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FEEDING FREQUENCY AND FEEDING REGIME IN CATFISH: EFFECTS ON NUTRIENT UTILIZATION, GROWTH, BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS

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Abstract: This study investigated growth, nutrient utilization, biochemical and haematological parameters of catfish fingerlings under varying feeding frequencies and regimes. Eight fish were put under different treatments; T (1–8) in triplicates. Fish were bulk weighed at the beginning of the experiment and on a weekly basis for 10 weeks. Significant effects ($p<0.05$) were recorded in mean weight gain (MWG), specific growth rate (SGR) and feed conversion ratio (FCR) across treatments. The control (T1) had the highest MWG (202.92±4.68) and SGR (4.24±0.05) while the lowest values were recorded in T4 (82.60±15.63 and 3.05±0.23 respectively). The best value for FCR was recorded in T6 (0.20±0.02), which differed significantly ($p<0.05$) from other treatments except T2. Significant differences ($P<0.05$) were shown in haemoglobin (Hb) and erythrocyte sedimentation rate (ESR). The highest value for Hb was recorded in T6 (10.8±0.3) and the lowest value in T8 (7.6±0.3). The utmost value for ESR was recorded in T8 (43.5±2.1) while the least value was observed in T6 (19.0±4.2) and no significant differences ($P>0.05$) were recorded in both PCV and WBC. The blood protein, albumin, creatine, urea, cholesterol, triglyceride and high density lipoprotein did not show significant differences ($P>0.05$) across treatments. Aspartate aminotransferase and alanine aminotransferase did not show significant differences ($P>0.05$) in values, while there was a reduction in values of chloride, potassium and sodium with a decrease in the number of feeding days. Results from this study confirmed that satiation feeding, two to three times daily, would enhance growth indices in *C. gariepinus* fingerlings.

Key words: feeding frequency, feeding regime, catfish, biochemical, haematological parameters.

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Introduction

Feeding is an important facet of aquaculture, especially when fish are raised under the intensive or semi-intensive system. The cost of feeding in most fish farm operations is roughly 60% of the total cost of production (Fagbenro et al., 2005). Therefore, more attention should be given to the optimum feeding management in order to reduce overfeeding, feed wastage, environmental pollution and increase fish production efficiency (Lee et al., 2000; Bolliet et al., 2001; Dwyer et al., 2002). One of the dilemmas faced by aquaculturists is balancing swift fish growth rate with the optimal use of feed because both underfeeding and overfeeding are harmful to the health and growth of fish, returns on investments and water quality (Priestley et al., 2006; Aderolu et al., 2010).

The sum of the daily feed intake, rate and time of feedings and presentation of the encoded ration are the major factors in feed management that influence the growth and feed conversion ratio (Jobling, 1995; Goddard, 1995). Feeding fish at a suitable frequency would enhance their growth and survival because their feed intake is regulated in relation to their energy demand (Ali et al., 2005; Schnaittacher et al., 2005). In addition, feeding at the optimal frequency can result in marvelous savings in the cost of feed (Davies et al., 2006).

To improve on fish culture and save the marine environment from eutrophication as a result of excess nutrients from aquaculture production, more information on the management strategies in the area of feeding frequency and regime to produce fish within the shortest possible time at a minimal cost with good quality feed is inevitable (Zakes et al., 2006).

Against this background, the present study investigated the nutrient utilization, growth performance, biochemical and haematological parameters of catfish, *C. gariepinus* fingerlings on varying feeding frequencies and regimes.

Materials and Methods

Experimental fish and rearing conditions

The experiment was carried out at the Nutrition Unit, Department of Marine Sciences, University of Lagos, Akoka-Lagos, Nigeria. One hundred and ninety two fingerlings of African catfish, *Clarias gariepinus* (average weight of 6.5g±0.1) were obtained from a fish farm in Ikorodu, Lagos state. The fingerlings were transported in aerated polyethylene bags and left to acclimatize to laboratory conditions in a flow-through system for two weeks. Thereafter, fish were raised in plastic tanks ($52 \times 33.5 \times 21$ cm^3) containing 30 L of borehole water under natural photoperiod of approximately 12/12 hour light/dark cycle and were fed a commercial catfish feed (Coppens®, Holland) of 2.0 mm in size (Table 1). The

cleaning of fish tanks was carried out daily by siphoning out residual feed and faecal matters while water in the tanks was changed thrice weekly. Water quality parameters were monitored twice weekly; temperature was measured with a mercury-in-glass thermometer (°C), and dissolved oxygen (DO) was determined using the DO meter, while the pH was determined with a pH meter for a period of ten weeks (Aderolu and Akpabio, 2009).

Table 1. Nutrient composition of experimental diet (Coppens % DM).

Contents	%
Crude protein	42
Carbohydrate	13
Crude fibre	1.50
Ash	9.00
Calcium	1.60
Phosphorus	1.10
Lysine	2.80
Methionine	0.90
$CuS0_4$	5 mg/kg
Selenium	0.3 mg/kg

Experimental design

Fish were raised under varying feeding regimes and frequencies; T1 (control) and T (2−8) (Table 2).

Table 2. Experimental feeding regimes and frequencies for catfish.

Experimental groups	Feeding regime	Feeding time	Feeding frequency
Group 1 (T1)	Fed to satisfaction	3 times/day 9am−1pm−5pm	Every day Monday−Sunday
Group 2 (T2)	Fed on 4% of body weight	3 times/day 9am–1pm–5pm	Every day Monday−Sunday
Group 3 (T3)	Fed to satisfaction only for 5 days of the week and starved for 2 days	3 times/day 9am–1pm–5pm	Monday–Friday (No feeding on Saturday and Sunday).
Group 4 (T4)	Fed to satisfaction only for 4 days and starved for 3 days subsequently	3 times/day 9am–1pm–5pm	Monday–Thursday (No feeding on Friday–Sunday)
Group 5 (T5)	Fed to satisfaction	2 times/day 9am–5pm	Every day (Monday–Sunday)
Group 6 (T6)	Fed on 4% of body weight	2 times/day 9am–5pm	Every day (Monday–Sunday)
Group 7 (T7)	Fed to satisfaction	3 times/day 9am–1pm–5pm	Fed on alternate days
Group 8 (T8)	Fed to satisfaction	3 times/day 9am–1pm–5pm	2 days of feeding, then 1 day of starvation

Each experimental setup had 8 fish in 3 replicates.

Each experimental setup had 8 fish in triplicate and prior to the commencement of the feeding experiment, fish were starved for a period of 24 hours. Feed allowance was measured using a digital scale (Camry EK5055 Max 5 kg/11lb d = 1 g/0.05 oz.) and recorded. Fish were bulk weighed at the beginning of the experiment and on a weekly basis with a digital scale throughout the experimental period.

Growth and nutrient utilization measurement

The following growth and nutrient utilization parameters were measured for this study:

Mean weight gain (MWG) (g) = Wf – Wi;

Voluntary feed intake (VFI) (%) = 100 x FI/[(Wi + Wf) × t];

Specific growth rate (SGR) (g) = (Log W2 – Log W1/T) × 100;

Relative growth rate (RGR) = (Weight gain/Initial body weight) × 100;

Feed conversion ratio (FCR) = Feed intake (FI) (dry weight in g)/Fish wet weight gain (g);

Protein efficiency ratio (PER) = Mean weight gain/Total protein intake;

Protein intake (PI) = Total feed intake/Protein content of feed.

Where: Wf = Mean final weight; Wi = Mean initial weight of fish, and T = Feeding trial period in days.

Histometry analysis

At the end of the 10^{th} week, 5 fish were collected from each treatment for histometry analysis. The fish were starved for 24 hours to ensure complete evacuation of the gut contents. Fish pooled from each treatment were bulk weighed, and their average values were recorded. Subsequently, they were slaughtered and dissected to remove the organs (gall bladder, liver, small intestine, large intestine, gills, kidney, stomach and head). Blood was removed from these organs with a dry towel, while organs collected from each treatment were thereafter bulk weighed and the average values recorded. Values for histometry analysis were calculated as follows:

$$\text{Histometry analysis} = \frac{\text{Organ weight x 100.}}{\text{Fish weight}} \qquad \text{Eq.(1)}$$

Blood collection and analysis

Samples of blood were taken from randomly picked fish from each experimental tank with a 5-ml syringe and transferred to EDTA bottles to prevent coagulation and to sterile plain sample bottles. Thereafter, the blood samples collected were taken to the laboratory for haematological analysis – packed cell

volume (PCV), white blood cell (WBC), erythrocyte sedimentation rate (ESR) and haemoglobin (Hb). Samples of blood in the plain bottles were spun at 3000 rpm to collect serum for biochemistry analysis – AST, ALT, LDL, HDL, Na, K, Cl, BCO_3, urea, creatine, cholesterol, triglyceride, albumin and protein.

Estimation of aspartate aminotransferase (AST)

The substrate (0.5 ml) and sodium azide were added in a test tube plus 0.5 ml of serum and put in a water bath at 37˚ C for 30 minutes. After this, 0.5ml of a 2,4-dinitrophenylhydrazine was added to the mixture, incubated for 20 minutes and 5ml of sodium hydroxide was added to the mixture which turned brown. It was placed in a spectrophotometer at 540nm and the results were read on the calibrated graph.

Estimation of alanine aminotransferase (ALT)

First, 0.5 ml of the substrate was put in a test tube containing 0.5ml of blood, and the mixture was put in a water bath at 37˚ C for 30 minutes, then 0.5ml of 2,4-dinitrophenylhydrazine was added to the solution and incubated for 20 minutes. Afterward, 5ml of sodium hydroxide was added to the mixture which turned brown. It was placed in a spectrophotometer at 540nm and results were read on the calibrated graph.

Calculation = ALT (U/I) = Change Abs/mm x 1750 Eq.(2)

Homogenization of liver sample for enzymatic assay

The liver sample was washed in an iced cold 1.15% KCl solution, blotted, weighed and homogenized with 0.1 M phosphate buffer (pH 7.2). The liver sample was blended along with the laboratory sand using a mortar and pestle. The homogenate was centrifuged at a speed of 2500 rpm for 15 minutes and the supernatant was decanted and stored at -21°C until the spectrophotometric determination of antioxidant enzyme activity was carried out using a UV-visible spectrophotometer (Habbu et al., 2008).

Estimation of glutathione (GSH) activity

The estimation of the reduced glutathione content of the liver as non-protein was carried out according to the method described by Sedlak and Lindsay (1968). To the homogenate, 10% TCA was added to the tissue homogenate and centrifuged. To read the absorbance, 1.0ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0) after which its absorbance was read at 412 nm.

Estimation of superoxide dismutase (SOD) activity

Superoxide dismutase activity was assessed by its ability to inhibit auto-oxidation of epinephrine which was determined by the increase in absorbance at 480 nm (Sun and Zigma, 1978). The reaction mixture (3ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of tissue homogenate and 0.03 ml of epinephrine while 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml of buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. The measurement of the change in absorbance at 480 nm for 5 minutes was used to determine the enzymatic activity.

Estimation of catalase (CAT) activity

Measurement of the decrease in absorbance at 240 nm due to the decomposition of hydrogen peroxide (H_2O_2) in a UV recording spectrophotometer was used to determine the catalase activity. The reaction mixture (3 ml) contained 0.1 ml of tissue homogenate in phosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 mM H_2O_2 in phosphate buffer pH 7.0. An extinction coefficient for H_2O_2 at 240 nm of 40.0 M-1cm-1 was used for the estimation (Aebi, 1984). The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per mg protein.

Estimation of glutathione S-transferase (GST) activity

Glutathione S- transferase activity was determined by the method according to Habig et al. (1974). The assay is based on the fact that all GSTs demonstrate a relatively high activity with 1-chlor-2, 4-dinitrobenzene as the second substrate. Therefore, the conventional assay for GST activity utilizes 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. The resulting conjugation of this substance with reduced glutathione allowed a shift of its absorption wavelength to a longer wavelength. The absorption increase at the new wavelength of 340nm provides a direct measurement of the enzymatic reaction (Habig et al., 1974). The medium for the estimation was prepared and allowed to run for 60 seconds each time before the absorbance was read against the blank at 340 nm at approximately 31°C using a spectrophotometer (Table 3).

Table 3. Glutathione S-transferase (GST) assay medium.

Reagent	Blank	Test
0.1M reduced glutathione (GSH)	30µl	30µl
20mM CDNB	150 µl	150 µl
0.1M phosphate buffer pH 6.5	2.82ml	2.79ml
Sample	-	30µl
Total mixture	3ml	3ml

The extinction coefficient of CDNB = 9.6 M-1cm-1 GHS-S-transferase activity = OD/min × 19.6 0.03ml/mg protein = µmole/min/mg protein.

Lipid peroxidation

The malondialdehyde (MDA), which is an index of lipid peroxidation, was estimated according to the method of Buege and Aust (1978). First, 1.0ml of the supernatant was added to 2ml of (1:1:1 ratio) TCA-TBA HCL reagent (thioarbituric acid 0.37%, 0.24N HCL and 15% TCA) and tricarboxylic acid-thioarbituric acid-hydrochloric acid reagent boiled at 100℃ for 15 minutes and allowed to cool. The flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes while the supernatant was removed and the absorbance read at 532nm against a blank. MDA was calculated using the molar extinction coefficient for the MDA-TBA- complex of 1.56×105M/Cm.

Statistical analysis

The experimental design was a complete randomised design. All data collected were subjected to analysis of variance (ANOVA). Data were reported as the mean ± standard error (n = 5). Comparisons among treatment means were carried out by Duncan's multiple range test (Duncan, 1955) at a significance level of $P < 0.05$. All computations were performed by the statistical package SPSS (IBM SPSS Advanced Statistics 20.0).

Results and Discussion

The results of the nutrient utilization and growth performance parameters of the experimental fish under different feeding regimes and frequencies are shown in Table 4. Significant differences ($P<0.05$) were recorded in average weight of fish across treatments. The control group T1 (where fish were fed to satiation three times a day and every day of the week) had the highest mean weight gain (MWG) (202.92±4.68) at the end of the experimental period. There was a significant difference ($P<0.05$) in the average weight gain of the control group when compared with other groups except in T5 (where fish were fed to satiation twice daily and every day of the week). The least value for mean weight gain was recorded in fish under T4 (82.60±15.63) regime. Previous studies have shown that feeding two or three times a day was sufficient for maximum growth of a number of fish species such as channel catfish, *Ictalurus punctatus* (Ruohonen et al., 1998) and sea bass, *Dicentrarchus labrax* (Tsevis et al., 1992*).* These reports are in agreement with the findings of the present study. Similarly, Garcia-Galano et al. (2003) reported that increased feeding frequency improved the growth in some fishes.

The specific growth rate (SGR) recorded the highest value (4.24±0.05) with fish in the control group which showed a significant increase ($p<0.05$) in comparison with fish fed under all other feeding regimes with the exception of T5 group. Also, the least value in SGR was recorded in T4 group where fish were fed

for 4 days and starved for 3 days which showed a significant decrease ($p<0.05$) in comparison with the control and other feeding regimes except in those fed in T7 group (alternate days). Similarly, the highest values for feed intake and relative growth rate were obtained in T1 group of fish. Studies conducted elsewhere on some fish species indicated that feed intake and growth rate generally increased with feeding frequency up to a given level (Bascinar et al., 2007; Aderolu et al., 2010). This is in agreement with the results of this study which proved that feeding frequency had a significant effect on feed intake and specific growth rate of *C. gariepinus* fingerlings.

Table 4. Growth performance and nutrient utilization of *C. gariepinus* fingerlings under different feeding regimes and frequencies.

	T1	T2	T3	T4	T5	T6	T7	T8
Parameters	Control	4% body weight daily	5 days of feeding 2 days off	4 days of feeding 3 days off	Twice daily	4% BW twice daily	Alternate days	2 days of feeding 1 day off
Final weight (g/fish) Wf	213.92± 4.56^{e}	134.58± 44.39^{abc}	133.54± 11.61^{bd}	93.61± 15.70^{a}	173.62± 13.47^{d}	126.36± 14.12^{cd}	108.94± 13.35^{ab}	153.40± 2.97^{cd}
Initial weight (g/fish) Wi	11.00± 0.13^{a}	11.00± 0.13^{a}	11.00± 0.13^{a}	11.00± 0.13^{a}	11.00± 0.13^{a}	10.92± 0.69^{a}	10.96± 0.144^{a}	11.00± 0.13^{a}
Mean weight gain (g/fish) MWG	202.92± 4.68^{e}	123.58± 44.31^{bc}	122.54± 11.57^{bc}	82.60± 15.63^{ab}	162.62± 13.57^{e}	115.44± 14.15^{bc}	97.98± 13.39^{bc}	142.40± 3.03^{cd}
Feed intake (g/fish)	127.81± 7.50^{e}	26.30± 8.14^{a}	99.39± 13.73^{c}	64.69± 10.58^{b}	123.29± 13.22^{d}	23.05± 1.61^{a}	73.70± 8.10^{b}	107.04± 13.09^{cd}
Voluntary feed intake (g/fish)	0.81± 0.03^{b}	0.26± 0.02^{a}	0.98± 0.06^{c}	0.88± 0.069^{bc}	0.95± 0.068^{d}	0.24± 0.02^{a}	0.88± 0.08^{b}	0.93± 0.10^{c}
Specific growth rate (%/day)	4.24± 0.05^{e}	3.52± 0.50^{bc}	3.56± 0.12^{bcd}	3.05± 0.23^{a}	3.94± 0.12^{de}	3.49± 0.16^{bc}	3.27± 0.18^{ab}	3.77± 0.04^{cd}
Relative growth rate (g/fish)	1844.60± 62.69^{e}	1121.31± 397.49^{abc}	1113.53± 102.53^{cd}	750.21± 136.77^{a}	1478.81± 138.148^{d}	1057.36± 132.75^{cd}	894.11± 127.02^{ab}	1294.36± 36.76^{cd}
Feed conversion ratio	0.63± 0.02^{b}	0.22± 0.02^{a}	0.81± 0.03^{c}	0.79± 0.08^{c}	0.76± 0.05^{c}	0.20± 0.02^{a}	0.76± 0.07^{c}	0.75± 0.08^{c}
Protein intake	3.04± 0.18^{e}	0.62± 0.19^{a}	2.37± 0.33^{c}	1.54± 0.25^{b}	2.93± 0.31^{d}	0.55± 0.38^{a}	1.75± 0.21^{c}	2.55± 0.31^{cd}
Protein efficiency ratio	66.78± 2.44^{a}	195.51± 16.46^{b}	51.98± 2.17^{a}	53.61± 5.25^{a}	55.58± 3.93^{a}	210.09± 16.04^{b}	55.36± 5.15^{a}	56.36± 6.02^{a}

All values in the same row with the different superscripts are significantly different ($P < 0.05$).

The highest FCR value was recorded in fish fed in T3 (0.81±0.03) showing a significant increase ($p<0.05$) when compared with T1, T2, and T6 groups. However, T6 group recorded a significantly lower ($p<0.05$) FCR value when compared with the control and other groups except for group T2. The lowest FCR value was obtained with fish fed on 4% body weight (T2 and T6), however, this

result could not be considered the best because fish were actually placed on restricted feeding and as such could not be regarded the best. The FCR value in T1 (fed to satiation three times daily) was significantly different ($P<0.05$) from values obtained in the other treatments and the FCR value obtained in T1 showed a high feed utilization which was expressed in good growth performance. The highest value (3.04±0.018) of protein intake (PI) was recorded in control group which differed significantly ($p<0.05$) when compared with other groups. The lowest value of PI was obtained in T6 group (0.55±0.38), this was followed by T2 (0.62±0.19), which was significantly lower ($p<0.05$) compared with the control and other groups. The protein efficient ratio was significantly high ($p<0.05$) in fish fed in T6 (210.09±16.04) and T2 (195.51±16.46) treatments, but was not significantly different among other feeding regimes. Previous studies have agreed that when an organism utilizes nutrients, particularly protein very well, it will positively enhance its growth rate (Sogbesan and Ugwumba, 2008). When fish are fed on percentage body weight, the restricted feed given seems to be better utilised as a result of insufficiency. This result is corroborated by the work of Daudpota et al. (2016) who observed improved protein efficiency ratio of juvenile Nile tilapia at the optimum feeding frequency of four times daily.

Histometry results of *C. gariepinus* fingerlings under different feeding regimes and frequencies (Table 5) revealed that the gall bladder of those of T4 showed the highest value (0.58±0.21) and significantly higher than those of other treatments except the control, T2, T5 and T7.

Table 5. Histometry analysis of *C. gariepinus* fingerlings under different feeding regimes and frequencies.

Organs	T1	T2	T3	T4	T5	T6	T7	T8
Gall bladder	0.47± 0.19^{ab}	0.41± 0.07^{ab}	0.36± 0.06^{a}	0.58± 0.21^{b}	0.38± 0.07^{ab}	0.28± 0.12^{a}	0.45± 0.17^{ab}	0.32± 0.24^{a}
Liver	1.11± 0.38^{ab}	1.29± 0.15^{ab}	1.43± 0.34^{b}	1.44± 0.34^{b}	1.10± 0.12^{ab}	0.92± 0.30^{a}	1.24± 0.51^{ab}	1.02± 0.38^{ab}
Small intestine	1.49± 0.12^{bc}	1.20± 0.54^{ab}	1.39± 0.26^{abc}	1.80± 0.20^{c}	1.12± 0.11^{ab}	1.21± 0.30^{ab}	1.09± 0.65^{ab}	0.99± 0.44^{a}
Large intestine	0.91± 0.11^{a}	0.86± 0.37^{a}	0.89± 0.16^{a}	0.86± 0.35^{a}	0.97± 0.29^{a}	0.72± 0.17a	0.90± 0.75^{a}	0.72± 0.23^{a}
Gills	3.48± 0.47^{ab}	3.21± 0.53^{ab}	3.47± 1.00^{ab}	3.96± 0.73^{b}	4.00± 0.73^{b}	3.27± 0.70^{ab}	2.98± 1.27^{ab}	2.57± 0.64^{a}
Kidney	0.43± 0.58^{a}	0.45± 0.16^{a}	0.59± 0.21^{a}	0.42± 0.49^{a}	0.52± 0.17^{a}	0.35± 0.09^{a}	1.59± 2.55^{a}	0.44± 0.29^{a}
Stomach	1.27± 0.07^{ab}	1.49± 0.18^{b}	1.53± 0.32^{b}	1.63± 0.36^{b}	1.47± 0.33^{b}	1.22± 0.35^{ab}	1.32± 0.53^{b}	0.89± 0.32^{a}
Head	12.85± 1.95^{a}	25.58± 18.22^{b}	16.76.± 4.04^{ab}	17.45± 6.08^{ab}	13.58± 6.01^{a}	15.12± 13.01^{ab}	10.33± 3.59^{a}	13.50± 4.57^{a}

All values in the same row with the different superscripts are significantly different ($P < 0.05$).

However, the lowest value was recorded in T8 (0.32±0.24) which was significantly lower than that of T4. The liver value showed a significant decrease ($p<0.05$) only in T6 (0.92±0.30) in comparison with other treatments. The value of small intestine showed a significant increase ($p<0.05$) in T4 when compared with other groups except T3. Similarly, the value of gill showed a significant increase ($p<0.05$) in T4 and T5 when compared with T8. Similarly, the value of stomach significantly decreased ($p<0.05$) in T8 when compared with the control and all other groups. There was also a significant increase ($p<0.05$) in the value of fish head in T2 group when compared with the control and other groups. The highest values for gall bladder, liver, small intestine and stomach were recorded in T4 (fed to satisfaction only for 4 days and subsequently starved for 3 days). This could be attributed to the long period of feed deprivation which could probably trigger lipolysis and transamination under such feeding regime.

The results of the haematological parameters are reported in Table 6. Out of the four parameters tested, haemoglobin (Hb), packed cell volume (PCV), white blood count (WBC) and erythrocyte sedimentation rate (ESR), significant differences ($P<0.05$) were recorded in the values of Hb and ESR. The T6 group recorded the highest value (10.8±0.3) for Hb, followed by T5 (9.7±0.0) and the least value was obtained in T8 (7.6±0.3). The ESR recorded a significant increase in T8 (43.5±2.1) and T2 (40.0±2.8), while the least value was found in T6 (19.0±4.2). However, significant differences ($P>0.05$) were not recorded in values of both the PCV and the WBC across groups. Heamatological tests can help in the diagnosis of nutritional, metabolic, hereditary, hormonal, neoplastic, drug-induced, stress, inflammatory or infectious disease states (Ochei and Kolhatkar, 2000). The objective of measuring Hb is to estimate the oxygen carrying capacity of blood, in addition to providing an assessment of erythropoietic status (Baker et al., 2000). Hence, the obtained result revealed that feeding regime (T8) led to a significant decrease ($p<0.05$) of Hb, which could reduce the oxygen carrying capacity of the fish. The ESR (erythrocyte sedimentation rate) is useful as a screening test for the presence of any chronic or acute condition which is marked by alteration in plasma protein concentrations. Serial estimation of ESR may also be used to monitor disease progression or treatment (Baker et al., 2000). The result from fish in T8 recorded a significant decrease ($p<0.05$) in ESR, attributing it to alteration in plasma protein which was occasioned by the feeding regime.

The results of the antioxidant enzymes of the fish liver are presented in Table 6. The analysed enzymes:glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and malondialdehyde (MDA) values recorded no significant differences ($P>0.05$) across the groups, though their values were higher in fish fed on percentage body weight and those starved for different day intervals. The importance of enzymatic and non-enzymatic antioxidant systems cannot be overemphasized in their role of managing the cellular response to

oxidative stress under physiological conditions. Consequently, GSH could be used to investigate modification in antioxidant enzymes due to stress in the organism (Dey and Lakshmanan, 2013). The observations from the present study did not show any significant difference in these enzymatic and non-enzymatic antioxidants, hence it could be deduced that the feeding regimes did not cause any oxidative stress in the physiology of the fish studied.

Table 6. Haematological parameters and antioxidant enzymes of *C. gariepinus* fingerlings under different feeding regimes and frequencies.

Parameters	T1	T2	T3	T4	T5	T6	T7	T8
PCV (%)	28.50±9.2	24.00±0.0	26.00±0.0	27.50±0.7	30.00±0.0	33.00±1.4	27.00±4.2	24.00±1.4
Hb (g/dl)	9.30±3.3^{ab}	7.70±0.0^{ab}	8.60±0.1^{ab}	8.90±0.2^{ab}	9.70±0.0^{ab}	10.80±0.3^{b}	8.70±1.4^{ab}	7.60±0.3^{a}
WBC (mm)	14,950.00±70.7	11,800.00±3959.8	11,200.00±2262.7	9,400.00±4808.3	13,800.00±311.3	15,600.00±3394.1	12,900.00±2121.3	13,500.00±35.5
ESR (mm/hr)	32.00±19.8^{ab}	40.00±2.8^{ab}	34.00±0.0^{ab}	37.50±2.1^{ab}	20.50±2.1^{a}	19.00±4.2^{a}	27.50±13.4^{ab}	43.50±2.1^{b}
GSH µmol/ml/mg pro	4.56±1.27	6.71±1.52	3.39±1.59	4.56±1.59	4.25±1.68	5.87±2.06	7.09±2.27	6.28±1.03
SOD µmol/ml/min/mg pro	8.01±.01^{ab}	10.62±2.14^{ab}	6.04±2.94^{ab}	9.34±4.89^{ab}	8.36±3.80^{ab}	10.97±2.30^{ab}	12.90±1.2^{ab}	13.54±1.36^{b}
CAT µmol/ml/min/mg pro	23.75±1.55	38.15±9.03	25.72±4.86	27.06±18.35	24.39±18.60	44.73±7.79	42.54±5.76	47.74±9.31
GST µmol/ml/mg pro	5.31±4.17	3.23±.65	1.84±.89	2.81±1.47	2.52±1.15	3.32±.71	3.89±.39	4.00±.40
MDA µmol/ml/mg pro	0.07±.02	0.03±.01	0.11±.11	0.18±.10	0.16±.09	0.14±.16	0.07±.02	0.04±.00

Values within the row with different superscripts are significantly different ($p<0.05$) from each other.

The results of metabolic enzymes and fish blood electrolytes are presented in Table 7. No significant differences ($P>0.05$) were observed in the values of serum protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), high density lipoprotein (HDL and sodium (Na). However, the low density lipoprotein (LDL) recorded the highest value in fish of T1 (54.50±50), followed by T3 (46.50±23.33), and the lowest value was recorded in T6 (12.00±8.49). The fish in the control group had the highest value (231.50±53.03) for sodium chloride which showed a significant increase ($p<0.05$) in comparison with all other treatments. Similarly, the highest value (30.50±2.12) of bicarbonate was obtained in T4 which differed significantly ($P<0.05$) when compared with other groups except T6. The values of creatine, urea, cholesterol, triglyceride and albumin recorded no significant difference ($P>0.05$) across all the groups.

Liver function tests (ALT, AST, protein and albumin) have been devised in the hope that they will serve as diagnostic aids when a metabolic process has been disturbed (Beker et al., 2000). The results on all the liver enzymes, protein and albumin showed no significant difference across all the feeding regimes, indicating that the regimes did not affect the metabolic process of the fish.

Table 7. Metabolic enzymes and blood electrolytes of *C. gariepinus* fingerlings under different feeding regimes and frequencies.

Parameters	T1	T2	T3	T4	T5	T6	T7	T8
Protein	3.45± .21	3.45± .07	4.10± .14	4.35± .49	3.70± .85	4.25± .49	4.15± .21	4.35± 1.34
LDL	54.50± 14.85^{b}	18.50± 7.78^{ab}	46.50± 23.33^{ab}	36.50± 17.68^{ab}	22.50± 7.78^{ab}	12.00± 8.49^{a}	42.50± 2.12^{ab}	22.00± 19.80^{ab}
HDL	97.50± 4.95	94.50± 13.44	68.00± 4.24	77.00± 2.83	92.50± 4.95	92.00± 25.46	94.00± 25.46	84.00± 11.31
AST	17.50± 14.85	23.00± 11.31	26.00± 25.46	26.50± 10.61	47.00± 16.97	54.50± 17.68	48.00± 8.49	22.50± 6.36
ALT	34.00± 22.63	23.00± 8.49	40.50± 30.41	17.50± 4.95	46.00± 39.60	45.00± 15.56	47.00± 7.07	31.50± 3.54
Na	231.50± 53.03	173.00± 9.90	180.00± 42.43	174.00± 5.66	187.50± 41.72	174.00± 8.49	190.00± 2.83	179.00± 15.56
K	3.75± .64^{ab}	3.35± .78^{a}	3.40± 0.00^{a}	3.75± .07^{ab}	5.20± 1.41^{b}	4.70± .28^{ab}	3.70± 0.00^{ab}	3.55± .07^{a}
Cl	231.50± 53.03^{b}	117.00± 15.56^{a}	134.00± 50.91^{a}	120.00± 0.00^{a}	118.50± 12.02^{a}	105.00± 7.07^{a}	134.00± 5.66^{a}	126.50± 7.78^{a}
BCO	20.50± .71^{a}	18.50± .71^{a}	22.00± 4.24^{a}	30.50± 2.12^{c}	24.00± 2.83^{ab}	29.50± 2.12^{bc}	21.50± .71^{a}	24.50± 3.54^{ab}
Urea	27.00± 18.38	13.00± 1.41	23.50± 7.78	29.00± 4.24	23.50± 12.02	27.50± 10.61	24.50± 12.02	27.00± 1.41
Creatine	0.90± .42	0.65± .07	0.80± .14	0.95± .07	0.80± .14	0.95± .21	0.80± .28	0.95± .07
CHOL	67.50± .71	96.50± 12.02	109.00± 8.49	66.00± 21.21	96.50± .71	102.00± 22.63	107.50± 36.06	86.00± 11.31
Trig	110.50± 34.65	103.50± 44.55	108.50± 9.19	128.00± 5.66	132.50± 17.68	108.50± 27.58	73.00± 7.07	119.50± 13.44
Albumin	2.20± .28	1.84± .06	1.60± .14	1.50± .14	1.85± .49	1.90± .42	2.00± .14	1.95± .35

This is supported by the findings of Delgiudice (1987) when wolf fish was starved over different periods of time. In contrast, Sridee and Boonanuntanasarn (2012) confirmed that long time duration of fasting could affect metabolic and liver functions. A decrease in ALT, AST and ALP could be an indication of transamination while an increase could indicate a distortion in liver activities or

damage to the liver or kidney tissues. Since the differences among the treatments were not significant, we can assume that none of the aforementioned happened and that the fish probably did not have enough nutrients in some of the feeding frequencies which could trigger a transamination reaction as seen in extremely starved fish (Kulkarni and Barad, 2015).

Metabolic enzymes and serum electrolytes were not significantly different among treatments because fish were not actually starved but probably did not get enough nutrients. Feed supplied is known to help maintain body metabolic activities before sufficient nutrients will be diverted for growth and reproductive activities. During starvation, stored nutrients are liberated for metabolic activities and as such lead to a decrease in their serum concentrations.

Lipids have important roles in virtually all aspects of life; serving as hormones, as an energy source, aiding digestion, and as structural components in cell membranes (Burtis et al., 2001). Nevertheless, the health importance of lipid profile is connected primarily with its role in coronary heart disease and varying lipoprotein disorders (Burtis et al., 2001).

Lipolysis is the breakdown of stored lipids to compensate for metabolic need during starvation; in this study, no significant difference in HDL suggested that the feeding regimes did not influence the lipid profile of the fish studied except in T6 which differed significantly ($p<0.05$) when compared with T1 and that fish were not overstarved since we did not have its breakdown, but instead we had a reduction in LDL, especially among T2 and T6 regimes. The maintenance of body fluid compartments is the primary function of Na^+, K^+,Cl^- and HCO_3^- electrolytes. They play the roles of water homeostasis, maintenance of pH, proper heart and muscle functioning, oxidation–reduction reactions, and they are cofactors for enzymes (Burtis et al., 2001). Also, the observed significant increase ($p<0.05$) in potassium in fish placed on T5 (fed to satiation 2 times every day) could probably be related to the significant increase in values recorded in gill and kidney weights for the same treatment.

Conclusion

The results obtained from the present study confirmed that there was a significant difference in nutrient utilization and growth of fish as a result of different feeding regimes and frequencies and that feeding to satiation two to three times daily would enhance growth in *C. gariepinus* fingerlings. In addition, variation in feeding regime and frequency could raise the antioxidant enzymes, especially in starved fish and those fed on a weight basis.

References

Aderolu, A.Z., & Akpabio, M.V. (2009). The feeding value of Mucuna seed meal in the diet of *C. gariepinus. African Journal of Aquatic Sciences, 34* (2), 131-135.

Aderolu, A.Z., Seriki, B.M., Apatira, A.L., & Ajaegbo. C.U. (2010). Effects of feeding frequency on growth, feed efficiency and economic viability of rearing African catfish (*C. gariepinus,* Burchell 1822) fingerlings and juveniles. *African Journal of Food Science, 4* (5), 286-290.

Aebi, H. (1984). Catalase in vitro. In: Colowick, S.P. and Kaplane, N.O. (editions). *Methods in Enzymology, 105*, 121-126.

Ali, M.Z., Hossain, M.A., & Mazid, M.A. (2005). Effect of mixed feeding schedules with varying dietary protein levels on the growth of sutchi catfish, *Pangasius hypophthalnus* (Sauvage) with silver carp, *Hypophthalmichthys molitrix* (Valenciennes) in ponds. *Aquaculture Research, 36*, 627-634.

Baker, F.J., Silverton, R.E., & Pallister, C.J. (2000). *Introduction to Medical Laboratory Technology*. (7th edition) Sam – Adex Printers, Felele Rab, Ibadan.

Bascinar, N.E., Cakmak, Y., & Cardar Aksunga, N. (2007). The Effect of Feeding Frequency on Growth Performance and Feed Conversion Rate of Black Sea trout (*Salmotrutta*, 1811). *Turkish Journal of Fisheries and Aquatic Science, 7*, 13-17.

Bolliet, V., Aranda, A., & Boujard, T. (2001). Demand-feeding rhythm in rainbow trout and consumption, feeding pattern and growth of juvenile yellowtail flounder (*Limanda ferruginea*). *Aquaculture, 213*, 279-292.

Buege, J.A., & Aust, S.D. (1978). Microsomal lipid peroxidation. *Methods Enzymology*, *52*, 302-310.

Burtis, C.A., Ashwood, E.R., & Bruns, D.E. (2001). *Tietz Fundamental of Clinical Chemistry.* (5th Edition) W.B. Saunders Company USA.

Daudpota, A.M., Abbas, G., Kalhoro, I.B., Shah, S.S.A., Kalhoro, H., Hafeez-ur-Rehman, M., & Ghaffar, A. (2016). Effect of feeding frequency on growth performance, feed utilization and body composition of juvenile Nile tilapia, *Oreochromis niloticus* (L.) reared in low salinity water. *Pakistan Journal of Zoology*, *48* (1), 171-177.

Davies, O.A., Inko-Tariah, M.B., & Amachree, D. (2006). Growth response and survival of *Heterobranchus longifilis* fingerlings fed at different feeding frequencies. *African Journal of Biotechnology, 5* (9), 778-780.

Delgiudice, G.D. (1987). Effects of feeding and fasting on wolf blood and urine characteristics, *Journal of Wild life Management*, *51*, 1-10.

Dey, A., & Lakshmanan, J. (2013). The role of antioxidants and other agents in alleviating hyperglycemia mediated oxidative stress and injury in liver. *Food Function*, *4*, 1148-1184.

Duncan, D.B. (1955). Multiple range and multiple F test. *Biometrics, 11*, 1-42.

Dwyer, K.S., Brown, J.A., Parrish, C., & Lall, S.P. (2002). Feeding frequency affects food consumption, feeding pattern, and growth of juvenile yellowtail flounder (*Limanda ferruginea*). *Aquaculture*, *213*, 279-292.

Fagbenro, O.A., Nwanna, L.C., Adeparusi, E.O., Adebayo, O.T., & Fapohunda, O.O. (2005). An overview of animal feed industry and dietary substitution of feedstuffs for farmed fish in Nigeria. In**:** *Crops: Growth, Quality and Biotechnology (current status and future prospects)* (Ramdane Dris, editor). WFL Publisher, Helsinki, Finland. pp. 91-107.

Garcia-Galano, T., Perez, J.C., Gaxiola, G., & Sanchez, Y.A. (2003). Effect of feeding frequency on food intake, gastric evacuation and growth in juvenile Snook, *Centropomus undecimalis* (BLOCH). *Revista de Investigaciones Marinas*, *24*, 145-154.

Goddard, S. (1995). *Feed Management in Intensive Aquaculture*. Chapman and Hall, New York.

Habbu, P.V., Shastry, R.A., Mahadevan, K.M., Hanumanthachar, J., & Das, S.K. (2008). Hepatoprotective and antioxidant effects of *Argyreia speciosa* in rats. *African Journal of Traditional Complementary and Alternative Medicines*, *5* (2), 158-164.

Habig, W.H., Pabst, M.J., & Jacoby, W.B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, *249*, 7130-7139.

Kulkarni, R.S., & Barad, V.S. (2015). Effect of starvation on haematological and serum biochemical changes in the fresh water fish, *Notopterus notopterus* (Pallas). *International Journal of Innovative Studies in Aquatic Biology and Fisheries*, *1* (1), 24-29.

Jobling, M. (1995). *Fish bioenergetics.* Chapman & Hall, London.

Lee, S.M., Hwang, U.G., & Cho, S.H. (2000). Effects of feeding frequency and dietary moisture content on growth, body composition and gastric evacuation of juvenile Korean rockfish (*Sebastes schlegeli*). *Aquaculture*, *187*, 399-409.

Ochei, J.O., & Kolhatkar, A.A. (2000). *Medical Laboratory Science: Theory and Practice.* Tata McGraw-Hill Publishing Company Limited.

Priestley, S.M., Stevenson, E.S., & Alexander, L.G. (2006). The influence of feeding frequency on growth and body composition of the common gold fish (*Carrassius auratus*). *Journal of Nutrition, 136* (7suppl), 2078S-2080S.

Ruohonen, K., Vielma, J., & Grove, D.J. (1998). Effects of feeding frequency on growth and food utilization of rainbow trout, *Oncorhynchus mykiss* fed low-fat herring or dry pellets. *Aquaculture, 165*, 111-112.

Schnaittacher, G., King, W., & Berlinsky, D.L. (2005). The effects of feeding frequency on growth of juvenile Atlantic halibut, *Hippoglossus hippoglossus* L. *Aquaculture Research*, *36*, 370-377.

Sedlak, J., & Lindsay, R.H. (1968). Estimation of total protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, *25*, 1192-1205.

Sogbesan, A.O., & Ugwumba, A.A.A. (2008). Nutritional evaluation of termite *Macrotermes subhyalinus* meal as animal protein supplements in the diets of *Heterobranchus longifilis* fingerlings. *Turkish Journal of Fish and Aquatic Science*, *8*, 149-157.

Sridee, N., & Boonanuntanasarn, S. (2012). The effects of food deprivation on haematological indices and blood indicators of liver function in *Oxyleotris marmorata*. *International Journal Medicine and Biological Science, 6* (5), 254-258.

Sun, M., & Zigma, S. (1978). An improved spectrophotometer assay of superoxide dismutase based on epinephrine antioxidation. *Analytic Biochemistry*, *90*, 81-89.

Tsevis, N., Klaoudatos, S., & Conides, A. (1992). Food conversion budget in sea bass, *Dicentrarchus labrax*, fingerlings under two different feeding frequency patterns. *Aquaculture, 101*, 293-304.

Zakes, A., Kowalska, A., Czerniak, S., & Demska-Zakes, K. (2006). Effect of feeding frequency on growth and size variation in juvenile pikeperch, *Sander lucioperca* (L). *Czech Journal of Animal Science*, *51* (2), 85-91.

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UČESTALOST HRANJENJA I REŽIM ISHRANE KOD SOMA: UTICAJI NA ISKORIŠĆENOST HRANLJIVIH MATERIJA, RAST, BIOHEMIJSKE I HEMATOLOŠKE PARAMETRE

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R e z i m e

Ovim istraživanjem ispitan je prirast, iskoristljivost hranljivih materija, biohemijski i hematološki parametri kod mlađi soma *C. gariepinus* pri različitoj učestalosti i režimima ishrane. Osam riba je stavljeno u različite tretmane, T (1–8), svaki u tri ponavljanja. Sve ribe su izmerene na početku ekperimenta, a zatim jednom nedeljno tokom 10 nedelja. Zabeležen je značajan uticaj tretmana ($p<0,05$) na srednju vrednost prirasta (engl. *mean weight gain* – MWG), specifičnu stopu rasta (engl. *specific growth rate* – SGR) i stope konverzije hrane (engl. *feed conversion ratio* – FCR). Kontrolni tretman (T1) je imao najviši MWG (202,92±4,68) i SGR (4,24±0,05), dok su najniže vrednosti zabeležene u tretmanu T4 (82,60±15,63 odnosno 3,05±0,23). Najbolja vrednost za FCR uočena je u tretmanu T6 (0,20±0,02) koji se značajno razlikovao ($p<0,05$) od drugih tretmana, osim tretmana T2. Značajne razlike ($p<0,05$) zabeležene su u vrednostima hemoglobina (Hb) i stopi sedimentacije eritrocita (ESR). Najviša vrednost za Hb uočena je u tretmanu T6 (10,8±0,3), a najniža u tretmanu T8 (7,6±0,3). Najviša vrednost za ESR zabeležena je u tretmanu T8 (43,5±2,1), dok je najniža vrednost uočena u tretmanu T6 (19,0±4,2), a značajne razlike nisu zabeležene ni za vrednosti PCV i WBC ($p>0,05$). Vrednosti proteina krvi, albumina, kreatina, uree, holesterola, triglicerida i lipoproteina visoke gustine nisu ispoljile značajne razlike ($p>0,05$) u različitim tretmanima. Takođe, nije bilo značajnih razlika ($p>0,05$) ni između vrednosti aspartat aminotransferaze i alanin aminotransferaze, dok su uočene smanjene vrednosti hlorida, kalijuma i natrijuma sa smanjivanjem broja preostalih hranidbenih dana. Rezultati ovog istraživanja su potvrdili da bi ishrana do sitosti, dva ili tri puta dnevno povećala indekse rasta kod mlađi soma *C. gariepinus*.

Ključne reči: učestalost hranjenja, režim ishrane, som, biohemijski, hematološki parametri.

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