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Optimizing ration level for young *Clarias gariepinus* Burch.

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Abstract: A 6-week growth trial was conducted to estimate the optimum ration level for young *Clarias gariepinus.* Fish were stocked in indoor 70 L polyvinyl flow-through (1-1.5 L/min) circular tanks at 22 $\pm$2 °C and fed experimental diet at varying levels daily at 0800 and 1600 h. Per cent live weight gain, weight increment (g fish^{-1} day^{-1}) and specific growth rate (SGR%) were higher ($P < 0.05$) in fish fed ration at 3% b.w. day^{-1}, whereas lowest ($P < 0.05$) values for these parameters were found at 1 % b.w. day^{-1}. Varying dietary intake had a significant ($P < 0.05$) influence on the body carcass composition. Whole body protein was found to be significantly ($P < 0.05$) higher in fish groups fed at 3 and 5% b.w. day^{-1}. Carcass fat increased with increasing ration level, with the lowest ($P < 0.05$) value obtaining in fish at 1 % b.w. day^{-1}. Moisture and ash contents were comparable ($P > 0.05$) in fish receiving ration at varying levels, excepting in those at 9% b.w. day^{-1} which exhibited significantly ($P < 0.05$) lower carcass moisture. It is thus evident that a ration level of 3% b.w. day^{-1} is optimum for young *C. gariepinus.*

Key words: *Clarias gariepinus*; catfish; optimum ration level; growth; conversion efficiency; weight increment.

1. Introduction

Growth and feed conversion in fish are markedly affected by quality and quantity of feed consumed (Omarov 1970; Elliott 1975a; Lovell 1989; Pickering 1993). For successful fish culture, it is important to work out the optimum ration size for different stages of the concerned species under culture conditions to obtain maximum growth and conversion rates (Huisman 1976; Reddy & Katre 1979; Machiels & Henken 1986; Hassan & Jafri 1994; Sampaio & Minillo 1995; Panda *et al.* 1999; Olurin *et al.* 2006; Adewolu *et al*. 2009). Knowledge of optimum ration is also considered important in determining the nutrient requirements of fish (Tacon & Cowey 1985; Talbot 1985).

Several reports have appeared in the past on influence of ration size on growth rate and feed utilization in different species of fish (Omarov 1970; Elliott 1975b; Allen & Wootton 1982; Joergensen & Jobling 1992; Sumagaysay 1993; Maekinen 1995; Medale *et al.* 1995; Hossain *et al.* 1998a; Adebayo *et al.* 2000; Graynoth & Taylor, 2000; Annappaswamy *et al.* 2001; Ng *et al.* 2000).

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Lately, *Clarias gariepinus* is being cultured in various parts of the country. Considerable information is available on the nutrition of this fish (Henken *et al.* 1986; Huisman & Richter 1987; Degani *et al.* 1989; Uys 1989; Hoffman & Prinsloo 1995; Awaiss & Kestemont 1998; Murty & Naik 1999). A quantitative estimate of maximum daily feed intake of *C. gariepinus* fingerling has been worked out by Hossain *et al.* (1998b). The present study was conducted to work out optimum ration- level for young *C. gariepinus* fed semi-purified test diet.

2. Materials and Methods

2.1 Source of fish stock/acclimation

Young *Clarias gariepinus*, obtained from local market, were transported to the laboratory in oxygen filled polythene bags, given a prophylactic dip in $KMnO_4$ solution (1:3000), and stocked in cement cisterns (1m x 1m x 1m) for acclimation. During the period of acclimation, fish were fed with minced meat twice daily. After a fortnight, desired number of fish were taken out and gradually acclimated to casein – gelatin based semi-purified experimental diet (Table 1),

Table 1. Ingredients & proximate composition of experimental diet.

Ingredients	g/100 g (as fed)
Casein (vitamin-free; 84.6% C.P.)	40.17
Gelatin (87.0% C.P.)	12.84
Dextrin	25.71
α- cellulose	7.28
Oil mix (2:1 corn and cod liver oil)	8.00
Vitamin mix	1.00
Mineral mix	3.00
Carboxymethyl cellulose	2.00
Proximate composition (%)*	
Crude protein	45.00
Crude fat	8.00
Carbohydrate	26.00
Energy (kJ/100g)	1509.34

* Hina (2001)

2.2 Preparation of experimental diet

Casein-gelatin based semi-purified test diet was prepared by calculating quantities of dietary ingredients on sensitive electronic balance (Sartorius MA-50, Germany). Gelatin was mixed in water in a stainless steel attachment of Hobart electronic mixer, with constant stirring, and heated to 80 °C. The mixer bowl was removed from heating, and quantities of casein, dextrin, minerals and α-cellulose were added to it, and the content blended in the mixer while still in lukewarm state. This was followed by the addition of vitamin mix and oil (2:1 corn and cod liver oil). After blending the mixture, carboxymethyl cellulose was added to it. The prepared diet, upon obtaining a bread dough-like consistency was poured into 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 refrigerator (-20 °C) in sealed polythene packs until used. The mineral and vitamin premixes (Table 2 & 3) were the same as used by Halver (1989).

Table 2. Composition of mineral mixture (Halver, 1989)

Mineral	g/100 g diet
Calcium biphosphate	13.48
Calcium lactate	32.40
Ferric citrate	02.97
Magnesium sulphate	13.70
Potassium phosphate (dibasic)	23.86
Sodium biphosphate	08.72
Sodium chloride	04.35
Aluminium chloride. $6H_2O$	00.015
Potassium iodide	00.015
Cuprous chloride	00.010
Magnesium sulphate. H_2O	00.080
Cobalt chloride. $6H_2O$	00.100
Zinc sulphate. $7H_2O$	00.300

Table 3. Composition of vitamin mixture (Halver, 1989) and incorporated with oil* (Hina, 2001).

Vitamin	g/100g diet
α- cellulose	8.00
Chloine chloride	0.50
Inositol	0.20
Ascorbic acid	0.10
Niacin	0.075
Calcium pentothenate	0.05
Riboflavin	0.02
Menadione	0.004
Pyridoxine. HCl	0.005
Thiamin. HCl	0.005
Folic acid	0.002
Biotin	0.001
α- tocopherol acetate*	0.04
Vitamin B-12 (10mg/500ml H_2O)	0.00001 (0.5ml)

2.3 Experimental design/feeding trial

Fish of desired size and number were sorted out from the acclimated lot and stocked in 70 L polyvinyl circular tanks (water volume 55 L), in triplicate. The tanks were supplied with ground water. The water exchange rate in each tank was maintained at 1-1.5 L min^{-1}. Prior to feeding faecal matter was siphoned off from the experimental tank. Feeding level and schedule was choosen after carefully observing the dietary intake as well as feeding behaviour of the fish. The moisture content in 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 recalculated for subsequent feeding. On the day of weekly measurements, no feed was offered to the fish and the tanks were thoroughly washed with $KMnO_4$ solution (1:3000). During the trial, the water temperature ranged between 22 ± 2 °C.

2.4 Proximate analysis

Proximate composition of fish was analysed using standard techniques (AOAC, 1995). The analysis was carried out in triplicate.

2.5 Estimation of moisture

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 hours. The crucible containing the dry sample was transferred to the desiccator, allowed to cool and reweighed. This process was repeated till a constant weight obtained. The loss in weight was expressed as per cent of moisture.

2.6 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; Yarco, India) for 3 to 4 hours, 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 and expressed in percentage.

2.7 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 a solvent. A weighed quantity of finely ground sample was taken in Whatman fat extraction thimble, cotton plugged, and introduced into the Soxhlet apparatus. A clean dry receiver flask was weighed and fitted to the Soxhlet assembly (Borosil, India) on a water bath for extraction. Extraction was carried out for 10-12hrs. Thereafter, the receiving 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 reweighed. The amount of fat extracted was expressed in percentage.

2.8 Estimation of crude protein

The technique employed for estimating the crude protein was based on a slight modification of Wong's micro-Kjeldahl method (Jafri *et al.* 1964). The principle involved digesting a known amount of sample in N-free sulphuric acid, in presence of potassium persulphate used as catalyst, which coverts the nitrogeneous compounds to ammonium sulphate. This was then treated with Nessler's reagent. The colour developed due to the formation of $(OHg)_2 NH_2I$ was measured by 1001 spectrophotometer (Milton Roy Company, USA). The optical density obtained was read off against a standard calibration curve of $(NH_4)_2 SO_4$ for nitrogen estimation. To calculate crude protein in the sample, the amount of nitrogen was multiplied with the conventional protein factor (6.25).

0.1g dry powdered sample was taken in a Kjeldahl flask with 5ml of N-free sulphuric acid (1:1), and 5ml potassium persulphate added to it. The volume was raised to 3ml with distilled water. The solution was then nesslerized using Bock and Benedict's Nessler reagent (Oser 1979), kept at room temperature for 10 min for complete colour development. A 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. 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).

2.9 Assessment of metabolizable energy

Metabolizable energy of the diets was calculated using physiological fuel values 3.5, 4.5 and 8.5 kcal g^{-1} for carbohydrate, protein and lipid, respectively (Jauncey 1982).

2.10 Assessment of growth and conversion efficiencies

Calculation of growth parameters and conversion efficiencies were made according to standard definitions (Hardy 1989; Hanley 1991).

Live weight gain (%) = $\dfrac{W_2 - W_1}{W_1} \times 100$

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

Where,
W_1 = Initial mass weight (g)
W_2 = Final mass weight (g)
D = Duration of the feeding trial (days)

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

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

2.11 Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at 0.05% significance level (Duncan 1955; Sokal & Rohlf 1981).

2.12 Chemicals and reagents

The following chemicals and reagents were used in the experiments: casein (vitamin-free; 84.6% C.P.), gelatin (87.0% C.P.), dextrin, α- cellulose, oil mix (2:1 corn and cod liver oil), and carboxymethyl cellulose (all Loba Chemie, Mumbai, India); and vitamin mix (α- cellulose, chloine chloride, inositol, ascorbic acid, niacin, calcium pentothenate, riboflavin, menadione pyridoxine. HCl, thiamine. HCl, folic acid, biotin, α- tocopherol acetate, vitamin B-12 (10mg/500ml H_2O)), petroleum ether and mineral mix (calcium biphosphate, calcium lactate, ferric citrate, magnesium sulphate, potassium phosphate (dibasic), sodium biphosphate, sodium chloride, aluminium chloride. $6H_2O$, potassium iodide, cuprous chloride, magnesium sulphate. H_2O, cobalt chloride. $6H_2O$ and zinc sulphate. $7H_2O$) (all Merck, Mumbai, India). All the chemicals and reagents used were of Analar grade.

3. Results

Results of 6-week feeding trial conducted to work out optimum ration size for *C. gariepinus* are given in Table 4.

Table 4. Results of feeding *C. gariepinus* at varying ration levels (% b.w. day^{-1})

	Ration Levels				
	1%	**3%**	**5%**	**7%**	**9%**
Initial individual weight (g)	12.81 ± 0.42	13.09 ± 0.57	11.90 ± 0.19	12.85 ± 1.14	12.05 ± 0.14
Final individual weight (g)	22.86 ± 0.48	30.70 ± 0.74	26.70 ± 0.90	26.40 ± 0.69	24.15 ± 0.04
Live weight gain (%)	78.59^{d} ± 2.14	134.94^{a} ± 5.96	124.38^{ab} ± 2.62	106.87^{bc} ± 8.33	100.62^{c} ± 1.49
Weight increment (g $fish^{-1}$ day^{-1})	0.23^{c} ± 0.01	0.41^{a} ± 0.03	0.35^{b} ± 0.02	0.32^{c} ± 0.01	0.28^{d} ± 0.01
Specific growth rate (SGR%)	1.37^{c} ± 0.02	2.02^{a} ± 0.16	1.92^{a} ± 0.02	1.71^{b} ± 0.14	1.65^{b} ± 0.02
Feed consumed (mg $fish^{-1}$ day^{-1})	594^{e} ± 6.00	630^{d} ± 4.00	683^{c} ± 13.00	713^{b} ± 9.50	800^{a} ± 19.99
Feed conversion ratio (FCR)	2.12^{ab} ± 0.16	1.29^{d} ± 0.09	1.67^{c} ± 0.05	1.92^{bc} ± 0.33	2.37^{a} ± 0.04
Protein efficiency ratio (PER)	1.04^{cd} ± 0.08	1.72^{a} ± 0.13	1.42^{b} ± 0.04	1.19^{bc} ± 0.21	0.93^{d} ± 0.02
Protein fed (g $fish^{-1}$ day^{-1})	0.22^{c} ± 0.02	0.24^{bc} ± 0.02	0.26^{bc} ± 0.01	0.27^{ab} ± 0.04	0.30^{a} ± 0.06

Results are mean ± SE of triplicate fish groups; values in each row with similar superscript are insignificantly (*P > 0.05)* different.

Per cent live weight gain, weight increment (g fish^{-1} day^{-1}) and specific growth rate (SGR%) were higher ($P < 0.05$) in fish fed ration at 3% b.w.day^{-1}, whereas lowest ($P < 0.05$) values for these parameters were found at 1 % b.w. day^{-1}. However, per cent live weight gain and SGR were insignificantly ($P > 0.05$) different in fish fed rations at 3 and 5% b.w. day^{-1}. Broken line regression analysis showed that a ration level of 3% b.w. day^{-1} is optimum with respect to per cent live weight gain (Fig. 1) and SGR (Fig. 2).

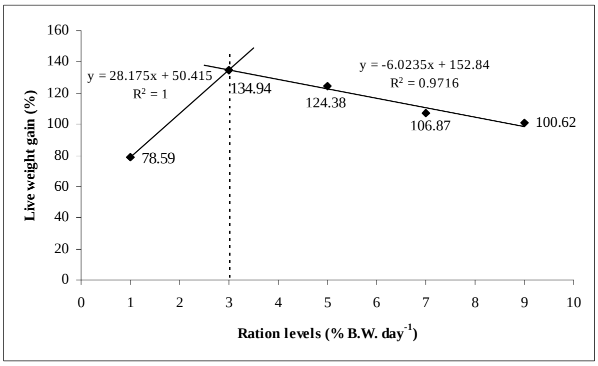

Figure 1. Broken line regression analysis of levels of ration vs. live weight gain (%) in *C. gariepinus*

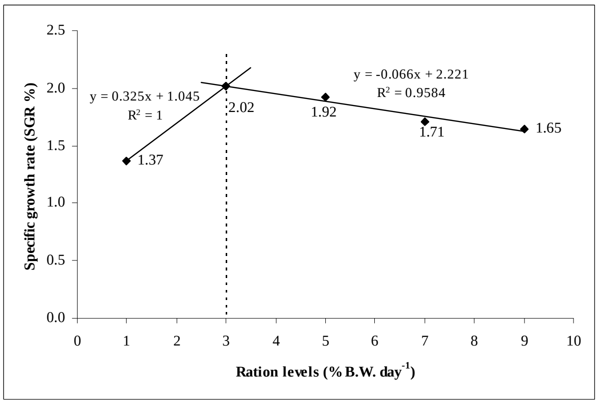

Figure 2. Broken line regression analysis of levels of ration vs. specific growth rate (SGR %) in *C. gariepinus*

Feed conversion ratio (FCR) and protein efficiency ratio (PER) were found better ($P < 0.05$) in fish receiving ration at 3% b.w. day^{-1}, as also evident from the broken line regression analysis (Fig. 3 and 4). Fish fed at 1 % b.w. day^{-1} exhibited poorest values for these parameters.

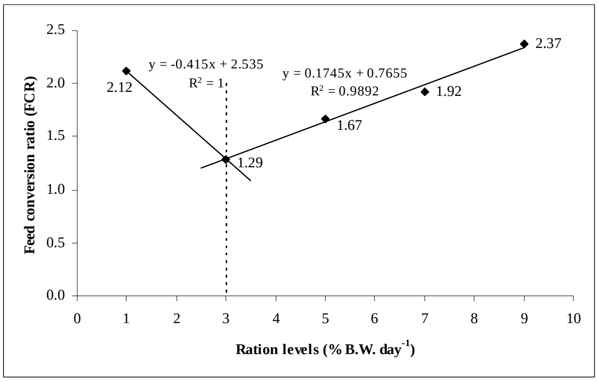

Figure 3. Broken line regression analysis of levels of ration vs. feed conversion ratio (FCR) in *C. gariepinus*

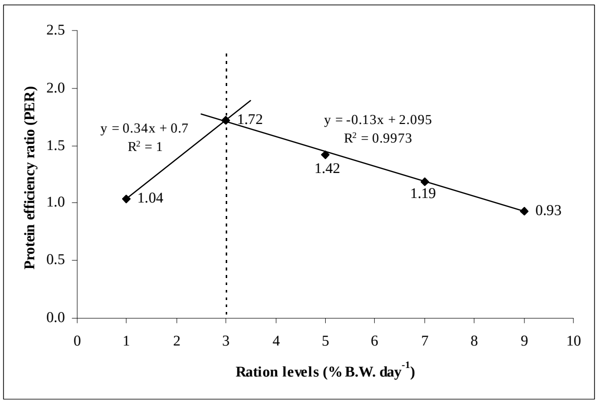

Figure 4. Broken line regression analysis of levels of ration vs. protein efficiency ratio (PER) in *C. gariepinus*

Carcass composition of fish was also influenced by varying levels of ration (Table 5). Higher ($P < 0.05$) carcass protein was obtained in fish fed at 3 and 5% ration levels and lowest ($P < 0.05$) in those at 1 % b.w. day^{-1}. Fat content increased with increasing ration levels. Lowest ($P < 0.05$) fat content was observed in fish fed at 1 % b.w. day^{-1}. Moisture and ash contents were comparable ($P > 0.05$) in fish receiving ration at varying levels, excepting in those at 9% b.w. day^{-1} which exhibited significantly ($P < 0.05$) lower carcass moisture.

Table 5. Carcass composition of *C. gariepinus* fed at different ration levels

Ration level % (b.w. day^{-1})	**Moisture** (%)	**Protein** (%)*	**Fat** (%)*	**Ash** (%)*
1.00	$75.66^a \pm 0.71$	$70.64^c \pm 0.03$	$13.18^c \pm 0.28$	$9.80^a \pm 0.29$
3.00	$75.38^a \pm 0.71$	$72.55^a \pm 0.15$	$14.40^b \pm 0.20$	$9.52^a \pm 0.23$
5.00	$75.51^a \pm 0.25$	$72.38^a \pm 0.06$	$14.59^b \pm 0.49$	$9.96^a \pm 0.14$
7.00	$75.81^a \pm 0.57$	$71.17^b \pm 0.07$	$16.12^a \pm 0.08$	$9.99^a \pm 0.10$
9.00	$73.34^b \pm 0.89$	$71.13^b \pm 0.19$	$17.24^a \pm 0.22$	$9.83^a \pm 0.11$

Dry-weight basis; results are mean ± SE of triplicate fish group; values in each column with similar superscript are insignificantly ($P > 0.05$*) different.

4. Discussion

It is evident from the results of the present study that *C. gariepinus* fed ration at 3% b.w. day^{-1} produced best growth and conversion efficiencies. The observed decrease in growth of fish fed ration at 1% b.w. day^{-1} suggests that at this level of feeding a major portion of nutrients get utilized for maintenance. Similar results have been reported in *C. batrachus* (Hassan & Jafri 1994). The findings of the present study are also in agreement with the observations on *Acipenser transmontanus* (Hung & Lutes 1987) and on *Sparus aurata* (Lupatsch *et al.* 1998). Feeding at 3% b.w. day^{-1} reportedly produced significantly higher growth in *C. gariepinus* x *H. bidorsalis* (Adebayo *et al.* 2000). In tropical bagrid catfish, *Mystus nemurus,* 2.5% ration level was found optimum with no significant improvement in weight gain beyond this level (Ng *et al.* 2000*).*

Feeding rate was reportedly influenced by fish Size, with the smaller fish consuming more feed than the larger ones (Cowx 1992). This also became apparent when results of the present study on young *C. gariepinus,* where 3% ration level produced best growth and conversion, is compared with those reported by Hina (2001) on fingerling of this species. Increasing level of ration beyond the optimum had a plateauing effect on growth of *Ictalurus punctatus* (Li & Lovell 1992).

A corollary to the present study was also evident in the work of Reddy & Katre (1979) who observed better conversion efficiency at ration level of 3% and poor FCR at ration levels above 3% in *Heteropneustes fossilis.* Similar observations have been made by other workers (Li & Lovell 1992; Hung *et al.* 1993; Hassan & Jafri 1994).

Higher carcass protein in *C. gariepinus* fed rations at 3 and 5% b.w. day^{-1} suggests that these feeding rates provided protein for both maintenance and growth. This finding is supported by observations made on other fishes (Hung & Lutes 1987; Brown *et al.* 1990; Hassan & Jafri 1994). Lower proportions of whole body dry matter, lipid and protein was, however, reported in *M. nemurus* at 5% ration level in comparison to 2.5% (Ng *et al.* 2000). In the present study, carcass fat increased with increasing levels of ration.

It may, therefore, be concluded that a ration level of 3% b.w.day^{-1} is optimum for young *C. gariepinus* as it produces higher growth and carcass protein, and better conversion efficiencies.

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