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The Optimal Stocking Density for Nile Tilapia (*Oreochromis niloticus*) Rearing under Different Stocking Sizes

Mayyar M. A. El-hanafy[1]*, Mahmoud F. Salem[2] and Ahmed F. Fath El- Bab[1]

1- Animal, Poultry and Fish Production Department, Faculty of Agriculture, Damietta University, Egypt

2- Aquaculture Research Unit. Sakha, Central Lab, of Aquaculture Research, Agriculture Research Center, Egypt.

Email address: ahfarouk74@gmail.com Mobile No: +20 1155302017
Email address: Salem_fouad@yahoo.com Mobile No: +20 1016463289
Corresponding author*: mayyar.elhanafy106@gmail.com Mobile No : 01066333945

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Oreochromis niloticus, Stoking density, stocking size growth parameters, feed utilization, chemical composition, and blood parameters.

Abstract:

This experiment was designed to study the main effect of stocking size and stocking density levels and their interactions on growth performance, feed utilization, proximate chemical analysis of whole-body fish, some biochemical, hematological parameters, and liver function, of Nile tilapia (*Oreochromis niloticus*) fingerlings. All stocked fingerlings in enclosures (hapas) were cultured for 84 days. A (2 × 3) factorial design involved a total of 240 fingerlings of two different stocking sizes – beginning weight (5.94±0.70g and 20.90±0.91g) that were stocked at three different stocking densities (5, 10 and 15 fish/m^3), respectively in twelve enclosures (hapas 1×2×1 m - 2m^3) (duplicated for each). At the end of this experiment, the results indicated that final BW, (WG, ADG, SGR, RGR) and feed utilization (FCR and PER) of fish increased with increasing stocking size. The stocking size 20.90 gm (W2) released the highest significantly (P<0.05) BW compared with the stocking size 5.94 gm (W1) fish groups. The final BW of *O. niloticus* increased with the decrease of stocking density levels, thus fish at stocking density 5 fish/m^3 (S1) showed the highest BW compared to the other two fish groups 10 and 15 fish/m^3. With regard to the effect of interaction between stocking size and stocking density on final BW, the highest final BW was recorded by fish (T4) while the lowest one was shown by fish in (T3). Chemical composition, RBCs, WBC, Hb, Tch ALT, and AST of *O. niloticus* were not affected by the two stocking sizes 5.94 and 20.90 g and the differences between the two groups were not significant While, these measurements were affected by stocking density levels (P>0.05).

INTRODUCTION

One of the most significant freshwater species that are cultivated worldwide is tilapia **(Wang *et al.*, 2008)**. Compared to commonly available strains of tilapia, genetically improved farmed tilapia (GIFT), an enhanced strain of Nile tilapia (*Oreochromis niloticus*), show greater rates of growth and survival **(Dey *et al.*, 2000).** Egypt often uses a variety of aquaculture techniques to raise genetically enhanced tilapia **(El-Sayed *et al.*, 2015; Zahran *et al.*, 2018)**. According to **Elsabagh *et al.* (2018)**, intense culture systems are currently widely used for tilapia culture and frequently result in stressful situations that hinder fish development and

well-being. Being very productive, highly suited to confined water, and one of the most significant and widely farmed fish species worldwide is tilapia. Due to the rising demand for tilapia in recent years, the Nile tilapia (*Oreochromis niloticus*) species is well-liked by farmers who are aware of its benefits, which include a fast growth rate, short feeding cycles, enhanced disease resistance, strong fertility, and a delicious taste **(Fitzsimmons, 2000)**.

To meet the needs of the expanding Egyptian population, aquaculture has emerged as the primary source of fish protein **(GAFRD, 2016)**. The primary determinants of fish productivity in pisciculture are the fish's beginning body weight and its source, daily feeding rate and stocking density, frequency of daily feeding (number of meals), food quality, and appropriateness of the rearing water **(Abdelhamid, 2019)**.

It is essential to create a system that will boost fish output vertically by implementing novel techniques related to fish nutrition, fish culture species, and fish stocking density. By using the discovered technology in aquaculture operations, fish farmers may be able to raise their productivity per unit area vertically. When it comes to aquaculture, stocking density should refer to the initial concentration of fish added to a system. But generally speaking, it refers to the fish density at any given period. According to **Liu and Chang (1992)**, it is regarded as one of the key elements influencing fish development, feed consumption, and gross fish output. According to **Chakraborty and Banerjee (2010)**, the profitability of a fish farm may be enhanced by fully utilizing available area for intensive culture, which maximizes fish output. Additionally, fish intensification through increased stocking density is deemed appropriate in addressing the issue of land scarcity. However, according to **Chang (1988)**, fish stocking density is a crucial component of aquaculture as it influences the amount of food that is naturally available, how well food resources are used, and the overall amount of fish produced in ponds.

The current study set out to assess the effects of stocking density on growth performance, feed consumption, chemical composition, and blood analysis in Nile Tilapia rearing under various stocking sizes in hapas in Egyptian circumstances.

MATERIALS AND METHODS

The present study was carried out at Fish incubation and hatchery station in Al-Khashaa, Kafr El-Sheikh Lakes and Fish Resources Protection and Development Authority, Egypt. The experiment started at 12th May 2022 and continued until 4th August 2022 (84 days). This experiment was designed to study the main effect of stocking size and stocking density levels and their interactions on growth performance, feed utilization, proximate chemical analysis of whole-body fish, some biochemical, hematological parameters, liver function, digestive enzymes analysis, and RNA extraction, and quantitative real-time PCR (qRT-PCR) of Nile tilapia (*Oreochromis niloticus*) fingerlings. The fingerlings were allowed to acclimatize for three days before the trial commenced. Mortalities encountered during this experimental period were replaced. All stocked fingerlings in enclosures (hapas) were cultured for 84 days.

Experimental fish and rearing management:

Nile tilapia, *Oreochromis niloticus* monosex fingerlings with an average initial body weight of (5.94±0.70 and 20.90±0.91) were obtained from Fish incubation and hatchery station in Al-Khashaa, Kafr El-Sheikh governate, Egypt. A (2×3) factorial design involved a total of 240 fingerlings of two different stocking sizes (5.94 g and 20.9 g) that were stocked at three different stocking densities (5, 10 and 15 fish/m^3), respectively in twelve enclosures (hapas 1×2×1m - 2m^3) (duplicated for each). The fingerlings were allowed to acclimatize for three days before the trial commenced. Mortalities encountered during this period were replaced.

During the acclimation and experimental periods, fish Were fed a commercial extruded diet contained 32% crude protein (Table, 1). The feeding rate was 3 % of biomass per day provided at equal quantities at (9:00, 13:00, and 16: 00 hr) by hand broadcasting. The feeding was carried out each day within the week with one day off to help improve the culturing environment of the fish **(Price and Morris, 2013)**.

Table 1 :Composition and chemical analysis of the experimental diet.

Feed ingredients	Diet
Fish meal (62%)	12
Yellow corn (7.5)	20
Soybean meal (44%)	42
Gluten (60%)	5
Rice bran (11%)	13
Sunflower oil	2.5
Fish oil	2.5
Vit. & Min. mixture*	3
Sum	100
Proximate analysis (dry matter basis)	
Crude protein (CP)	31.09
Ether extract (EE)	13.16
Crude fiber (CF)	9.57
Ash	10.01
NFE**	36.17
Gross energy, MJ/kg***	448.68

* Mixture of vitamins and minerals/kg premix: The following are the amounts of vitamin D (0.8 million IU), A (1.33g), D3, 1.68g, E (6.66g), C (16.8g), k (0.8g), B1, 0.4g, Riboflavin 3.75g, B6 2.45g, B12,.33mg, NI (9.42g), Pantothenic acid (12.42g), Folic acid (0.68g), Biotin (16.6mg), BHT (0.5g), Mn (14.7g), Zn (31.6g), Fe (18.3g); 1, 0.62g; Selenium, 0.22g, and Co, 6.8 mg. ** Determined by subtracting [100-(CP+ EE+ CF+ Ash)] from the nitrogen free extract (NFE). *** Based on their chemical makeup, the gross energy value was estimated by Jobling (1983). For protein, fat, and NFE, they are 5.64, 9.44, and 4.11 Kcal/g, respectively.

Ten individuals of fish in each hapa were sampled randomly once every two weeks, and the individual body weight (BW), and body length (BL) were taken using an inscribed ruler and a digital weighing scale respectively. Each fish was then returned into the hapa after sampling, and then the amounts of feeding were adjusted according to the change in weight.

Water quality:

Table 2 shows the average value of each experimental enclosures (hapas) water quality measures during the course of the 12-week trial. During the trial, water quality parameters were recorded twice a week. The parameters included water temperature (28.4 to 28.8 °C), pH (7.4 to 75), salinity (27.5 to 27.7 ppt), NO_2 (0.033 to 0.035 mg/L), NO_3 (0.034 to 0.036 mg/L), NH_3^+ (0.039 to 0.04 mg/L), DO_2 (4.6 to 4.9 mg/L), K^+ (2.1 to 2.2), Na^+ (215.7 to 218.7), Mg_2^+ (57.7 to 58.3), Ca_2^+ (68.2 to 69.9), SO_4 (169.7 to 173.6), Cl^- (172.6 to 174.3), HCO_3- (4.1 to 4.4), and CO_3^{2-} (0.1). According to **(Boyd, 1990; 1998 and 2016)**, every physicochemical feature assessed in water was deemed suitable for Nile tilapia development.

Table 2 :Over the course of the 12-week investigation, the average value of the water quality metrics across all experimental ponds

Parameters	Range during the experiment.
water temperature	28.4 to 28.8 °C
Ph	7.4 to 7.5
Salinity	27.5 to 27.7 ppt
No_2	0.033 to 0.035 mg/L
No_3	0.034 to 0.036 mg/L
NH_3	0.039 to 0.04 mg/L
O_2	4.6 to 4.9 mg/L
K	2.1 to 2.2
Na	215.7 to 218.7
Mg	57.7 to 58.3
Ca	68.2 to 69.9
So4	169.7 to 173.6
Cl	172.6 to 174.3
Hco_3	4.1 to 4.4
$Co3^{-2}$	0.1

Growth parameters and efficiency of feed:

Records of live body weight (BW/g) and body length (BL/cm) of fish were measured in all fish for each pond and registered every 14 days (biweekly) during the experimental period (12 weeks – 84 days).

Growth performance parameters were measured by using the following equations:

Condition factor (K):

$K = (W/L^3) \times 100$

Where: W = weight of fish in grams and L = total length of fish in "cm". **(Ricker, 1975)**.

Weight gain (WG) = final weight (g) – initial weight (g) **(Ricker, 1975)**.

Specific growth rate (SGR):

$$\textbf{SGR} = \frac{\text{LnW2} - \text{LnW1}}{t} \times 100$$

Where: Ln = the natural log; W_1 = first fish weight; W_2 = the following fish weight in grams and t = period in days. **(Ricker, 1975)**.

Relative growth rate (RGR):

$$\textbf{RGR} = \frac{(W2 - W1)}{W2} \times 100.$$

Where: W_1 = Initial fish weight; W_2 = the Final fish weight in grams. **(Ricker, 1975)**.

Feed conversion ratio (FCR):

FCR = Offered feed (g)/Weight gain (g). **(Pillay, 1990)**.

Protein efficiency ratio (PER):

PER = Weight gain (g)/Protein ingested (g). **(Pillay, 1990)**.

Sampling, analytical procedure and measurements:

Fish were sampled at the beginning and at the end of the trial from each treatment, dried and immediately stored at −20°C pending analyses. Diets and carcass samples were submitted to proximate composition analysis. The crude protein, lipid, and ash content were determined using the Association of Official Analytical Chemists standard techniques **(AOAC, 2007).** The moisture content was determined by drying the samples until obtaining a consistent weight using a drying oven (GCA, model 18 EM, Precision Scientific group, Chicago, IL, USA) at 85 ◦C for 24 hr.

Blood sampling protocol:

Before blood was collected from the fish at the end of the experiment, they were given anesthesia using 100 µg mL−1 MS222 (Tricine methane sulfonate, Sigma-Aldrich Co. LLC). Blood samples were taken from three fish per hapa, or six fish per treatment, at random. Using sterile 2.5 mL syringes, blood samples were extracted from the caudal vein and divided into equal portions. The first component was kept for haematological measures in a heparinized tube, and the second half was left to coagulate at room temperature for thirty minutes before being refrigerated for three hours at 4 °C. After that, serum was extracted from the clotted samples and stored at -20 °C until further biochemical analysis. The samples were centrifuged at 3000 rpm for 10 minutes at 4 °C.

Hematological examination of blood:

Following the completion of this experiment, a random sample of five fish per hapa was taken for blood examination. Triclone methane sulphonate (25 mg/L) was used to anesthetize the fish (MS-222). After being extracted from the caudal vein, the blood was placed in sterile tubes and let to stand slantwise for three hours. Samples were centrifuged at 5000 revolutions per minute (rpm) for 10 minutes at 4 ◦C. Using the diluting fluids for red blood cells (RBC) and white blood cells (WBC), total erythrocyte and leucocyte counts were ascertained. In a sterile test tube, around 20 µl of blood and 4000 µl of the diluting solution were combined. Using the cyanmethemoglobin technique, the hemoglobin (Hb) content of blood was measured using Drabkins Fluid (Qualigens Chemicals) **(Anderson, *et al*. 2010).**

At 540 nm, the absorbance was measured spectrophotometrically, and the final concentration was determined by comparing it to the standard of cyanmethemoglobin (Qualigens Chemicals). The formula for calculating Hb content was then as follows: Hb (g/dl) = [OD (T) / OD (S)], where OD (T) stands for test absorbance and OD (S) for standard absorbance. Atomic absorption spectrophotometry was used to assess the levels of total cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) using Crest Biosystems® kits.

Statistical analysis:

The data homogeneity and normalcy were assessed using the Shapiro-Wilk test. Additionally, two-way ANOVA statistical analysis approach was used to all calculated and estimated data, and Tucky's range test was used to confirm any discrepancies between the means. $P < 0.05$ served as the tested threshold of significance. The findings are shown as mean± pooled SE values **(SAS, 2012)** was used for all statistical analyse

RESULTS AND DISCUSSION

Effect of stocking size and stocking density on growth performance of *O. niloticus*:

Body weight and length:

Results of the effect of stocking size (initial weight) levels and of stocking density levels and their interactions on body weight (BW) of *Oreochromis niloticus* are presented in Table 3.

Results in Table 3 indicated that final BW of fish increased with increasing stocking size. The stocking size 20.90 gm (W2) released the highest significantly (P<0.05) BW compared with the stocking size 5.94 gm (W1) fish groups.

As described in table (3) final BW of *O. niloticus* significantly (P<0.05) increased with the decrease of stocking density levels, thus fish at stocking density 5 fish/m^3 (S1) showed the highest BW compared to the other two fish groups 10 and 15 fish/m^3 .

With regard to the effect of interaction between stocking size and stocking density on final BW, the highest final BW was recorded by fish T4 which containing stocking size 20.90 gm and stocking density 5 fish/m^3, while the lowest one was shown by fish in T3 which containing stocking size 5.94 gm and stocking density 15 fish/m^3.

Data in table 3 showed that, at the end of the experiment, fish at stocking size 20.90 g was recorded the longest (P<0.05) final BL (17.95 cm), while those at stocking size 5.94 g was recorded the shortest BL (P<0.05) one (15.98 cm).

Table 3: Effect of stocking size and stocking density levels on body weight (BW) and length (BL) of *O. niloticus*.

Parameters Treatments	Initial weight	Final weight	Initial length	Final length
Effect of Stocking size levels				
Stocking size 5.94 gm (W1)	5.94±0.70^b	56.41±1.50^b	7.07±0.096^b	16.98±0.11^b
Stocking size 20.90 gm (W2)	20.90±0.91^a	77.32±2.65^a	11.21±0.091^a	17.95±0.10^a
P Value	0.0001	0.0001	0.0001	0.0001
Effect of stocking density levels				
Stocking density 5 fish/m^3 (S1)	13.47±1.77	71.57±1.36^a	9.34±0.50	17.53±0.28^a
Stocking density 10 fish/m^3 (S2)	13.49±1.22	68.39±0.97^a	9.00±0.35	17.22±0.16^a
Stocking density 15 fish/m^3 (S3)	13.41±0.97	61.61±0.63^b	9.17±0.29	16.18±0.15^b
P-Value	0.75	0.0001	0.23	0.0001
Effect of Stocking size and stocking density levels				
W1+ S1 (T1)	5.78±0.34	60.87±0.56	7.26±0.25	16.43±0.18^c
W1+ S2 (T2)	5.93±0.34	58.79±0.53	6.97±0.18	16.37±0.13^c
W1+ S3 (T3)	6.007±0.30	53.33±0.44	7.077±0.13	15.18±0.10^c
W2+ S1 (T4)	21.16±0.57	82.27±0.56	11.41±0.21	18.63±0.18^a
W2+ S2 (T5)	21.07±0.72	78.99±0.53	11.04±0.16	18.07±0.13^a
W2+ S3 (T6)	20.82±0.70	70.89±0.41	11.26±0.13	17.18±0.10^b
P-Value	0.10	0.006	0.94	0.24

Means with the same letter in each column are not significantly differences (P < 0.05).

Results in Table3 also revealed that fish at stocking density with 5 fish/m^3 had longer final BL than those of fish at stocking density 10 and 15 fish/m^3. Analysis of variance showed that, decreasing

of stocking density level had significant effect ($P<0.001$) on the final BL of fish (Table, 3).

As shown in the same Table, the effect of interaction between stocking size and stocking density on final BL, the highest final BL was recorded by fish T4 which containing stocking size 20.90 gm and stocking density 5 fish/m3, while the lowest one was shown by fish in T3 which containing stocking size 5.94 gm and stocking density 15 fish/m^3.

Generally speaking, the growth metrics demonstrated that fingerlings in all treatments gradually grew from the beginning to the end of the trial. The final fish weight was considerably greater at 5 followed by 10 fish/m^3 stocking density compared to 15/m^3 stocking density for either 6 g or 20 g at stocking size, regardless of the stocking size (6 g or 20 g).

Additionally, 20 g at 5 and 10 fish/m^3 considerably outperformed than other treatments.

The observed decrease in growth rate and feed use by fish at density (15/m^3) may have been caused by social interaction resulting to competition

for feed and space, which in turn increased energy need. The findings of **(Mensah *et al.*, 2013; Addo, 2021)** were comparable.

Compared to all other treatments, the 20 g at 5/m^3 often produced the greatest average end weight. This is consistent **Zannatal *et al*. (2014)**, who showed that, the fish's body weight during the growing stage is influenced by its beginning weight (stocking size).

Ndwiga (2015) discovered that, even at the maximum stocking density of 90 fish/m^3, the measured ultimate weights and lengths were noticeably low. Numerous factors were cited as causes of these outcomes. Fish crowded out by high densities may have had a negative impact on their appetite, which in turn may have led to inadequate feeding and low feed conversion. The fish may have been stressed as a result of space limitations and other unfavorable physical and chemical circumstances brought on by the hapa nets' reduced capacity. The same results on 20g *O. niloticus* supplied in hapa nets at the same densities were reported by **(Cato and Brown, 2013)**. Poor development might have resulted from an accumulation of ammonia caused by uneaten meals. According to research by **Youssouf (2007)**, inadequate development is brought on by the buildup

of harmful ammonia and nitrite from uneaten food, which in turn causes stress.

Condition factor:

It is often thought that condition factor (K) reflects features of the environment, such as habitat quality, water quality, and availability of prey, in addition to fish traits, such as growth, health, and reproductive status **(Liao *et al.*, 1995).**

At the end of this experiment, the average values of K as affected by stocking size 5.94 and 20.90 gm decreased from 1.42 to 1.34 respectively, with significant differences ($P<0.05$) due to treatment effect on K values (Table, 4).

With respect to the effect of interaction between stocking size and stocking density on the K values, data illustrated in table 4 showed that at the experimental end, K value ranged between 1.29 and 1.53 with no clear direction, whereas the highest average K value was recorded by T3 at stocking size 5.94 gm and stocking density 15 fishes/m3 and the lowest value was recorded by T4 stocking size 20.90 gm and stocking density 5 fishes/m3 and the differences between K values are significant.

Table 4: Effect of stocking density and stocking size levels on condition factor (K) of *O. niloticus*

Parameters Treatments	Initial K	Final K
Effect of stocking size levels		
Stocking size 5.94 gm (W1)	1.81±0.1[a]	1.42±0.02[a]
Stocking size 20.90 gm (W2)	1.53±0.044[b]	1.34±0.016[b]
P Value	0.009	0.0001
Effect of stocking density levels		
Stocking density 5 fish/m^3 (S1)	1.54±0.12	1.33±0.04[b]
Stocking density 10 fish/m^3 (S2)	1.75±0.097	1.34±0.026[b]
Stocking density 15 fish/m^3 (S3)	1.66±0.079	1.46±0.029[a]
P-Value	0.43	0.0001
Effect of stocking size and stocking density levels		
W1+ S1 (T1)	1.63±0.226	1.39±0.041
W1+ S2 (T2)	1.89±0.17	1.35±0.035
W1+ S3 (T3)	1.82±0.14	1.53±0.034
W2+ S1 (T4)	1.45±0.08	1.29±0.03
W2+ S2 (T5)	1.61±0.09	1.34±0.03
W2+ S3 (T6)	1.5±0.06	1.40±0.02
P-Value	0.89	0.05

Means with the same letter in each column are not significantly differences (P < 0.05).

The range of 1.29 to 1.53, which **Bagenal and Tesch (1978)** regarded as optimal for Nile tilapia fish, was reflected in the mean condition factor values reported for the sizes (6 g and 20 g). The mean condition factor value was found to be more significant for the 15 fish/m^3 stocking density than for the 5 and 10 fish/m^3 stocking densities. The 6 fish/m^3 stocking density recorded a higher significant mean condition factor value than the 3 fish/m^3 stocking density, according to **(Addo, 2021)**. These results are consistent with that finding.

This went against what **Duodu (2014)** said, who claimed that high stocking density was a chronic stressor associated with aquaculture that slowed development. This might be the reason for variations in the length range, sample size, and water quality. Mature stages and the time of year might also be important variables.

Weight gain and average daily gain:

Results presented in Table (5) showed that, averages of weight gain (WG) as affected by stocking size were 50.47 and 56.42 g for two stocking size

5.94 and 20.90 g, respectively, and the differences between means were significant ($p<0.001$) and the same trend was also observed for average daily gain (ADG), where the averages were 0.62 and 0.67 g for the two stocking sizes 5.94 and 20.90, respectively.

As show in this Table, weight gain (WG) as affected by stocking density were 58.1, 55.89 and

48.20 g for three stocking density 5, 10 and 15 fish /m^3, respectively, and the same trend was also observed for average daily gain (ADG), where the averages were 0.69, 0.66 and 0.57 g for the three stocking sizes 5, 10 and 15 fish /m^3, respectively.

The interaction between the stocking size and stocking density on weight gain (WG) and average daily gain (ADG) of *O. niloticus* are shown in Table 5. Results showed that *O. niloticus* in T4 recorded the highest WG and the best ADG. The average WG and ADG were almost significantly increased with each increase of stocking size and decreased of stocking density.

Table 5: Effect of stocking size and stocking density levels on wight gain (WG), Average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR) of *O. niloticus*

Parameters Treatments	WG/g	ADG/g	SGR (% d^{-1})	RGR (g/g)
Effect of stocking size levels				
stocking size 5.94 gm (W1)	50.47±0.15[a]	0.62±0.006[a]	2.68±0.02[a]	8.67±0.16[a]
stocking size 20.90 gm (W2)	56.42±0.63[b]	0.67±0.008[b]	1.55±0.011[b]	2.67±0.03[b]
P Value	0.0001	0.0001	0.0001	0.0001
Effect of stocking density levels				
Stocking density 5 fish/m^3 (S1)	58.1±0.6[a]	0.69±0.007[a]	2.21±0.16[a]	6.26±0.84[a]
Stocking density 10 fish/m^3 (S2)	54.9±0.50[b]	0.66±0.006[b]	2.15±0.11[b]	5.89±0.56[a]
Stocking density 15 fish/m^3 (S3)	48.20±0.51[c]	0.57±0.006[c]	2.03±0.086[c]	5.17±0.401[b]
P-Value	0.0001	0.0001	0.0001	0.0001
Effect of stocking size and stocking density levels				
W1+ S1 (T1)	55.09±0.61[b]	0.66±0.008[b]	2.81±0.034[a]	9.59±0.3
W1+ S2 (T2)	52.87±0.56[b]	0.63±0.007[b]	2.74±0.032[a]	9.04±0.28
W1+ S3 (T3)	47.32±0.47[c]	0.56±0.005[c]	2.60±0.025[a]	7.97±0.185
W2+ S1 (T4)	61.11±0.50[a]	0.73±0.01[a]	1.63±0.01[b]	2.92±0.03
W2+ S2 (T5)	57.93±0.55[b]	0.68±0.007[b]	1.57±0.012[b]	2.73±0.03
W2+ S3 (T6)	50.07±0.40[b]	0.59±0.005[b]	1.45±0.009[b]	2.39±0.02
P-Value	0.03	0.04	0.77	0.006

Means with the same letter in each column are not significantly differences (P < 0.05).

The early spawning may have contributed to the notable differences in average daily gain (ADG) and mean weight gain (WG) at 20g stocking densities. The current findings are in line with those of **(De Graaf, *et al*., 1996 & Addo, 2021)**, who noted that the growth rate of adult tilapia drops in the presence of recruits and that fewer fish of a marketable size may be caught. It is possible that their weight increase was impacted by competition for the meal meant for the stocked adult. Similar to this, the high stocking density which inhibits fish growth by competing with other fish for food and space may be the cause of the difference in the 6 g stocking densities **(Islam, 2002)**

Specific growth rate (SGR % d^{-1}) and relative growth rate (RGR g/g):

As presented in Table (5) showed that, averages of Specific growth rate (SGR) as affected by stocking size were 2.68 and 1.55 g for two stocking size 5.94 and 20.90 g, respectively, and the differences between means were significant ($p<0.001$) and the same trend was also observed for relative growth rate (RGR), where the averages were 8.67 and 2.67 for the two stocking sizes, respectively.

As shown in this Table, SGR as affected by stocking density were 6.26, 5.89 and 5.17 for three stocking density 5, 10 and 15 fish /m^3, respectively, and the same trend was also observed for RGR,where the averages were 6.26, 5.89 and 5.17 for the three stocking sizes, respectively.

The interaction between the stocking size and stocking density on SGR and RGR of *O. niloticus* are shown in Table 5. Results of this table showed that *O. niloticus* in T1 recorded the highest SGR and the best RGR. The average SGR and RGR were almost significantly decreased with each

increased with each of stocking size and stocking density.

n this study, the stocking sizes and densities had a substantial and independent impact on the specific growth rate (SGR) and relative growth rate (RGR). Compared to the 20 g stocking size, the 6 g stocking size was much larger. This was This was in line with the findings of **(Addo, 2021)** which showed that the 2 g stocking size was substantially greater than the 10 g stocking size. However, these findings run counter to those of **Zannatal *et al.* (2014)** and **Abdel-Hakim *et al.* (2001)**, who hypothesized that a large beginning weight (stocking size) of fish affects the body weight over the growth phase, leading to a greater specific growth rate. Comparing fish supplied at densities of 10 and 15 fish/m^3, those stocked at 5 fish/m^3 showed greater mean SGR and RGR. This was consistent with **Islam's (2002)** finding that increased SGR is typically the result of lower stocking densities.

Effect of stocking size and stocking density on feed utilization of *O. niloticus*:

Feed conversion ratio (FCR):

Results of Table 6 showed the FCR values for *O. niloticus* when stocked with the two stocking sizes 5.94 and 20.90 g. Averages of FCR at the end of the experiment were 1.19 and1.84 with significant differences among these means (Table, 6). Data obtained indicated that the best FCR was recorded by fish stocked by 20.90 g.

As described in Table 6, FCR was significantly affected by stocking density in tilapia diets and the obtained results indicated that, FCR increased with increasing stocking density. The highest FCR was recorded by fish stocked at 15 fish/m^3 than the 5 and 10 fish/m^3. The differences among treatments were significant ($P<0.001$).

Table 6 also indicated that, FCR values as affected by interaction of stocking size and stocking density ranged from 1.17 and 1.97. The best FCR was recorded by fish of T6 which *O. niloticus* stocked at 20.90 g and stocking density 15 fish/m^3, and differences among treatments were significant ($P<0.001$).

The food conversion ratio appears to have gone up as stocking density has gone up. That is, because to competition for food and space, fish became less effective at using food for somatic development as stocking densities increased (**Ronald et al., 2014**). They were both in accordance with the suggestion given by **Bag and Moulick (2016)** that an FCR value of less than 2.0 is regarded "good" in the aquaculture business, despite the fact that there were no appreciable variations between them.

It appears that the mean feed efficiency values of all the treatments were significant. Higher feed efficiency, however, did not significantly affect their increase in body weight **(Miura et al., 2012)**

Table 6: Effect of stocking size and stocking density levels on feed conversion ratio (FCR) and Protein efficiency ratio (PER) of *O. niloticus.*

Parameters Treatments	FCR	PER
Effect of stocking size levels		
Initial weight 5.94 gm (W1)	1.19±0.008[b]	2.82±0.02[a]
Initial weight 20.90 gm (W2)	1.84±0.023[a]	1.82±0.015[b]
P Value	0.0001	0.0001
Effect of stocking density levels		
Stocking density 5 fish/m^3 (S1)	1.45±0.11[b]	2.39±0.14[a]
Stocking density 10 fish/m^3 (S2)	1.49±0.08[b]	2.24±0.10[a]
Stocking density 15 fish/m^3 (S3)	1.59±0.08[a]	2.22±0.09[b]
P-Value	0.0001	0.0001
Effect of stocking size and stocking density levels		
W1+ S1 (T1)	1.17±0.008	2.85±0.021
W1+ S2 (T2)	1.17±0.007	2.84±0.016
W1+ S3 (T3)	1.21±0.016	2.76±0.04
W2+ S1 (T4)	1.73±0.02	1.93±0.01
W2+ S2 (T5)	1.81±0.03	1.85±0.02
W2+ S3 (T6)	1.97±0.02	1.69±0.01
P-Value	0.0001	0.093

Means with the same letter in each column are not significantly differences (P < 0.05)

Protein efficiency ratio (PER):

As illustrated in Table 6, averages of protein efficiency ratio (PER), were significantly (P<0.05) increased with decrease stocking size. As shown in table 6, PER values were improved with decreasing stocking density and the differences among three stocking densities were significant.

Data in Table 6 also showed that PER values were improved for fish stocked at size 5.94 and 5 fish/m^3 and the highest PER value was recorded by fish stocked in T1.

In comparison to the other two stocking densities 15 fish/m^3, the protein efficiency ratio (PER) of *O. niloticus* at the 5 and 10 fish/m^3 showed superior PER, indicating that 10 fish may be employed as a likely stocking density. This is comparable to observations made for *O. aureus* stocked at 30 fish/m^3 in marine hapas, where stressors including toxic ammonia and nitrite impacted the growth performance **(Anderson *et al*., 2010)**.

Effect of stocking size and stocking density on proximate analysis of *O. niloticus.*

After the trial ends, chemical analysis is commonly done to assess how feed affected the composition of the fish. Fish body composition is influenced by both exogenous (diet composition, feeding frequency, temperature, etc.) and endogenous (size, sex, and life cycle stage) elements **(Hepher, 1990)**. It should be highlighted that while other endogenous parameters were kept constant throughout the investigation, the feed's composition is the only one that may have affected the fish's chemical makeup.

Proximate analysis of tilapia *O. niloticus* as affected by stocking size is presented in Table 7. it is clear that increased of stocking size (20.90 g) significantly increased dry matter (P<0.01), lipid (P<0.001) and protein content (P<0.05) while ash content was not significantly affected (Table, 7).

Results of proximate analysis table 7 showed that, decreasing stocking density increased dry matter and protein content (P<0.05) altered dry matter, lipid, crude protein or ash contents, while lipid content was not significantly affected. But increasing stocking density increased ach content (P<0.05) (Table, 7).

The interaction stocking size and stocking density (Table, 7) showed that, proximate analyses of whole fish had no clear direction.

In order to verify the nutritional condition of *O. niloticus* at each of stocking size and stocking density, an analysis of their proximate body composition was conducted. The findings showed

that, in comparison to fish in the other groups, fish in the fish group 15 fish/m^3 had considerably less protein and more fat. The fish may have used surplus metabolic energy to maintain normal metabolic activity, which might be one explanation. African catfish (*Clarias gariepinus*) and Gangetic mystus (*Mystus cavacius*) may have different body compositions depending on stocking densities, according to research by **Ok´e and Goosen (2019)** and **Rahman *et al.* (2020)**. Low protein and high fat percentage had a significant impact when the density 15 fish/m^3. in Similar study, rainbow trout effectively use protein as fuel for development and adaptation to harsh environments like dense stocking **(Refaey *et al.*, 2018)**. In teleosts, growth, body fat buildup, and reproduction are all included in net energy for production (NEp) **(Liu *et al.*, 2016).** In the high-density group, excessive energy was stored as body fat in an overcrowded setting where performance was subpar.

Table 7: Effect of stocking size and stocking density levels on proximate analysis of *O. niloticus*

Parameters Treatments	Moisture	Protein	Lipid	Ash
Effect of stocking size levels				
Initial weight 5.94 gm (W1)	11.13±0.33^{a}	53.14±0.47^{b}	8.81±0.11^{b}	19.32±0.26
Initial weight 20.90 gm (W2)	9.82±0.12^{b}	56.21±0.34^{a}	10.48±0.09^{a}	19.91±0.31
P Value	0.013	0.024	0.017	0.006
Effect of stocking density levels				
Stocking density 5 fish/m^3 (S1)	13.02±0.16^{a}	56.60±0.58^{a}	9.53±0.14	20.41±0.22^{a}
Stocking density 10 fish/m^3 (S2)	9.15±0.11^{b}	54.05±0.52^{b}	9.61±0.15	20.22±0.31^{a}
Stocking density 15 fish/m^3 (S3)	9.25±0.21^{b}	50.22±0.63^{b}	9.71±0.17	18.11±0.29^{b}
P-Value	0.028	0.096	0.033	0.039
Effect of stocking size and stocking density levels				
W1+ S1 (T1)	12.98±0.93^{a}	55.07±0.46^{a}	8.72±0.05^{ab}	17.26±0.38^{c}
W1+ S2 (T2)	9.81±0.40^{b}	54.61±0.39^{c}	8.97±1.17^{ab}	21.34±1.46^{a}
W1+ S3 (T3)	12.61±0.72^{a}	49.76±0.60^{abc}	8.74±0.30^{ab}	21.13±1.23^{a}
W2+ S1 (T4)	13.07±1.10^{a}	58.13±1.57^{a}	10.74±0.35^{a}	19.01±0.79^{b}
W2+ S2 (T5)	8.49±0.71^{bc}	53.48±2.06^{bc}	10.15±0.09^{ab}	19.19±0.14^{b}
W2+ S3 (T6)	7.90±0.91^{bc}	51.03±2.50^{abc}	10.55±0.69^{a}	19.77±1.44^{ab}
P-Value	0.0116	0.54	0.67	0.046

Means with the same letter in each column are not significantly differences (P < 0.05).

A study in largemouth bass, which was further examined using histology and revealed significant injury in the liver tissue, supported the idea that oxidative stress could cause hepatic dysfunction and hepatotoxicity in fish, as indicated by the higher lipid levels **(Perez-Jimenez *et al.*, 2017)**. It has been suggested that the liver is a key indicator for the detection of liver disease and function as it regulates body metabolism in response to varying dietary lipid levels and plays a significant role in maintaining metabolic homeostasis. A prior study **(Zhou *et al.*,**

2020) that suggested high food fat levels stress fish and severely lower immunity lends weight to this. Furthermore, appreciable increases in fat content in the high-density group could be a symptom of fat buildup, which might cause fish health to decline. High stocking density has been linked to fatty breakdown in common carp (*Cyprinus carpio*), according to **Zhao *et al*. (2019).**

Effect of stocking size and stocking density on some hematological indices of *O. niloticus*.

Fish are classified based on physiological condition and stress level, which are often represented by biochemical blood markers that offer crucial information about fish health **(Birnie-Gauvin *et al*., 2017)**.

Red Blood Cell Count (RBCs), White Blood Cell (WBC), Hemoglobin (Hb) and Total cholesterol (Tch) of Nile tilapia *O. niloticus* were not affected by two stocking sizes 5.94 and 20.90 g and the differences between two groups were non significance (Table, 8) .

Results in Table 8 indicated that RBCs, WBC, Hb and Tch of fish increased with increasing stocking density. The S1and S2 fish groups released the highest significantly (P<0.05) RBCs, WBC and Hb compared with the S3 fish group. while, the total cholesterol (Tch) value increased with increasing in stocking density of *O. niloticus*.

With regard to the effect of interaction between stocking size and stocking density (Table, 8) on RBCs, WBC, and Hb of Nile tilapia, *O. niloticus*, the highest values RBCs, WBC, and Hb were recorded by fish fed T4 which containing stocking size 5.94g at density 5 fish/m^3, while the lowest one was shown by fish fed the control diet T6. whereas, the highest value Tch was recorded by fish fed T6 which containing stocking size 20.90 g at density 15 fish/m^3 of *O. niloticus*.

Generally, blood parameters were significantly affected by the difference in stocking density in this study, the RBC, WBC, and Hb contents of fish blood decreased with increasing stocking density. A study conducted by **Mazumder *et al*. (2019)** demonstrated a similar reduction in the RBC, WBC, and Hb contents of the silver carp cultured at high stocking density. Thus, stocking density affects the fish welfare in *O. niloticus*, indicated by the biochemical parameters. Moreover, higher plasma lipid indicates tissue damage and health deterioration **(Hoseini *et al*., 2018a, 2018b)**. In contrast, lower stocking density minimizes the stress conditions and actively mobilizes the total cholesterol to cope with the extra energy balance. Similar trends in cholesterol and triglyceride variations with stocking density were also observed in Nile tilapia in a study conducted by **Wu *et al*. (2018)**

Table 8: Effect of stocking size and stocking density levels on of Blood Cell Count (RBCs), White Blood Cell (WBC), Hemoglobin (Hb) and Total cholesterol (Tch) of Nile tilapia *O. niloticus*

Parameters Treatments	RBC (×10^6 µl)	WBC (×10^3 µl)	Hemoglobin (g/dl)	Total cholesterol (mg/dl)
Effect of stocking size levels				
stocking size 5.94 gm (W1)	105.9±1.3	215.45±2.4	5.2±0.1	198.7±3.2
stocking size 20.90 gm (W2)	107.04±1.1	217.83±1.7	5.3±0.1	202.9±2.9
P Value	0.4	0.85	0.58	0.00
Effect of stocking density levels				
Stocking density 5 fish/m^3 (S1)	112.76±1.4	214.9±3.4	5.4±.09	202.5±2.8
Stocking density 10 fish/m^3 (S2)	109.39±1.1	209.14±2.7	5.2±.07	205.1±2.3
Stocking density 15 fish/m^3 (S3)	95.77±2.1	189.62±2.6	4.8±.06	214.7±1.9
P-Value	0.11	0.18	0.08	0.09
Effect of stocking size and stocking density levels				
W1+ S1 (T1)	108.68±0.92	215.17±2.42	5.3±0.02	200.6±2.81
W1+ S2 (T2)	106.49±0.89	212.29±1.98	5.2±0.02	201.9±2.46
W1+ S3 (T3)	100.18±1.05	202.53±2.66	5.1±0.01	206.7±2.07
W2+ S1 (T4)	109.9±1.41	216.36±2.21	5.4±0.03	203.7±1.77
W2+ S2 (T5)	107.72±0.96	213.48±1.91	5.3±0.02	205.1±2.14
W2+ S3 (T6)	101.41±0.98	203.72±1.48	5.0±0.02	209.8±2.03
P-Value	0.58	0.96	0.33	0

Means with the same letter in each column are not significantly differences (P < 0.05).

Effect of stocking size and stocking density levels on liver function of *O. niloticus*.

Because they regulate the transfer of the amino group function from alpha-amino acids to alpha-keto acids, the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are significant indicators of liver health and function. Animal blood has high levels of ALT and AST, mostly as a result of liver cell injury **(Kumar *et al*., 2011)**.

Table 9 presents the findings of the ALT and AST values as influenced by stocking density and size as well as their interactions. As shown in this table, ALT values increased with increasing the stocking size, being 92.83 and 109.67 for fish stocked at 5.94 and 20.90 g, respectively. Analysis of variance (Table, 9) indicated that the differences in ALT values attributed to stocking size effect were significant (P<0.05).

In Table 9, the results regarding the impact of stocking density on ALT u/L values demonstrate that fish stocked at a density of 15 fish/m^3 had the highest (P<0.05) ALT value (125.08 u/L), while fish stocked at a density of 5 fish/m^3 had the lowest (P<0.05) ALT value (76.75 u/L). These findings highlight the detrimental effects of increasing stocking density on the preservation and enhancement of liver cells. The fish of T6 (stocking density: 15 fish/m^3) and stocking size: 20.90 g had the highest (P<0.05) ALT levels (143.50 u/L). Conversely, the fish with the lowest (P<0.05) ALT levels, 76.3 u/L, were supplied at a weight of 20.90 g and a density of 5 fish/m^3.

Table 9: Effect of stocking size and stocking density levels on liver function of Nile tilapia *O. niloticus*

Parameters Treatments	AST (U/L)	ALT(U/L)
Effect of stocking size levels		
Initial weight 5.94 gm (W1)	92.83±5.52^b	55.67±4.18
Initial weight 20.90 gm (W2)	109.67±12.61^a	56.17±4.81
P Value	0.005	0.85
Effect of stocking density levels		
Stocking density 5 fish/m^3 (S1)	76.75±0.63^c	42.5±1.5^b
Stocking density 10 fish/m^3 (S2)	102.13±5.08^b	61±2.67^a
Stocking density 15 fish/m^3 (S3)	125.08±11.38^a	64.25±1.49^a
P-Value	0.0002	0.0008
Effect stocking size and stocking density levels		
W1+ S1 (T1)	77.5±0.5	43.1±2.1
W1+ S2 (T2)	94.5±3.5	62.3±4.2
W1+ S3 (T3)	106.5±4.5	62.2±1.4
W2+ S1 (T4)	76.3±1.1	42.1±3.2
W2+ S2 (T5)	109.5±5.5	60.0±5.1
W2+ S3 (T6)	143.5±8.5	66.5±1.5
P-Value	0.019	0.56

Means with the same letter in each column are not significantly differences (P < 0.05).

As can be seen from the results in Table (9), the AST values for fish fed at 5.94 and 20.90 g, respectively, were 55.67 and 56.17 u/L. These findings showed that two stocking sizes had little influence on AST readings.

The AST values for fish stocked with 5, 10, and 15 fish/m^3 were 76.75, 102.13, and 125.08 u/L, respectively, as Table (9) illustrates. According to the findings, AST levels rose as stocking density increased. The interplay between stocking density and size (Table, 9) revealed that the AST of *O. niloticus*, the Nile tilapia, lacked a distinct direction. Higher plasma lipid levels corroborate a rise in ALT and AST, which point to tissue damage brought on by stress **(Mirghaed *et al*., 2017)**.

CONCLUSION

Fish final weight, condition factor, weight increase, and specific growth rate were significantly impacted by the growth parameters seen in both stocking size and density independently at various probability levels. With the exception of T3 and T6, the final mean weights of the fish varied considerably.

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This suggests that prospective farmers can engage in small-scale enterprises that are financially feasible given the current circumstances. Small-scale pond farmers could be advised to raise tilapia with a stocking size of 20.90 g and a density of 5 fish/m^3 in order to get faster growth and more benefits in a shorter amount of time. Farmers might also raise 5.94 g at 10/m^3, though, as the yield was somewhat greater when stocking at 10/m^3.

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الملخص العربي

الكثافة المثلي لرعاية أسماك البلطي النيلي تحت أحجام تخزينية مختلفة

ميار مجدي أبو الخير الحنفي[1] - محمود فؤاد سالم[2] و احمد فاروق فتح الباب[1]

1- قسم الانتاج الحيوانى و الداجني و السمكي- كلية الزراعة-جامعة دمياط
2- رئيس بحوث المعمل المركزي لبحوث الثروة السمكية - وحدة بحوث بسخا - كفر الشيخ

أجريت هذه التجربة لدراسة التأثير الرئيسي للوزن الابتدائي ومستويات كثافة التخزين وتداخلاتهما على أداء النمو، والاستفادة من العلف، والتحليل الكيميائي للجسم ، وبعض المعايير البيوكيميائية والدموية ووظائف الكبد لأسماك البلطي النيلي. حيث تم استزراع جميع الإصبعيات المخزنة في هابات لمدة 84 يومًا. تم تصميم تجربة عاملية (2 × 3) بإجمالي 240 إصبعية مقسمة لحجمين تخزين 5.94 جم و 20.9 جم وتم تخزينها بثلاث كثافات تخزين مختلفة (5، 10 و 15 سمكة/م^3) في اثنتي عشرة (هابة 1×2×1 م - 2 م^3) (مكررة لكل منها). وكانت أهم النتائج المتحصل عليها كالتالى:

أشارت النتائج إلى أن وزن الجسم النهائي يزداد مع زيادة حجم التخزين. حيث حقق حجم التخزين 20.90 جم أعلى معدل لوزن الجسم وزيادة الوزن ومعدل الزيادة اليومية ومعدل النمو النوعى ومعدل النمو النسبى ومعدلى التحويل الغذائي كفاءة البروتين للأسماك مقارنة بمجموعات الأسماك حجم التخزين 6.00 غم. أما بالنسبة لكثافة التخزين فقد زاد وزن الجسم النهائي لأسماك البلطى النيلى مع انخفاض مستويات كثافة التخزين، وبالتالي أظهرت الأسماك ذات الكثافة التخزينية 5 سمكة / م 3 أعلى وزن للوزن مقارنة بمجموعتي الأسماك الأخريين 10 و 15 سمكة / م 3. وفيما يتعلق بتأثير التداخل بين حجم التخزين وكثافة التخزين على وزن الجسم النهائي، فقد سجلت المعاملة الرابعة عند وزن 20.90 جم وكثافة 5 سمكة/م^3 أعلى وزن للوزن النهائي للأسماك في حين سجلت الأسماك أقلها في المعاملة الثالثة عند وزن 5.94 جم وكثافة 15 سمكة/م3. لم يتأثر التركيب الكيميائي، كرات الدم الحمراء، وكرات الدم البيضاء ونسبة الهيموجلوبين والكلوستيرول الكلى ووظائف لأسماك البلطى النيلى بحجمي التخزين 5.94 جم و 20.90 جم. بينما تأثرت هذه القياسات بكثافة التخزين.

وبناءا على ما سبق يتضح أن إستزراع أسماك البلطي النيلى بحجم تخزين 20.90 جم عند كثافة 5 سمكات/م^3 من أجل الحصول على نمو أسرع وفوائد أكثر في فترة زمنية أقصر. كما يمكن للمزارعين أيضًا زيادة 5.94 جرام بمعدل 10/م^3، حيث يكون المحصول أكبر إلى حد ما عند التخزين بمعدل 10/م^3