

## FEEDING FREQUENCY AND FEED INTAKE IN THE AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL 1822)

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### Abstract

Triplicate groups of subadult catfish (*Clarias gariepinus*; 102.18±30.48 g) were fed a purified diet to satiation twice or three times a day during the daylight hours for 26 days. Fish fed twice a day consumed 1.42±0.49% of their body weight per day. Those fed three times a day consumed 1.27±0.27%. The fish fed twice a day exhibited better growth and food conversion. Despite the use of purified diets, performance indices for the group fed twice per day were good, compared to previously reported data. The specific growth rate was 1.24±0.08%, the weight gain was 38.51±2.96% and the food conversion ratio was 0.72±0.13.

### Introduction

Growth is affected by numerous factors. Feed composition, ration size and feeding frequency are among the most important factors of a biological nature (Jobling, 1998). A comprehensive understanding of gastric evacuation and return of appetite enables maximization of feed utilization and beneficial effects on growth (Jobling, 1986; Jobling et al., 1995).

Although piscine gastric evacuation and return of appetite are controlled by mechanisms similar to those in mammals (pH, osmotic pressure, fatty acid anions and certain aromatic amino acids; Jobling, 1986), they are also regulated by factors that are not always conclusive and may even be contradictory in their effects (Talbot et al., 1984; dos

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Santos and Jobling, 1991,1992; Santulli et al., 1993; Buckel and Conover, 1996). Therefore, gastric evacuation in fish is monitored through X-radiography of markers (barium sulfate, iron powder) incorporated in fish feeds even though their passage rate may differ from that of other ingested components (Jørgensen and Jobling, 1988, 1992).

Only recently have radiographic methods been employed in catfish nutrition. Hossain et al. (1998a) showed that various size "ballotini" (glass beads), incorporated into the diet, do not have any effect on feed preference or evacuation rate in African catfish fingerlings (3-4 g). Following similar X-radiography techniques, the same authors were able to quantify the maximum daily feed intake of catfish fingerlings (Hossain et al., 1998b) and concluded that African catfish follows a diel cycle with distinct crepuscular peaks of feeding activity.

The present study forms the second stage of a more comprehensive investigation aimed at refining the nutritional profile and improving the cost-effectiveness of artificial diets for African catfish *Clarias gariepinus*. The aim was to quantify the maximum ration size and optimum feeding frequency of subadult catfish fed purified diets that would later be employed in protein:energy (P/E) experiments. Although *C. gariepinus* is a nocturnal feeder (Bruton, 1979a; Britz and Pienaar, 1992) adoption of a feeding regime during the dark hours was considered inexpedient, mainly for practical reasons. Hand-feeding at regular intervals and direct observations of whether or not food is eaten is a suggested practice in such studies (Jobling, 1998). Therefore, it was decided to investigate the ration size and feeding frequency on the basis of hand-feeding at distinct time intervals during the light hours of the day.

#### Materials and Methods

**Feed.** The nutrient profile and raw materials of the purified diet (Table 1) were carefully selected to match the profiles of diets to be used in subsequent protein:energy (P/E) experiments. After careful weighing, the ingredients were mixed in a Hobart A200 Mixer and

the necessary oils and hot water (approximately one-third of the total weight of the prepared diet) were added. A 3 mm die (food mincer attachment) created long strings of pellets that were dried overnight at 40°C to a final moisture level of 10-15%. The purified and semi-purified ingredients used in the diet were purchased from Sigma-Aldrich Ltd, (casein, gelatin, dextrin, a-cellulose, carboxymethylcellulose, zein and gluten). Fish oil was provided by BOCM-PAULS (Renfrew, Glasgow) and vegetable oil was a rapeseed pure vegetable oil.

A sample of the diet was ground with a mortar and pestle and passed through a 1 mm sieve in preparation for analysis. Analyses were performed on a dry matter basis in triplicate.

The moisture content in the raw materials and prepared diet was determined by the standard procedure (oven drying at 135°C for 2 hours; AOAC 1990, method 930.15). The total nitrogen content in raw materials was estimated by the Kjeldahl method (Tecator application note; AN 30/87) using mercury tablets as a catalyst (each tablet contained 1 g sodium sulfate and the equivalent of 0.1 g mercury as mercuric oxide; Fisher, Leicestershire, UK). To convert the determined nitrogen of the raw materials into crude protein the following factors were used: casein 6.38, gelatin 5.55, zein 5.70, gluten 5.70 (Osborne and Voogt, 1978; Takeuchi, 1988). To determine crude protein in the prepared diets, a weighted average was used, taking into account the percent of each material in the final formulation.

Crude lipid in the raw materials and the final diet was determined as initially described in the EEC Methods (Official Journal of the European Community, 18.1.84, no. L15/29) and applied according to the Tecator application note 92/87: samples were subjected to sequential hydrolysis (3.3 N hydrochloric acid) and extraction (petroleum spirit B.P. 40-60°C) in a Tecator Soxtec System HT 1043 extraction unit.

Crude fiber was determined in a Tecator Fibertec System M/1020 Hot Extractor Unit as described by Tecator application note 01/78.

Table 1. Composition of the purified diet.

<i>Ingredient</i>	<i>g/100 g feed, dry matter</i>
Casein	27.5
Gelatin	4.5
Gluten	8.5
Zein	3.5
Dextrin	36.0
$\alpha$ -cellulose	3.0
Carboxymethylcellulose	1.0
Rapeseed pure vegetable oil	5.0
Fish oil (BOCM-PAULS aquaculture grade)	5.0
Vitamin premix*	2.0
Mineral premix*	4.0
<i>Proximate composition</i>	%
Dry matter	85.82
Crude protein	41.32
Crude lipid	10.75
Carbohydrates	42.71
Crude fiber	5.22
Ash	4.17
Gross energy	23.06 kJ/g
Protein:energy ratio	19.89 mg/kJ

\* Vitamin premix (g/100 g premix): cyanocobalamin (B12) 0.000125, ascorbic acid 3.75, cholecalciferol (D) 0.0004, tocopherolacetate (E) 0.7, vitamin K 0.15, thiamine hydrochloride (B1) 0.425, riboflavin (B2) 0.3, pyridoxine hydrochloride (B6) 0.125, calcium pantothenate 0.525, niacinamide 1.25, biotin 0.009, folic acid 0.1, choline chloride 7.4, myoinositol 0.25, ethoxyquin 0.0019, vitamin A 0.008,  $\alpha$ -cellulose 85

Mineral premix (g/100 g premix):  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ , 72.77;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 12.75; NaCl, 6; KCl, 5;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 2.5;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.55;  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.25;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.078;  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.047;  $\text{CaIO}_3 \cdot 6\text{H}_2\text{O}$ , 0.029;  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.012

Carbohydrates were determined by the anthrone-sulfuric acid reagent method as initially described by Morris (1948) and based on the methodology employed by Good et al. (1933), McCreedy et al. (1950), Seifter et al. (1950) and Hassid and Abraham (1957): soluble sugars were extracted by stirring the sample in water and hot (60°C) 80% ethanol. The sugar-free residue was treated with water and 52% perchloric acid to hydrolyze the remaining starch. Both fractions (soluble sugars and hydrolyzed starch) were reacted with 0.2% anthrone-sulfuric acid reagent kept in a hot water bath (100°C) for 7.5 min. Their carbohydrate content was spectrophotometrically calculated using a glucose standard (Sigma). Total carbohydrates were estimated by adding the determined glucose of the soluble sugar fraction to the respective hydrolyzed starch fraction, after converting the glucose reading of the latter into starch by multiplying by 0.9 (Hassid and Abraham, 1957).

Ash was determined by incineration in a muffle furnace at 600°C for two hours (AOAC 1990, method 942.05). Gross energy was determined based on the organic carbon content in the sample as initially described by Salonen et al. (1976). Thirteen materials were used in calibration of the method with direct energy determination by bomb calorimetry. An average value of 47.08 kJ/g organic carbon for the thirteen materials was considered as the future reference conversion factor. A similar conversion factor was established for aquatic invertebrates by Salonen et al. (1976), i.e., 46 kJ/g organic carbon. Ross (1982) established a conversion factor of 43 kJ/g for organic carbon, based on samples of *Littorina rudis* (Maton).

*Fish.* Fish originated from the African catfish stock of the Institute of Aquaculture, University of Stirling, Scotland. Thirty-four African catfish with an average weight of 102.18±30.48 g were stocked at an average density of 11.58±5.58 g per liter in six 50-l cylindrical tanks following a completely randomized experimental design (Zar, 1996). Animals were individually marked by intra-peritoneal injection of electronic tags (Avid

Plc, East Sussex, UK). Fish were anesthetized with benzocaine (ethyl para-aminobenzoate) at a concentration of 0.05 g/l (Ross and Ross, 1983). Before initiation of the experiment, fish were acclimatized to the purified diet for ten days.

*Feeding.* Groups 1, 3 and 5 were fed twice a day, approximately every 12 hours, i.e., shortly after the lights went on at 08:00 and half an hour before the lights went off at approximately 19:30. Groups 2, 4 and 6 were fed three times a day, approximately every six hours: shortly after the lights went on, midway between the first and last feedings, and half an hour before the lights went off. The size of the ration was determined experimentally. Groups of fish were fed a pre-weighed ration and each tank was monitored for 10 minutes. If the entire ration was consumed, an additional ration was administered and monitored 5-10 minutes later. After every meal any uneaten food was removed, dried and subtracted (on a dry matter basis) from the quantity administered. This practice was followed to ensure that all fish in the tank would be adequately fed since catfish is an extremely aggressive and territorial species creating strong behavioral and feeding hierarchies within any group or population (Hecht and Appelbaum, 1988). The experiment lasted 26 days.

*Culture system and water quality.* The experimental tanks were part of a recirculating water system. Water temperature was kept constant at 26-27°C. Water from the header tank of the system was sampled weekly to assess water quality parameters. Samples for water quality analyses were filtered through a glass microfiber GF/C 2-micron filter paper and a Technicon II Autoanalyzer was used to determine total ammonia, nitrites and nitrates employing spectrophotometric methods as described by Golterman (1978) for total ammonia and Strickland and Parsons (1972) for nitrites and nitrates.

*Feed and growth parameters.* Evaluation of the feeding frequency was based on the following indices: specific growth rate (SGR; Steffens, 1989), thermal unit growth coefficient (TGC; Cowey, 1992), percent weight

gain (PWG; Steffens, 1989), food conversion ratio (FCR; Steffens, 1989) and feed intake (FI). As fish were individually tagged, average SGR, TGC and PWG were calculated as the arithmetic mean of the individual values for each fish within the group. In contrast, FCR and FI were based on cumulative tank data by computing the total rations and collective weight gains of each tank.

**Statistical analysis.** The SPSS for Windows Statistical Software Package was used for statistical evaluation of the results. Duncan's multiple range test was used for multiple comparisons. For statistical evaluation of the weight gain, percentages were arcsine transformed (Zar, 1996).

### Results

**Water quality.** During the 26 days of the experiment, four samplings from the header tank of the recirculated water system were performed. The following values were recorded: nitrites  $0.2 \pm 0.05$  ppm, nitrates  $19.28 \pm 4.84$  ppm, unionized ammonia  $0.75 \pm 0.06$  ppm and pH  $7.07 \pm 0.05$ . These values are quite acceptable for African catfish as it has been shown that they tolerate levels up to 8.8 ppm  $\text{NH}_4^+$ , 10-15 ppm  $\text{NO}_2^-$  and 300 ppm  $\text{NO}_3^-$  even in the larval stage (Viveen et al., 1986). Oxygen was kept at 4-4.5 mg/l, a level that is not considered critical for the air breathing African catfish in the size range used in this experiment (Haylor and Oyegunwa, 1993).

**Feed and growth parameters.** The average weights of the six fish groups did not significantly differ ( $p < 0.05$ ; Table 2). The average feed intake for the groups fed twice a day ( $1.42 \pm 0.49\%$  of the body weight per day) was significantly higher ( $p > 0.05$ ) than for the groups fed three times a day ( $1.27 \pm 0.27$ ) and generated the following best fit regression ( $r^2 = 0.59$ ):  $Y = 0.8031 x X^{-0.871}$ , where  $X =$  g live fish weight and  $Y =$  g dry ration consumed (shown in g/live fish weight/day). Compared to the performance of the groups fed three times a day, groups fed twice daily had a significantly higher average SGR ( $1.24 \pm 0.08$ ), PWG ( $38.51 \pm 2.96\%$ ) and TGC ( $0.0193 \pm 0.001$ ) and a significantly lower FCR ( $0.72 \pm 0.13$ ), implying better feed utilization.

Table 2. Performance indices (with standard deviations in parentheses) of African catfish (n = 4-6) fed twice a day (groups 1,3,5) or three times a day (groups 2,4,6) for 26 days.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Initial weight (g)	96.81 <sup>a</sup> (28.75)	99.70 <sup>a</sup> (36.25)	95.33 <sup>a</sup> (32.68)	102.86 <sup>a</sup> (15.08)	96.37 <sup>a</sup> (38.65)	122.25 <sup>a</sup> (29.09)
Initial stocking density (g/l)	11.61	7.97	11.43	10.28	13.49	14.67
Final weight (g)	135.45 <sup>a</sup> (47.01)	122.86 <sup>a</sup> (44.07)	134.68 <sup>a</sup> (45.06)	127.41 <sup>a</sup> (24.36)	129.36 <sup>a</sup> (46.64)	151.58 <sup>a</sup> (39.42)
Weight gain (%)	37.99 <sup>a</sup> (13.7)	23.38 <sup>b</sup> (1.18)	41.7 <sup>a</sup> (5.99)	23.35 <sup>b</sup> (6.98)	35.84 <sup>a</sup> (12.99)	23.78 <sup>b</sup> (11.97)
Specific growth rate (%/day)	1.22 <sup>a</sup> (0.37)	0.81 <sup>b</sup> (0.36)	1.27 <sup>a</sup> (0.16)	0.8 <sup>b</sup> (0.22)	1.24 <sup>a</sup> (0.36)	0.81 <sup>b</sup> (0.37)
Thermal growth coefficient	0.019 <sup>a</sup> (0.007)	0.012 <sup>b</sup> (0.0012)	0.021 <sup>a</sup> (0.003)	0.013 <sup>b</sup> (0.004)	0.018 <sup>a</sup> (0.005)	0.013 <sup>b</sup> (0.006)
Feed conversion ratio*	0.74 <sup>b</sup>	1.49 <sup>a</sup>	0.68 <sup>b</sup>	1.55 <sup>a</sup>	0.76 <sup>b</sup>	1.64 <sup>a</sup>
Food intake (% body weight/day)*	1.46	1.37	1.38	1.29	1.42	1.16

Values in a row with the same superscript are not significantly different ( $p > 0.05$ ).

\*Standard deviations not shown because of calculation method.

### Discussion

Our regression equation is not applicable to smaller catfish. However, Hossain et al. (1998b) established the feed consumption, expressed as 'return of appetite', for smaller fingerlings with a mean weight of  $0.95 \pm 0.1$  g. Using radiography methods and estimation of stomach contents after the first feeding, they identified the feed intake of catfish fed a commercially pelleted trout diet three times daily as 1.53% body weight per day for a 6-hour deprivation (between-meals) period and 2.68% for a 12-hour deprivation period. Hogendoorn et al. (1983) used various feeding levels depending on the water temperature and the size of the experimental fish. Their levels ranged 5-8% for small fish (1-2 g) to 0.14-5% for larger fish (130-200 g) following a nocturnal 12-hour continuous feeding strategy. Baras et al. (1998) used a regression similar to ours to estimate their daily food ration size (also in % of the body weight per day) in another African catfish, *Heterobranchus longifilis*.

*C. gariepinus* is characterized by quite variable growth rates, particularly during the early life cycle. Live weight increases twenty to fifty fold, dry matter content changes considerably and the SGR decreases dramatically (Verreth and den Biemen, 1987). Hogendoorn (1980) reported a rapid decrease in SGR during the first 28 days of feeding, from 85% to less than 20% of the body weight per day. As a result, our equation and all previously reported equations describing daily feed intake are of limited value as they apply to a particular fish size, feeding frequency and experimental period. Consequently, fixing the feeding level as a percent of the body weight per day for considerably longer periods could only be a poor approximation of feed requirements (Haylor, 1992).

Despite our short trial and the use of a purified diet, SGR, PWG and FCR in this experiment were similar to those observed in other experiments with Clariid catfish of a similar size. For example, Baras et al. (1998), testing diurnal, nocturnal and 24-hour feeding patterns at stocking densities of 3.4 and 13.6 g/l for *H. longifilis* of an average weight of 85 g

for a 14-day experimental period, recorded weight gains of 11.85-41.53%, SGRs of 0.8-2.5%/day and FCRs of 0.8-3.25.

Hogendoorn (1981), experimenting with *Clarias* catfish fingerlings (0.5-10.0 g) fed a commercial diet ("Trouvit 0", Trouw and Co, Netherlands) for four weeks, obtained a much higher weight gain (208-735%) but a similar FCR (0.75-1.36) and established that the best feeding regime was at satiation on a continuous 24-hour basis with the second best being a nocturnal regime on a continuous 12-hour basis (at a stocking density of 0.8-1.5 g/l). Later on (and by adopting the nocturnal 12-hour continuous feeding strategy during a 28-day period), he established appropriate feeding levels (in terms of percent body weight per day) for size groups of 1-200 g at a temperature of 20-35°C and stocking density of 0.83-12 g/l (Hogendoorn et al., 1983). The weight gain of the large size groups ranged 6.6-164% and the SGR 0.23-1.95%/day. However, extrapolation of these results to larger fish and higher stocking densities was arbitrarily rather than experimentally substantiated.

Uys (1989) adopted a feeding frequency of three times daily (sunrise, noon and sunset) in outdoor tanks with feeding levels based on "visually determined satiation" (no stocking densities stated). He concluded, "physiological data (quick and strong digestive response subsequent to feeding) provided no evidence that nocturnal feeding schedules would have to be implemented in commercial culture, whereas behavioural data might indicate otherwise". Britz and Pienaar (1992) and Bruton (1979a) observed that African catfish is a nocturnal tactile feeder characterized by a distinct crepuscular activity pattern. However, if food or prey is available only during light hours, *C. gariepinus* adopts a daytime searching and feeding pattern (Bruton 1979b; Britz and Pienaar, 1992).

Hossain et al. (1999) confirmed the crepuscular tactile feeding nature of the species by showing that "voluntary food intake in African catfish follows a diel cycle". They demonstrated that although the majority of food was ingested during darkness, two distinct peaks

were observed, one at the onset of the dark phase (20:00-23:00) and the second before the onset of the light phase (06:00-08:00). Both of these peaks are quite close to the feeding times employed in the present study.

Despite the multitude of feeding levels, feeding frequencies, type and nutrient composition of feed applied by other researchers for various sizes and stages of maturity, results of this experiment are useful for subsequent P/E experiments with subadult African catfish. Further, it is suggested that when (for practical reasons) a continuous nocturnal feeding regime cannot be implemented, a 12-hour interval feeding regime during the light phase may be the best option, for either experimental or commercial operations.

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