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Optimum Feeding Rate, Energy And Protein Maintenance Requirements Of *Clarias Gariepinus* (Burchell 1822)

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ABSTRACT

Optimum feeding rate, energy and protein maintenance requirements were reported in the fingerling of Clarias gariepinus (Burchell 1822), fed with purified diet (45% C.P.; 360.5 kcal/100g energy) at 0-10% Body Weight day^{-1}. A linear increase (r = 0.99) was observed in daily average growth increment up to a ration level of 8% Bw. day^{-1} but maximum conversion efficiencies were obtained at 4% Bw. day^{-1}, corresponding to protein and energy intakes of 0.05g/fish day^{-1} and 0.39 kcal/fish day^{-1} respectively. The study indicates that a ration level of 4% Bw day^{-1} is optimum for this species at 25±1° C. Poor Food Conversion Ratio and daily average growth increment obtained at a ration level of 2% Bw day^{-1} suggested that this level approximated the maintenance requirements of the fish. Body moisture, fat and ash contents were significantly (P<0.05) affected by ration levels whereas variations in protein were insignificant (P>0.05).

INTRODUCTION

Optimization of feeding rate of cultured fish is important to achieve efficient production. Since feeding rate in fish affects their nutrient requirements, knowledge of optimal feeding rate is considered a prerequisite to nutrient requirement estimates (Tacon and Cowey, 1985; Talbot, 1985). Ration level in fish is also reported to influence fish growth, utilization efficiencies and chemical composition (Reddy and Katre, 1979; Reinitz, 1983). Several factors, including fish size, feeding level and water temperature influence optimum feed requirements. In commercial culture, although control of environmental temperature may not be feasible, ration size can be manipulated to maximize production. Although some information is available on the nutrition of *Clarius gariepinus* (Henken *et al.*, 1985, 1987; Degani *et al.*, 1989; Uys, 1989; Mybenka and Agua, 1990; Hoffman and Prinsloo, 1995; Awaiss and Kestemont, 1998; Murty and Naik, 1999), there is almost no information available on the optimal feeding rate of this species. Henken *et al.* (1985) have examined the effect of feeding level on the apparent digestibility of this fish. A quantitative estimate of maximum daily feed intake of *C. gariepinus* fingerling has also been made by Hossain *et al.* (1998).

The present study deals with the effect of feeding rate on growth and body composition of fingerling C. *gariepinus*, leading to the estimation of its optimal ration level, and energy and protein maintenance requirements. The information will be of interest to fish nutritionists and farmers.

MATERIALS AND METHOD

Source of fish stock / acclimation

Fingerlings of *C. gariepinus* were obtained from a fish farm in Rampur district, Uttar Pradesh. They 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 (1x1x1m) 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 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 a-cellulose were added to it. Finally the content blended in Hobart mixer while still in 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 at 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 (Table 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 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.

Estimation of Moisture

For moisture estimation a weighed 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.

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.

Estimation of crude fat

For estimating the crude fat, continuous soxhlet extraction technique was employed. Petroleum ether (40-60° 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.

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 persulphate is used as a catalyst, which converted 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 (NHg21) was measured spectrophotometrically. The optical density obtained was read off against a standard calibration curve of $(NH_4)_2SO_4$ 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). A 0.1g dry powdered sample 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 m1 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).

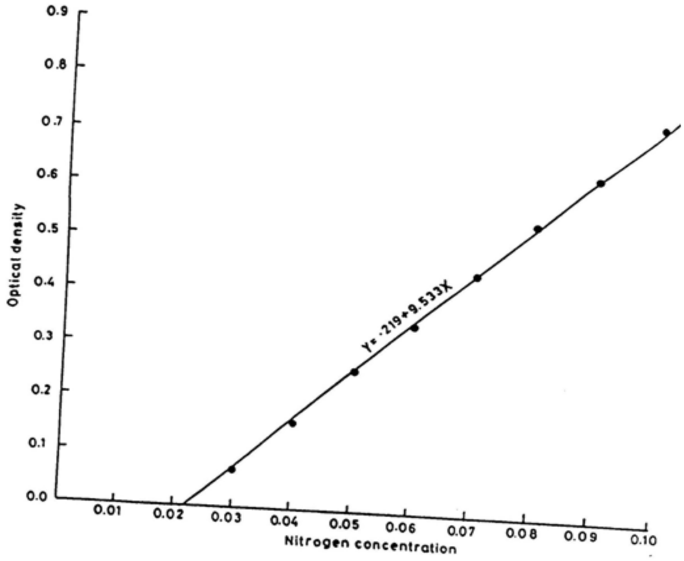

Fig. 1 Calibration curves of nitrogen

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).

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).

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

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)

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

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

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

Protein productive value (%) =100x (Final wet weight x Final per cent body protein)-(Initial wet weight x Initial per cent body protein) / (Amount of diet fed/No. of fish per trough)x % crude protein in diet.

Energy conversion efficiency (%)

= 100x [(Final wet weight x Final body crude energy (kcal g^{-1})]-[(Initial wet weight x Initial body crude energy (kcal g^{-1})]/ Amount of diet fed/No. of fish per tank x Total energy in diet (k cal g^{-1})].

Preparation of experimental diet for the experiment

Crude protein (45%) diet with 360.5 kcal /100g digestible energy (Tables 1 & 3) was prepared using the method as described earlier under the General Methodology section . 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). An energy to protein ratio of 8.13 kcal/g was maintained in the diet.

Table 1. Composition of mineral mixture*

Mineral	g/100g
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_20	0.015
Potassium iodide	0.015
Cuprous chloride	0.010
Manganous sulphate H_2O	0.080
Cobalt chloride .6H_20	0.100
Zinc sulphate. 7H_20	0.300

*Halver (1989).

Table 2. Composition of vitamin mixture*

Vitamin	g/100g
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-HCI	0.005
Thiamine-HCL	0.005
Folic acid	0.0015
Biotin	0.0005
a -tocopherol acetate	0.040
Vitamin B_{12} (10 mg /500 ml H_20)	0.00001(0.5 ml)

*Halver (1989).

Table 3. Ingredient and proximate composition of experimental diet

Ingredients	g/100g (as fed)
Casein (Vitamin free; 84.6% C.P)*	40.17
Gelatin (87.0% C.P)*	12.84
Dextrin	25.71
a-Cellulose	7.28
Oil mix (2:1 corn and cod liver oil)	8.00
Vitamins mix.	1.00
Mineral mix.	3.00
Carboxymethyl cellulose	2.00

Proximate composition% (calculated)

Crude protein	45.00
Crude fat	8.00
Carbohydrate	26.00
Energy (kcal/100g)	360.50
E/p ratio (kcal/g)	8.01

*Loba Chemie, India

Feeding trial

Details of acclimation of fish and general experimental design was described above. A 3x2 factorial design of experiment was used. Fish (6.14± 0.13 cm, total length; and 1.70±0.01g weight) were stocked in triplicate groups of 15 fish each in circular polyvinyl troughs (water volume 55 L; water exchange rate 1Umin). Water temperature over the experimental period was 25±1°C. Fish were fed ration levels at 0-10% of body weight per day, on dry to wet weight basis, twice daily at 0800 and 1600h for 4 weeks. Mass weights of fish were taken weekly and ration size recalculated for subsequent feeding. No unconsumed feed was noticed in experimental troughs initially but in the last week of experiment fish took relatively longer time to consume their daily ration and some unconsumed feed accumulated in higher ration groups.

Estimation of gross energy and proximate analysis

For initial and final carcass composition, fish were taken out from the acclimated stock and at the end of feeding trial from each trough and analysed for their proximate carcass composition, using standard techniques explained above. Gross energy in the tissue was calculated using physiological fuel values similar to those used for the energy estimates in the diet.

Statistical analysis

Comparison among different treatment means were made by one way analysis of variance (Snedecor and Cochran, 1967) and Duncan's multiple range test (Duncan, 1955). A significance level of $P<0.05$ was used. Regression and correlation coefficient (r) were calculated to establish the relationship between various parameters.

RESULTS

Results of the 4 weeks feeding trial was summarized in Tables 4-6. A linear increase (r=0.99) in average daily growth (Figs 2-3) was obtained by feeding fish with varying ration levels up to 8% Bw. day^{-1}, corresponding to protein and energy intakes of 0.15g and 1.16 kcal energy/ fish day^{-1}, respectively. In the linear growth portion, average daily growth increment of fish (Y) over increasing levels of protein and energy (X) was described by the equations Y=0.021+4.476 X for protein and Y⁼0.028+0.556 X for energy. Fish fed 10% Bw. day^{-1} produced insignificant ($P>0.05$) difference in growth compared with those fed 8% Bw. day^{-1}. A continuous loss in weight was noticed in starved fish. A linear increase (r=0.95) in specific growth rate (SGR%) was also evident with increase in ration levels up to 8% Bw. day^{-1} (Fig. 4).

Table 4 Growth of *C. gariepinus* fed varying levels of experimental diets

Feeding rate			Total diet fed (g/fish)	Initial average weight (g)	Final average weight (g)	Growth increment (g/fish day^{-1})	Gross growth efficiency (%)
%(Bw. day^{-1})	Protein (g/ fish day^{-1})	Energy kcal/g per fish day^{-1})					
0.00	0.00	0.00	0.00	1.67 ±0.00	1.16 ±0.05	-0.06^{e} ±0.00	-
2.00	0.02	0.15	2.09	1.72 ±0.01	2.79 ±0.03	0.11^{d} ±0.00	107.87^{b} ±3.68
4.00	0.05	0.39	5.38	1.72 ±0.01	4.85 ±0.03	0.29^{c} ±0.00	122.00^{a} ±0.85
6.00	0.09	0.73	10.07	1.73 ±0.00	7.46 ±0.01	0.51^{b} ±0.00	118.97^{a} ±0.43
8.00	0.15	1.16	16.09	1.70 ±0.01	9.90 ±0.17	0.73^{a} ±0.01	106.84^{b} ±0.90
10.00	0.18	1.46	20.32	1.69 ±0.01	9.82 ±0.14	0.74^{a} ±0.01	83.84^{c} ±0.89

Results are mean ± SE of triplicate fish groups

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

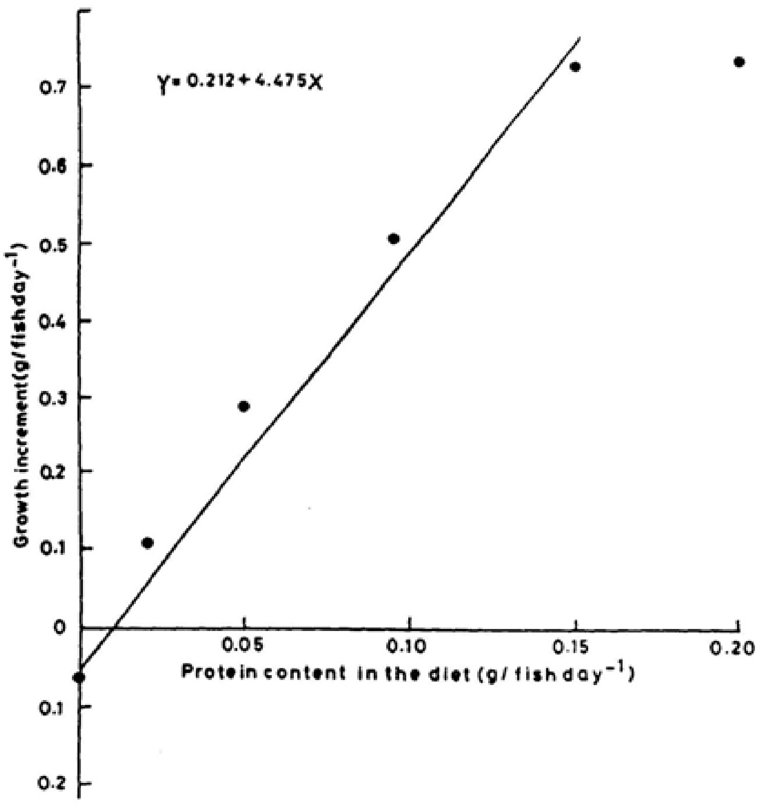

Fig. 2 Growth increment in *C. gariepinus* fed with varying levels of dietary protein (g/fish day^{-1})

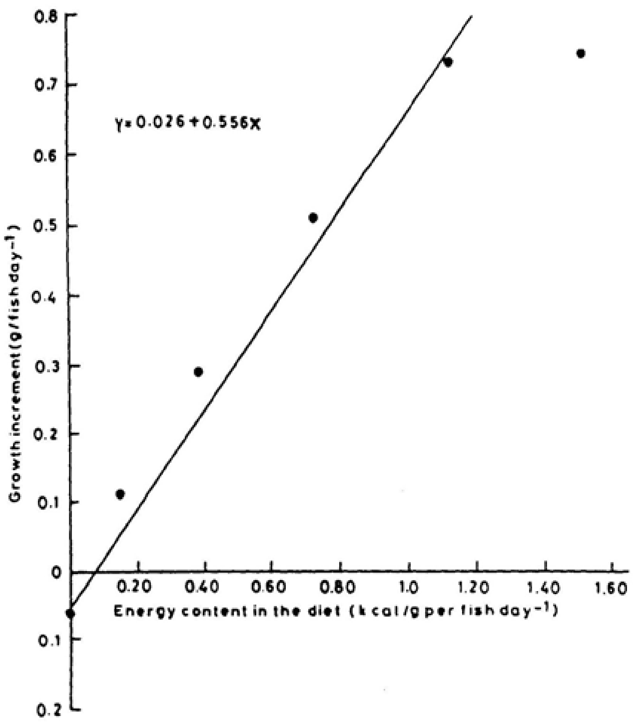

Fig. 3 Growth increment (g/fish day^{-1})in *C. gariepinus* fed with varying levels of dietary energy)k cal/g per fish day^{-1})

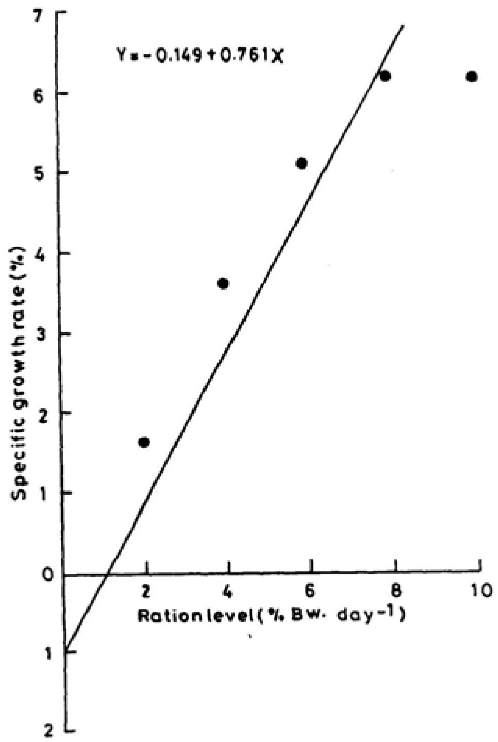

Fig. 4 Specific growth rate (%) in *C. gariepinus* fed with varying ration levels

Highest gains in body protein and energy were observed by feeding fish with 0.15 g protein/fish day^{-1} and 1.16 kcal/fish day^{-1} but maximum gross growth efficiency, protein and energy conversion efficiencies were discernible at the ration level of 4% Bw corresponding to protein and energy intakes of 0.05 g and 0.39 kcal/fish day^{-1}. When the same parameters were compared with those at 6% Bw. day^{-1}, corresponding to 0.09 g protein/ fish day^{-1} and 0.73 kcal energy/fish day^{-1}, no significant difference was observed.

The best feed conversion ratio (FCR) was obtained in fish fed 4% Bw. day^{-1} (0.05g protein/ fish day^{-1} and 0.39 kcal energy/fish day^{-1}) FCR was poor above and below this feeding level (Table 5). FOR plotted against ration levels produced a typical U-shaped curve (Fig. 5).

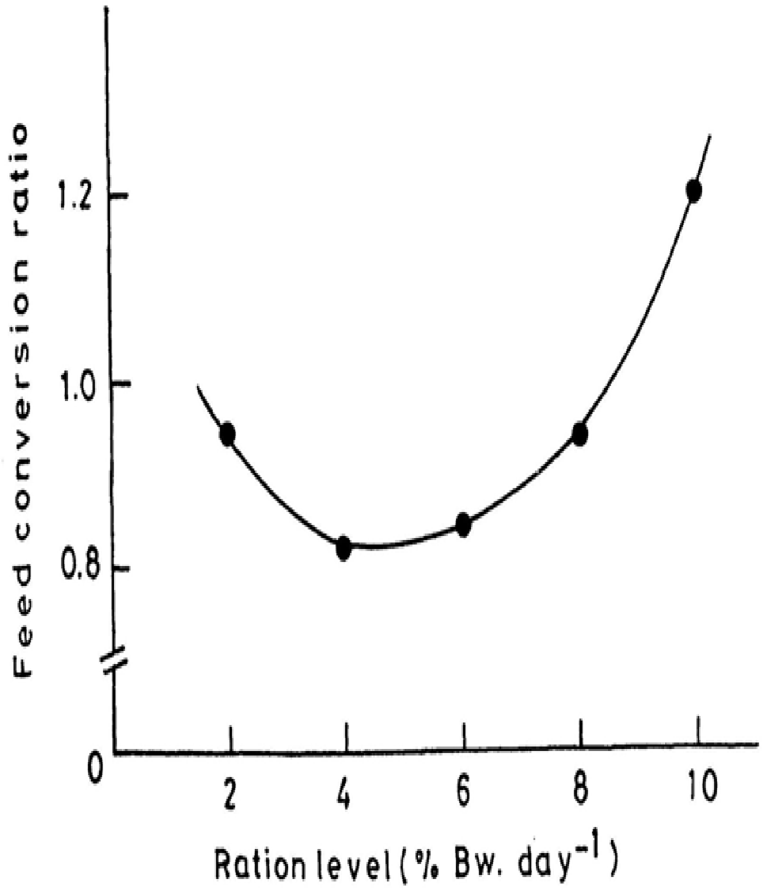

Fig. 5 Effect of ration levels (% B.W. day^{-1}) on feed conversion ratio in *C. gariepinus*

Table 5: Conversion efficiencies in *C. gariepinus* fed varying levels of experimental diets

Feeding Rate			Conversion efficiency (%)	
B.W. day^{-1}	Protein (g/fish day^{-1})	Energy (kcal/g per fish day^{-1})	Energy	Protein
0.00	0.00	0.00	-	-
2.00	0.02	0.15	13.80^{c} ±0.67	17.33^{c} ±0.51
4.00	0.05	0.39	18.79^{a} ±0.37	21.86^{a} ±0.09
6.00	0.09	0.73	18.69^{a} ±0.31	21.16^{a} ±0.10
8.00	0.15	1.16	17.19^{b} ±0.14	19.16^{b} ±0.32
10.00	0.18	1.46	13.50^{c} ±0.02	14.98^{d} ±0.07

Values are mean ±SE of triplicate fish groups

Means is each column with similar superscript are insignificantly different (P>0.05)

The proximate composition of fish fed varying ration levels has been given in Table 6. The body composition varied with levels of feeding. Changes in moisture, lipid and ash percentages were significant (P<0.05), whereas variations in protein content were insignificant (P>0.05). Moisture and ash decreased with increase in ration levels from 2 to 8% Bw. day^{-1}. An increasing trend was also observed in body fat and energy (kcal g^{-1} dry matter) contents. No significant difference in the proximate composition was observed between the fish fed 8% Bw. day^{-1} and those fed 10% Bw. day^{-1}. In starved fish, compared to crude protein, fat was greatly reduced, whereas moisture and ash contents registered an increase.

Table 6 Proximate composition and energy content in the carcass of *C. gariepinus* fed varying levels of experimental diets

Feeding rate			Proximate composition (g/100g dry matter)				
% (Bw. day^{-1})	Protein (g/fish day^{-1})	Energy (kcal/gper fish day^{-1})	Moisture (g/100g, wet weight)	Crude protein	Crude fat	Ash	kcal g^{-1} dry matter
0.00	0.00	0.00	80.12^{a} ±0.13	79.04^{a} ±0.43	10.00^{e} ±0.58	8.93^{a} ±0.11	0.87^{e} ±0.01
2.00	0.02	0.15	78.93^{b} ±0.11	69.72^{a} ±0.44	12.01^{d} ±0.12	8.60^{ab} ±0.12	0.95^{d} ±0.01
4.00	0.05	0.39	77.01^{c} ±0.32	69.68^{a} ±0.42	16.00^{c} ±0.58	8.25^{bc} ±0.14	1.09^{c} ±0.02
6.00	0.09	0.73	76.68^{c} ±0.15	69.48^{a} ±0.39	18.50^{b} ±0.29	7.92^{c} ±0.05	1.13^{b} ±0.01
8.00	0.15	1.16	76.23^{c} ±0.01	69.32^{a} ±0.44	20.03^{a} ±0.01	7.75^{c} ±0.15	1.17^{a} ±0.00
10.00	0.18	1.46	76.25^{c} ±0.13	69.16^{a} ±0.07	20.00^{a} ±0.13	7.75^{c} ±0.26	1.17^{a} ±0.01
Initial	-	-	79.02 ±0.28	68.72 ±0.18	11.00 ±0.10	8.57 ±0.21	0.94 ±0.01

Values are mean ±SE of triplicate fish groups

Means in each column with similar superscript are insignificantly different ($P>0.05$)

DISCUSSION

It is apparent from the results of the present study that, over the experimental period, fingerling C. *gariepinus* fed ration at 2% Bw. Day^{-1} increased in its live weight by 0.11g/fish day^{-1} whereas those receiving ration at 4% Bw. day^{-1} increased by 0.29 g/fish day^{-l}. Although significant growth improvement was noticeable at higher ration levels, highest conversion values were achieved at a ration level of 4%, which was not significantly different from the values achieved when fish were fed at 6% Bw. Day^{-1}. This indicates that between a feeding rate of 4 to 6% Bw. Day^{-1}, a larger portion of dietary nutrients were utilized by fish for their growth.

Poor daily average growth increment and FCR in fish fed 2% Bw. Day^{-1} suggests that this ration level approximates only the maintenance requirement of nutrients, wherein a major portion of ingested nutrients is utilized to maintain life and a smaller portion available for growth. Hassan and Jafri (1994) obtained comparable results on the other *Clarias* species, C. *batrachus.* The findings also seem in agreement with the observations of Hung and Lutes (1987) on *Acipenser transmontanus* and Lupatsch *et al.* (1998) on *Sparus aurata.*

A positive effect of feeding rate on growth has been shown in striped bass (Hung *et al.*, 1993) and in channel catfish (Li and Lovell, 1992). In these fishes, either a linear increase or plateauing effect was noted on the growth while feed conversion ratio reduced with increased ration. Similar pattern was observed in *C. gariepinus* during the present study. When FCR was plotted against ration level, a typical U-shaped curve was obtained, indicating that ration level of 4% Bw. day^{-1} is optimum for the fingerling *C. gariepinus.* Similar results have been reported in other fish species (Hassan and Jafri, 1994; Panda *et al*, 1999). Poor FCR at lower and higher ration levels can be the result of loss of nutrients and wastage of food, as fish took longer time to consume food to reach satiation (Tvenning and Giskegjerde, 1997). Although maximum growth improvement (g/fish day^{-1}) was observed in *C. gariepinus* at a ration level of 8% Bw. day^{-1}, which was not significantly different from the growth increment obtained when fish were fed at a ration level of 10% Bw. day^{-1}, maximum gross growth efficiency was obtained when fish received ration at 4% Bw. day^{-1}.

A gradual decline in conversion efficiency was noticed in fish fed at higher ration levels, thus feeding fish beyond a ration level of 4% Bw. day^{-1} appears a wastage. A general reduction in conversion efficiency of fish at higher ration levels has also been reported by Hassan and Jafri (1994) in *C. batrachus.*

Several factors, including growth and diet, are known to influence the proximate composition of fish. Proximate composition of fish is also influenced by varying ration levels, In the present study, moisture, fat and ash contents of fish were significantly influenced by feeding rates. However, no significant change was observed in protein content. When fish were starved, the amount of moisture and ash increased, whereas a distinct decrease in fat was noticeable over their initial values. Similar changes in proximate composition vis-a-vis ration level were noticed in *C. batrachus* (Hassan and Jafri, 1994). A net loss of energy in starving *C. gariepinus* indicates that both lipid and protein get catabolized during starvation, but there seems to be a preferential catabolism of body lipid. A slightly lower percentage of body fat was observed in fish fed lower ration levels, though at the same time the fish could manage to maintain a relatively higher and constant amount of protein in their body tissue over the initial value, suggesting that in this fish body fat is preferred as an energy source over protein. This finding finds support in the studies made on other fishes (Hung and Lutes, 1987; Brown *et al.*, 1990; Hassan and Jafri, 1994).

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