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Original Article

Effect of re-feeding regime under different stocking density of Nile tilapia, *Oeochromis niloticus* on growth performance, nutritional efficiency and fish body composition

Ashraf Y. El-Dakar[1], Aya Y.Mouhmed[2], A. Elgamal[3], Mohamed Abd ElBaky Amer [3], Shymaa M. Shalaby[4] , Mohamed F. Abdel-Aziz[1]

[1] Department of Aquaculture and Biotechnology, Faculty of Aquaculture and Marine Fisheries, Arish University, Arish, Egypt.
[2]Faculty of Agriculture, Suez canal University, Ismailia, Egypt
[3]Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
[4]Aquaculture Department, Faculty of Fisheries, Suez University, Suez, Egypt.

ABSTRACT: A factorial rearing trial 4x2 was done to investigate the compensatory growth of Nile tilapia (*Oreochromis niloticus*) in structural size and live weight in response to different deprivation periods and re-feeding. Four treatments were evaluated. The control group was fed to satiation two times a day and one-day starvation and re-feeding six days (1/6) throughout the experiment. The other three treatment groups were starved for 2, 3, and 4 days and re-feeding 7, 11, and 14 days, (2/7),(3/11), and (4/14) respectively. *O. niloticus* were constructed in this experiment with initial primary weights of 21.62 ± 0.08 g. Fish were randomly fed twice daily and starved for four days and were constructed for fourteen days in two replicates to assess the cost of feeding, growth performance, feed use, and survival rate for 84 days in 16 fiberglass tanks. The results showed that the best final body weight and feed conversion ratio in the feeding regime system was 3/11 compared to the control. Feed restriction significantly ($P \leq 0.05$) reduced the cost of feed. The lowest feeding cost was obtained by *O. niloticus* fish starved for three days compared to starving for two days, four days, and control. The cost of feeding was reduced by 30% when the feeding was limited to three days starving and re-fed eleven days compared with those fed six days and starved one day.

Key word: Feeding regime, Starvation, Compensatory growth, *Oreochromis niloticus*.
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1. INTRODUCTION

Feed is the most expensive component of aquaculture enterprise accounting for 60% of the operating cost depending on the intensity of production (Marimuthu *et al.*, 2010 and El-Sayed *et al.*, 1999). Successful cultured fish production, therefore, requires the availability of running cost and optimal feeding practices to ensure the best growth rates and feed efficiencies (Gao and Lee 2012, El-Dakar *et al.*, 2021, Abdel-Aziz *et al.*, 2021, 2016a).

Correspondence :
Ashraf Y. El-Dakar
Faculty of Aquaculture and Marine fisheries, Arish, Egypt
Mail: ayegypt99@gmail.com;
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Nile tilapia, (*Oreochromis niloticus*), one of the most popular and widely cultured species globally is in a similar situation. Its feeding usually involves two or more meals per day which further increases the feeding cost. Unfortunately, many *O. niloticus* farmers especially in developing countries lack enough income and knowledge of feeding their fish. This scarcity of income coupled with the low price of the cultured *O. niloticus* in the local markets has weakened the development of its production. One potential way of reducing feed costs is to take advantage of the compensatory growth that is usually decreased feeding days. It is a phase of fast growth that occurs after the re-feeding of fish following a period of feed deprivation or after abnormal conditions or increase in appetite (Choi *et al.*, 2021); an accelerated growth rate induced through a period of resources restriction (Nicieza and Alvarez, 2009). The compensatory growth may identify as hyperphagia usually elevate the appetite of fish and encourage them to consume more feed (Cho *et al.*, 2006). Starvation and re-feeding resulted in a rapid growth rate of fish and a reduction in nutrient intake (Mirea *et al.*, 2013) which led to reduce in feed costs as well as proper feed management. Compensatory growth has been applied for many fish-cultured species including European minnow carp, Phoxinus phoxinus (Russell *et al.*, 1992) hybrid tilapia, *O.mossambicus X O.niloticus* (Wang *et al.*, 2000), Atlantic salmon, *Salmo Salar*, (Johansen *et al.*, 2001). However, the full compensatory growth technique has been found in relatively few fish species e.g. Sunfish (Hayward *et al.*, 1997) and gibel carp (Xie *et al.*, 2001). On the other hand, intensive systems are one of the means for maximizing productivity, exploiting the available land area, and optimizing water utilization. But there is an inversely related between higher densities and water quality, growth rate, and survival rate of cultures aquatic animals (Abdel-Aziz *et al.*, 2016b and Hassan *et al.*, 2022). Therefore, the present study aimed to determine the starvation period of Nile tilapia reared under the compensatory growth condition with different stocking densities and their effects on growth performance, feed conversion ratio, body composition, and cost-benefit analyses.

2. MATERIALS AND METHODS

2.1.Site of work

The present work was conducted in Central Laboratory for the Wet Fish Lab., Fish Research Center, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.

2.2.Experimental conditions

A factorial trial (4 x2) of replicates was designed to investigate response to four different of deprivation periods and re-feeding under two different stocking densities on compensatory growth of Nile Tilapia (*Oreochromis niloticus)*. Treatments were formed as follows:- The control group was fed to satiation two times a day and one day starvation and re-feeding six days(1/6) throughout the experiment. The other three treatment groups were starved for 2, 3, and 4 days and re-feeding 7, 11, 14 days,(2/7), (3/11) and (4/14) respectively. Sixteen Fiberglass tanks (240 L) were used and equipped with aeration with blower (1HP). Tow stocking density were used in the experiment 10and 20 fish/tank or (42 fish/m^3) and (84 fish/m^3) respectively by starvation factors and re-feeding. The initial of average weight was 21.62 ± 0.08 g. A commercial floating diet containing 30% CP with diameter 2mm was used in this experiment Table 1. The water was exchanged at rate of 20% of water volume after the siphoning process every two days.

2.3.The diet used

The basal diet (30% CP) contained ingredients of Soybean meal 48%, Wheat milling by-product, Wheat bran, Yellow corn, Corn gluten 60%, Soy oil, NaCl , Calcium monohydrate, Choline chloride, Vitamin & minerals mixture.

Table 1: Proximate composition of the Commercial diets (g/kg diet).

Proximate analysis*% On DM basis*	
Dry matter	89.84
Crude protein	29.7
Crude fiber	4.65
NFE	47.45
Ash	11.54
Ether extract	6.66

2.4. Water quality indices

Temperature, pH and dissolved oxygen were recorded every day and determined using ZWQ500 Multi-Parameter Water Quality Meter. Total ammonia nitrogen was measured every week by chemical analysis of (APHA, 1992).

2.5. Determinants of growth and feed utilization

Growth performance records of initial body weight (IBW) and final body weight (FBW) of each individual fish were measured in all fish for each tank. Weight gain (WG), specific growth rate (SGR %), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and condition factor (K), were calculated using the following equations: WG=FBW (g)-IBW (g). SGR% = (ln FBW-ln IBW)/t×100; where: FBW is final body weight (g); IBW is initial body weight (g); ln= natural logarithmic; t=time in days. FCR=Feed intake (g)/WG (g). PER=WG (g)/protein intake (g). PPV% = protein gain (g)/protein intake (g) 100.

2.6. Sampling and chemical analysis

All the experimental *O. niloticus* were taken from each tank for individual weight and length measurements every 14 days. Individual *O. niloticus* weight was measured using an electronic weighing balance (model number YP5001N, made in China) to nearest 0.01 g. The length was measured using a ruler to the nearest 0.01 cm from the tip of the snout to the end of the tail, with mouth closed and the tail lobes pressed together. After the measurements, *O. niloticus* were released back to their respective treatment tanks. At the end of study four fishes were taken from each tank to analyses whole body composition according to methods of (AOAC, 2010). In the same methods were used to analyses the diet content of nutrition compounds. Gross energy GE was estimated for diets using the factors 5.64, 9.44, and 4.22 Kcal for CP, EE, and carbohydrates, respectively (NRC, 1993).

2.7.Statistical analysis

Data were analyzed by Two-way analysis of variance (ANOVA). The differences among groups were determined using Waller–Duncan test at $P \leq 0.05$ as the significance level using SPSS Statistical Package Program v.17 and means was showed with (± SE) standard error.

3. RESULTS AND DISCUSSION

3.1.Water quality

Table (2) shows the water quality parameters during the experimental period. Results showed that no significant differences ($P \leq 0.05$) were found in water dissolved oxygen, temperature, pH and total ammonia. These values were 4.99 – 5.32 ppm for DO, 27.12 -27.26 ^{o}C for temperature, 6.90 – 8.20 for pH, 0.17 - 0.18 for total ammonia.

Table 2. water quality parameters throughout the experimental period (means ±SE)

Item	**Density**	**Parameters**			
		DO (mg/L)	**temperature (C^{o})**	**pH**	**TAN, ppm**
1/6	**Low**	**5.00±0.13**	**27.12±0.21**	**7.55±0.22**	**0.18±0.01**
1/6	**High**	**4.99±0.1**	**27.14±0.32**	**8.20±0.13**	**0.17±0.01**
2/7	**Low**	**5.05±0.12**	**27.22±0.23**	**6.90±0.14**	**0.17±0.02**
2/7	**High**	**5.16±0.2**	**27.18±0.17**	**8.12±0.22**	**0.17±0.01**
3/11	**Low**	**5.32±0.3**	**27.15±0.30**	**7.40±0.12**	**0.18±0.01**
3/11	**High**	**5.08±12**	**27.23±0.20**	**7.92±0.11**	**0.18±0.02**
4/14	**Low**	**5.25±14**	**27.13±0.19**	**7.50±0.23**	**0.18±0.01**
4/14	**High**	**5.21±0.21**	**27.26±0.32**	**8.00±0.24**	**0.17±0.01**

Values are means±S.E. of duplicate groups of two tanks

All tested water quality criteria were suitable and within the acceptable limits for rearing the Nile tilapia, *O. niloticus*. The present results are in agreement with those obtained by Abdel-Hakem *et al*., (2009) who studied the effect of feeding regimes on growth performance of hybrid tilapia, O. *niloticus* and O. *aureus*, They reported that water quality was maintained at quite acceptable levels throughout the experimental. These findings supported control group GC did not affected on water quality parameters specially water temperature, DO, mg/l, pH and total ammonia, mg/l.

3.2.Growth performance: -

Growth performance of Nile tilapia fed different feeding regime was illustrated in the Table (3).

Table 3. Effect of different starvation regimes on growth performance of Nile tilapia fingerlings under different densities.

Item	Density	IBW, g	FBW, g	WG, g	SGR% /day	SR, %
1/ 6	Low	21.26	56.35^{a}	35.08^{a}	1.04^{a}	88
1/6	High	21.82	49.45 ^{b}	27.63^{c}	0.86^{b}	80
2/ 7	Low	21.30	54.70^{a}	33.40^{ab}	1.00^{a}	85
2/7	High	21.80	41.49^{c}	19.70^{c}	0.69^{c}	79
3/ 11	Low	21.48	58.06^{a}	36.58^{a}	1.06^{a}	87
3/11	High	21.81	49.88^{b}	28.07^{b}	0.88^{b}	82
4/ 14	Low	21.68	49.41^{b}	27.73^{b}	0.87^{b}	85
4/14	High	21.81	40.26^{c}	18.44^{c}	0.65^{c}	80
PSE*	-	0.08	0.19	0.27	0.01	-

Values are means±S.E. of duplicate groups of two tanks. (a, and b) Average in the same row having different superscripts significantly different at ($P \leq 0.05$)

*Pooled standard error

1 /6 means one day starvation and six day feeding ,2/7 means two day starvation and seven day feeding ,3/11 means three day starvation and eleven day feeding, 4/14 means four day starvation and fourteen day feeding.

The average weight of fish at the start of the experiment was 21.62 ± 0.08 g/fish. However, at the end of the experiment, fish body weight was differed by feeding regime. In case of low density (42/m^3), fish fed on the feeding regime system (FRS) of 1/6 was insignificantly ($P \geq 0.05$) with those fed on the feeding regime system of 3/11(Table 3). Body weight of the feeding regime system of 1/6 and 3/11 gave high significantly differences ($P<0.05$) than those feeding regime system of 2/7.

The lowest final body weight was obtained from fish fed the feeding regime system of 4/14. At the higher stocking density (84 fish/m^3), fish fed the feeding regime system (FRS) of 1/6 and FRS 3/11 gave the highest body weight, however the lowest value of body weight was recorded to fish fed the feeding regime system of 4/14 (Table 3). The same trend was found for total weight gain and specific growth rate (SGR).

Starvation followed by re-feeding regimes as FRS_3 (3/11) resulted in highest body weight than other tested feeding regimes. It indicated that fish reared in the same condition able to compensate the growth retardation during the feed starvation period of 1, 2 and 4 days and came to almost the same final weights on the control group. The present results are in agreement with those obtained by (Wang *et al*.,2000, Christensesn and Mclean, 1998, Ali *et al*., 2003,and Abdel-Hakem *et al*., 2009).

Partial compensatory growth was observed with hybrid tilapia (Christensens and Mclean, 1998) stand a period of feeding retraction could ration to a normal growth. In contrast, study of Limbu and Jumanne (2014) revealed that feed restriction did not effect on performance of Nile tilapia due to compensate growth (CG) which is defined of unusually fast growth should by individuals encountering abundant food following a period of feed deprivation.

Fig. (1) and (2) showed the effect of compensatory growth on growth of Nile tilapia. Fish fed (FRS) 3/11 was better than the control group and other feeding regime system.

Fish group fed FRS 3/11 under low density was the best feeding system in a hall time followed by FRS 1/6, and FRS 2/7. The lowest value of body weight was recorded to fish fed on FRS 4/14. However, no significant differences ($P \leq 0.05$) were observed among fish groups fed FRS 1/6, 2/7, 3/11, and 4/14.

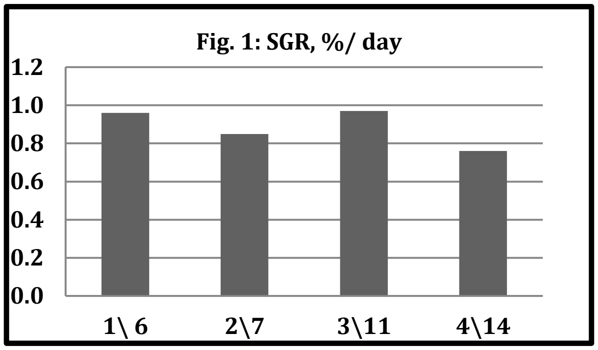

Figure 1: Effect of compensatory growth on specific growth rate of Nile tilapia regardless of stocking density.

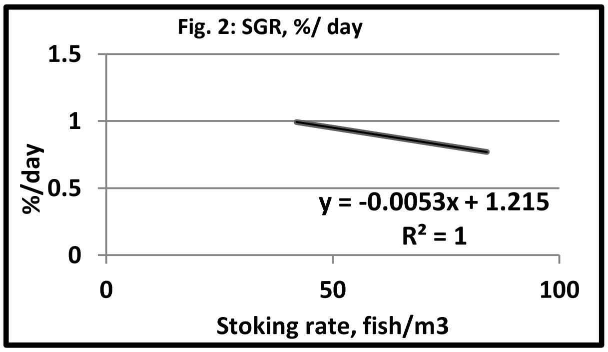

Figure 2: Effect of stoking rate on specific growth rate of Nile tilapia regardless of re-feeding.

This mean that starvation fish to three days then re-feeding for eleven days gave similar results to fish fed the FRS 1/6 that mean starvation one day and re-feeding six days , recording save one day feed and increase one day starvation than control group (2/12). The above observation didn't record in case of FRS 2/7 and FRS 4/14.

Results appeared that the best SGR was recorded in fish fed FRS 3/11 compared to control group (Table 3). The lowest SGR was recorded in fish fed FRS 4/14. It was illustrated that SGR values of fish fed feeding regime system of 1/6 and 3/11 higher significantly ($P<0.05$) than those fed feeding regime system of 2/7 and 4/14.

In the present study feeding regime systems 1/6, 2/7 and 3/11, respectively didn't effect on SGR, while feeding regime system 4/11 under high stocking density was recorded the lowest SGR of Nile tilapia (Table 3).

Stocking density has a strong effect on CG of Nile tilapia. Specially with increasing starvation on days. The present results indicated that SGR of fish were starved for two days then re-feed for 7 days (FRS 2/7) under low stocking density was significantly higher than the same feeding system under higher stocking density (1.00 vs 0.65%/day/fish), respectively.

Feed intake of fish fed feeding regime system 2/7 under low or high density was 76.42 and 90.54 g/fish respectively. This indicated that fish that reared at a lower density failed to assimilate feeds to good growth which focuses on the importance of the duration as the frequency of feed during re-alimentation may be very important for the regulation of catch-up growth and other parameters in Nile tilapia (Yousif, 2002).

Regarding, the values of survivability were observed as 88%, 85%, 87% and 85% in treatments 1/6, 2/7, 3/11 and 4/14 respectively in low stocking density and the values survivability were observed as 80%, 79%, 82% and 80%in treatments 1/6, 2/7, 3/11 and 4/14 respectively in height stocking density. The total feed intake in different treatments ranging from 73.75±1.70 to 102.85±1.70 in 3/11 and 1/6 respectively (table 4 and Fig 3).

Table 4. Effect of different starvation regimes on feed and nutrient intake of Nile tilapia fingerlings.

Item	Density	Feed intake g/fish	DM g/fish	CP intake g/fish	GE intake kcal/fish	DE intake kcal/fish
1/6	Low	100.88^{a}	90..63^{a}	26.92^{a}	4.45	3.96
1/6	High	102.85^{a}	88.96^{a}	26.42^{a}	4.35	3.69
2/7	Low	76.42^{c}	68.66^{c}	20.39^{b}	4.28	3.64
2/7	High	90.54^{ab}	81.34^{b}	24.16^{ab}	4.05	3.45
3/11	Low	73.75^{c}	66.26^{c}	19.68^{c}	4.31	3.68
3/11	High	81.03^{b}	72.80b^{c}	21.62^{b}	4.25	3.64
4/14	Low	79.52^{b}	71.44^{bc}	21.22^{b}	4.26	3.60
4/14	High	90.80^{ab}	81.78^{b}	24.23^{ab}	4.15	3.59
*PSE	-	1.70	1.46	0.19	0.03	0.001

Values are means of duplicate groups of two tanks. (a, and b) Average in the same row having different superscripts significantly different at ($P\leq 0.05$)

*Pooled standard error

1 /6 means one day starvation and six day feeding ,2/7 means two day starvation and seven day feeding ,3/11 means three day starvation and eleven day feeding, 4/14 means four day starvation and fourteen day feeding.

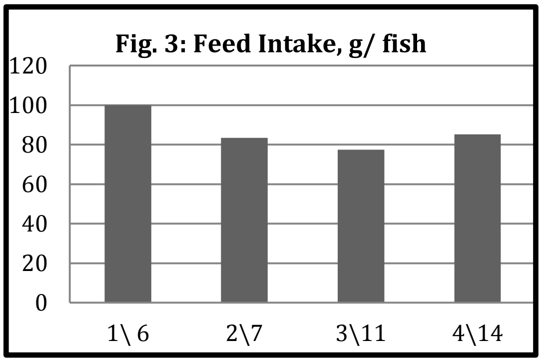

Figure 3. Effect of re-feeding on feed intake of Nile tilapia regardless stocking density.

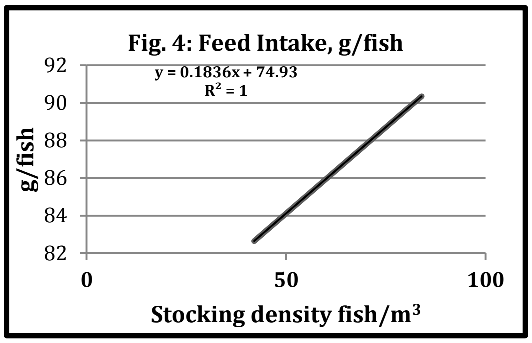

Figure 4. Effect of stoking rate on feed intake of Nile tilapia regardless of re-feeding.

The highest feed intake was recorded to 1/6 feeding system under high density while the lowest feed intake was recorded to 3/11 feeding system under low density. Yousif (2002) reported that increasing the number of fish (density) adversely affect fish growth. The present study found that higher stoking density adversely affect the growth of Nile tilapia, corresponds to our result similar results were reported by Breine *et al*. (1996). Social interactions through competition for food and/or space can negatively affect fish growth, hence higher stocking densities leads to increased stress and that resulting increase in energy requirements causing a reduction in growth rates and food utilization (Fig, 4). This explanation is in conformity with the study was done by (Aksungur *et al.*, 2007).

The FCR values in treatment 1/6, 2/7, 3/11 and 4/14 were significantly different ($P<0.05$) (table 5 and Fig. 5).

Feed conversion ratio significantly ($P<0.05$) affected by feeding regime and stocking densities. The best FCR was recorded to fish fed 3/11 under low density. In general, high FCR may be due to the quality of feed ingredients used in the feed formulation, subsequently, inefficiency of fry to convert feed into flesh (nutrient digestibility and absorption), environmental factors etc (Liti *et al.*, 2006).

Table 5. Effect of different starvation regimes on feed and nutrient intake of Nile tilapia fingerlings.

Item		Feed utilization		
		FCR	PER	PPV %
	Density			
1/6	Low	2.88[bc]	1.31[a]	22.35[ab]
1/6	High	3.73[b]	1.05[a]	17.42[b]
2/7	Low	2.30[c]	1.63[a]	28.93[a]
2/7	High	4.60[a]	0.81[b]	14.55[c]
3/11	Low	2.01[d]	1.86[a]	35.11[a]
3/11	High	2.90[bc]	1.30[a]	23.51[ab]
4/14	Low	2.87[bc]	1.31[a]	23.72[ab]
4/14	High	4.94[a]	0.76[c]	13.02[c]
*PSE	-	0.05	0.01	0.36

Values are means± S.E. of duplicate groups of two tanks. (a, and b) Average in the same row having different superscripts significantly different at (P≤0.05)
1 /6 means one day starvation and six day feeding ,2/7 means two day starvation and seven day feeding ,3/11 means three day starvation and eleven day feeding, 4/14 means four day starvation and fourteen day feeding.
*Pooled standard error

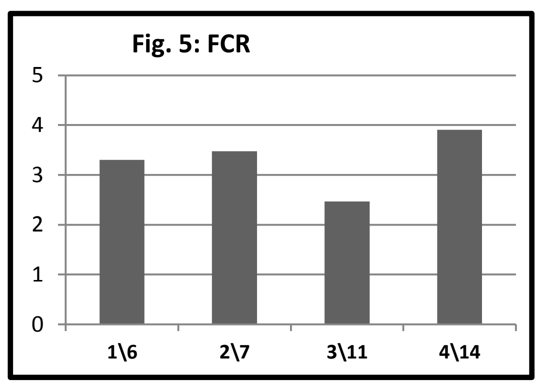

Figure 5: Effect of re-feeding on FCR of Nile tilapia regardless of stoking rate.

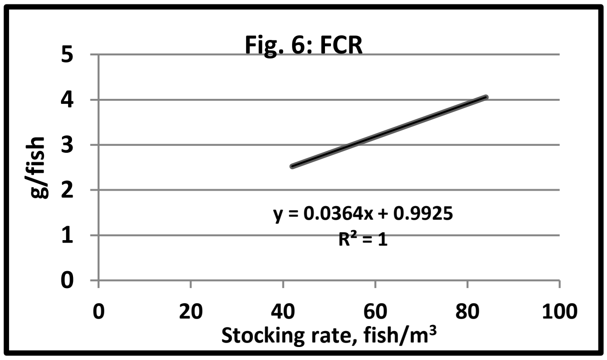

Figure 6: Effect of stoking rate on FCR of Nile tilapia regardless of re-feeding**.**

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. Feed intake of the treatment 3\11 73.75 g vs 80.03 g/fish indicated those reared lower density they successful assimilation of this feed resulted in good growth emphasizing the importance of duration as the frequency of feed during re-feeding may be very important for regulating catch-up growth and other factors in Nile tilapia (Yousif, 2002).

Moreover, Niazie *et al.* (2013) FCR value was significantly increased indicating fish high density that leading to increased metabolism then their requirements of energy increase. Similar trends were observed by (Larsen *et al.*, 2012, Abdel-Aziz *et al.*, 2016b).

These finding are completely in agreement with our result as showed Fig. 6. Protein efficiency ratio (PER) significantly affected by different feeding regime systems and stocking density (Table 5). The highest PER was recorded to 3/11 feeding system under low density while the lowest PER was recorded to 4/14 feeding system under high density. Results in this study are in agreement with those obtained by Ali *et al.* (2003) they explained that the compensatory growth may be an internal adjustment mechanism for animal to adapt to often dramatically varied environment.

The animal that withstands a period of nutrition restriction could retain to a normal growth. In contrast, the results of Wang *et al*. (2000) and Christensen and Mclean (1998) revealed that hybrid tilapia reared in sea water and Mozambique tilapia had a partial compensatory growth.

3.3. Whole body composition

Values of body composition of the fish are showed in Table (6). There were significant differences between Dry matter, crude protein, lipids, ash and gross energy in initial fish body composition and final fish body composition. But there are no significant differences between the treatments ($P \geq 0.05$) in both low and high stocking density.

Table 6. Effect of different starvation regimes on body composition of Nile tilapia fingerlings

Item		DM %	% On DM basis			GE
			CP	Lipids	Ash	kcal/fish*
start		**23.56**	**61.16**	**12.48**	**25.53**	**923**
	Density					
1/6	Low	25.85	62.32	13.71	23.35	940
1/6	High	25.80	60.75	14.62	24.14	916
2/7	Low	25.80	63.84	13.62	21.99	963
2/7	High	25.39	62.83	13.63	22.94	949
3/11	Low	27.65	62.26	13.93	23.30	939
3/11	High	26.29	62.36	14.03	22.97	941
4/14	Low	26.50	62.29	13.39	23.63	940
4/14	High	25.27	61.94	13.80	23.61	935
**PSE	-	0.25	0.28	0.10	0.21	4.15

1 /6 means one day starvation and six day feeding ,2/7 means two day starvation and seven day feeding ,3/11 means three day starvation and eleven day feeding, 4/14 means four day starvation and fourteen day feeding.

*GE means gross energy kcal. g-1

**Pooled standard error

4. CONCLUSION

The results of the current study summarized that re-feeding for 11 days after a 3-day starvation period is considered the best feeding system that achieves the best compensatory growth and the best FCR when tilapia are raised at 42 or 84 fish / m^3. Also, compensatory growth as a result of starvation and re-feeding is negatively affected by an increase in fish density.

Furthermore, fish body composition was not affected by differences of feeding regimes or stoking rates.

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