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Olele NF
Department of Fisheries, Delta State University, Asaba Campus, Nigeria.

Ikenga EE
Department of Fisheries, Delta State University, Asaba Campus, Nigeria.

Bratte L
Department of Animal Science, Delta State University, Asaba Campus, Nigeria.

Adaka GS
Department of Fisheries and Aquaculture Technology, Federal University of Technology, PMB 1526, Owerri, Imo State.

Correspondence
Olele NF
Department of Fisheries, Delta State University, Asaba Campus, Nigeria.

Effects of dietary protein levels and stocking densities on growth, and survival of monosex *Oreochromis niloticus*

Olele NF, Ikenga EE, Bratte L, Adaka GS

Abstract
Crude protein rations and stocking densities influence growth, and nutrient composition of fish, depending on environmental factors etc. 240 fish samples, with weight and size ranges between 10.01-12.11 g and 8.0-9.7cm respectively were hand-sexed. They were acclimated in 100 liters plastic bowl and fed compounded feed, 5% body weight, twice daily for 90 days. The experiment was 2 x 3 x 4 factorial. Data obtained revealed that the highest body weight (T_{C2} - 16.72±6.52) was obtained at 30% crude protein at a stocking density of 20 fish samples. The highest feed conversion ratio (T_{C3} -19.57±0.96) was obtained at 30% crude protein at a stocking density of 30 fish samples. The highest and lowest protein efficiency ratios were obtained at 35% crude protein diet (T_{D1} -1.76±0.48^{a}) and 30% crude protein diet (T_{C3}-0.35±0.12^{c}). Fish survival at dietary rations and stocking densities was 100%. The study indicates that *Oreochromis niloticus* fed 30% crude protein diet at a stocking density of 20 fish/100 liters bowl resulted in the highest growth.

Keywords: Dietary protein, stocking density, growth, survival, *Oreochromis niloticus*.

Introduction

Animal protein is the most essential and costliest component in fish feed composition, because it accounts for about 60% or more of the cost of fish feeds (Yassir, Mohsen, Mohamed, 2010) [21]. Optimal use of low-cost dietary protein is therefore necessary for cost-effective production of fish in aquaculture. The level of dietary protein resulting to maximum growth in tilapia species depends on protein quality, energy content of the diets, physiological state of the fish, size, their production state and condition of the environment (Yassir, Mohsen, Mohamed, 2010) [21]. In aquaculture, fish size and production are important to meet market demands as both factors determine the price the fish may be sold. Since increasing stocking density eliminates land shortage issue, it will be an important factor to be considered for growth performance (Yassir *et al*., 2010) [21]. *Oreochromis niloticus* is not only notable for its ability to mature at small size (8.0-10.0 cm) but also at a young age (that is when only three months old). (Kamal, Kurt, Michael, 2010) [7].

Commercial tilapia production generally requires the use of monosex (male) population. Mixed-sex population develops great size disparity among harvested fish, which affects market price (Mallya 2007) [8]. Moreover, the presence of female tilapia leads to uncontrolled reproduction, excessive recruitment of fingerings, competition for food, and stunting of the original stock, preventing them from reaching table size (Mohsen, Mohammad, Medhat, 2008) [11]. In mixed-sexed population of tilapia species, the weight and invariably the size of recruits may constitute up to 70 percent of the total harvest weight, hence they make minimal contribution to fisheries production for human consumption as a result of their stunted growth (Mohsen *et al*., 2008) [11].

The fact that much research has not been done to improve the size and weight of this favoured species of tilapia fingerlings in this part of the globe is the reason for this study. The experiment therefore evaluated the effects of protein rations and stocking densities on growth, and survival of monosex *Oreochromis niloticus*.

Materials and Methods

Collection and sexing of experimental fish

Fish samples were harvested from the Departmental Research Fish Farm at Musa Camp, Asaba Campus, Asaba. A total number of 240 hand-sexed *Oreochromis niloticus* having a body weight range of 0.01– 2.11g and total length range of 8.0 – 9.7cm was used for the study. Sexing was done twice by using a solution of 1g Methylene blue mixed in 0.5g of Sodium Chloride (NaCl) to ensure that all fish samples used for the study were monosex (Male). Sexed fish samples were transported to the project site beside Fishery Department at Delta State University, Asaba Campus.

Acclimation

Fish samples were acclimated for 7 days in twelve 100 litres bowls measuring 76 cm in diameter before the commencement of the experiment. Fish samples were fed with compounded diet morning and evening with plankton inclusion. The plankton used was cultured from the poultry droppings collected from the University poultry farm. The dropping were tied in 25 kg sack and introduced into 1000 liters tank filled with water to produce plankton for the experimental fish. From this 1000 liters tank containing grown plankton, 70 liters surface water samples was collected using a bowl to scoop surface water from the tank. Fish samples were randomly distributed in triplicate (1-3) at four crude protein rations labeled A-D. The four treatments were dietary crude protein rations of 20%, 25%, 30% and 35%. Each of these crude protein treatments had a stocking density of 10, 20, and 30.

Feed consumed and meristic characteristics

Fish samples were fed the various crude protein rations, 5% of their body weight with plankton inclusion. Bi weekly measurements of body weight (g) and total length (cm) were conducted throughout the 3 months of study, by using analytical weighing balance (Model- METLAR 200) and a calibrated meter rule respectively. Both measurements were recorded before the commencement of the experiment and during the experiment.

Growth parameter

Specific growth rate (SGR).

This was calculated from data on the changes of body weight over the given period.

$$SGR = \frac{100 \times \text{Ln (final body weight)} - \text{Ln (Initial body weight)}}{\text{Rearing period in days}}$$

Where:

Ln = natural logarithm. (Solomon and Boro, 2010) [19]

Feed utilization

Feed Conversion Ratio (FCR)

This was calculated according to Akinwole and Faturoti (2006) [1] thus;

$$FCR = \frac{\text{Feed intake}}{\text{Weight gain}}$$

Protein Efficiency Ratio (PER) - This was calculated according to Mohsen (2012) [10] thus;

$$PER = \frac{\text{Weight gain}}{\text{Protein intake}}$$

Survival Rate (SR)-

The survival rate, SR was calculated as total fish number harvested/total fish number stocked expressed in percentage according to Akinowole and Faturoti, (2006) [1] thus

$$SR = \frac{\text{Total fish number harvest}}{\text{Total fish number stocked}} \times \frac{100}{1}$$

Determination of water quality parameters

Some water quality parameters such as Hydrogen ion Concentration (pH), Dissolve Oxygen (DO), and Temperature, were monitored throughout the experiment. The pH was determined with a pH meter, model PHS-25, Techmel and Techmel USA. Dissolved oxygen was conducted with the use of dissolve oxygen meter, Msodel-Labtech. Temperature was determined using temperature meter (Model- 611-R).

Proximate analysis of feed

Feed from each treatment were chemically analyzed according to Association of Analytical Chemist (AOAC, 1990) [2] for protein, fat, ash, and moisture contents. Crude protein was estimated using a kjedahl apparatus and multiplying nitrogen content by a constance, 4.37. Total lipid content was determined by ether extraction for 7 hours at 70^0C. Ash content was determined by combusting samples in a furnace at 500^0C for 30 minutes. Moisture content was estimated by heating samples in an oven for 24 hours at 105 ^{0}C.

Statistical Analysis and Experimental Design

Data collected were analysed using SPSS version 20 of (2011) Statistical Package. Significantly different means were separated by Duncan's Multiple Range Test using the same package. The experiment was a 2 by 3 by 4 factorial with dietary protein at four inclusion rations (20%, 25%, 30%, and 35%), and stocking density in triplicates (of 10, 20, and 30) per 100 liters of water.

Results

Effect of dietary protein rations (with plankton inclusion) on body weight and total length of *Oreochromis niloticus*

The effect of dietary protein levels on body weight and total length of *Oreochromis niloticus* is presented in Table 2. Body weight was significantly different amongst the treatments. The highest body weight for all treatment was 13.48±4.62 (as recorded in T_C, fed 30% crude protein). Total length was also significantly different; as the highest total length recorded was 10.96±1.97 (in treatment T_C fed 30% crude protein).

Effect of stocking density on body weight and total length of *Oreochromis niloticus*

The effect of stocking density on body weight and total length of *Oreochromis niloticus* is shown in Table 3. Values in the table revealed high significant ($P<0.01$) differences in the body weight of fish at different stocking densities. The highest value in body weight (12.77±4.30) was recorded at a stocking density of 20 fish/100 liters of water, while the least (11.26±1.75) was recorded at a stocking density of 30 fish/100 liters of water. Also, there was a significant difference in the total length of fish at different stocking densities. The total length value at a stocking density of 20 fish (10.69±1.76) was significantly different from those at stocking densities of 10 and 30 fish/100 liters of water (10.46±1.09 and 10.43±0.89 respectively). The total length of fish at stocking densities of 10 and 30 fish samples were however not significantly different from each other.

Growth parameter, feed utilization and survival rate

Table 4 revealed that the highest specific growth rate (SGR) value (2.67±0.39) was recorded at treatment C_2 while the least (0.21±0.13) was recorded in treatment A_1. The food conversion ratio (FCR) revealed that there were significant differences among the various treatments. The highest feed conversion ratio (19.57±0.96) was observed in treatment C_3 while the least was recorded in treatment A_1 (7.11±0.21). Also the highest protein efficiency ratio (PER) was obtained at treatment D_1 (1.76±0.48) while the least value was obtained at treatment C_3 (0.35±0.12) (Table 4). The survival rate (SR) varied positively amongst various treatments. The mortality rate was very minimal amongst treatments (Table 4) where 100% survival was observed in treatments A_1, B_1, C_1, D_l, C_2, B_3, C_3 while the least survival was recorded in treatment A_2 (93.33±3.33) Table 4.

Water quality parameters

The monthly mean results of water quality parameters monitored during the course of the experiment are shown in Table 5. The table revealed that Dissolved Oxygen ranged between 5.9-6.9mg/l. Temperature ranged between 25.6 ^{0}C-27.7 ^{0}C, and pH ranged between 6.1-6.9 throughout the duration of the study.

Proximate composition of randomly selected samples from each treatment (A-D)

The proximate composition of randomly selected fish from treatments A-D (fed 20%, 25%, 30%, 35% crude protein rations) is presented in Table 6.

Treatments C and D had crude protein values of 56.57±1.14^a and 56.22±2.00^a respectively which was not significantly different. Also treatments A and B had crude protein levels of 54.12±1.15^b and 53.98±1.14^b respectively, which was slightly different although the difference was not statistically significant. Sample B had the least crude protein value (53.98±1.14) which was not significantly different from treatment B (53.98±1.14). However treatments C and D were significantly different from treatments A and B. The fat content was not significantly different in all treatments. Treatment A had the least composition of ash (2.98±0.33) while other treatments (B-D), although higher in fat content, were not significantly different from each other. The crude fiber composition for all treatments was not significantly different from each other. For moisture content, treatment C (12.06±0.35) was significantly different from those of treatments (A=11.98±1.18; B= 11.78±1.05 and D= 11.96±0.88).

Table 1: Feed composition at various crude protein inclusions

	A	B	C	D
Feed Ingredients	**20% CP**	**25% CP**	**30% CP**	**35% CP**
Fishmeal	8.1	11.7	15.3	18.9
Groundnut cake(GNC)	16.1	23.3	30.5	37.7
Maize	68.3	57.5	46.7	35.9
Premix	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5
Oil	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5
Starch (Iso-caloric)	3.5	3.5	3.5	3.5

Key: Treatments A-D are the different treatment options.

Table 2: Effect of dietary protein rations (with plankton inclusion) on body weight and total length of *Oreochromis niloticus*

Dietary crude protein levels (%)				
Parameters	**(T_A) 20**	**(T_B) 25**	**(T_C) 30**	**(T_D) 35**
Body weight (g)	10.84±2.02^d	11.35±1.36^c	13.48±4.62^a	11.98±1.65^b
Total length (cm)	10.22±1.01^c	10.32±0.85^c	10.96±1.97^a	10.56±0.72^b

Means with same superscripts on the horizontal row are not significantly different (P<0.01).

Key: T_A = Treatment A, T_B = Treatment B, T_C = Treatment C, T_D =Treatment D.

Table 3: Effect of stocking density on body weight and total length of *Oreochromis niloticus*

Stocking densities/100L water			
Parameters	**10**	**20**	**30**
Body weight (g)	12.25±1.63^b	12.77±4.30^a	11.26±1.75^c
Total length (cm)	10.46±1.09^b	10.69±1.76^a	10.43±0.89^b

Means with same superscripts on the horizontal row are not significantly different (P<0.01).

Table 4: Mean production parameters of *Oreochromis niloticus* for all treatments at different stocking densities.

	TRTS	Stocking Densities	SGR	SR	FCR	PER
20% CP	A_1	10	0.21±0.13^c	100.0±0.00^a	7.11±0.21^c	0.53±1.13^c
25% CP	B_1	10	1.57±0.38^a	100.0±0.00^a	9.20±0.17^b	0.75±0.20^c
30% CP	C_1	10	0.91±0.64^b	100.0±0.00^a	10.20±0.71^a	1.38±0.24^b
35% CP	D_1	10	0.31±0.27^c	100.0±0.00^a	10.11±0.22^a	1.76±0.48^a
20% CP	A_2	20	0.49±0.29^c	93.33±33.3^b	12.10±0.22^c	0.38±0.99^b
25% CP	B_2	20	1.26±0.14^b	96.66±1.67^b	13.17±0.11^b	0.82±0.16^a
30% CP	C_2	20	2.77±0.39^a	100.0±0.00^a	17.51±0.33^a	0.44±0.15^b
35% CP	D_2	20	0.54±0.35^c	96.66±1.66^b	13.11±0.25^b	0.44±0.15^b
20% CP	A_3	30	0.29±0.16^c	96.66±3.33^b	13.29±0.57^c	0.73±1.09^b
25% CP	B_3	30	1.19±0.65^b	100.0±0.00^a	15.02±0.51^b	0.90±0.25^a
30% CP	C_3	30	2.67±0.43^a	100.0±0.00^a	19.57±0.96^a	0.35±0.12^c
35% CP	D_3	30	0.58±0.39^c	98.61±0.51^b	14.53±0.91^c	0.69±0.16^b

Mean with same superscript on the vertical row are not significantly different at (P<0.01)

Key: Where SGR: Specific Growth Rate, SR: Survival Rate, FCR: Feed Conversion Ratio, PER- Protein Efficiency Ratio, and ± Standard error of the mean.

Table 5: Data on water quality parameters

Dissolved Oxygen (mg/l)												
Crude protein level	20% (A)			25% (B)			30% (C.)			35% (D)		
Stocking Density	(10)	(20)	(30)	(10)	(20)	(30)	(10)	(20)	(30)	(10)	(20)	(30)
Month 1	6.7	6.5	6.3	6.1	5.9	6.2	6.2	6.2	6.1	6.3	6.8	6.2
Month 2	6.6	6.3	6.4	6.2	6.3	6.3	6.0	5.9	6.1	6.4	6.3	6.2
Month 3	6.8	6.9	6.9	6.6	6.6	6.5	6.3	6.1	6.1	5.8	6.5	6.2

Temperature (°C)												
Crude protein level	20% (A)			25% (B)			30% (C.)			35% (D)		
Stocking Density	(10)	(20)	(30)	(10)	(20)	(30)	(10)	(20)	(30)	(10)	(20)	(30)
Month 1	25.9	26.7	26.9	26.3	26.8	26.1	27.6	26.9	26.9	26.4	26.1	26.5
Month 2	27.7	26.8	26.8	27.5	28.0	27.5	28.2	25.6	25.9	26.9	27.1	27.2
Month 3	27.4	27.7	27.1	26.9	26.6	26.7	27.1	26.5	26.8	26.8	27.1	25.8

pH (mg/l)												
Crude protein level	20% (A)			25% (B)			30% (C.)			35% (D)		
Stocking Density	(10)	(20)	(30)	(10)	(20)	(30)	(10)	(20)	(30)	(10)	(20)	(30)
Month 1	6.3	6.8	6.5	6.4	6.9	6.7	6.6	6.6	6.6	6.3	6.3	6.2
Month 2	6.5	6.5	6.3	6.3	6.2	6.1	6.7	6.1	6.4	6.5	6.3	6.3
Month 3	6.6	6.7	6.6	6.9	6.3	6.7	6.6	6.2	6.8	6.5	6.6	6.4

Table 6: Proximate composition of randomly selected fish from each treatment (A-D)

Sample	Crude Protein	Fat	Ash	Crude Fibre	Moisture	NFE
A	$54.12±1.15^b$	$16.35±1.52^a$	$2.98±0.33^b$	$1.08±0.33^a$	$11.98±1.18^b$	26.97
B	$53.98±1.14^b$	$15.78±0.88^a$	$3.15±0.34^a$	$1.10±0.33^a$	$11.78±1.05^b$	25.99
C	$56.57±1.14^a$	$16.38±0.87^a$	$3.25±0.33^a$	$1.09±0.32^a$	$12.06±0.35^a$	22.71
D	$56.22±2.00^a$	$16.48t1.31^a$	$3.19±0.57^a$	$1.15t1.52^a$	$11.96±0.88^b$	22.71

Treatments with same superscript on the vertical row are not significantly different ($P<0.05$). NFE (nitrogen free extract) = 100 – (protein + lipid + ash + fiber).

Discussion

Optimum protein level and stocking density

Dietary protein rations have often been implicated and considered of primary importance in fish feeding, thus sufficient supply of dietary protein is needed for rapid growth (Mohsen *et al*., 2008) [11]. Intensive culture of tilapia species require the formulation of efficient food with optimum protein rations and energy input, to meet their requirements during grow-out period (Yassir *et al*., 2010) [21]. As tilapia culture continues to expand worldwide, there is a growing pressure to minimize production cost associated with diet supplementation. Optimization of diet rations and stocking density are two mechanisms that could be enforced, if both goals are to be met (Mohsen *et al*., 2008) [11]. Particular attention has been paid to stocking density as one of the key factors to influence the perceived level of stress in fish. Stocking density is an important aspect of fish culture, and it is necessary to find a balance between the maximum profit and the minimum incidence of behavioral disorder (Mohsen 2012) [10]. It has been demonstrated that rearing fish at inappropriate stocking densities may impair their growth and reduce their immune competence, due to factors such as clustering, stress and the deterioration of water quality. These factors affect feed intake and conversion efficiency of the fish (Ellis, North, Scott, Bromage, Porter, and Gadd. 2002) [4]. Understanding the protein requirement of Nile tilapia in relation to its stocking density may help to curb stress and enhance growth. Protein inclusion represents about 60% or more of the cost of fish feeds (Yassir *et al*., 2010) [21]. Thus, optimal utilization of dietary protein is essential for economical production.

Previous literatures have showed the effect of dietary protein levels on the growth of Nile tilapia at different locations with conflicting results. According to Mohsen *et al*., 2008 [9], the dietary protein requirements for several species of tilapia ranged between 20% and 56%. While this researcher maintained that 35% resulted to the best growth performance, Mohammed (2009) [9] reported a value of 30% (for 160 fish/tank) for the same reason. Yassir *et al*., (2010) [21] opined that a diet of 45% crude protein at a stocking density of 15 fish/100 liters may be more potent than other levels for Nile tilapia growth. Based on a previous studies, Balarin and Halfer (1982) [3] made a generalized conclusion on this fish species that fish weight of 5-25 g required diet with 25-35% crude protein. It was observed in this study that there was significant increase in body weight in treatment C_2 ($16.72±6.52^a$), fed 30% crude protein at a stocking density of 20. Mohammed (2009) [9], reported that the best growth was realized at a diet containing 30% crude protein for Nile tilapia at a stocking density of 160 fish per tank. The result of this study is in contrast with that reported by Yassir *et al*., (2010) [21] where 45% crude protein at a stocking density of 15 fish per100 liters resulted in the best growth. Ghazalah *et al*., (2010) [6] also reported values of 34-36% crude protein for the same fish species. EI-Sayed (2002) [5], reported a value of 30% crude protein for five (5) fries resulting in the best growth performance of the same fish species. Mohsen (2012) [11] reported that the best growth performance of Nile tilapia was obtained when the fish were reared at a stocking density of 150 fish/m^3 and fed on 45% crude protein diet. Studies concerning the relationship between stocking density and growth of tilapia have shown that optimal stocking density for obtaining the highest possible fish yields depended on the rearing capacity, quantity and quality of food available (Omar *et al*., 2005) [15].

In this study, rations containing 20, 25 and 35% crude protein (when compared to 30% crude protein), led to poor and reduced growth performance. Since tilapia is herbivores, the plankton inclusion would have aided their growth.

Conclusion and Recommendation

The overall results in this study indicates that Nile tilapia, which fed on 30% CP diet (at a density of 20 samples/100 liter bowl) resulted to the best growth performance. The increased stocking density and the introduction of natural food (plankton) for this type of fish may have aided their growth performance. Hence, samples fed 30% CP ration could cope with stress induced by increased stocking density.

It is recommended that fish farmers adopt the use of 30% crude protein at a stocking density of 20 fingerlings.

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