

## OPTIMUM DIETARY PROTEIN REQUIREMENT OF GENETICALLY MALE TILAPIA (*OREOCHROMIS NILOTICUS*) CULTURED IN FLOATING HAPA SYSTEM

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### ABSTRACT

*The study was conducted to investigate the optimum dietary protein level needed for growing genetically male tilapia, Oreochromis niloticus. Diets containing crude protein levels 40, 42.5, 45, 47.5 and 50% were formulated and tried in triplicates. Test diets were fed to 20 fish/1m<sup>3</sup> floating hapa at 5% of fish body weight daily for 24 weeks. Survival of fish was not affected by dietary protein levels. Growth rate varied inversely with dietary protein levels to a maximum at 40%. Protein utilization and turnover decreased with increasing protein levels in diets. Quadratic regression analysis of growth indices against protein inclusion levels indicated that the optimum dietary protein required for maximum growth was 40%. This result also paralleled the least values of protein and calorie deposited for 40% protein level whereas the other levels were significantly ( $p < 0.05$ ) higher. The study concludes that 47.5% and 50% protein levels may depress tilapia growth and feed utilization, while 40% protein gave optimum growth.*

**Keywords:** Genetically male tilapia, Dietary protein levels, Growth, Protein requirement

### INTRODUCTION

Aquaculture is an integral component of the overall agricultural production system in Nigeria. The country with hundreds of rivers and ponds is notable for being a fish-loving nation where fish plays an important role in the diets, constituting the main and often irreplaceable animal protein source in both urban and rural households (Otubusin, 2011). The major fish species cultured in Nigeria include catfishes, tilapia and carp. Tilapia is one of the most widely cultured fish in the world. Currently, farmed tilapia represents more than 75% of world tilapia production (FAO, 2013), and this contribution has been exponentially growing in recent years. Several factors have contributed to the rapid global growth of tilapia. Among these are: genetic improvement, ease of culture, highly adaptable to a wide range of environmental conditions (Ponzoni *et al.*, 2008). However, a major problem in tilapia culture is that females grow slower than males. Early

sexual maturation diverts energy from growth to reproduction and unwanted breeding results in overcrowding and competition. The most effective solution to this problem is to produce and grow only male fish. Researches have addressed this problem in an innovative way through the application of basic genetics, to develop a unique product in genetically male tilapia (GMT) (Mair *et al.*, 1997). The GMT so developed has proved to be excellent production fish in both extensive and intensive systems using ponds, raceways, cages and tanks (Ek Nath *et al.*, 2007). They are now in use in more than 20 countries around the world (Gupta and Acosta, 2004; Gupta *et al.*, 2004) including Nigeria. In formulating diet for fish it is important to meet all nutritional requirements since lack of quality feed for economic production adversely affects growth rate, disease manifestation and total harvest (Alatise *et al.*, 2006). Dietary protein is used by fish for growth, energy and body maintenance. Therefore, understanding protein requirement

for maximum growth of any species of fish is a step forward in developing cost-effective feed for fish farming and this has to do with determining the optimum amount required to produce maximum growth rate. Protein requirements of catfish had been widely reported (Jamabo and Alfred-Ockiya, 2008; Diyaware *et al.*, 2009). However, little information on nutritional research with respect to genetically male tilapia had been established. Therefore, the present study had been designed to determine protein requirement of rearing the YY-male tilapia for maximum production.

## MATERIALS AND METHODS

**Collection of Fish Sample:** The GMT fingerlings were procured from Durante Fisheries Industries, Ibadan, Oyo State, Nigeria. Fish were transported in oxygen bags to Department of Zoology, University of Uyo, Uyo, Akwa Ibom State, Nigeria where the experiment was conducted.

**Experimental Design:** A complete randomized block design (CRBD) consisting of five treatments (blocks) replicated thrice was used for the study. Five outdoor concrete tanks (8 x 5 x 1.65m<sup>3</sup>) at Vika Farms Limited, Mbak Etoi, Uyo, Akwa Ibom State, latitude 5° 3' North and longitude 7° 56' East was used. The experimental design had fifteen 1 x 1 x 1m<sup>3</sup> hapa placed on the concrete tanks at the rate of three hapa per tank. Each hapa was rigged and suspended to maintain a depth of 0.75m in water and a free board of 0.25m. The float lines were tied to the four corners of each compartment using kuralon rope (Number 15) as described by Otubusin (2000).

**Diets Preparation:** Diet compositions for the feeding trial are presented in Table 1. All ingredients were carefully weighed out, mixed, made into pellets using 2 mm meat mincer, air-dried and labelled separately according to diets. Proximate analysis was done on the dietary ingredients and the resultant experimental diet (AOAC, 2004).

**Fingerling Rearing:** Each hapa was randomly stocked with GMT (2.00 ± 0.01g) at 20 fish/1m<sup>3</sup> /hapa and raised for 24 weeks. The stocked fish were fed at 5% of their body weight three times daily. The stocked fish (20%) were sampled fortnightly. Fish weights were measured using a Furi Digital Balance (Model: FEJ-6000) to the nearest 0.1g.

**Growth Performance:** The following variables were calculated: Mean weight gain (MWG) (g) = final weight (g) – initial weight (g), Average daily growth (ADG) = MWG (g)/length of feeding trial (days), Specific growth rate (SGR, %/day) = 100(ln W<sub>2</sub> – lnW<sub>1</sub>)/T<sub>2</sub>-T<sub>1</sub>, Where: W<sub>2</sub> = Weight at time T<sub>2</sub>; W<sub>1</sub> = Weight at time T<sub>1</sub>, Feed conversion ratio (FCR) = Total dry feed fed (g)/MWG (g), Protein index (PI) = Survival (W<sub>1</sub>-W<sub>0</sub>)t and Percentage survival rate (%SR) = 100(number at end of feeding trial/number at start of feeding trial).

**Protein Requirement Determination:** The curvilinear plateau analysis between protein inclusion levels and selected growth parameters [average daily growth (ADG), specific growth rate (SGR) and protein index (PI)] was used to determine the nutritional requirement of GMT as described by Aksnes *et al.* (1996). The second degree polynomial analyses on the growth parameters were generated to give quadratic prediction equations for the best performing diet. Test of significance for the relationship between protein inclusion levels and ADG, SGR and PI was done and values of correlation coefficients (r) obtained were used to assess the relationship between protein inclusion levels and nutritional response of catfish.

**Proximate Analysis of Fish Carcass:** Proximate analysis of fish carcass was done according to standard AOAC method (AOAC, 2004). Moisture Content was done by oven-drying to a constant weight; Total ash by muffle furnace combustion; Crude fibre by trichloroacetic acid method; Lipid content by soxhlet extraction method; Protein by micro-kjeldahl method, Carbohydrate was calculated as difference obtained after subtracting moisture, total organic nitrogen (protein), ether

**Table 1: Proportion of dietary ingredients (% dry matter), cost and proximate composition of experimental diets fed to genetically male tilapia cultured in floating hapa system**

Ingredients (g)	40.0%	42.5%	45.0%	47.5%	50.0%
Fishmeal	172.10	186.00	200.00	215.30	230.60
Soyabean	172.00	186.00	200.00	215.30	230.60
Cornmeal	239.70	182.00	124.30	67.50	11.20
Groundnut cake	345.10	375.00	404.60	431.00	456.60
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30	0.30
Fish Premix*	0.50	0.50	0.50	0.50	0.50
Fish oil	70.00	70.00	70.00	70.00	70.00
<b>Cost</b>					
₦/kg	217.50	222.70	227.80	233.80	239.90
₹/kg	1.09	1.11	1.14	1.17	1.20
<b>Proximate Composition</b>					
Protein	38.83	40.05	43.43	45.01	48.25
Carbohydrate	27.84	25.98	21.54	18.99	15.39
Fat	8.38	8.40	8.64	8.75	8.87
Moisture	14.10	14.00	13.82	13.75	13.72
Ash	6.48	7.11	7.82	8.49	8.61
Fibre	4.72	4.74	4.75	4.76	4.78

\*Pfizer livestock product; 1kg fish premix contains: Vitamin A: 10,000,000 I.U.D; D3: 2,000,000 I.U.D; E: 23,000mg; K3: 2,000mg; B1: 3000mg; B2: 6,000mg; niacin: 50,000mg; calcium pathonate: 10,000mg; B6: 5000mg; B12: 25.0mg; folic acid: 1,000mg; biotin: 50.0mg; choline chloride: 400,000mg; manganese: 120,000mg; iron: 100,000mg; copper: 8,500mg; iodine: 1,500mg; cobalt: 300mg; selenium: 120mg; antioxidant: 120,000mg.

extract, ash and fibre from 100%. Caloric value was estimated based on physiological fuel values (0.2364 KJ/g for protein; 0.3954 kJ/g for lipid and 0.1715 kJ/g for carbohydrate) as described by (Henken *et al.*, 1986).

**Data Analysis:** Data analyses were carried out using Statistical Package for Social Sciences (SPSS 19.0, 2010 version).

## RESULTS

The results of growth performances and survival rates of GMT fed diets containing different protein levels for 24 weeks indicated that all the growth indices were highest in diet containing 40% protein. There were no significant differences ( $p > 0.05$ ) in food conversion ratio and survival rate in all the treatments. Fish fed the 40% protein diet had the best growth performance ( $p < 0.05$ ), with a mean weight gain of  $190.27 \pm 29$ g, average daily growth of

2.26g/day, specific growth rate of  $5.43 \pm 0.02\%$ /day and protein efficiency ratio of  $13.33 \pm 0.16$ . The least weight gain ( $156.07 \pm 1.7$ g) and poorest specific growth rate ( $5.20 \pm 0.02$ ) was observed in fish fed 50% protein diet (Table 2). Generally, there was an inverse relationship between protein level and growth performance of fish.

The results of proximate composition of fish carcass revealed inverse relation between protein content in diet and lipid content in fish muscle. However, linear relation existed between protein in diet and protein and gross energy contents in fish muscle (Table 3).

The results of the analyses between protein inclusion levels and average daily growth (ADG:  $y_{\max} = 2.26$ g/day at  $x_{\max} = 40\%$  protein). Similar results were obtained for specific growth rate (SGR:  $y_{\max} = 5.43\%$ /day at  $x_{\max} = 40\%$  protein) and protein index (PI:  $y_{\max} = 2.23$ kg/day at  $x_{\max} = 40\%$  protein). Results of the second degree polynomial analyses on these

**Table 2: Growth response of genetically male tilapia fed diets with varied crude protein levels for 24 weeks**

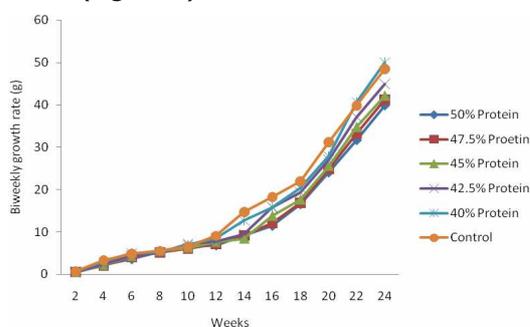
Variables	40%	42.5%	45%	47.5%	50%
<b>MWG</b>	190.27±2.9 <sup>e</sup>	171.8±0.5 <sup>d</sup>	166.6±0.2 <sup>c</sup>	160.93±1.3 <sup>b</sup>	156.07±1.7 <sup>a</sup>
<b>ADG</b>	2.26±0.03 <sup>c</sup>	2.04±0.02 <sup>b</sup>	1.96±0.01 <sup>b</sup>	1.92±0.01 <sup>b</sup>	1.86±0.02 <sup>a</sup>
<b>SGR</b>	5.43±0.02 <sup>c</sup>	5.32±0.01 <sup>b</sup>	5.30±0.01 <sup>b</sup>	5.3±0.01 <sup>b</sup>	5.20±0.01 <sup>a</sup>
<b>FCR</b>	0.14±0.00	0.14±0.00	0.14±0.00	0.13±0.00	0.14±0.00
<b>PER</b>	13.33±0.16 <sup>d</sup>	13.47±0.39 <sup>e</sup>	10.52±0.5 <sup>c</sup>	10.14±0.4 <sup>b</sup>	9.03±0.44 <sup>a</sup>
<b>PI</b>	4.71±0.03 <sup>b</sup>	5.05±0.17 <sup>c</sup>	4.32±0.30 <sup>a</sup>	4.13±0.19 <sup>a</sup>	3.97±0.18 <sup>a</sup>
<b>% SR</b>	100±0.00	100±0.00	100±0.00	93.33±1.67	100±0.00

**Key:** MWG = Mean weight gain (g), ADG = Average daily growth, SGR = Specific growth rate, FCR Feed conversion ratio, PER = protein efficiency ratio, PI = Protein index, %SR = Percentage survival rate

**Table 3: Proximate composition of the carcass of genetically male tilapia fed diets with varied crude protein levels for 24 weeks**

Variables	40%	42.5%	45%	47.5%	50%
<b>Crude protein</b>	15.17±0.16 <sup>a</sup>	15.87±0.43 <sup>b</sup>	16.49±0.01 <sup>c</sup>	16.85±0.16 <sup>d</sup>	17.55±0.15 <sup>e</sup>
<b>Crude lipid</b>	5.99±0.05 <sup>c</sup>	6.17±0.19 <sup>c</sup>	5.85±0.08 <sup>c</sup>	5.58±0.03 <sup>b</sup>	5.08±0.06 <sup>a</sup>
<b>Ash</b>	2.81±0.10 <sup>b</sup>	2.48±0.28 <sup>a</sup>	3.08±0.06 <sup>b</sup>	3.03±0.03 <sup>b</sup>	3.38±0.18 <sup>c</sup>
<b>Gross energy</b>	6.0±0.02 <sup>a</sup>	6.27±0.06 <sup>b</sup>	6.29±0.02 <sup>c</sup>	6.32±0.04 <sup>c</sup>	6.32±0.11 <sup>c</sup>

growth parameters gave quadratic prediction equations: ADG:  $y = -0.8148x^2 + 1.6477x + 0.234$ ; SGR:  $y = -0.1871x^2 + 1.0326x + 0.3412$ ; PI:  $y = -0.8328x^2 + 1.6739x + 0.4023$ . Test of significance for the relationship between protein inclusion levels and ADG, SGR and PI gave high correlation coefficients (ADG:  $r = 0.9549$ ; SGR:  $r = 0.9764$  and PI:  $r = 0.9586$  at  $p < 0.05$ ). This high correlation ( $r > 0.9$  at  $p < 0.05$ ) proved a strong relationship between protein inclusion level of 40% and growth performance of GMT. This showed that growth rate varied inversely with the amount of protein in diet. The biweekly growth curve indicated that fingerlings of GMT required 40% protein in the diet for optimum growth (Figure 1).

**Figure 1: Growth curve of genetically male tilapia fed diets containing different levels of protein over a 24-week growth trial**

## DISCUSSION

The whole body composition of genetically male tilapia was influenced ( $p < 0.05$ ) by dietary protein levels in diets. Fish fed 40% protein diet had lower carcass protein and higher carcass lipid than those fed 45%, 47.5% or 50% protein diets. These results were similar to those obtained by Al-Hafedh (1999). Ash content was unaffected by dietary protein levels and followed no particular trend. Similar result was presented by Khattab *et al.* (2000) who reported that ash content was unaffected by protein level in Nile tilapia collected from fish ponds. In this study, high protein level (50% protein) did not significantly enhance fish growth. These results were in agreement with Hamza and Kenawy (1997) who reported that 40% protein was more potent than other levels for Nile tilapia growth. Many authors obtained conflicting results from their studies on the effect of dietary protein level on the growth of Nile tilapia. Abdelghany (2000) reported that optimum dietary protein level for growth of Nile tilapia fry was 30% crude protein. Al-Hafedh (1999) concluded that better growth of Nile tilapia was obtained at dietary protein levels of between 40% and 45%. However, this study

revealed that GMT would grow optimally at 40% protein level. Food conversion ratio was not affected by protein levels. This trend was not in agreement with that obtained by (Khattab *et al.*, 2000). Protein efficiency ratio, productive value and index are commonly used as indicators of protein quantity, quality and amino acid balance in fish diet. These parameters were used to assess protein utilization and turnover, where they were related to dietary protein intake and its conversion into fish gain and protein gain. Results showed that PER, PPV and PI were significantly affected by protein level in diets. This indicated that protein utilization decreased with increasing dietary protein levels in diets. These results may be due to the fact that major part of weight gain is related to the deposition of protein, and the protein accretion is a balance between protein anabolism and catabolism. Furthermore, gastric emptying rate or solubility of the protein has been shown to affect the utilization of dietary protein (Epse *et al.*, 1999). The results of this study concluded that a diet containing 40% protein would be adequate and suitable for tilapia growth and optimum performance.

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