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Effects of stocking density and feeding levels on growth and survival of Nile tilapia (*Oreochromis niloticus* L.) fry reared in an earthen pond, Khartoum, Sudan.

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Abstract

The present study was conducted over a 40 days period based on a 3 × 3 factorial design with three rearing densities (*D*1 = 100, *D*2 = 200, and *D*3 = 300 fish/m^3) and three levels of feeding rates (25%, 30%, and 35% of body weight) using feed having 36.8% crude protein (CP) to ascertain the effects of stocking density and feeding levels on the growth and survival of fry *Oreochromis niloticus L.* cultured in hapas in an earthen pond. Fish of average initial weight 1.1 g ± 0.03 were stocked at three different rates: 6, 12 and 18 fish per hapa (30×40×50cm about 60 litre each). Fish of each density were fed on the above experimental diets till satiation twice daily, 6 days a week, for 6 weeks. The growth parameters were inversely affected by stocking density and feeding level with positive effect on their interaction. The average final weights varied significantly (P < 0.05) and varied at *D*1 from 2.30 to 4.23 g, at *D*2 from 2.80 to 2.97 g and *D*3 from 2.63 to 2.90 g. Fish stocked at 100/ m3 with feeding level 25% exhibited the highest average weight gain (3.17 ± 0.03 g) whiles; fish stocked at 300/ m3 with feeding level 35% recorded the lowest average weight gain (1.60 ± 0.07 g). The lowest feeding rate with lowest stocking density recorded the best feed conversion ratio, protein efficiency ratio and relative growth rate, with significant differences. Condition factor and fish survival in different experimental groups were significantly affected by rearing densities and protein levels. The overall results recorded here reveal that the best growth performance of *Oreochromis niloticus* was obtained when the fish fed on the 25% of body weight diet and when reared at a stocking density of 100 fish/ m^3.

Keywords: Growth rate; Feed conversion ratio; Weight gain; Condition factor.

Introduction:

In the view of cultured fish species, tilapia is one of the most commercially important and widely used fish in the global aquaculture production particularly in tropical and subtropical areas (Gjedrem, 2005; El-Sayed, 2006). *Oreochromis niloticus* is by far the most important tilapia species and distributed throughout the world (El-Sayed, 2006). The importance of *Oreochromis niloticus* seams from its biological reasons: fast growth, high food conversion ratio, readily accepting artificial feeds, ease of breeding in captivity, disease resistance, high fecundity; social reasons: good table food quality, good market price; and physical reasons: tolerant to a wide range of environmental conditions (El-Sayed, 2006; Ashagrie *et al.*, 2008). Stocking density is an important indicator that determines the economic viability of the production system (Aksungur *et al*., 2007). Therefore, Particular attention has been paid to stocking density as one of the key factors to influence the perceived level of stress in fish (Ellis *et al*. 2002). Knowing the best densities for a species is a critical factor for good husbandry practices and creating efficient culture systems. It has been demonstrated that rearing fish at inappropriate stocking densities may impair the growth and reduce immune competence due to factors such as clustering stress and the deterioration of water quality, which can affect both the feed intake and conversion efficiency of the fish (Ellis *et al*. 2002).

Protein is the main constituent of the fish body thus sufficient dietary supply is needed for optimum growth. It is the most expensive macronutrient in fish diet (Pillay, 1990). So, the amount of protein in the diet should be just enough for fish growth where the excess protein in fish diets may be wasteful and cause diets unnecessarily expensive (Ahmad, 2000). Understanding the protein requirement of Nile tilapia in relation to its rearing density may lead to the coping with the stress caused and enhancing their growth and production. The objectives of this study are to investigate the effects of stocking density and feeding levels on the Growth, survival, feed conversion ratio (FCR) and body composition of Nile tilapia fry.

Materials and Methods

Experimental site:

The present study was carried out at Khartoum State Fish Hatchery, General Department of Fisheries and Water Resources, Sudan. The hatchery center is situated to northwest direction of Khartoum, the capital city of Sudan at 25 km distance. The mean annual temperature of the area is 36.4°C.

Fish and culture facilities

Three groups in triplicate hapas of healthy fish of mixed-sex Nile tilapia, *Oreochromis niloticus L.* fries used in the present study were obtained from pond at Khartoum State Fish Hatchery, General Department of Fisheries and Water Resources, Sudan.

Polyethylene bags containing approximately 3 litres of water were used to transport about 50 individuals of *Oreochromis niloticus* fingerlings from hatchery to the pond, provided with pure oxygen using oxygen cylinder. At the study pond, the polyethylene bag containing the fish was immersed into the pond for about 20 minutes to acclimatize the fish to the new water condition. Moreover, the polyethylene bag was tied off and allowed a flow of water into the bag to make an equilibrium condition, and then the fish were counted and immersed into hapas with scoop net. The fish were let in hapas for three days until they become more active and stopped mass mortality due to stress during transportation. All hapas was covered with mosquito net to protect them from birds.

At the ends of the conditioning period, the fish were netted, weighed collectively, and the average initial weight was recorded. The fingerlings were reared for 40 days (26 April 2013 to 5 June 2013). Water quality parameters including temperature, dissolved oxygen (DO), pH, conductivity, nitrates (NO_3-N), ammonia (NH_4-N) and turbidity were monitored weekly.

Experimental design

The experiment designed to study the effects of stocking density and feeding levels on fish survival, growth rates, feed utilization efficiency and body composition. (324) fingerlings (1.1g average weight) stocked in (27) rearing hapas in three groups (D_1, D_2 and D_3) each group have nine hapas (30×40×50cm about 60 litre each), mounted in a fertilized earthen pond of dimensions 75 × 25× 1.2 m with the aid of nylon ropes tied to the edges of the hapas, stretched and fastened to sticks placed at vantage points. The fingerlings were stocked at three stocking densities (100, 200 and 300 fish/m^3) corresponding to (6, 12, and 18 fish per hapa, each triplicate) and fed with locally available ingredients feed having

36.8% crude proteins at three feeding levels (25, 30 and 35% BW day-[1]) manually, twice a day at (10:00am, and 16:00pm) for 40 days. Fish were weighed collectively at 10-day intervals, and their average weights recorded. Feeding and feed supplements The experimental diet was formulated from local materials based on the use of fish meal, peanut meal, Wheat bran, and Dura flour (Table 2). Experimental diets were fed as a powder spread on centre of each hapa by a small plate to avoid feeding competition with exterior fish. The feeding rate was (25, 30 and 35 %) of the body weight of the fish per day throughout the experiment. The nutritional composition of the diet used for the experiment was 94.5 dry matter (DM) which contains 36.8 crude protein (CP), 9.2 crude fiber (CF), 7.0 ether extract (EE), 28.08 nitrogen-free extract (NFE) and 15.9 ash table (2). The amount of the feed was adjusted once in 10days interval based on the body weight of the fish. Thus the amount of daily supplementary feed (DSF) was calculated using the average body weight (ABW), the total number of the fish (N) and the feeding rate per day (FR /d) using DSF = ABW × N × FR /d.

Table 1: Ingredients and proximate analysis of the experimental diets

Feed ingredients	Amount in feed (%)
Fish meal	33.4
Peanut meal	33.4
Wheat bran	15.6
Dura flour	15.6
Starch	2.0
Total	100
Proximate analysis	
Dry matter (DM)	94.50
Crude protein (CP)	36.83
Crude fibre (CF)	12.20
Ether extract (EE)	7.00
Ash	15.89
Nitrogen-free extract (NFE)	28.08

* Nitrogen free extract (NFE) + crude fibre (CF) = 100 - (% (CP) + % (EE) + % ash).

Water quality parameters

During the experiment, water quality parameters were measured biweekly. Temperature was measured using digital thermometer, dissolved oxygen (DO) concentration with dissolved oxygen test kit (BIOTECH), pH was measured using HI96107 pH meter (HANNA), conductivity with the CD601 conductivity meter (Milwaukee), and nitrates (NO3-N) and ammonia (NH4-N) using test kits (API). Aquarium Pharmaceuticals, turbidity was measured with Secchi Disc

Sampling of fish and measuring of growth parameters

About 30% of fish from each hapa were randomly sampled every 10days with scoop net for their body weight and body length measurement. Length and weight of the fish were measured using ruler and digital weight balance (Lutron GM-600.0g×0.1g), respectively. Mortality of the fish was also recorded throughout the experiment.

Growth rates

At the ends of the experiment, all fry in each hapa were netted, weighed and their individual lengths recorded, and finally frozen for final body composition analyses. The fry was sent to Fisheries Laboratory, Department of Fisheries Science and Wildlife (DFSW), College of Veterinary Medicine and Animal Production (CVMAP), Sudan University of Science and Technology (SUST). Initial body analyses were performed on a pooled sample of 50 gram fingerlings, which was frozen prior to the study.

Growth performance was determined and feed utilization was calculated as described by Sveier *et al.* (2000) as follows:
Weight gain = W2 – W1 Where: W1 and W2 are the initial and final fish weight, respectively. Specific growth rate was computed as (SGR): $SGR = \frac{\ln W2 - \ln W1}{T} \times 100$

Where: W1 = initial weight (g) at stocking, W2 = final weight (g) at the end of experiment, InW2 – InW1 = natural logarithms of both the final and initial weight of fish, T = duration (in days) of trial (Ogunji *et al.*, 2008; Effiong et al., 2009).
Relative growth rate (RGR): RGR (%) = [(Wf – Wi) / Wi] x 100
Where Wf = Final average weight at the end of the experiment and Wi = Initial average weight at the beginning of the experiment. Feed conversion ratio (FCR) = Feed intake / Weight gain Protein efficiency ratio (PER) = Weight gain / Protein intake
Survival of the fish was monitored to determine the survival rate (SR) of various treatments using the formula (Ogunji *et al.*, 2008):

$$SR\% = \frac{\text{Initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish stocked}} \times 100$$

Condition factor (k) =100 (Wt/L3). Where Wt is fish body weight (g), L is total length (cm) according to Hengsawat *et al.* (1997).

Statistical analysis

The data obtained were subjected to two-way analysis of variance (ANOVA) to test the effect of feeding levels and rearing densities as Factorial in Complete Randomized Design. Duncan's multiple range tests was used to compare between means at P≤0.05 using Sigma Stat Program version 3.5.

Results and discussion Growth

performance and survival:

Averages of final body weight and body length were affected by stocking density and feeding level of Nile tilapia fry as illustrated in (Table 3). Analysis of variance showed significant differences between all treatments for average of final body weight of *Oreochromis niloticus* fry. From the data presented it can be seen that increasing feeding rate from 25 to 35% BW day^{-1} and increasing stocking density from 100 to 200 fish / m^3 resulted in a significant (P<0.05) reduction in growth performance. However, analyses of variance on the interaction between feeding level and stocking density per hapa were statistically insignificant (P<0.05). In many cultivated fish species, growth and feed utilization are inversely related to rearing density, and this is mainly attributed to social interactions such as competition for food and/or space that can negatively affect fish growth (Canario *et al.* 1998). Similar results for Nile tilapia were obtained by (Huang and Chiu, 1997), (El-Sayed, 2006), and (Ayyat *et al.*, 2011) who found that the increase of stocking density inversely affected the growth of Nile tilapia. Ridha, (2006) reported that a density of 200 fish /m^3 significantly decreased the growth performance of Nile tilapia compared with a density of 125 fish /m^3. Similarly, (Aksungur *et al.*, 2007) explained that, higher stocking densities leads to increased stress resulting in increased energy requirements and causing a reduction in growth rates and food utilization. On the contrary, our findings does not support (Osofero *et al.*, 2009) who reported increased fish production with increased stocking density, attributed to good feed quality and favorable physico-chemical conditions.
Dietary protein is always considered to be of primary importance in fish feeding (Jauncey and Ross, 1982), thus sufficient supply of dietary protein is needed for rapid growth. In the present study, results revealed that the optimum feeding level for Nile tilapia fry reared at *D*1, *D*2 and *D*3 was 25%. Similarly, Magouz (1990) reported that the dietary protein level for *O. niloticus* could be reduced from 40% to 33% without any significant effect on

growth of fish. On the other hand Santiago *et al*. (1982) reported that the optimum dietary crude protein level for *O. niloticus* fry was between 35 to 40%.

These results disagree with Abdel-Tawwab *et al*. (2010) who found that the best growth of Nile tilapia fry was obtained at a high dietary protein level (45%) rather than 25% or 35% protein.

The specific growth rate (SGR) obtained in this study ranged from 2.08 to 3.45% with insignificant (P > 0.05) differences among the trails suggesting that higher stocking densities of *O. niloticus* have apparent effect on the growth of the fish. The results of SGR in this study are higher than those obtained in other studies. For instance, Iluyemi *et al*. (2010) reported SGR of range 0.77 to 1.49% and that of Attipoe *et al*. (2009) with SGR range 0.43 to 0.53.

The condition factor and survival rates were affected significantly by the rearing density, suggesting that there was a competition for space. Moreover, the low survival rate of Nile tilapia at high density indicates that stocking density inversely affected the growth of

Oreochromis niloticus. These results are the same as report by Osofero *et al*. (2009) in a study on the effects of stocking density on growth and survival of *O. niloticus*, they found out an inverse relationship between survival rate and stocking density.

Table 2: Growth parameters and survival of *Oreochromis niloticus* affected by feeding levels and rearing density:

Stocking density	Feeding level	WG (g)	DWG (g)	SGR (%)	SR (%)	K	Initial (g)	Final (g)
25	6	3.17	0.09	3.45	98.15	1.39	1.07	4.23
	12	1.90	0.06	2.84	83.33	1.68	0.90	2.80
	18	1.90	0.06	2.66	83.33	1.74	1.00	2.90
30	6	2.00	0.06	2.45	75.00	1.41	1.20	3.20
	12	1.93	0.06	2.65	80.55	1.61	1.03	2.97
	18	1.70	0.05	2.34	88.89	1.99	1.10	2.80
35	6	1.30	0.04	2.08	88.89	1.54	1.00	2.30
	12	1.80	0.05	2.57	75.00	1.68	1.00	2.80
	18	1.60	0.05	2.33	84.26	1.74	1.03	2.63
Main effects								
Feeding level	25	2.22±0.09a	0.07±0.03a	2.81±0.09a	87.65±3.1a	1.58±0.07a		
	30	2.07±0.09b	0.06±0.03a	2.76±0.09a	76.85±3.1b	1.69±0.07b		
	35	1.88±0.09b	0.05±0.03b	2.45±0.09b	71.29±3.1b	1.71±0.07b		
	sig	0.019	0.019	0.023	0.005	0.415		
Stocking density	6	2.59±0.09a	0.08±0.07a	3.04±0.09a	79.01±3.1a	1.58±0.07a		
	12	1.76±0.09b	0.05±0.07b	2.47±0.09b	77.78±3.1a	1.75±0.07b		
	18	1.78±0.09b	0.05±0.07b	2.52±0.09b	79.01±3.1a	1.65±0.07a		
	Sig	0.001	0.001	0.001	0.948	0.293		
interaction	sig	0.013	0.013	0.048	0.069	0.045		

(WG): Weigh Gain, (DWG): Daily Weight Gain, (SGR): Specific Growth Rate, (SR): Survival Rate and (k): Condition factor.

Feed utilization:

FCR recorded here was significantly affected by rearing density and feeding levels and their interaction. The optimum feed utilization for Nile tilapia was obtained at a 25% BW day-^{1} diet under lower density, as shown in (Table 4). Abdel-Tawwab *et al*. (2010) reported that feed intake increased and FCR decreased with increasing the dietary protein level. The affected feed utilization in the present study is expected; as the number of fish stocked in an aquarium increases, the amount of feed available to each fish decreases (Chang, 1988). The FCRs (3.03 to 6.20) in the current study though high, because it is slightly higher than the recommended FCR of 1.5 for aquaculture (Stickney, 1979), they are lower for *O. niloticus* fed on a commercially prepared diet in a study by Siddiqui *et al*. (1991) who reported FCR values ranging from 3.7 to 4.9. The slight differences could stem from the differences in feed sources, environmental conditions and the particular strain of species used. This explanation is in agreement with Guimaraes *et al*. (2008) that efficient utilization of diets may vary even within a single species because of the particular strain of fish used and the environmental factors. Ali and Al-Asgall (2001) reported that elevated crude fiber (beyond 10.5%) content for fish diets may exert a negative effect on the digestibility of nutrients. In this study, the higher FCR recorded could be attributed to the higher crude fibre content (12.20%) of the feed.

The protein efficiency ratio (PER) decreased with increasing dietary protein levels and fish density. The maximum PER values were obtained when fish at lower density were fed on 25% BW day-^{1} diet. These results may have been obtained because weight gain is related to the deposition of protein, and protein accretion is a balance between protein anabolism and catabolism. In this concern, (Abdel-Tawwab *et al*. 2010) found that PER decreased with increasing dietary protein content. Moreover, fish density could affect the efficiency of feed utilization; as the number of fish stocked in a pond increases, the amount of feed available to each fish decreases (Chang 1988).

Table 3: Feed utilization of *Oreochromis niloticus* affected by feeding levels and rearing density

Feeding levels	Stocking Densities	Feed Utilization Parameters		
		FCR	**PER**	**RGR**
	6	3.03	0.09	298.18
25%	12	4.10	0.05	211.11
	18	4.20	0.05	190.00
	6	4.10	0.05	166.67
30%	12	4.13	0.05	189.26
	18	6.20	0.05	154.55
	6	6.20	0.04	130.00
35%	12	6.10	0.05	180.00
	18	6.17	0.04	154.85
Main effect				
Feeding level	25	4.44±0.04a	0.06±0.003a	214.1±10.5a
	30	5.52±0.05b	0.07±0.003a	203.8±10.5b
	35	5.54±0.04c	0.05±0.003b	168.1±10.5c
	Sig	0.001	0.019	0.017
Stocking density	6	4.20±0.04a	0.07±0.003a	242.1±10.5a
	12	5.13±0.04b	0.05±0.003b	170.1±10.5b
	18	6.18±0.04c	0.05±0.003b	174.3±10.5b
	Sig	0.001	0.001	0.001
Interaction	sig	0.01	0.013	0.031

(FCR): Feed Conversion Ratio, (PER) Protein Efficiency Ratio and (RGR): Relative Growth Rate.

Conclusion:

In conclusion, the overall results of this study demonstrated that increasing rearing density and feeding level in fingerlings of *O. niloticus* resulted in heterogeneous growth rates. Feed conversion ratios, specific growth rate and survival rates were affected by stocking densities and feeding level in this study.

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الكثافة التخزينية ومستوى التغذية و أثرهما على نمـو وحياتية يرقات أسماك البلطي النيلي المستزرعة في حوض ترابي ، الخرطوم ، السودان

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المستخلص :

أجريت الدراسة لمدة 40 يوماً إعتماداً على طريقة تعدد العوامل (3×3) بواسطة ثلاثة كثافات تخزينية (ك1=100 ، ك2=200 ، ك3=300 سمكة/م^3) وثلاثة معدلات تغذية (25% ، 30% ، 35% من وزن السمكة) باستخـــدام عليقة تحتوي على 36.8% بروتين للتحقق من تأثير مستوى الكثافة التخزينية ومعدلات التغذية على النمو والإعاشة لزريعة البلطي النيلي المستزرعه في هابات داخل أحواض ترابية . أدخلت الأسماك بالوزن الإبتدائي 1.1 ± 0.03 جرام في ثلاثة كثافات مختلفة 6 ، 12 ، 18 سمكة / الهابا (30 × 40 × 50 سم ، حوالي 60 لتر) . أعطيت أسماك التجربة هذه العلائق مرتين في اليوم ولمدة 6 أيام في الأسبوع لفترة 6 أسابيع . دلت نتائج معدلات النمو والإعاشة على وجود فروق معنوية (5%) عند رعاية زريعة البلطي النيلي في كثافات تخزينية مختلفة وفي معدلات تغذية مختلفة ، حيث تتأثر عكسياً مع كل من الكثافة التخزينية ومعدل التغذية . سجلت الاختلافات في الكثافات التخزينية في (ك1 من 2.30 إلي 4.23 جرام ، ك2 من 2.80 إلي 2.97 جرام و ك3 من 2.63 إلي 2.90 جرام . الزريعة التي تمت رعايتها في كثافة 100سمكة / م^3 بمعدل تغذية 25% سجلت أعلى متوسط في الزيادة الوزنية (3.17 ± 0.03 جرام) بينما سجلت الزريعة المرباه في كثافة 300 سمكة / م^3 بمعدل تغذية 35% أقل متوسط في زيادة الوزن (1.60 ± 0.07 جرام) . معامل الحالة ومعدل الإعاشة في كل مجموعات التجربة تأثرت باختلاف الكثافة ومعدل التغذية بفروق معنوية (5%) . النتائج التي سجلت في هذه الدراسة تشير إلي أن أفضل معدل نمو وإعاشة حدث عندما تمت رعاية أسماك التجربة في كثافة تخزينية 100سمكة / م^3 بمستوى تغذية 25% من وزن السمكة .