at both the energy levels and at each protein levels. Growth rate was reduced with increase in energy density of the diet. Specific Growth Rate (SGR %) exhibited a linear negative correlation with E/P ratio within isocaloric diets. Mean feed consumption, Feed Conversion Ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and energy conversion efficiency (ECE) were influenced both by dietary energy and protein level in the diets within isocaloric diets. PER and PPV exhibited a positive relation, whereas FCR and ECE showed an increased correlation with E/P ratios. Carcass composition was influenced by both dietary energy and protein. This study indicates that for optimum growth and better feed conversion practical diets for *C. gariepinus* fingerlings should contain 45% crude protein and 360.5 kcal/100g energy.

INTRODUCTION

The determination of protein requirement is a prerequisite for the development of a nutritionally balanced diet for the culture of a particular fish species. Higher levels of dietary protein, besides adding to the cost, also result in water quality deterioration due to excessive excretion of ammonia (Beamish and Thomas, 1984). The dietary protein requirement of fish is influenced by a delicate balance of dietary energy to protein ratio besides other factors. Provision of sufficient amounts of non-protein energy in the diet results in greater utilization of protein for growth (Wilson, 1989, De Silva and Anderson, 1995).

African catfish, *Clarias gariepinus* (Burchell, 1822) is regarded as an important species for aquaculture. Although extensive work has been done on various aspects of nutrition of *C. gariepinus* (Henken *et al.*, 1985, 1987; Huisman and Richter, 1987; Degani *et al.*, 1989; Uys, 1989; Mybenka and Agua, 1990; Hoffman and Prinsloo, 1995; Awaiss and Kestemont, 1998; Murty and Naik, 1999). However, a very little information exists on the effect of dietary energy and protein interaction on the growth and utilization efficiencies of this species (Machiels and Henken, 1985; Henken *et al.*, 1986; Pantazis and Jauncey, 1996). The present study deals with the effects of dietary energy and protein levels, and their interaction on growth and utilization

efficiencies of *C. gariepinus* fingerling.

MATERIALS AND METHODS

Source of fish stock / acclimation

Fingerlings of *C. gariepinus* were obtained from a fish farm in Rampur district, Uttar Pradesh. These were transported to the Research Station in oxygen filled polythene bags, given a prophylactic dip in KMnO_4 solution (1:3000) and stocked in flow-through (1-1.5L/min) outdoor cement cisterns (1x1x1 m) for a week. During this period fish were fed to satiation on minced meat twice daily at 0800 and 1600h. After a week, a desired number of fish were taken out and acclimated on casein-gelatin based semi-purified diet (H-440) for two weeks in high density polyvinyl circular tanks (water volume, 70L) fitted with flow-through system (1L/min).

Preparation of experimental diet

For experimental diets, were formulated using semi-purified ingredients which contained three levels of protein (25, 40 and 45%) where each protein level containing two levels of energy (360.8 and 406.7 k cal/100g). Diet with 50% crude protein and 406.5 k cal/100g was used as control (Tables 1 & 2). Dietary energy was calculated using physiological fuel values, 3.5, 4.5 and 8.5 kcal/g for carbohydrate protein and fat, respectively (Jauncey, 1982). At each protein level, two different levels of lipids (8 and 16%) were used. The amount of dextrin and α -cellulose were altered to obtain the desired levels of energy in the diet.

Table 1. Composition of mineral mixture*

Mineral	g/100 g
Calcium biphosphate	13.48
Calcium lactate	32.40
Ferric citrate	2.97
Magnesium sulphate	13.70
Potassium phosphate (Dibasic)	23.86
Sodium biphosphate	8.72
Sodium chloride	4.35
Aluminium chloride .6H ₂ O	0.015
Potassium iodide	0.015
Cuprous chloride	0.010
Manganous sulphate H ₂ O	0.080

Cobalt chloride .6H ₂ O	0.100
Zinc sulphate. 7H ₂ O	0.300
*Halver (1989).	

Table 2. Composition of vitamin mixture*

Vitamin	<u>g/100g</u>
Choline chloride	0.500
Inositol	0.200
Ascorbic acid	0.100
Niacin	0.075
Calcium Pantothenate	0.050
Riboflavin	0.020
Menadione	0.004
Pyridoxine-HCl	0.005
Thiamine-HCL	0.005
Folic acid	0.0015
Biotin	0.0005
α-tocopherol acetate	0.040
Vitamin B ₁₂ (10 mg /500 ml H ₂ O)	0.00001(0.5 ml)
*Halver (1989).	

Preparation of experimental diets

Casein-gelatin based semi-purified diets were used for different experiments. Calculated quantities of dietary ingredients were weighed on a sensitive electronic balance (Precisa-120A). For preparation of diet, a weighed quantity of gelatin was mixed in a known quantity of water in stainless steel attachment of Hobart electric mixer with constant stirring, and heated to 80°C. The mixer bowl was then removed from heating and weighed quantity of casein, dextrin, minerals and α-cellulose were added to it. The content was then blended in Hobart mixer under lukewarm state. This was followed by the addition of vitamin mix and oil (2:1, corn and cod liver oil). The mixture was again blended and in the end carboxymethyl cellulose was added to it. The prepared diet obtained a bread dough like consistency, was poured into a teflon-coated pan and placed in a refrigerator to jell. The prepared diet was in the form of moist cake which was cut into small cubes and stored in the

refrigerator (-20°C) in sealed polythene packs until used. The mineral and vitamin premixes used (Tables 1-2) were the same as given by Halver (1989).

General experimental design / feeding trial

Fish of desired size and number were sorted out from the acclimated stock and stocked in triplicate groups in 70L polyvinyl circular flow-through (water volume 55L) troughs. The troughs were provided with groundwater. The water exchange rate in each trough was maintained at 1-1.5Umin. Each morning faecal matter was siphoned off from the experimental troughs before feeding. Feeding level of fish and feeding schedule was chosen after carefully observing the dietary intake as well as feeding behaviour of fish. Fish were fed experimental diets in the form of moist cake six days a week. The moisture content of the diet was estimated and the ration level calculated as dry feed to wet fish weight. Mass weight of fish was taken weekly and amount of ration readjusted for subsequent feeding. On the day of weekly measurements, no feed was offered to fish, when troughs were also thoroughly washed and rinsed with KMnO_4 solution. A record of daily dissolved oxygen and water temperature was maintained.

Proximate analysis

Proximate analysis of carcass was made using standard techniques (AOAC, 1984). The analyses were carried out in triplicate runs.

(i) Estimation of Moisture

For moisture estimation a weighted quantity of finely ground/homogenized sample was taken in a pre-weighed silica crucible and placed in an oven (100°C) for 24 h. The crucible containing dried sample was directly transferred to a desiccator, allowed to cool and reweighed. This process was repeated till a constant weight was obtained. The loss in weight was expressed as percent of moisture.

(ii) Estimation of ash

A known quantity of finely powdered sample was taken in a pre-weighed silica crucible and incinerated in a muffle furnace (600°C) for 2-3h till the sample became free of carbon. The crucible containing the incinerated sample was transferred to a desiccator, cooled and reweighed. The quantity of ash was calculated in percentage.

(iii) Estimation of crude fat

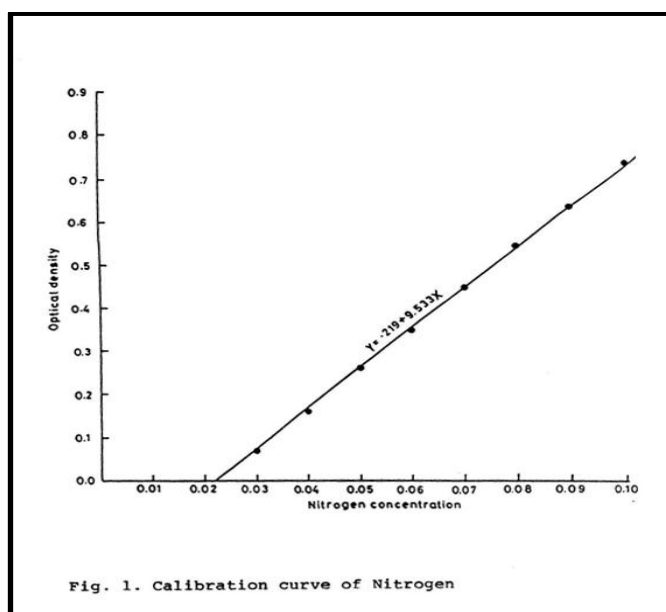
For estimating the crude fat, continuous soxhlet extraction technique was employed. Petroleum ether ($40-60^{\circ}\text{C}$ B.P.) was used as solvent. A weighted quantity of finely ground sample was taken in Whatman fat extraction thimble, cotton plugged and introduced into the soxhlet apparatus. A clean dry soxhlet receiver flask was weighed and fitted to the soxhlet assembly on a water bath for extraction. Extraction was carried out for 10-12h. Thereafter, the flask was removed and kept in a hot air oven (100°C) to evaporate the solvent traces. The flask was then cooled in a desiccator and then reweighed. The amount of fat extracted was expressed in percentage.

(iv) Estimation of crude protein

The technique employed for estimating the crude protein was based on a slight modification of Wong's micro-Kjeldahl method, as adopted by Jafri (1965). The principle involved digesting a known amount of sample in N-free sulphuric acid, in presence of potassium per-sulphate was used as a catalyst, which converts the nitrogenous compounds to ammonium sulphate. This was then treated with Nessler's reagent. The colour developed due to the formation of a complex compound ($\text{NHg}21$) was measured spectrophotometrically. The

optical density obtained was read off against a standard calibration curve of $(\text{NH}_4)_2\text{SO}_4$ for nitrogen estimation. To calculate the total crude protein in the sample, the amount of nitrogen was multiplied with the conventional protein factor (6.25).

Dry powdered sample (0.1g) was taken in a Kjeldahl flask with 5 ml of N-free sulphuric acid (1:1), and 5 ml potassium persulphate added to it. The volume was raised to 3 ml with distilled water. The solution was then nesslerized using Bock and Benedict's Nessler reagent, allowed to stand for 10 min before measuring the absorbance with a blank. The blank was prepared in the same manner using distilled water in place of aliquot. The amount of nitrogen was obtained by reading the optical density against the standard calibration curve (Fig. 1). The nitrogen value was multiplied with 6.25 to obtain the amount of crude protein. The spectrophotometric measurements were made on microprocessor- controlled split beam spectronic 1001 spectrophotometer (Milton Roy Company, USA).



(v) Estimation of gross energy

Gross energy was calculated using fuel values 3.5, 4.5 and 8.5 kcal/g for carbohydrate, protein and lipid, respectively (Jauncey, 1982).

(vi) Assessment of growth and conversion efficiencies

Calculation of growth parameters and conversion efficiencies were made according to standard definitions (Millikin, 1983; Tabachek, 1986; Parazo, 1990).

$$\text{Increase in live weight (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

$$\text{Specific growth rate (\%)} = \frac{\log_e W_2 - \log_e W_1}{D} \times 100$$

Where,

W_1 = Initial mass weight (g)

W_2 = Final mass weight (g)

D= Duration of the feeding trial (days)

$$\text{Feed conversion ratio} = \frac{\text{Total feed intake (g)}}{\text{Live weight gain (g)}}$$

$$\text{Gross growth efficiency (\%)} = \frac{\text{Live weight gain (g)}}{\text{Total feed intake (g)}} \times 100$$

$$\text{Protein efficiency ratio} = \frac{\text{Live weight gain (g)}}{\text{Total protein intake (g)}}$$

$$\text{Protein productive value (\%)} = 100 \times \frac{(\text{Final wet weight} \times \text{Final per cent body protein}) - (\text{Initial wet weight} \times \text{Initial per cent body protein})}{(\text{Amount of diet fed/No. of fish per trough}) \times \% \text{ crude protein in diet}}$$

$$\text{Energy conversion efficiency (\%)} = 100 \times \frac{[(\text{Final wet weight} \times \text{Final body crude energy (kcal g}^{-1})] - [\text{Initial wet weight} \times \text{Initial body crude energy (kcal g}^{-1})]}{(\text{Amount of diet fed/No. of fish per tank} \times \text{Total energy in diet (kcal g}^{-1})]}$$

Feeding trial

Sources of fish, details of their acclimation, and general experimental design are described under the General Methodology section. Young *C. gariepinus* (6.03 ± 0.13 cm; 1.30 ± 0.00 g) were selected from among the acclimated fish and stocked in 70L polyvinyl circular troughs (water volume 55L), fitted with flow-through system (1 L/min of groundwater), in triplicate groups of 15 fish each, and fed control or one of the six experimental diets for 4 weeks. Average water temperature during the experimental period was $26 \pm 1^\circ\text{C}$.

Fish were fed till satiation at 0800 and 1600h. Uneaten feed, if any, was collected over a fine mesh sieve and dried overnight (100°C) for calculating the quantity of feed consumed. The performance of diet, in terms of weight gain and nutrient utilization, was evaluated using standard definitions.

Gross energy and proximate analysis

Healthy fish (20 numbers) were randomly taken out from the acclimated fish stock for the analysis of initial carcass composition, using standard techniques as followed earlier. At the end of the feeding trial, 10 fish from each trough were again taken randomly, pooled and analyzed for their final carcass composition. Gross energy was calculated using physiological fuel values.

Statistical analysis

Statistical evaluation through one-way analysis of variance (Snedecor and Cochran, 1967) and Duncan's multiple range test (Duncan, 1955) was done to test the difference between treatment means ($P < 0.05$). Relationship between specific growth rate (%) vs. E/P ratio and dietary protein vs. weight gain (%)

was established by linear regression analysis (Snedecor and Cochran, 1967).

RESULTS

Results of feeding of *C. gariepinus* with varying energy and protein ratios are given in Table 2 and 3. A significant influence of energy and protein was evident on fish growth in terms of per cent live weight gain. At each protein level, a general decline in per cent live weight gain was noticed with increasing dietary energy. Weight gain (%) was found to be directly related (360.8 kcal/100g: $r = 0.99$, $n = 3$ and 406.7 kcal/100g: $r = 0.96$, $n = 4$ including control) to dietary protein levels (Fig. 2). Diets with lower energy levels (360.8 kcal/100g) produced best growth. Maximum growth was observed at 45 % crude protein level with 360.5 kcal/100g energy.

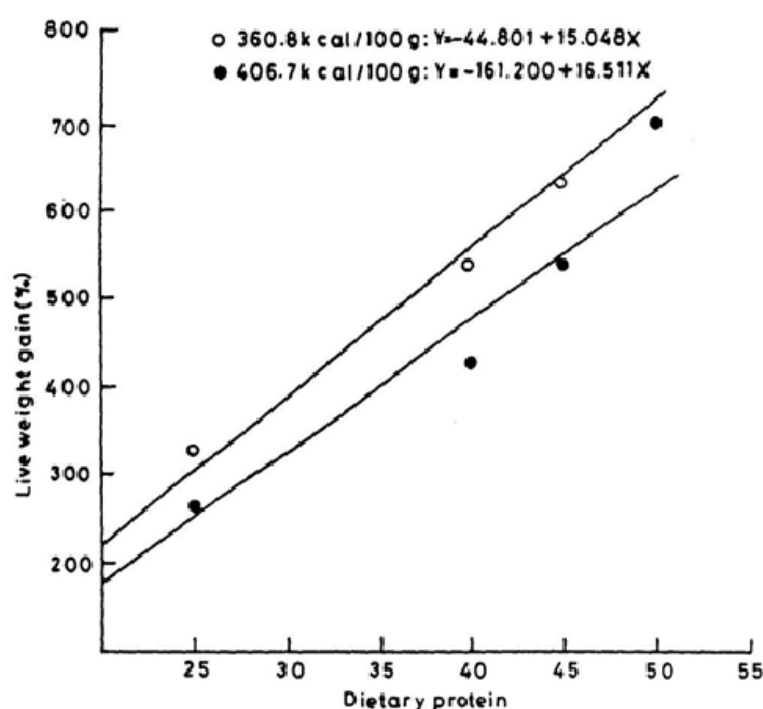


Fig. 2 Effects of dietary protein levels on live weight gain (%) of *C. gariepinus* fed experiments

Specific growth rate (SGR%) showed a negative linear correlation (Fig. 3) with E/P ratios (360.5 kcal/100g : $r = -0.99$, $n = 3$ and 406.7 kcal/ 100g: $r = -0.95$, $n = 4$, including the control diet).

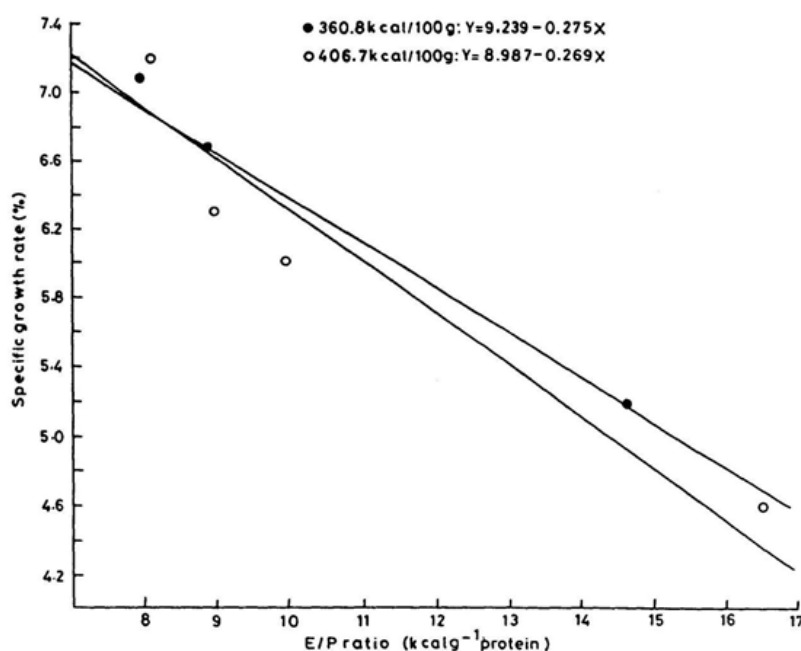


Fig. 3 Effects of dietary E/P ratios on specific growth rate (%) of *C. gariepinus* fed experiments

The daily mean feed consumption (mg/fish day⁻¹) was found to be influenced by both protein and energy levels of the diet. In general, at a given protein level feed consumption is reduced with increase in dietary energy. Influence of dietary protein on feed consumption was positively linear (360.8 kcal/100: $r = 0.99$, $n = 3$ and 406.7 kcal/100g : $r = 0.95$, $n = 3$) at a given energy level. Correlation analysis between E/P ratio and feed consumption produced a negative linear effect ($r = -0.84$, $n = 6$). Fish fed 45% crude protein at lower energy density (360.5 kcal/100g) consumed maximum feed (539.4mg/fish day⁻¹) while those fed with 25% crude protein and higher dietary energy (406.7 kcal /100g) consumed the minimum (375.8mg/fish day⁻¹). The percent live weight gain (Y) showed a positive correlation with daily mean feed consumption (X) ($Y = -410.65 + 1.934X$: $r = 0.96$, $n = 6$) (Fig. 4).

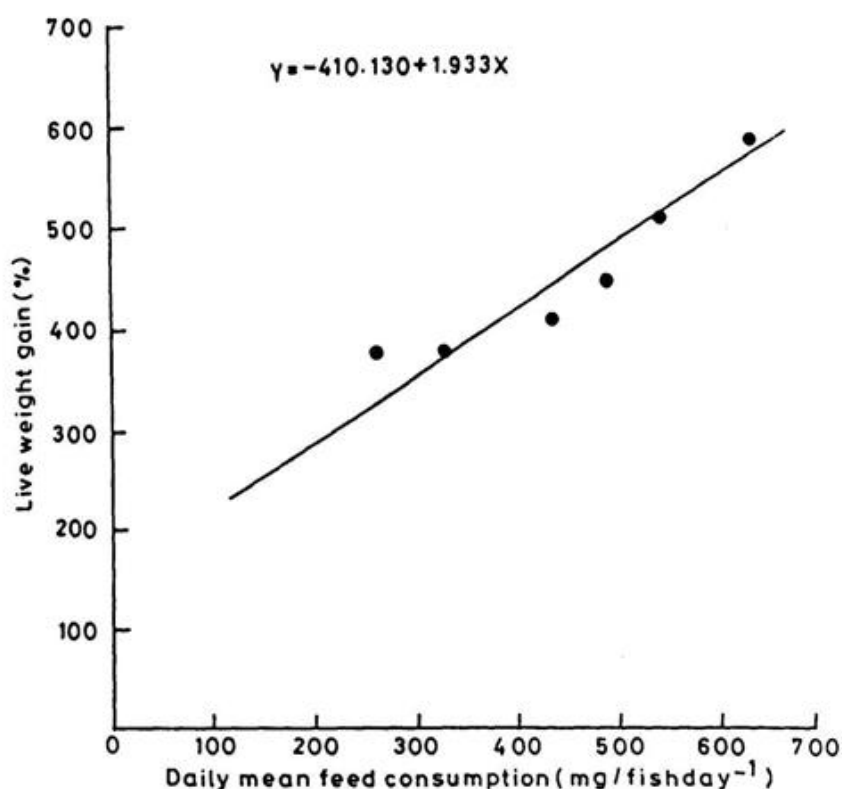


Fig. 4 Relationship between live weight gain (%) and daily mean feed consumed (mg/fish day⁻¹ in *C. gariepinus* fed experiments

Feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and energy conversion efficiency (ECE) were altered by both energy and protein contents of the diet. Best feed conversion was noticed in the group fed 45% crude protein and low energy. At a given energy level, FCR improved with decreasing E/P ratios (360.8 kcal/100g: $r = 0.99$, $n = 3$ and 406.7 kcal/100g : $r = 0.99$, $n = 4$). Poor FCR and lower values of PER, PPV and ECE were observed with increase in dietary energy content at a given protein level. Both PER (360.8 kcal/100g: $r = -0.99$, $n = 3$ and 406.7 kcal/100g $r = 0.99$, $n = 4$) and PPV (360.8 kcal/100g: $r = -0.99$, $n = 3$ and 406.7 kcal/100g: $r = -0.96$, $n = 4$) showed inverse relationship with dietary protein. An opposite trend was observed in ECE at both the energy levels (360.8 kcal/100g: $r = 0.99$, $n = 3$ and 406.7 kcal/100g: $r = 0.98$, $n = 4$) when isocaloric diets were compared. PPV and PER were maximum 30.71 and 3.62, respectively, at the lowest protein level (25%) with 366.6 kcal/100g diet, while maximum ECE (21.15%) was observed at 45% crude protein with 360.5 kcal/100g (Table 3).

Moisture, crude protein, fat and ash in fish tissue were found to vary significantly among the various treatment groups (Tables 3 & 4)). At the end of the feeding trial, fish in all the experimental groups showed higher percentages of lipid and lower percentages of moisture, protein and ash, compared to their initial values. A decrease in moisture, protein and ash was observed with increase in dietary energy.

*Loba Chemie, India.

Table 04. Body composition of *C. gariepinus* fed experimental diets.

Diet	Energy (Kcal/100g)	Moisture (g/100g, wet weight)	g/100g, dry matter		
			Protein	Fat	Ash
Initial		80.91 ± 0.12	69.36 ± 0.32	10.00 ± 0.00	8.52 ± 0.01
25% crude protein					
Diet I	366.56	76.12 ^a ± 0.13	65.56 ^a ± 0.16	19.99 ^d ± 0.11	7.20 ^{ab} ± 0.12
Diet II	413.00	76.04 ^a ± 0.30	63.80 ^b ± 0.12	23.37 ^b ± 0.04	6.89 ^{de} ± 0.05
40% crude protein					
Diet III	355.31	75.70 ^a ± 0.06	65.80 ^a ± 0.49	19.59 ^d ± 0.05	7.18 ^{abc} ± 0.02
Diet IV	402.31	74.5 ^{bc} ± 0.09	61.36 ^d ± 0.05	23.18 ^c ± 0.05	6.76 ^e ± 0.02
45% crude protein					
Diet V	360.50	76.50 ^a ± 0.52	65.68 ^a ± 0.05	19.84 ^d ± 0.49	7.28 ^a ± 0.05
Diet Vi	405.00	75.48 ^{ab} ± 0.60	62.12 ^c ± 0.02	25.40 ^a ± 0.06	7.05 ^{bed} ± 0.03
Control	406.50	73.97 ^c ± 0.03	58.00 ^e ± 0.14	22.62 ^c ± 0.22	7.01 ^{ed} ± 0.01

Results are mean ± SE of triplicate fish groups

Values in each column with similar superscript are insignificantly different (p>0.05)

Survival in each treatment was over 90%, and no specific pattern of mortality was noticeable to which treatment levels could be related.

DISCUSSION

The results of the present study indicates that, as in many other fish species (Halver, 1989; De Silva and Anderson, 1995), that growth of *C. gariepinus* is also significantly influenced by the levels of energy and protein in the diet. Fish fed with 360.5 kcal/100g energy and 45% crude protein (E/P ratio 8.01) diet, produced maximum growth which was not significantly produced by the control diet with high energy (406.5 kcal/100g) and high protein (50% C.P.) having an E/P ratio of 8.13. When specific growth rate (SGR%) was plotted against E/P ratio at both the energy levels (360.8 kcal/100g and 406.7 kcal/100g) also intersected at or near 8.01. A negative correlation existed between E/P ratio and SGR (%), indicating that higher E/P ratios do not prove beneficial to fish in terms of growth. This fact is justified by a negative correlation observed between E/P ratio and the amount of feed consumed by the fish.

A positive correlation observed between the amount of feed consumed and gain in live weight percent indicates that the amount of feed consumed by the fish has a direct bearing on weight gain. Page and Andrews (1973) and Lovell (1979) made a similar observation on channel catfish. Hassan, M.A.(1993) noted that in *C. batrachus* high digestible energy to protein ratio causes cessation of feeding before sufficient amount of protein is consumed by fish resulting in decreased fish growth. These observations strengthen the fact that feed consumption in fish is determined by energy density of the diet. A corollary to the above fact was also evident in the work of Jantrarotai *et al.* (1998) on hybrid *Clarias*, whereas Li and Robinson (1999) on juvenile channel catfish and Hernandez *et al.* (2001) on sharpnose seabream.

A general reduction in percent gain in live weight with increase in dietary energy content at each protein level and a direct relationship with dietary protein content noted during the present study on *C. gariepinus*, conform to the findings of Hassan (1993) on *C. batrachus*, and McGoogan and Gatlin III (1999) on *S. ocellatus*. The observation that feed intake in *C. gariepinus* fingerling is significantly influenced by the levels of dietary energy and protein emphasizes that both energy and protein are important considerations while formulating the feed for fish. The data indicate that, irrespective of the protein content of diet, feed consumption gets reduced with increase in dietary energy levels. In *C. gariepinus* feed consumption was more pronounced in the groups fed low energy diets. Influence of protein and energy content on feed consumption has also been reported in other fishes (Tabachek, 1986; De Silva *et al.*, 1991; Hassan, 1993; Pullin *et al.*, 1996; Jantrarotai *et al.*, 1998; and Hernandez *et al.*, 2001). Influence of both energy and protein contents on FCR, which reflects the overall performance of diet in terms of live weight gain, was also evident during the present study on *C. gariepinus*. Best FCR was obtained in fish groups fed diets containing 45% crude protein with 360.5 kcal/100g energy. Machiels and Henken (1985) and Uys (1989) also noted a dietary protein requirement of 40% or more for this species.

Protein utilization in fish is known to be influenced by the quality of protein in the diet besides the levels of dietary energy (Steffens, 1981). In *C. gariepinus*, a negative correlation was evident between dietary protein and PER/PPV, with the highest values of both PER and PPV obtaining at lowest protein level (25% C.P.) Increase in dietary energy at each protein level, however, resulted in a reduction in both PER and PPV. Decreasing E/P ratio in isocaloric diets reflected a positive correlation with PER and PPV, indicating that more the dietary energy available g^{-1} protein, the greater is the utilization of protein for growth, this being measurable by PER and PPV. A negative correlation was observed between E/P ratio and ECE. Low ECE noted in the fish fed diet with high E/P ratio indicates that more energy is utilized to meet the energy demands of the body and little get deposited. Highest ECE was observed at 45% crude protein and 360.5 kcal/100g energy, and this was not significantly different from the value obtained with the control diet (50% crude protein and 406.5 kcal/100g energy), indicating that E/P ratio of 8.01 is adequate to meet the energy and protein requirements of fingerling *C. gariepinus*. On the basis of weight gain and values of FCR, PER, PPV and ECE obtained by feeding fish with a diet of varying E/P ration, it may be summarized that a 45% crude protein diet with 360.5 kcal/100g energy is optimum for this fish.

The data on body composition of *C. gariepinus* reveals that proximate composition of fish is markedly influenced by dietary energy level. An increase in body lipid content observed in *C. gariepinus* with increase in dietary energy was also reported in other fish species (Zeitler *et al.*, 1984; Hassan, 1993; Catacutan

and Coloso, 1995; Hossain *et al.*, 1998). Similarly, the inverse relationship noted between dietary energy (lipid) and body moisture, protein and ash of *C. gariepinus* was also seen in other fish species (Parazo, 1990; El Sayed and Tehsima, 1992; Hassan, 1993; Panda *et al.*, 1999).

It is evident from the present study that to produce sufficient weight gain and better feed conversion, the diet of *C. gariepinus* should contain 45% crude protein and 360.5 kcal/100g of energy.

The dietary protein component can further be reduced at the same energy level to reduce feed cost. Reduced growth in fish fed low protein diet (25% C.P.) is compensated by relatively high protein retention. Therefore, it is suggested to make a compromise between growth rate and cost effectiveness of the diet while formulating feed for *C. gariepinus*.

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