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Effects of Feeding Frequency on Growth and Nutrient Utilization of *Oreochromis Niloticus* (Linnaeus 1757) Fingerlings

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I. Introduction

Tilapia is a global commodity reaching a production of 3.5 million tonnes in 2010 (Rana 2013). It is one of the most productive and internationally traded food fish in the world (Modadugu and Belen 2004). They are a major protein source in many of the developing countries. The commodity is not only the 2nd most important farmed fish globally, next to carp in global production (Fitzsimmons *et al*., 2013) but also described as the most important aquaculture species of the 21st century (Shelton 2002). Its species have since been introduced in different parts of the world to improve fisheries or to develop aquaculture (Lèveque, 2002). There are about 70 species of tilapias, most of them native to Western rivers of Africa (Anon 1984). Out of these, nine species are used in aquaculture worldwide among which are *Oreochromis niloticus* and *Tilapia zillii* (Hepher and Pruginin 1981, Mair 2001, FAO 2002).

The global tilapia production (*Oreochromis niloticus* inclusive) in 2010 was approximately 3.5 million metric ton and should have increase to more than 3.7 million mt in 2012 (Fitzsimmons 2013).

One of the most crucial elements influencing the ability of cultured fish to exhibit its potential for growth and reproduction is nutrition (Başçınar 2007). A good nutrition in animal production stem is essential to economically produce a healthy, high quality product (Nekoubin, and Sudagar 2012). They are greatly influenced by factors such as feed quality, fish behaviour, daily ratio size, feed intake and water temperature. Feed is the most expensive component in intensive aquaculture (El-sayed, 2004), accounting for 40-60% of the aquaculture production (El-sayed, 1999 and Anderson *et al.,* 1997 and, ADCP, 1983). Aqua-feed alone represent the largest single cost item in management of semi-intensive and intensive farming operations. Feed quality and feeding strategy are of great importance in fish nutrition (Güroy *et al*., 2006).

In addition, both over- and underfeeding can be detrimental to the health of the fish and may cause a marked deterioration in water quality, reduced weight, poor food utilization, and increased susceptibility to infection (Priestley *et al*., 2006). Feed consumption rate is dependent on species type, dietary composition, fish size, meal size, environmental conditions and feeding frequency (Reche 2000). The growth of fish at all stages is largely dependent by ration, the kind of food, feed intake, feeding frequency and its ability to absorb the nutrients. Among these, feeding frequency is an important aspect for the survival and growth of fish at the early stage (Shaoushaki *et al*., 2012). The objective of this study therefore is to evaluate effect of *O. niloticus* fed pelleted diet to satiation at different feeding frequencies and determine the effect on growth and nutrient utilization.

II. Materials and Methods

a) Diet formulation and preparation

Feedstuffs were purchased from Metrovet Agroallied shop, Ado Ekiti and were separately milled to small particle size (< 250 μm) using pulverizing machine (Model BCC-2516). The ingredients weighed on a Metler top-loading balance (Model PB-8001). The isonitrogenous diet was formulated (Table 1) at 350g crude protein and 18.5KJ gross energy/g^{-1} diet, respectively. The feedstuffs were thoroughly mixed in a Hobart A-200T pelleting machine. Hot water was added at intervals to gelatinize starch. The diet was pelletized using a die of 0.8 mm diameter. The diet was air-dried at ambient temperature for 72 hours; broken, sieved into

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small pellet sizes, packed in air-tight plastic containers, labelled and stored. Prior to storage, the diet were analyzed for proximate composition according to standard AOAC methods AOAC (1990). Crude protein was determined using a Kjeltec Auto 1003 Analyser after digestion with concentrated H_2SO_4 in a digester. Crude lipid was estimated by extracting in chloroform: methanol (2:1) using a Soxlet extraction HT6 unit. Crude fiber was determined using a Fibretec System 1020 Hot Extractor and ash content was determined by igniting at 550°C in a muffle furnace for 12 hours. Gross energy content was determined using a ballistic bomb calorimeter (Gallenkamp Co. Ltd., Loughborough, England).

b) Experimental system and animals

O. niloticus fingerlings were obtained from a reputable fish farm in Ekiti State, Nigeria, and acclimated for 14 days in concrete tanks (2m x 2m x 1.25m) of 400L capacity. Two-third of each concrete tank was filled with water. Continuous aeration was provided using a blower and air stones (Tecas air pump AP-3000; two ways). The fish were acclimatized in the concrete tanks for 7days while being fed on a commercial pelletized diet (30% crude protein). Four treatments namely, daily feeding frequencies of once (F1) at 0900hr, twice (F2) at 0900 and 1700 h, three (F3) at 0900, 1300 and 1700 h and four times at 0900, 1200, 1500 and 1800 h, respectively. After acclimation, twenty (20) *O. niloticus* (3.40g $\pm$ 0.04) were randomly stocked in each concrete tank (2m x 2m x 1.25m) supplied with 400 litres of fresh water (water temperature, 27°C; pH, 7.3; alkalinity, 50ppm; dissolved oxygen, 7.6-7.9 mg/L). Each treatment was replicated thrice. Feeding commenced a day after stocking and lasted 58 days. The fish were hand-fed to apparent satiation at different feeding frequencies (once (FF1) at 0900hr, twice (FF2) at 0900 and 1700 h, three (FF3) at 0900, 1300 and 1700 h and four times (FF4) 0900, 1200, 1500 and 1800 h, respectively. All fish were removed from each concrete tank every fortnight and batch weighed. Mortality was monitored daily and recorded. Growth performance and nutrient utilization indices were determined as final fish weight (g), survival (%), specific growth rate (SGR, % day-1) and feed conversion ratio (FCR). Growth parameters were calculated as:

[1] % weight gain (%. fish^{-1}) = [(final wt. – initial wt.)/initial wt.)] x 100

[2] weight gain (g) = (final wt. – initial wt.)

[3] specific growth rate (%. day-^{1}) = [(ln final wt. – ln initial wt.)/no of days] x 100

[4] feed conversion ratio = feed intake (g)/body weight gain (g)

Water temperature and dissolved oxygen were measured daily using a combined digital YSI DO meter (YSI model 57); pH was monitored weekly using an electronic pH meter (Metler Toledo 320 model). Two weeks before the completion of the feeding trial, faeces were collected from each tank, 8 h after each feeding daily. The ashes were digested by acid insoluble ash (AIA) as described by Halver *et al.* (1993). The value obtained for AIA was used as indicator in the calculation of digestibility coefficient. The digestibility coefficient was calculated as follows:

Digestibility = 100-100(%AIA in feed) × (% nutrient in faeces)/(%AIA in faeces)(% nutrient in feeds).

All data obtained were subjected to one-way Analysis of Variance (ANOVA) test using the SPSS Version 11. Fisher's pairwise comparison was used in comparing differences among individual mean.

III. Results

Crude protein of 35% was used in the formulation of the experimental diets for *Oreochromis niloticus* fingerlings (Table 1). The proximate composition of the experimental diet are; the crude protein is 355.0± 0.50, crude fibre 17.8±1.20, lipid 102.2±0.26, ash 129.8±1.53, moisture 57.0±1.21 while Nitrogen Free Extract (NFE) is 318.2(g/kg^{-1} DM) (Table 2).

At the expiration of 58days, *O. niloticus* fed thrice a day, Treatment 3 (F3) had significant (P<005) the highest weight gain (19.33g $\pm$ 0.67) and protein intake (114.42 $\pm$ 10. 57) than the other diets (Diets 1, 2 and 4) (Table 3). The least weight gain was evident in fish fed once per day, Treatment 1(F1) 9.33 $\pm$ 0.33). *O. niloticus* survival was not significantly affected by the treatments. Water quality during the feeding trial was within the acceptable range for tilapia culture (water temperature, 27 °C; pH, 7.3; alkalinity, 50 ppm; dissolved oxygen, 7.6 - 7.9 mg/L).

Table 1 : Ingredients composition of the basal diet (35% crude protein)

Ingredient	g/kg Diet
Menhaden fish meal	150
Soybean meal	450
Yellow maize	250
Cod liver oil	40
Vegetable oil	60
Vitamin-mineral mix	30
Corn starch	20

[1]Fish pre-mix. Colborne Dawes Nutrition Ltd., United Kingdom.: vitamin A, 1600 IU; vitamin D, 2400 IU; vitamin E, 160 mg; vitamin K, 16 mg; thiamin, 36 mg; riboflavin, 48 mg; pyridoxine, 24 mg; niacin 288 mg; panthotenic acid, 96 mg; folic acid, 8 mg; biotin, 1.3 mg; cyanocobalamin, 48 mg; ascorbic acid, 720 mg; choline chloride, 320 mg; calcium 5.2 g; cobalt, 3.2 mg; iodine, 4.8 mg; copper, 8 mg; iron, 32 mg; manganese, 76 mg; zinc, 160 mg; Endox (antioxidant) 200 mg.

Table 2 : Proximate composition of the experimental diets

Proximate composition (g/kg^{-1} DM)	Diet
Crude protein	355.0
Ether extract	102.2
Crude fibre	17.8
Ash	129.8
Moisture	57.0
Nitrogen free extract	318.2
Gross energy (KJ g^{-1})	18.5

Table 3 : Growth performance and nutrient utilization of *Oreochromis niloticus* fingerlings fed at different feeding frequencies

	Treatment 1 FF1	Treatment 2 FF2	Treatment 3 FF3	Treatment 4 FF4
Weight Gain	9.33±0.33^{d}	13.67±0.33^{c}	19.33±0.67^{a}	17.67±0.33^{b}
Percentage Weight Gain	53.38±8.34^{b}	50.00±8.39^{b}	57.09±7.97^{b}	78.44±3.44^{a}
Specific Growth Rate	0.76±0.09^{a}	0.72±0.09^{a}	0.81±0.08^{a}	0.61±0.03^{b}
Protein Intake	21.32±2.74^{c}	66.64±7.76^{b}	114.42±10.57^{a}	95.71±15.45^{ab}
Survival Rate	73.33±3.33^{b}	83.33±3.33^{ab}	90.00±5.77^{a}	86.67±3.33^{ab}
Feed Conversion Ratio	14.16±2.19^{a}	18.57±2.01^{a}	13.96±1.66^{a}	6.53±0.84^{b}
Gross Feed Conversion Efficiency	7.44±1.23^{b}	5.52±0.62^{b}	7.40±0.78^{b}	15.85±2.08^{a}

Mean and Standard Error having the same superscripts are not significantly different.

Table 4 : Carcass composition of *Oreochromis niloticus* fingerlings fed at different feeding frequencies

	Initial	Treatment 1 FF1	Treatment 2 FF2	Treatment 3 FF3	Treatment 4 FF4
Moisture Content	3.67	4.19	4.24	4.25	4.35
Ash(%)	29.63	16.40	15.04	16.05	16.05
Lipid(%)	10.90	20.57	18.67	21.29	20.19
Crude Protein (%)	38.24	40.23	55.17	57.25	54.62
Nitrogen Free Extract	17.56	18.61	6.88	1.16	4.79

IV. Discussion

The crude protein of 35% used in the formulation of the experimental diets for *Oreochromis niloticus* fingerlings (Table 1) falls within the recommended ranges of 25-35% crude protein requirement for Tilapia species (Santiago and Lovell 1988), and 30%-35% recommended by N.R.C (1981) and N.R.C (1983), and satisfied the nutrient requirements for tilapias (Jauncey, 2000). *O. niloticus* fed thrice daily frequency, Treatment 3 (FF3) had significantly (P<005) the highest weight gain (19.33g ± 0.67) and protein intake (114.42 ± 10. 57) than the other daily frequencies (FF1, FF2 and FF4) .Also the FCR of *O. niloticus* fed daily frequency FF3 (13.96± 1.66) is better than FF1, FF2 and FF4 (Table 3). The least weight gain was evident in fish fed once per day, Treatment 1(FF1) 9.33 ± 0.33. This study corroborate a similar study by Başçınar (2007) where growth, feed consumption and conversion ratios of Black Sea trout (*Salmo trutta labrax*) subjected to daily feeding frequencies showed that fish fed trice daily frequency had better final live weight and SGR value than all the other frequencies when evaluated. *O. niloticus* survival was not significantly affected by the treatments. The trend in the proximate analysis of the fish carcass in all

the treatments(Table 4) are similar to that obtained by El-Sayed (1998) where the effects of total replacement of animal protein sources in *Oreochromis niloticus* was investigated. An appreciable increase in crude protein and lipid were reported in the carcass of *Oreochromis niloticus* fed Poultry by Product (PBP) as against the other diets. Increase in crude protein and lipid of the carcass of fish is a pointer to the superiority of the fish quality Sahu *et al*., (2000). The water quality during the feeding trial was within the acceptable range for tilapia culture (Ross, 2000) and do not differ significantly among treatments. Acceptance of the diets was good and fish became accustomed to the diets within the first week.

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