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EFFECT OF FEEDING RATE, FREQUENCY AS WELL AS STOCKING DENSITY, ON REPRODUCTIVE PERFORMANCE OF NILE TILAPIA BROODSTOCK KEPT IN HAPAS SUSPENDED IN EARTHEN POND

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SUMMARY

This study investigated the effects of three stocking densities (4, 6 and 8 fish/m^3), two levels of feeding (0.5 or 1% BW/day) and two feeding frequency (once or twice daily) on the fecundity, spawning frequency, egg weight and number of Nile tilapia, *Oreochromis niloticus* (L.) broodstock to improving hatchery seed production. The trial started at 19/5/2010 and continued 110 days in a commercial hatchery in Fayoum governorate, 3600 fish at a rate of 1 ♂: 3♀ were stocked in 24 hapa suspended in earthen pond. Eggs were collected every 7 days directly from the mouths of incubating females. Higher eggs weight was observed in treatments with lower levels of stocking density (4 fish/m^3) and feeding rate of 1.0% BW and twice daily feeding. Stocking density interacted with feeding rates and frequency improving egg weight, egg number, FCR and economic efficiency. This suggests that feeding rate (1% BW/day), lower stocking density (4 fish/m^3) and twice daily feeding could be adopted as a management strategy to improve current tilapia hatchery seed production.

Key words: *Stocking density, feeding rate, feeding frequency, reproductive performance, Nile tilapia and hapa.*

INTRODUCTION:

Tilapias have a long history of aquaculture and are known to have been cultured in ponds thousands of years ago in ancient Egypt and Rome. Due to their economic importance, the familiarity with their life cycle, and the relative simplicity of reproducing and maintaining these species, tilapias became some of the more extensively studied fish in the last half century (Kocher and Kole, 2008).

Tilapias are easily bred in captivity, and in the tropics they can breed all year round. In subtropical regions, the breeding season is limited by water temperature and may range from 5 to 7 months. Fry production can be practiced in a wide range of facilities (earthen, concrete, and plastic-lined ponds; fine-mesh cages; aquaria; and tanks). Due to their investment in parental care, fecundity is relatively low compared to most fishes and ranges from several hundred to several thousand per spawn, depending on the species and size of the breeding female (Kocher and Kole, 2008).

The intensive farming of tilapia is rapidly expanding and the need to produce sufficient quantities of quality fry is becoming crucial to meet increasing global demands for stocking tilapia farms (Ng and Wang, 2011).

Fine meshed nylon net cages 'hapas' have been used for collected the fry from broodstock. Hapa nursing of small fry to larger, more predator-resistant fingerlings has been the focus for intensification of aquaculture in most countries (Barman and Little, 2011). The high degree of control of broodfish that is possible using fine meshed nylon net cages or 'hapas' has made them popular for both breeding and nursing tilapia seed-stock at several levels of intensity (Beveridge, 2004; Guerrero and Garcia, 1983; Hughes and Behrends, 1983; Little *et al.*, 1993; Mair and Little, 1991) and can result in high quality mixed-sex fish (Little, 2004) of the same age and size (Dan and Little, 2000; Little *et al.*, 2003).

Stocking density can influence seed production in tilapia in culture conditions (Hughes and Behrends 1983; Guerrero and Guerrero, 1985; Khater and Ali, 1988: Obi and Shelton, 1988). The effect of various stocking densities and sex ratios on hatchery production of tilapia was reported in land-based (concrete tanks) and lake-based (hapa nets) systems (Bautista *et al.*, 1988). Low broodstock densities and low sex ratios generally resulted in higher fingerling production (Hughes and Behrends, 1983).

Hughes and Behrends (1983) suggested that 5 fish/m^3 for the optimum seed production of Nile tilapia in suspended hapa. On the other hand, only 29% of Nile tilapia females reared in hapas in ponds in Thailand spawned when broodstock density was 10 fish/m3, compared to 39% and 42% at 5 and 2.5 fish/m^3 (Bevis, 1994). However, Ridha and Cruz (1999) found that 4 fish/m3 had better seed production and spawning synchrony than 8 and 12 fish/m3.

Izquierdo *et al*. (2001) sited that Broodstock nutrition is one of the most important factors limiting fish fry production and larval quality. Gunasekera *et al.* (1997) pointed out to the critical role of nutrition to brooding females, in supplying the essential nutrients required for gonadal development as well as the performance of their seeds and larvae. Macintosh and Little (1995) fed brooding females at a rate of 1.5 % of the total fish biomass/day. Abou-Zied (2006) reported that the 1.0% feeding rate is the best for tilapia broodstock.

The present study aimed to investigate the effects of three stocking densities (4, 6 and 8 fish/m^3), two levels of feeding (1 or 2%BW/day) and two feeding frequency (once or twice daily) on the fecundity, spawning frequency, egg weight and number of Nile tilapia, *Oreochromis niloticus* (L.) broodstock to improving hatchery seed production.

MATERIALS AND METHODS:

Experimental design

In 3×2×2 factorial design, using 24 hapa (7× 5× 0.0.75 m) with a net water volume of 25 m^3/hapa, three different broodstock densities (4, 6 and 8 fish/ m^3) were combined with two different feeding rate (0.50 and 1.0%) and two feeding frequency (once and twice daily) to obtain 12 experimental treatments with two replicate. Brood-fish were stocked at a ratio of three females to one male (1♂: 3♀). The experimental treatments were assigned to this histogram as follows: -

Treatment	Stocking density, fish No/m^3	Feeding frequency	Feeding rate, % of body weight	Fish No/hapa	
				Total	Female
T_1	4	1	0.50	100	75
T_2	4	2	0.50	100	75
T_3	4	1	1.0	100	75
T_4	4	2	1.0	100	75
T_5	6	1	0.50	150	112
T_6	6	2	0.50	150	112
T_7	6	1	1.0	150	112
T_8	6	2	1.0	150	112
T_9	8	1	0.50	200	150
T_{10}	8	2	0.50	200	150
T_{11}	8	1	1.0	200	150
T_{12}	8	2	1.0	200	150

Experimental fish

This study was conducted at a commercial hatchery with production capacity of 7 million fry annually in Fayoum Governorate at 19/5/2010 and continued for 110 days. Nile tilapia *O. niloticus*, broodstock were obtained from hatchery earthen ponds and manually selected, sexed and transferred to hapas, that held in earthen pond. The average weight of broodstock was 170 ± 5.43 g.

A total number of 2696 females and 904 males were counted, batch weight and stocked in each hapa at a rate of 4, 6 and 8 fish/ m^3.

Experimental diets

Broodstock were fed a commercial diet (Table 1) which contained 27.48 % CP. Eggs were collected every 7 days intervals.

Data obtained were spawned females number/hapa, % spawned females, seed weight/hapa, seed number/hapa, seed weight/female, absolute fecundity (Rana, 1990), number of spawning/female/period, broodstock weight at the start and at the end, daily gain, % SGR, feed intake and feed conversion ratio (FCR) with a simple economic evaluation.

Statistical analysis was performed using SPSS, 1997. Statistical significant between treatments was evaluated at the 5 % probability level. General liner model (ANOVA) and regression analysis were used.

Table (1). Chemical analysis of used diet, on DM basis.

Items	%
Crude protein, CP	27.48
Ether extract, EE	10.77
Ash	6.34
Crude fiber, CF	5.02
Nitrogen free extract. NFE[1]	40.42
GE, kcal/g*	4.433

1, Calculated by differences * Calculated according to NRC, 1993.

RESULTS AND DISCUSSIONS:

1- Effect of different densities

1.1. Spawning parameters

Table 2 shows the effect of broodstock density on reproductive performance, there are significant differences in spawned female number through the experiment and this is natural because of the different densities (4, 6 and 8/ m^3) used. Also, significant differences between spawned female%, egg weight/female, fecundity, and spawning frequency were found and the low density (4/ m^3) was the best one followed by 6/m^3 and 8/ m^3 respectively. Egg weight, egg number and system productivity were insignificant although it increased fish number in 6/m^3 and 8/m^3 the differences between these data was slightly. These results were agreement with these results obtained by (Hughes and Behrends 1983; Khater and Ali, 1988 and Ridha and Cruz, 1999) and reflected that low density resulted in better reproductive performance and took advantage benefit from surrounding circumstances like, oxygen and better water quality than other higher densities.

1.2. Feed conversion

The main important character of feed utilization is feed conversion ratio (FCR) especially with egg production (Excluding fish weight) and total conversion but FCR with weight gain is not important because the purpose of the experiment was spawning. Table 2 showed the effect of different densities of broodstock FCR for egg production which significantly higher with low density which showed the best followed by other densities (6/ m^3 and 8/ m^3) respectively. These results reflect the best benefit low density of feed than high density.

1.3. Economic evaluation

Regarding economic evaluation of broodstock spawning as shown in Table 2. the Table showed that the number of fry was selling was higher with high density thus the total costs was increased with high density. Comparing net returns the low density was the best than high density. Costs of produced 1000 fry was lower with low density

Table (2). Effect of brood stock density on reproductive performance of Nile tilapia through the experimental period.

Parameters	Broodstock density/ m^3 4	6	8	SED
Spawning parameters				
Spawned females number/hapa/period (1)	**277.75[c]**	**339.63[b]**	**435.00[a]**	**21.62**
Spawned females %(2)	**24.69[a]**	**20.22[b]**	**19.33[b]**	**1.32**
Egg weight, g/hapa/period	**1954**	**1989**	**2060**	**238.28**
Egg No/hapa (3)	**197678**	**201144**	**209601**	**23847**
Egg weight, g/ female/period	**26.05[a]**	**17.76[b]**	**13.73[b]**	**2.59**
Absolute fecundity (4)	**699[a]**	**588[b]**	**479[c]**	**45.0**
System productivity(5)	**72**	**73**	**76**	**8.67**
No of spawning/ female per period .	**3.70[a]**	**3.03[b]**	**2.90[b]**	**0.20**
Hatching %	**81.66**	**81.98**	**81.85**	**0.36**
Feed conversion				
Total feed used, kg/hapa	**14.438[c]**	**21.656[ab]**	**28.875[a]**	**4.00**
FCR				
Excluding egg weight (6)	**6.53**	**6.55**	**5.72**	**0.90**
Excluding fish weight (7)	**7.44[c]**	**10.75[b]**	**13.79[a]**	**1.25**
Including fish weight and egg weight(8)	**3.46**	**4.03**	**4.03**	**0.50**
Economic efficiency				
Fry selling No/hapa	**129000**	**132000**	**137000**	**---**
Total costs, L.E	**1026**	**1201**	**1376**	**---**
Fry selling, L.E	**4514**	**4617**	**4800**	**---**
Net return, L.E	**3488**	**3416**	**3424**	**---**
Total selling/total costs %	**438.74**	**383.41**	**347.76**	**---**
Costs of produced 1000 fry, L.E	**8.58**	**9.47**	**10.36**	**---**

* Average in the same row having different superscripts differ significantly $P \leq 0.05$.
* SED is the standard error of difference
* No of collection = 15, Experimental period = 110 days
(1) = Spawned females number/hapa × No of collection
(2) = Spawned females number/hapa × 100/ No of female per hapa.
(3) = Egg weight per hapa, g × egg No per g
(4) = (3) / Spawned females number per hapa
(5) = Egg No/m/day.
(6) = Total feed offered per hapa/ (1).
(7) = Total feed offered per hapa/ egg weight per hapa.
(8) = Total feed offered per hapa/ (Total gain per hapa + egg weight per hapa).
- Feed price 3500 L.E / ton - Price of 1000 fry = 35 L.E
- Costs including (pumps, hapas, caretaker's laborer and laboratory house, land rental, fuel, electricity, feeds and workers' salaries…)

2. Effect of feeding rate

2.1. Spawning parameters

In effect of feeding rate number of broodstock was equal and the results were shown in Table 3 which showed that the 1.0% feeding rate was the best rate significantly than 0.5% in most measures except spawned female number was high at 1.0% but insignificant. This reflects that brood benefit from food at a rate of 1.0% better than 0.50% because a part of food may be loosed during throw the food in hapas or stay in any corner of hapa, but 1.0% when losing any part the remains enough to broodstock feeding, or low feeding was not enough. These results were agreement with the results obtained by Macintosh and Little (1995) and Abou-Zied (2006).

2.2. Feed conversion

Table 3 showed the effect of feeding rate on broodstock feed utilization expressed as feed conversion ratio for egg (FCR Excluding weight). Significant effect was found and the best value was recorded with 1.0% feeding rate which explain that the broodstock high utilized from feed at a rate of 1.0% than 0.50%.

2.3. Economic evaluation

Regarding economic evaluation of broodstock spawning as shown in Table 3. the Table showed that the number of fry was selling was increased number with high feeding rate thus the total costs was increased. Comparing net returns the high feeding rate 1.0% was the best than low feeding rate 0.50%. Costs of produced 1000 fry was lower with 1.0% feeding rate.

Table (3). Effect of broodstock feeding rate on reproductive performance of Nile tilapia through the experimental period.

Parameters	Feeding rate %		SED
	0.5	1.0	
Spawning parameters			
Spawned females number/hapa/period (1)	**321.67**	**379.92**	**36.82**
Spawned females % (2)	**19.60^b**	**23.23^a**	**1.51**
Egg weight, g/hapa/period	**1655^b**	**2347^a**	**148.6**
Egg No/hapa (3)	**$16794b5^b$**	**237670^a**	**14772**
Egg weight, g/ female/period	**15.73^b**	**22.63^a**	**3.21**
Absolute fecundity (4)	**533^b**	**645^a**	**57.29**
System productivity (5)	**61^b**	**86^a**	**5.37**
No of spawning/ female per period.	**2.94^b**	**3.48^a**	**0.23**
Hatching %	**81.90**	**81.75**	**0.36**
Feed conversion			
Total feed used, kg/hapa	**14.438^b**	**28.875^a**	**2.65**
FCR			
Excluding egg weight (6)	**5.22^b**	**7.31a**	**0.59**
Excluding fish weight (7)	**8.89^b**	**12.43a**	**1.29**
Including fish weight and egg weight (8)	**3.19^b**	**4.48a**	**0.31**
Economic efficiency			
Fry selling No /hapa	**110000**	**155000**	**---**
Total costs, L.E	**1176**	**1226**	**---**
Fry selling, L.E	**3851**	**5436**	**---**
Net return, L.E	**2675**	**4210**	**---**
Total selling/total costs %	**330.04**	**449.90**	**---**
Costs of produced 1000 fry, L.E	**10.97**	**7.97**	**---**

* Average in the same row having different superscripts differ significantly P≤0.05.
* SED is the standard error of difference
* No of collection = 15, Experimental period = 110 days
(1) = Spawned females number/hapa × No of collection
(2) = Spawned females number/hapa × 100/ No of female per hapa.
(3) = Egg weight per hapa, g × egg No per g
(4) = (3) / Spawned females number per hapa
(5) = Egg No/m/day.
(6) = Total feed offered per hapa/ (1).
(7) = Total feed offered per hapa/ egg weight per hapa.
(8) = Total feed offered per hapa/ (Total gain per hapa + egg weight per hapa).
- Feed price 3500 L.E / ton - Price of 1000 fry = 35 L.E
- Costs including (pumps, hapas, caretaker's laborer and laboratory house, land rental, fuel, electricity, feeds and workers' salaries…)

3. Effect of feeding frequency

3.1. Spawning parameters

In feeding frequency number of broodstock was equal and the results is shown in Table 4. feeding frequency insignificantly affect on reproductive performance except egg weight and number per hapa and system productivity were better with the twice daily feeding than once daily. The stomach of tilapia fish is small and increased feeding number lead to improved all production parameters and complete useful of food to reconstruction of the ovaries after spawning to enter the following.

3.2. Feed conversion

Table 4 showed the effect of feeding frequency on broodstock feed utilization expressed as feed conversion ratio for weight (FCR Excluding egg). Significant effect was found and the best value was recorded with twice daily feeding which explain that the broodstock high utilized from feed than once daily feed.

3.3. Economic evaluation

Regarding economic evaluation of broodstock spawning as shown in Table 4. the Table showed that the number of fry was selling was increased with twice feeding daily. Comparing net returns the twice daily feeding was the best than once. Costs of produced 1000 fry was lower with twice daily feeding.

Table (4). Effect of brood stock feeding frequency on reproductive performance of Nile tilapia through the experimental period.

Parameters	Feeding frequency/ day		SED
	Once	Twice	
Spawning parameters			
Spawned females number/hapa/period (1)	332.25	369.33	38.65
Spawned females % (2)	20.29	22.53	1.68
Egg weight, g/hapa/period	1765^{b}	2237^{a}	198.82
Egg No/hapa (3)	179661^{b}	225954^{a}	20089
Egg weight, g/ female/period	16.72	21.65	3.45
Absolute fecundity (4)	546	631	60.37
System productivity (5)	65^{b}	82^{a}	7.30
No of spawning/ female per period .	3.04	3.37	0.25
Hatching %	82.06	81.60	0.34
Feed conversion			
Total feed used, kg/hapa	21.656	21.656	4.06
FCR			
Excluding egg weight (6)	7.38^{a}	5.16^{b}	0.57
Excluding fish weight (7)	11.83	9.49	1.40
Including fish weight and egg weight (8)	4.45a	3.23b	0.32
Economic efficiency			
Fry selling No /hapa	118000	147000	---
Total costs, L.E	1201	1201	---
Fry selling, L.E	4130	5158	---
Net return, L.E	2929	3947	---
Total selling/total costs %	344.79	435.14	---
Costs of produced 1000 fry, L.E	10.62	8.32	---

* Average in the same row having different superscripts differ significantly P≤0.05.
* SED is the standard error of difference
* No of collection = 15, Experimental period = 110 days
(1) = Spawned females number/hapa × No of collection
(2) = Spawned females number/hapa × 100/ No of female per hapa.
(3) = Egg weight per hapa, g × egg No per g
(4) = (3) / Spawned females number per hapa
(5) = Egg No/m/day.
(6) = Total feed offered per hapa/ (1).
(7) = Total feed offered per hapa/ egg weight per hapa.
(8) = Total feed offered per hapa/ (Total gain per hapa + egg weight per hapa).
- Feed price 3500 L.E / ton - Price of 1000 fry = 35 L.E
- Costs including (pumps, hapas, caretaker's laborer and laboratory house, land rental, fuel, electricity, feeds and workers' salaries…)

4. Regression analysis

Table 5 shows the regression analysis between fecundity, spawning frequency and egg weight as a dependant factor and broodstock density as independent factor (X)

Significant negative relationship was found between fecundity, spawning frequency and egg weight and broodstock density. This means that decreasing broodstock density increased fecundity, spawning frequency and egg weight**.**

Table (5). Regression analysis between egg weight, fecundity and spawning frequency, (Ŷ) and broodstock densities, (X)

(ŷ)	a	b	r	n
Egg weight/female	**36.959**	**– 3.078**	**0.682 ****	**24**
Fecundity	**922.23**	**– 55.53**	**0.731 ****	**24**
Spawning frequency	**4.417**	**- 0.201**	**0.642****	**24**

5. Interaction between density, feeding rate and frequency

The impact of overlapping factors is the important for the breeders to get the best output results of the interaction of these factors. Sometimes increased number of brood/m^3 lead to increased number of fry but this is difficult applying because high costs thus the breeders selected the best interaction which lead to increased number of fry with kept on low costs resulted a best economic evaluation.

Regarding to interaction of study factors. From the previous results low stocking density, 1.0% feeding rate and twice daily feeding was the best resulted. The reaction between these factors produced the best treatment was shown in Table 6. The best reproductive performance results was significantly obtained with treatment 4, 3, 2 and 12 (density 4/ m^3 + 1.0% feeding rate+ twice daily feed) (density 4/ m^3 + 1.0% feeding rate+ once daily feed) (density 4/ m^3 + 0.50% feeding rate+ twice daily feed) and (density 8/m^3 + 1.0% feeding rate+ twice daily feed) for spawned female %. But the best egg weight obtained with treatments 4 and 12. System productivity which increased with high density was the best with low density when reacted with other factors treatment 4. (density 4/ m^3 + 1.0% feeding rate+ twice daily feed) recorded the best treatments.

Regarding to the economic evaluation treatments 4, 8, 3 was the best treatments economically although these results obtained with low density but maintenance of target production of fry from hatchery.

Finally from these results to improved reproduction efficiency and best economically, suggesting low stocking density from (4-6 fish/m^3) and 1% feeding rate of broodstock body weight twice daily in a commercial hatchery

Table (6). Effect of interaction between broodstock density, feeding rate and frequency on Nile tilapia spawning.

Parameters	Treatment= (density 4, 6, 8) × (feeding rate 0.5, 1) × (feeding frequency, 1, 2)											
	1	2	3	4	5	6	7	8	9	10	11	12
Spawned females number	235	257.5	290.5	328	302.5	355	348	353	352.5	427.5	465	495
Spawned females %(1)	20.89	22.89	25.82	29.16	18.01	21.13	20.71	21.01	15.67	19.00	20.67	22.00
Egg weight/hapa, g	1222	1866	2005	2721	1426	1934	2191	2404	1514	1969	2232	2528
Egg No/hapa (2)	123950	187913	203450	275400	144950	196050	221700	241875	157230	197580	226687	256905
Egg weight/ female, g	16.29	24.88	26.74	36.28	12.73	17.27	19.56	21.46	10.09	13.13	14.88	16.85
Absolute fecundity (3)	527.5	730	700	840	477.5	552.5	637.5	684.5	446.5	461.5	487.5	518.5
System productivity	45.07	68.33	73.98	100.1	52.71	71.29	80.62	87.95	57.17	71.85	82.43	93.42
No of spawning/ female per period.	3.13	3.43	3.87	4.37	2.70	3.17	3.11	3.15	2.35	2.85	3.10	3.30
Total feed used, kg/hapa	9625	9625	19250	19250	14438	14438	28875	28875	19250	19250	38500	38500
FCR excluding fish weight(4)	7.88	5.16	9.64	7.08	10.26	7.47	13.18	12.08	12.75	9.85	17.28	15.31
Fry No after sexing/hapa	81.000	123.000	134.000	178.000	95.000	129.000	145.000	159.000	103.000	129.000	149.000	167.000
Total costs, L.E	1009	1009	1042	1042	1176	1176	1226	1226	1342	1342	1410	1410
Fry selling, L.E	2835	4305	4690	6230	3325	4515	5075	5565	3605	4515	5215	5845
Net return, L.E	1826	3296	3648	5188	2149	3339	3849	4339	2263	3173	3805	4435
Total selling/total costs %	281.0	426.7	450.1	597.9	282.7	383.9	413.9	453.9	268.6	336.4	369.9	414.5
Costs of produced 1000 fry	12.46	8.20	7.78	5.85	12.38	9.12	8.46	7.71	13.03	10.40	9.46	8.44

* No of collection = 15, experiment period = 110 days

(1) = Spawned females number/hapa × 100/ No of female per hapa.

(2) = Egg weight per hapa, g × egg No per g

(3) = (2) / Spawned females number per hapa

(4) = Total feed offered per hapa/ egg weight per hapa.

- Price of 1000 fry = 35 L.E
- Costs including (pumps, hapas, caretaker's laborer and laboratory house, land rental, fuel, electricity, feeds and workers' salaries…)
- Treatments 1, 2, …. 12 as shown in histogram (1).

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تأثير معدل وعدد مرات التغذية و كثافة التخزين على الأداء التناسلى لأمهات البلطى النيلى المسكنة فى هابات معلقة فى حوض ترابى

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أجريت هذه الدراسة لتقييم تأثير ثلاثة كثافات (4، 6، 8 سمكات/م^3) ومستويين تغذية (1، 2% من وزن الجسم) و عدد مرات التغذية (مرة أو مرتان يوميا) على الخصوبة، عدد مرات التفريخ، وزن و عدد البيض لأمهات البلطى النيلى لتحسين إنتاج المفرخ من الزريعة.

بدأت التجربة فى 2010/5/19 واستمرت لمدة 110 يوم فى مرخ تجارى بمحافظة الفيوم. خزنت 3600 أم من الذكور والإناث فى 24 هابة مثبتة فى حوض ترابى. جمع البيض كل 7 أيام من فم الإناث الحاضنة. أعلى وزن بيض تم الحصول عليه مع المعاملات المنخفضة ى كثافة التخزين (4 سمكات/م^3) و 1% معدل تغذية مرتين يوميا.

تفاعل كثافة التخزين مع معدل وعدد مرات التغذية أدى لتحسين وزن البيض وعدد البيض ومعدل التحويل الغذائى وكذلك الكفاءة الاقتصادية. لذلك يقترح استخدام كثافة التخزين المنخفضة (4 سمكات/م^3) مع معدل تغذية 1% مرتين يوميا يعتبر إداريا استيراتيجية لتحسين إنتاج المفرخ من الزريعة.