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Effect of Diel Rhythms of Feeding on Growth Performance of Walking Catfish, *Clarias batrachus* Fingerlings

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Abstract

Clarias batrachus fingerlings were maintained under laboratory conditions and fed on a 40% soybean based protein diet on a circadian pattern selecting six different time intervals, *viz.*, 08^{00}h, 12^{00}h, 16^{00}h, 20^{00}h, 24^{00}h and 04^{00}h. The results indicated that body weight gain (live weight gain and specific growth rate) in *C. batrachus* on a time restricted meal is optimal when the food is made available at mid-night *viz.*, 24^{00}h. Better accumulation of protein with less excretion of ammonia in fish body was also reported at this time of feeding. The study concluded that the growth rates of fish fed during night time (mid-night) had significantly low food conversion ratios and food wastage. Therefore, it is beneficial to feed *C. batrachus* at mid-night.

Keywords: *Clarias batrachus,* circadian pattern, live weight gain, mid-night, time restricted meal

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Introduction

The circadian rhythm of activity in fish has been widely studied. It is known to be synchronized by daily oscillations of environmental cues and light is generally thought to be the main factor (Muller, 1978). The different intensity of light between the light and dark phases being more important than the intensity or the wavelength of the light (Molina et al., 1990).

Unlike numerous studies devoted to the feeding periodicities of fish (Jacob & Nair, 1983 and Walsh et al., 1988), only a few reports describe diel rhythms of feeding activity in fish held under controlled environments. However, the latter only can give substantive information about feeding rhythms as the food availability is controlled and influence of ecological factors such as interspecific relations (competition and predation) is avoided. From ecological studies, fish feeding pattern can be classified into three categories, viz., nocturnal (night feeding), diurnal (day feeding) and crepuscular (twilight feeding) and similar patterns are evident in captive fish.

A lot of work has been done on Indian major carp species but much less work on catfishes. Therefore, an attempt was made to study the circadian rhythms of feeding activity in the catfish *Clarias batrachus* under controlled conditions. Since water pollution is an important limiting factor, which affects growth and survival of a species, the effects of different timings of feeding on post-prandial excretory levels of ammonical nitrogen (NH_4-N) and ortho-phosphate (o-PO_4) in the treated water was also studied.

Materials and Methods

Specimens of *C. batrachus* were procured from Sultan Singh Fish Farm, Nilokheri, and Haryana, India, with mean body weight 6.56 ± 0.13 g were used in the studies. Fingerlings were placed in transparent glass aquaria (60×30×30 cm) kept in laboratory where the temperature was maintained at 25 ± 1^0C and the lighting scheduled at 12 h of light alternating with 12 h of darkness. The fingerlings were acclimatized for 15 days prior to the initiation of experiment. The water was renewed daily with chlorine free water. After acclimatization, three replicates for each treatment containing 10 fingerlings each were maintained in glass aquaria. The fingerlings were fed on processed soybean (PS) based diet containing 40% protein. The ingredient and proximate composition of the feed is presented in Table 1.

Table 1. Ingredient (%) and proximate composition (% dry weight basis) of the experimental diet

Ingredients (%)	
Groundnut oil Cake (GNOC)	59.0
Rice bran (RB)	5.6
Processed soybean (PS)	28.4
Binder[a]	5
Chromic oxide[b] (Cr_2O_3)	1
Mineral premix[c] and amino acids (MPA)	1
Proximate composition (%)	
Crude protein	40.25
Crude fat	9.50
Crude fibre	7.25
Ash	6.50
Nitrogen free extract (NFE)	36.50
Gross energy KJ g^{-1}	19.53

a - used is carboxyl methyl cellulose to make the diets water stable
b - used as an external indigestible marker for estimating apparent digestibility.
c - Each kg contains copper – 312 mg; cobalt – 45 mg; magnesium - 2.114 g; iron -979 mg; zinc - 2.13 g; iodine 156 mg; DL-Methionine - 1.92 g; L-lysine mono hydrochloride - 4.4 g; calcium 30% and phosphorous - 8.25%

Seven treatments (Table 2) containing 10 fish each with three replicates were fed with the formulated diet at 5% level of body weight for 120 days. This period was considered enough to study the best time of feeding catfish to produce the best growth without polluting the water. Initial weight of the test fish for each treatment was recorded at the start of the experiment. Fish were weighed together on every 20[th] day and the feeding rates were adjusted accordingly. Faeces were siphoned off from culture tanks after two hours of feeding. In addition, approx. 40-50% of culture water was replaced daily with fresh dechlorinated water. The pooled faecal samples were dried in an oven maintained at 60^0C for subsequent analysis. At the termination of experiment, fish from all the treatments were weighed and processed for carcass composition.

The experimental diets, faecal samples and fish carcass were analyzed according to AOAC (2000).

Table 2. Treatment groups

Sl. No.	Treatment / Feeding Time
Group 1	Control (feeding *ad libitum*)
Group 2	Feeding at 08^{00}h
Group 3	Feeding at 12^{00}h
Group 4	Feeding at 16^{00}h
Group 5	Feeding at 20^{00}h
Group 6	Feeding at 24^{00}h
Group 7	Feeding at 04^{00}h

Chromic oxide levels in the diets as well as in the faecal samples were estimated spectrophotometrically (Spyridakes et al., 1989). Live weight gain (g), growth percent gain, specific growth rate (SGR, % d^{-1}), protein efficiency ratio (PER) and gross conversion efficiency (GCE) were calculated using standard methods (Steffens, 1989). Apparent nutrient digestibility (APD) of the diets were calculated (Cho et al., 1982).

The various water quality parameters like dissolved oxygen (DO), pH, temperature, conductivity, free carbon dioxide, total alkalinity, total hardness, ammonical nitrogen (NH_4-N) excretion and orthophosphate (o-PO_4) production were analyzed according to APHA, (1998) to see the influence of compounded feeds on pollution of rearing water in the aquaria.

Duncan's multiple range test and multivariate analysis was applied to find out the significant differences between different treatments.

Results and Discussion

Highest value of DO was observed in the fish group fed at mid-night (24^{00}h). This indicated that when catfishes were fed at mid-night, feed was better utilized with less deterioration of the water quality. These results are in agreement with those of Jindal et al. (2007a, b). pH remained alkaline. Water temperature fluctuated between 25.2 to 25.6^{O}C while all other parameters remained in the optimal range (Table 3).

Significantly ($p< 0.05$) low values of NH_4-N and o-PO_4 were observed in the fish fed at mid night (24^{00}h) as shown in the Table 3. After mid-night, these values started increasing. This showed that fishes are active at mid-night and utilized feed better at this time with lesser excretion of ammonia and phosphorous (Jindal et al., 2007b; 2009). This is in agreement with field studies in which diel rhythms appear to be directly related to the day/night alteration (Boujard & Leatherland, 1992).

Fish survival in different dietary treatments was high and varied between 93 to 98% (Table 4). This revealed that processed soybean have no negative effect on the survival of fish. A significantly ($p < 0.05$) high live weight gain, length gain and SGR were observed in fingerlings fed at mid-night with lowest FCR and food wastage. The results showed clearly negative correlation between FCR values and live weight gain.

The results should be viewed in the light of data documenting the fact that food offered at different times in the circadian cycle encounters temporally different biologic systems. It has been reported that if food availability is restricted daily to one or another fraction (*e.g.*1 or 4 h) of daily routine, then better growth of fish was observed. The best consumption of food occurs if food is given at the right time according to the nocturnal or diurnal habitat of species (Noeske et al., 1985, Sundararaj et al., 1982, Hossain et al., 2001 and Jindal et al., 2009).

The data on carcass composition (Table 5) indicates that the fish fed at mid-night had high retention of protein and fat. Further, accumulation of carcass protein paralleled with that of weight gain; indicated that growth was mainly due to the accumulation of protein. These results are supported by Kalla (2001) and Jindal et al. (2007 a,b).

The growth and digestibility parameters, protein accumulation and fat deposition in the fish carcass were found to be negatively correlated with NH_4-N excretion and o-PO_4 production. These results are in agreement with those of Mazid et al. (1994), Jindal et al. (2007 a, b; 2009).

The results clearly indicate that meal timings play an important role in food utilization and growth in *C. batrachus.* When feed was given at different timings of light/dark cycle, the group of fish which was fed at mid-night shows better growth performance paralleled with better accumulation of carcass protein in fish body. These results concluded

Table 3. Water quality parameters at different treatments

Parameters	Treatments						
	Control	08^{00} h	12^{00} h	16^{00} h	20^{00} h	24^{00} h	04^{00} h
Dissolved oxygen (DO) mg l^{-1}	5.4 ±0.000	5.2 ±0.200	5.4 ±0.200	5.6 ±0.200	5.4 ±0.000	6.0 ±0.200	5.8 ±0.200
pH	7.75 ±0.005	7.60 ±0.100	7.85 ±0.005	7.40 ±0.000	7.55 ±0.000	7.70 ±0.100	7.70 ±0.005
Water temperature (^{0}C)	25.5 ±0.100	25.2 ±0.150	25.6 ±0.100	25.5 ±0.000	25.4 ±0.100	25.5 ±0.100	25.5 ±0.005
Conductivity micro (μ) mhos cm^{-1}	0.53 ±0.001	0.53 ±0.015	0.54 ±0.055	0.49 ±0.002	0.47 ±0.005	0.54 ±0.005	0.51 ±0.005
Free Carbon dioxide (Free CO_2) mg l^{-1}	16.6 ±0.200	16.3 ±0.100	16.2 ±0.200	16.2 ±0.000	16.1 ±0.100	16.4 ±0.000	16.2 ±0.200
Total alkalinity (mg l^{-1})	218 ±2.000	214 ±2.000	211 ±1.000	215 ±1.000	210 ±2.000	212 ±0.000	217 ±1.000
Total hardness (mg l^{-1})	222 ±2.000	218 ±2.000	216 ±0.000	218 ±0.200	213 ±1.000	217 ±1.000	219 ±1.000
NH_4-N excretion (mg $100g^{-1}$ BW of fish)	2.147 ±0.055	2.040 ±0.010	1.841 ±0.045	1.504 ±0.030	1.369 ±0.015	1.211 ±0.040	1.578 ±0.015
o-PO_4 excretion (mg $100g^{-1}$ BW of fish)	1.158 ±0.050	1.023 ±0.025	0.979 ±0.075	0.815 ±0.055	0.650 ±0.030	0.535 ±0.040	0.758 ±0.045

* All values are mean ± S.E. of mean of 3 observations * BW = Body Weight

Table 4. Growth performance indicates of *Clarias batrachus* fingerlings on diel rhythms feeding

Parameters	Treatments						
	Control	08^{00} h	12^{00} h	16^{00} h	20^{00} h	24^{00} h	04^{00} h
Survival (%)	93	95	96	95	98	98	95
Live Weight gain (g)	7.37^{A} ±0.003	8.95^{B} ±0.016	9.11^{B} ±0.004	10.54^{C} ±0.003	14.33^{D} ±0.041	16.21^{E} ±0.012	9.78^{B} ±0.021
Fish length gain (cm)	3.10^{AB} ±0.000	3.35^{ABC} ±0.005	3.50^{BC} ±0.000	3.85^{C} ±0.005	4.09^{D} ±0.001	4.30^{E} ±0.010	3.90^{C} ±0.002
Growth (% gain in BW)	158.94^{AB} ±3.800	177.32^{ABC} ±2.020	188.88^{BC} ±5.590	218.98^{D} ±3.360	283.41^{E} ±7.360	333.93^{F} ±2.685	167.09^{ABC} ±6.875
Growth day^{-1} (% BW)	0.885^{A} ±0.001	0.940^{B} ±0.003	0.971^{B} ±0.002	1.045^{C} ±0.005	1.172^{D} ±0.003	1.251^{E} ±0.003	0.978^{B} ±0.001
Specific growth rate SGR (% d^{-1})	0.413^{AB} ±0.060	0.443^{ABC} ±0.025	0.467^{BC} ±0.085	0.504^{D} ±0.050	0.584^{E} ±0.016	0.637^{F} ±0.030	0.426^{AB} ±0.024
Food Consumption day^{-1} (% BW)	4.730^{A} ±0.001	4.745^{A} ±0.050	4.765^{B} ±0.001	4.765^{B} ±0.050	4.775^{C} ±0.050	4.795^{D} ±0.002	4.780^{C} ±0.003
Feed Conversion ratio (FCR)	5.345^{A} ±0.006	5.050^{B} ±0.002	4.915^{B} ±0.009	4.565^{C} ±0.002	4.075^{D} ±0.009	3.840^{E} ±0.002	4.560^{C} ±0.125
Protein efficiency ratio (PER)	0.184^{A} ±0.000	0.223^{B} ±0.002	0.227^{B} ±0.001	0.263^{C} ±0.002	0.357^{D} ±0.001	0.405^{E} ±0.003	0.270^{C} ±0.002
Gross conversion ratio (GCE)	0.187^{A} ±0.002	0.197^{A} ±0.000	0.203^{B} ±0.004	0.219^{C} ±0.002	0.245^{D} ±0.004	0.261^{E} ±0.015	0.215^{CC} ±0.004
Apparent protein digestibility (APD) %	82.72^{A} ±0.000	85.78^{B} ±0.001	85.96^{B} ±0.000	86.80^{C} ±0.002	87.05^{D} ±0.000	88.15^{E} ±0.000	85.81^{B} ±0.002

* BW = Body Weight; * Duration of experiment = 120 days
* Mean with the same letter in the same row are not significantly (P>0.05) different
* All values are Mean ± S.E. of three observations

Table 5. Proximate carcass composition (% wet weight) of *Clarias batrachus* fingerlings

Carcass composition (%)	Treatment						
	Control	08^{00} h	12^{00} h	16^{00} h	20^{00} h	24^{00} h	04^{00} h
Moisture	66.78^{A} ±0.0135	65.96^{B} ±0.575	64.86^{C} ±0.150	64.41^{C} ±0.250	63.86^{D} ±0.290	63.13^{D} ±0.518	64.88^{C} ±0.321
Crude protein	19.53^{A} ±0.315	21.51^{B} ±0.150	21.52^{B} ±0.210	22.16^{C} ±0.075	23.06^{D} ±0.075	24.51^{E} ±0.045	21.41^{B} ±0.175
Crude Fat	3.69^{A} ±0.145	4.02^{B} ±0.006	4.22^{C} ±0.003	4.32^{C} ±0.004	4.75^{D} ±0.195	5.02^{E} ±0.003	4.34^{C} ±0.005
Total Ash	3.34^{A} ±0.002	3.59^{B} ±0.008	3.58^{B} ±0.005	3.63^{B} ±0.005	3.80^{C} ±0.001	3.85^{C} ±0.006	3.62^{B} ±0.005
Nitrogen Free extract (NFE)	6.65^{A} ±0.515	4.91^{B} ±0.165	5.82^{C} ±0.445	5.47^{D} ±0.135	4.52^{E} ±0.190	3.56^{F} ±0.120	5.76^{C} ±0.125
Gross Energy (KJg^{-1})	7.218^{A} ±0.128	7.517^{B} ±0.483	7.753^{C} ±0.150	7.887^{D} ±0.570	8.108^{E} ±0.620	8.378^{F} ±0.190	7.763^{C} ±0.240

* Mean with the same letter in the same row are not significantly (P>0.05) different
* All values are Mean ± S.E. of three observations

that the growth rates of fish fed during night time (mid-night) follow their feed demand significantly higher with the lowest food conversion ratios and food wastage with better accumulation of carcass protein in fish body. Therefore, it is beneficial to feed *C. batrachus* at mid-night.

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