J. Agric. Sci. Mansoura Univ., 31 (9): 5689 - 5704, 2006

A STUDY ON NILE TILAPIA BROODERS DURING WINTERING BY ADDING DIETARY FATS OR VITAMINS

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ABSTRACT

This study was carried out during 2004/2005 on Nile tilapia fish. Fats or vitamins were added to the brooders' diet. This work was evaluated via fish weight, egg production, economic efficiency, fish body composition and blood analysis. From the obtained results, it could be concluded that Nile tilapia brooders used in hatching could be fed before spawning season a diet contains 25% crude protein and supplemented with 5% of a mixture 1:1 of fish oil and sunflowers oil. This dietary treatment was the best concerning % laying brooders, fry production, profitability, economic efficiency and immunity (high WBCs and % lymphocytes).

Keywords: Nile tilapia – Over wintering – Oils – Vitamins – Reproduction – Performance.

INTRODUCTION

Delgado *et al.* (2003) cited that fish are an important source of protein, especially in developing countries. Fish are accounted for 20 % of animal-derived protein in low-income, food-deficit countries, compared with 13 % in the industrialized countries (Bardach *et al.*, 1972). Tilapia fish are favorable for their rapid growth, beneficial of a wide variety of natural and artificial foods, tolerance for a wide range of environmental conditions, resistance for diseases and stresses, and prolification in capture (El-Sayed and Teshima, 1991). Currently, tilapia culture is widely practiced In many tropical and subtropical regions of the world. Tilapias are the third largest group of farmed finfish species, only after carps and salmonids, with an average annual growth rate of about 11.5%. The global production of farmed tilapia has increased more than three-folds since 1984, 98.6% of farmed tilapia were produced in developing countries in 1995 (FAO, 1997). However, 54% of the Egyptian fish production came from the fish culture (GAFRD, 2005). Many of the problems associated with tilapia farming arise from the difficulty of producing large numbers of fry (Bhujel, 2000). This is almost certainly true under the hot temperate climatic conditions that prevail in several tilapia producing countries such as Egypt, their homeland. Under these conditions, temperature drops during winter months below the range within which tilapia can be grown economically. Therefore, the present study was decided to try to overcome the over wintering problem in Nile tilapia fish under the Egyptian conditions. It was carried out to improve tilapia fish performance during hatching as well as throughout the over wintering period. These aims were tried to be reached via feed additives, namely lipids (fish oil and sunflowers oil) and vitamins (C and E) in a brooder trial aiming to elevate reproduction of fish and for increasing deposit fat in the fish body during winter (fasting

season) as well as for elevating the immunity to resist cold weather and consequently improving performance of fish.

MATERIALS AND METHODS

Similar body weight and sized Nile tilapia brooders were selected in June and July from the Fish Hatchery of the Integrated Fish Farm, Al-Manzalah. Each sex of the selected brooders was kept in a separate out-door concrete tank (15 x 3 x 0.75 m) provided with feed, oxygen and changing the rearing water till the start of the experiment. The hatching hall of Al-Manzalah Hatchery was used. Fifteen fiberglass tanks (1 x 1 x 0.5 m) were used. Each tank was constructed with an upper irrigation open, an under drainage, an air stone (connected to an air plower (pump) of 5 horse working via a timer for the adjustment of the required level of oxygen), and a net cover to prevent jumping of fish out of the tanks. The brooders fish were transformed from the out-door concrete tanks into the hatching hall. Abnormal fish were separated and removed. Weight, total length and depth of the individual fish were measured. Thereafter, the apparently-healthy fish were distributed into the in-door fiber glass tanks at a stocking density of 6 fish/tank (four females plus two males) on the 31th of July 2004. Some fish were kept frozen for chemical analysis. The experiment started at the following day (1st August 2004). At the start, the fish were treated with potassium permanganate (1 g/tank of 0.25 m^3) for one hour with aeration. Al-Manzalah powdered feed (25.0% crude protein, 6.7% crude fat, 7.7% crude fibers and 2567 Kcal gross energy/kg feed) was used as a basal ration. It consisted of soybean meal, decorticated cottonseed meal, yellow corn, fish meal, rice bran, and molasses at 15, 15, 27.5, 9.5, 31, and 2 %, respectively. The dietary ingredients as well as the dietary additives were purchased from the local market. Five treatments (T, each in triplicate tanks) were conducted, namely: T_1: Fed the basal diet plus fifty grams of fish oil / kg diet; T_2: Fed the basal diet plus fifty grams of sunflowers oil / kg diet; T_3: Fed the basal diet plus twenty five grams of each of fish oil and sunflower oil / kg diet; T_4: Fed the basal diet plus 200 mg of each of vitamins E and C / kg diet; and T_5: Fed the basal diet without additives, control.

One kilogram of each experimental diet was properly hand mixed with its proper supplement (T_4 additives were dissolved in 50 ml water for homogenization, then air dried for 24 hours). The experimental diets were kept in a refrigerator for use. After consuming this quantity, another kilogram of each experimental diets was mixed and refrigerated with the same manner. All environmental factors were fixed, but the only variable was the tested feed additives. One third of the tank's water was changed daily morning at a rate of 3 l/min. Feed was offered twice daily at 9 a.m. and 1 p.m., 6 days a week. The brooders were fed daily at a rate of 2% of their body weight from the 1st of August to the 23rd of October 2004, then reduced to 1% from the 24th of October till the 23rd of December 2004. Thereafter, feed stop was followed from the 24th of December 2004 till the 31th of January 2005. The feeding rate was considered during the experiment according to the physiological state and the environmental temperature, particularly,

during winter (fasting season). Three hatching cycles were carried out to study the effect of different dietary additives on the reproductive performance of Nile tilapia fish. Sex separation was followed in each treatment to stop the uncontrolled hatching. Fish weight was recorded after each spawning cycle as well as monthly during the over wintering. It was noticed that the water temperature was not enough cold throughout the over wintering phase, so there was no mortality among fish brooders. Yet, it affected only the feed consumption; therefore, an artificial (man made) cold shock was done using ice blocks for 30 hours, continuously to reduce the water temperature from 17°C to 8°C.

Water parameters were measured one time throughout the entire experiment (except the temperature), since the hatching hall is working under complete control; therefore, the water quality criteria were approximately constant to great extent throughout the work. But these parameters were measured twice during the emergency of the main irrigation during November and the start of wintry block at the end of January. Since there were changes in the water quality criteria; therefore, it prevented to enter the hatching hall. Water temperatures were measured daily at sunrise and sunset using an alcoholic – mercuric thermometer, made in China. The pH values were measured using a pH meter, P 4000, USA. Salinity and turbidity were estimated by a salinometer model 115, USA. Dissolved oxygen concentration was estimated using an oxygen meter model 50 B, USA. Total ammonia level was determined according to the Nessler's method using DR 2010 spectrophotometer, USA. Toxic ammonia level was calculated from the level of total ammonia {Toxic ammonia (NH_3) = A x 1.2 x total ammonia/100}, where A = factor related to the temperature and pH values at the test time (Boyd, 1992).

Blood samples were withdrawn from three fish per treatment into heparinzed test tubes for carrying out a blood profile including hematological and biochemical parameters using Swelab Cell AC 920 auto counter; Swelab instrument AB Stockholm, Sweden; and spectrophotometer, Spekol 11, Carlzeis Jena, Germany. Proximate chemical analysis of whole body of fish as well as of fish flesh (before and after the treatments) and feed used in the experiment was under taken using the official methods of analysis (AOAC, 1990). Economic efficiency was calculated to appoint the most profitable treatment during the pre-spawnin. So, the prices of feed and feed additives consumed during the hatcheries tested were calculated besides the prices of the produced fry to calculate the economic efficiency. All numerical data collected were statistically analyzed by SAS (1996) for analysis of variance procedure, Chi–square and t-test. Duncan's (1955) multiple range test was carried out for proving significance between means, when f-test was significant.

RESULTS AND DISCUSSION

During the critical period of irrigation, i.e. during November and the beginning of the wintry block, the fish rearing water parameters tested were: pH (8.7), dissolved oxygen (2 mg/l), salinity (1.8‰), total ammonia (3.4 mg/l), toxic ammonia (0.55 mg/l) and total dissolved solids elevated from 807 to

2003 mg/l. However, the water quality criteria were controlled as given in Table 1, except during the wintry block as mentioned above. Averages of water temperatures (daily measured from 1/8/2004 till 31/1/2005) along the study period are given too in Table 1. From these averages, it was clear that the temperature was high; therefore, it did not affect the experimental brooders; hence, there were no mortality among the fish. Yet, it affected only feed intake of the fish; thus, it was a must to do artificial cooling of the water using ice blocks. At the 10th of December, it was the beginning of Alarbe'enia (the 1st of Kihak), where the lowest water temperature usually along the year (but in indoor tanks, it was 17.5°C). At the 18th of January, it was the end of Alarbe'enia and the beginning of Alghetas (the indoor water temperature was 11.5°C). At the end of January, water level was decreased for the beginning of the wintry block; so, the water quality changed to pH 8.45, total ammonia 1.344 mg/l, toxic ammonia 0.012 mg/l, dissolved oxygen 2.5 mg/l, salinity 1.8 g/l, and outdoor water temperature 20°C.

Table (1): Water quality criteria during the study.

Criteria	Treatments No.				
	1	2	3	4	5
Temperature, °C	24	24	24	24	24
pH value	7.42	7.68	7.74	7.69	7.72
Dissolved oxygen, mg/l	9.5	6.8	8.3	8.5	9.1
Total ammonia, mg/l	0.896	0.672	0.896	1.12	0.583
Unionized ammonia, mg/l	0.014	0.017	0.035	0.028	0.023
Salinity, g/l	0.9	0.9	0.9	0.9	0.9

Months	August	September	October	November	December	January
Water temperature, °C	28.77	27.38	24.92	21.63	16.37	14.19

The first experimental diet (T_1) contained 5% fish oil, the second (T_2) contained 5% sunflowers oil, the third (T_3) contained 2.5% fish oil and 2.5% sunflowers oil, the fourth (T_4) was supplemented with 200 mg vitamin E plus 200 mg vitamin C/kg, whereas the fifth diet (T_5) served as a control without any additives. Proximate chemical analysis of the experimental diets is given in Table2. There were significant differences among the diets for all their components, except crude protein. Although moisture content of T_4 was the highest (for dissolving vitamins powder in 50 ml water/kg feed), even it still below the tolerance value (12%) for preserving and storage of dried feed (Abdelhamid, 2000).

Table (2): Means ± standard errors of proximate analysis (%) of the experimental diets.

Treatments No.	Moisture	Protein	Fat	Ash
1	$6.30^{d} \pm 0.100$	$25.05^{a} \pm 0.250$	$10.95^{c} \pm 0.350$	$8.75^{a} \pm 0.250$
2	$7.50^{cd} \pm 0.300$	$25.55^{a} \pm 0.150$	$13.65^{a} \pm 0.350$	$6.00^{bc} \pm 0.500$
3	$8.30^{bc} \pm 0.300$	$26.10^{a} \pm 0.700$	$12.30^{b} \pm 0.300$	$7.00^{b} \pm 0.000$
4	$11.20^{a} \pm 0.800$	$26.90^{a} \pm 0.100$	$7.50^{d} \pm 0.500$	$6.75^{b} \pm 0.250$
5	$9.60^{b} \pm 0.000$	$26.75^{a} \pm 0.250$	$7.15^{d} \pm 0.150$	$5.00^{c} \pm 0.500$

a – d: Means in the same column with different letters differ significantly ($P \leq 0.05$).

However, this diet (T_4) was the highest containing crude protein and contained also significantly lower crude fat and ash percentages. Generally, all the experimental diets contained suitable composition (25.05 – 26.90% crude protein, 7.15 – 13.65% ether extract, and 5.00 – 8.75% ash) for rearing Nile tilapia fish (Abdelhamid, 2003).

The brooders weight ranged between 139.3 and 144.3 g (without significant differences among treatment), their total length ranged 20.11 – 20.51 cm (with significant differences among treatments), and their depth did not significantly differ (6.061 – 6.100 cm) and had low coefficient of variance (2.895%) at the beginning of the experiment (Table 3). There were significant differences between males and females, in favor of males for live body weight and depth, but for females in the total length (Table 3). However, Nile tilapia fish are among the fish species characteristic with bigger males than females (Abdelhamid, 2003). Additionally, it is clear that fish oil addition to Nile tilapia brooders is not recommended, since T_1 was responsible for the lowest live body weight and length (Table 3). El-Sayed and Teshima (1991) came to the conclusion that tilapia require n-6 essential fatty acids, while n-3 essential fatty acids may not be required.

Table (3): Means ± standard errors of live body measurements for the experimental brood fish at the beginning of the study.

Treatments No.	Fish body measurements		
	Weight, g	Total length, cm	Depth, cm
1	$139.3^{a} \pm 1.346$	$20.11^{b} \pm 0.085$	$6.067^{a} \pm 0.037$
2	$142.0^{a} \pm 1.406$	$20.27^{ab} \pm 0.125$	$6.067^{a} \pm 0.044$
3	141.0 ± 1.561	$20.17^{b} \pm 0.151$	$6.061^{a} \pm 0.039$
4	$140.8^{a} \pm 1.688$	$20.12^{b} \pm 0.099$	$6.067^{a} \pm 0.043$
5	$144.3^{a} \pm 1.320$	$20.51^{a} \pm 0092$	$6.100^{a} \pm 0.060$
Females	$140.5^{b} \pm 0.822$	$20.30^{a} \pm 0.056$	$6.028^{b} \pm 0.022$
Males	$143.3^{a} \pm 1.066$	$20.10^{b} \pm 0.104$	$6.160^{a} \pm 0.035$
Total	141.5 (CV = 4.156)	20.24 (CV = 2.144)	6.072 (CV = 2.895)

a – b: Means in the same column with different letters differ significantly ($P \leq 0.05$). CV = coefficient of variance.

Table 4 cleared that during the 1st and the 2nd hatching, T_5 and T_2 were responsible for the heaviest ($P \leq 0.05$) brooders, while at the 3rd hatching there were no significant ($P \geq 0.05$) differences among treatments in brooders weight. Yet, males were heavier ($P \leq 0.05$) than females along the three hatchings. Moreover, after the three hatchings and sex separation, there were no significant ($P \leq 0.05$) effect of the dietary treatments on brooders weight during the following four months (October – January) as shown from Table 5. However, fat containing diets led to apparently lower feed consumption; thus, the residues increased in the tanks causing white rot on their floor. Whereas T_4 and T_5 consumed all the diet offered. At 23/10 and till 23/11 the feeding rate was reduced from 2 to 1% daily, of the fish biomass, for the decreased consumption in T_1, T_2 and T_3. Thereafter, the feed was offered only according to consumption and water temperature. End December, the feeding was completely stopped for decreasing water

temperature to 15°C. During January, fish of all treatments were weak, particularly the females.

Generally, mean brooders' weight, regardless to the dietary treatments (Table 5), decreased after the 3rd spawning due to depletion of the stored nutrients in the seeds of the three spawning. Moreover, the second treatment (sunflowers oil) was responsible for the significantly heaviest body weight after the different spawning (Table 4). In this concern, Ng and Chong (2004) describe how tilapia respond in term of growth performance, feed utilization and fillet quality to various dietary vegetable oils when used to replace fish oils in feeds. Yet, T_3 gained more (Table 6).

Table (4): Means ± standard errors of brooders weight (g) as affected by the dietary treatments (15/8/2004 – 23/9/2004) and before the over wintering of the experimental brood fish as well as by the sequence of spawning.

Treatments No.	1st hatching (15/8/2004)	2nd hatching (2/9/2004)	3rd hatching (23/9/2004)
1	$135.2^{c} \pm 1.689$	$137.7^{c} \pm 1.868$	$140.9^{a} \pm 2.583$
2	$141.0^{ab} \pm 1.380$	$145.5^{a} \pm 1.979$	$144.8^{a} \pm 2.345$
3	$137.8^{bc} \pm 1.272$	$140.4^{bc} \pm 1.298$	$143.9^{a} \pm 2.505$
4	$140.3^{ab} \pm 1.783$	$141.3^{bc} \pm 1.285$	$140.6^{a} \pm 3.072$
5	$141.8^{a} \pm 1.555$	$142.9^{ab} \pm 1.344$	$144.0^{a} \pm 2.387$
Female	$137.3^{b} \pm 2.497$	$137.8^{b} \pm 0.801$	$136.4^{b} \pm 0.836$
Male	$143.1^{a} \pm 1.229$	$149.1^{a} \pm 1.024$	$155.4^{a} \pm 0.993$
Mean (CV%)	137.3 (4.056)	137.7 (3.891)	136.4 (4.272)

a – c: Means in the same column with different letters differ significantly ($P \leq 0.05$). CV = coefficient of variance.

Table (5): Means* ± standard errors of brooders monthly weight (g) after sex separation.

Treatments No.	Weight (g) at			
	23/10/2004	23/11/2004	23/12/2004	23/1/2005
1	160.7 ± 9.57	162.7 ± 9.35	159.2 ± 9.24	154.0 ± 8.34
2	159.6 ± 13.9	157.1 ± 13.4	157.7 ± 13.0	150.7 ± 13.6
3	168.4 ± 6.12	170.5 ± 6.24	171.4 ± 5.94	164.4 ± 6.09
4	168.4 ± 13.9	169.1 ± 15.4	167.8 ± 14.8	159.8 ± 14.1
5	156.2 ± 14.26	155.5 ± 14.5	154.1 ± 14.4	147.4 ± 13.9

*** There were no significant ($P \geq 0.01$) differences among treatments for the four weights.**

Cold tolerance in the tropical-subtropical tilapias genus (*Oreochromis*) is a trait of economic importance where growing seasons are 6-8 months long and over wintering of stocks is a necessity. Standardized short- and long-term cold-tolerance tests have been evaluated for quantifying cold tolerance within and among populations. These tests will allow selection of superior individuals and/or families which can subsequently be evaluated for their breeding value (Behrends *et al.*, 1990). However, stocking density had no significant effect on the survival rate. Significantly better specific growth rate, condition factor and feed conversion were observed at a water flow rate of 0.2L / kg fish/min than at 0.1L / kg fish/min. Significantly higher mean

individual weight gain, specific growth rate, and survival rate were observed at 1.0%/day than at the 0.75%/day feeding rate (Cruz and Ridha, 1994). Generally, fish are sensitive for temperature variations, since they could not control their body's temperature. At a sudden low temperature (as during seasonal winds), cold bite (sting) can occur with skin discoloration and erosions followed by depressing growth and resistance then death (Abdelhamid, 2003).

Table (6): **Changes in live body weight (g) of the Nile tilapia brooders tested as affected by the dietary treatments.**

Treatments No.	Average initial body weight	Average final body weight	Changes in body weight
1	139.3	154.0	14.7
2	141.9	150.7	8.8
3	141.0	164.4	23.4
4	140.8	159.8	19.0
5	144.3	147.4	3.1

In accordance with the present results, Abdelhamid *et al.* (1995) reported that there were insignificant differences among treatments (0 – 1600 ppm ascorbic acid supplementation) in growth performance of Nile tilapia fish. The same trend was found in a case of dietary supplementation of Nile tilapia brood stock with both vitamins C and E (Abdelhamid *et al.*, 1999-a & b). Eid (1995) obtained opposite results to the present data concerning fish growth, since he found that the best growth performance of Nile tilapia fingerlings occurred in group of fish fed diets containing either fish oil or corn oil. Gaber (1996) reported significant effect of different dietary oil sources on Nile tilapia growth performance and feed utilization. However, Abdelghany (1998) and El-Saidy and Gaber (1998) found that ascorbic acid supplementation to diets improved Nile tilapia growth. In disagreement with the present results, El-Saidy *et al.* (1999) mentioned that increasing dietary energy (oil) improved growth performance of Nile tilapia. In agreement with the present results, El-Husseiny *et al.* (2001-a) found that plant oil was more better for tilapia growth than fish oil. Goda (2002), Magouz *et al.* (2002) and El-Kholy *et al.* (2003) came to the conclusion that vegetable oils have certain advantages to fish oil. They are cheaper, available in large quantities and less subjected to oxidation than non-hydrogenated fish oil. Moreover, they permit reasonable growth for Nile tilapia fish.

Either of absolute and relative fecundities did not differ significantly by the effect of the experimental dietary treatments; so, they had low CV percentages being 33.136 and 32.728%, respectively. Yet, the hatching sequence had significantly affected either of fecundities, since the 1st hatching was significantly better than the 2nd and 3rd hatchings (Table 7). These significances in fecundity among hatchings consequently affected the egg production and diameter (Tables 8 and 9). The best egg production (Table 8) and egg diameter (Table 9) were found throughout 1st and 2nd spawning in groups from brooders fed T_1 (fish oil supplemented). Yet, the

best brooders weight at the 2nd and 3rd hatchings (Table 4) was recorded for T_2. Regardless to hatching sequence, the allover egg production and egg diameter did not differ significantly among dietary treatment; yet, T_3 gave the best production but egg diameter was higher in T_1 (Table 10). Generally, the control diet (T_5) was the worst in most cases, egg production of the 1st and 3rd hatchings (Table 8), egg diameter of the 2nd hatching (Table 9), and egg production and diameter, regardless to spawning sequence (Table 10). Otherwise, there were no remarkable changes among the experimental dietary treatments concerning egg production and diameter.

Table (7): Absolute (count/fish) and relative (count/kg fish body weight) fecundity for the experimental brooders fish (means ± SE)

Treatments No.	Absolute fecundity	Relative fecundity
1	$864.9^{a} \pm 87.88$	$6598.0^{a} \pm 683.1$
2	$850.2^{a} \pm 108.9$	$6167.6^{a} \pm 778.1$
3	$839.7^{a} \pm 59.72$	$6186.2^{a} \pm 419.6$
4	$769.6^{a} \pm 90.33$	$5798.7^{a} \pm 648.2$
5	$784.3^{a} \pm 113.5$	$5761.7^{a} \pm 816.5$
1st hatching	$1136.0^{a} \pm 75.27$	$8398.0^{a} \pm 553.1$
2nd hatching	$626.46^{b} \pm 25.06$	$4633.3^{b} \pm 198.6$
3rd hatching	$705.23^{b} \pm 38.33$	$5351.7^{b} \pm 278.4$
Total	825.42 (CV = 33.136)	6146.6 (CV = 32.728)

a – b: Means in the same column with different letters differ significantly ($P \leq 0.05$). CV = coefficient of variance.

Table (8): Means* ± standard errors of seed (egg) production (count/fish) as affected by the dietary treatments of the experimental brood fish and by the sequence of spawning.

Treatments No.	1st hatching	2nd hatching	3rd hatching
1	714.6 ± 202.8	446.3 ± 106.0	280.7 ± 107.2
2	417.0 ± 188.9	147.3 ± 77.56	285.8 ± 103.1
3	683.9 ± 165.1	355.3 ± 93.64	515.3 ± 134.0
4	487.2 ± 189.7	330.7 ± 101.1	272.5 ± 92.86
5	363.0 ± 172.9	182.3 ± 77.97	173.7 ± 90.85
Mean (CV%)	533.1 (119.8)	292.4 (109.0)	305.6 (121.0)

*** There were no significantly ($P \geq 0.05$) differences among treatments. CV = coefficient of variance.**

Table (9): Means* ± standard errors of seed (egg) diameter (mm) as affected by the dietary treatments of the experimental brood fish and by the sequence of spawning.

Treatments No.	1st hatching	2nd hatching	3rd hatching
1	0.683 ± 0.244	0.858 ± 0.260	0.575 ± 0.245
2	0.561 ± 0.239	0.283 ± 0.191	0.142 ± 0.142
3	0.280 ± 0.189	0.292 ± 0.197	0.422 ± 0.220
4	0.138 ± 0.138	0.425 ± 0.222	0.283 ± 0.191
5	0.288 ± 0.195	0.150 ± 0.150	0.283 ± 0.191
Mean (CV%)	0.390 (181.8)	0.402 (178.4)	0.341 (204.0)

* There were no significantly ($P \geq 0.05$) differences among treatments. CV = coefficient of variance.

Table (10): Means* ± standard errors of egg parameters, regardless to spawning sequence.

Treatments No.	Production, No./ fish	Diameter, mm
1	1441.5 ± 294.0	2.117 ± 0.364
2	850.08 ± 242.0	0.986 ± 0.389
3	1554.5 ± 293.5	0.993 ± 0.392
4	1090.3 ± 263.9	0.847 ± 0.391
5	718.92 ± 270.9	0.722 ± 0.448
Mean (CV%)	1131.1 (83.13)	1.133 (121.6)

* There were no significantly ($P \geq 0.05$) differences among treatments. CV = coefficient of variance.

The percent of eggs-producing brooders was reduced from the 1st to the 3rd (except for T_2) spawning. T_3 was the best in this respect (61.11% of the 12 female brooders spawned), but T_5 (the control, 30.55% of the 12 female brooders spawned) was the worst (Table 11). However, all the incubated eggs had been hatched (100% hatchability rate). Chi-square test for the brooders % lay eggs as affected by the dietary treatment revealed no significant ($P \geq 0.05$) effect for the three individual hatchings; yet, there was a significant ($P \leq 0.05$) effect of dietary treatments ($P < 0.05$) on % laying brooders as average of the three hatchings altogether. In this respect, Abdelhamid *et al.* (1999-b) reported that, relative fecundity significantly improved by dietary vitamins C and E supplementation. Therefore, they recommended the dietary addition of these vitamins for Nile tilapia brood stock for one month, at least, before the spawning for increasing fecundity, eggs number, eggs diameter and larval number.

Table (11): Percent of eggs-producing brooders.

Hatchings	Treatments No.					
	1	2	3	4	5	Average
1	58.33	33.33	66.66	41.66	33.33	46.7
2	66.66	25.00	58.33	50.00	33.33	46.7
3	41.66	41.66	58.33	50.00	25.00	43.3
Average	55.55	33.33	61.11	47.22	30.55	45.6

An improvement in brood stock nutrition and feeding has been shown to greatly improve not only egg and sperm quality but also seed production. Gonadal development and fecundity are affected by certain essential dietary nutrients, especially in continuous spawners with short vitellogenic periods. Thus, during the last two decades, more attention has been paid to the level of different nutrients in brood stock diets. Lipid and fatty acid composition of brood stock diet have been identified as major dietary factors that determine successful reproduction and survival of offspring. Some fish species readily incorporate dietary unsaturated fatty acids into eggs, even during the course of the spawning season. Highly unsaturated fatty acids (HUFA) with 20 or more carbon atoms affect, directly or through their metabolites, fish maturation and steroid genesis. In some species, HUFA in brood stock diets increases fecundity, fertilization and egg quality. As in higher vertebrates, vitamin E deficiency affects reproductive performance, causing immature gonads and lower hatching rate and survival of offspring. Ascorbic acid has also been shown to play an important role in salmonid reproduction, where the dietary requirement of brood stock was higher than that of juveniles (Izquierdo *et al.*, 2001). However, Khalil *et al.* (2001) reported significant values of correlation coefficient between body weight of females and eggs weight or eggs number. They obtained the best egg production in the 2nd spawning.

From Table 12, it is clear that T_3 was the most economic, whereas the control (T_5) was the worst. Where, each one LE input (in feeding) introduced 75.76, 41.81, 79.74, 58.75 and 37.65 LE output (in fry buying) from T_1, T_2, T_3, T_4 and T_5, respectively. These results (superiority of T_3) are associated and dependent on the results in Table 11 (concerning the % of egg-producing brooders), Table 6 (concerning the changes in live body weight, since heavy-body females lay more eggs than the lower body weight females, Abdelhamid, 2003) and in Table 10 (concerning egg production).

Table (12): Economic efficiency* of over wintering Nile tilapia brooders on the experimented dietary treatments.

Items	Treatments No.				
	1	2	3	4	5
Feed intake, Kg	2.284	2.378	2.318	2.231	2.264
Price of 1 Kg feed, LE	4.970	5.130	5.023	4.992	5.060
Feed cost, LE	11.350	12.200	11.646	11.136	11.456
Price of sailing fry, LE	809.90	510.10	928.65	654.20	431.35
Economic efficiency	75.76	41.81	79.74	58.75	37.65

Price of one ton of the basal diet = 2235 LE, fish oil 10 LE/Kg, sunflower oil 6 LE/Kg, vitamin E 2% 50 LE/Kg, and vitamin C 20% 30 LE/Kg. 1000 fry price was 50 LE. *Economic efficiency = Price of buying fry/price of feeding.

Although there were slightly differences among treatments in moisture content of fish muscles, there were no significances among dietary treatments in muscular contents of protein, fat and ash on dry matter basis as shown from Table 13. Chemical analysis of whole body of Nile tilapia brooders after the over wintering period (184 days) revealed significant differences among the experimental dietary treatments in moisture, protein,

fat and ash percentages (Table 14). The highest moisture percent was recorded in T_1, whereas the highest protein and ash and lowest fat percentages on dry matter basis were determined in fish fed T_4. But the lowest protein and ash and highest fat contents were estimated in fish of T_3. This means that the best feed additives to overcome wintering stress for Nile tilapia are a mixture of vitamin C plus vitamin E, followed by oil addition, whether fish or sunflowers oils. Generally, and although these significances in chemical composition of the whole fish body, CV% was very low, being 1.82, 3.22 and 4.82% for protein, fat and ash percentages, respectively (Table 14). The positive effect of the vitamins C and E mixture may be due to the synergistically interaction between them to prevent lipid oxidation in fish. Since ascorbic acid as a reducing (antioxidant) agent could react with intermediates of α-tocopherol to effect a reduction to its initial state. Thus, ascorbic acid would or could have a sparing effect on tissue vitamin E levels and the vitamin E requirement level of fish (Shiau and Hsu, 2002). Moreover, dietary vitamin E supplementation increased the antioxidant capability of tilapia tissues against lipid peroxidation (**Hung *et al.*, 2003**).

Table (13): Proximate chemical analysis* (% dry matter basis) of the fish muscles before and after the experiment (means ± SE).

Treatments No.	Moisture	Protein	Fat	Ash
Before:	77.38 ± 0.04	75.75 ± 0.30	4.25 ± 0.75	20.00 ± 0.45
After: 1	79.15 ± 0.15	92.50 ± 0.50	3.40 ± 0.50	4.00 ± 0.00
2	78.45 ± 0.25	91.25 ± 1.25	3.50 ± 0.50	5.25 ± 0.75
3	77.30 ± 0.10	91.50 ± 0.00	4.25 ± 0.75	4.25 ± 0.75
4	78.40 ± 1.00	92.20 ± 0.50	4.30 ± 0.00	3.50 ± 0.50
5	78.15 ± 0.15	91.00 ± 0.50	4.25 ± 0.25	4.75 ± 0.25
Mean (CV%)	78.29 (0.85)	91.69 (1.049)	3.96 (16.94)	4.35 (17.43)

*There were no significant ($P \geq 0.05$) differences among treatments. CV = coefficient of variance.

Table (14): Proximate chemical analysis (% dry matter basis) of the whole fish body before and after the experiment (means ± SE).

Treatments No.	Moisture	Protein	Fat	Ash
Before:	70.89 ± 0.11	53.60 ± 0.40	22.00 ± 0.50	24.40 ± 0.10
After: 1	75.25^{a} ± 0.75	61.00^{ab} ± 0.50	18.00^{c} ± 0.50	21.00^{ab} ± 1.00
2	72.10^{bc} ± 0.90	60.50^{ab} ± 1.00	20.00^{b} ± 0.00	19.50^{b} ± 1.00
3	70.30^{c} ± 0.30	56.75^{c} ± 0.25	27.50^{a} ± 0.50	15.75^{c} ± 0.25
4	73.90^{ab} ± 0.60	61.25^{a} ± 1.33	15.75^{d} ± 0.75	23.00^{a} ± 0.50
5	72.10^{bc} ± 0.60	58.25^{bc} ± 0.25	20.00^{b} ± 0.00	21.75^{ab} ± 0.25
Mean (CV%)	72.73 (1.28)	59.55 (1.82)	20.25 (3.22)	20.20 (4.82)

a–c:Means in the same column with different letters differ significantly ($P \leq 0.05$).CV = coefficient of variance.

The present results disagreed with those of Eid (1995), who found that the highest protein and fat contents in fish body were recorded in group of fish fed diets containing fish oil (5%) or corn oil (5%) or combination of fish oil (2.5%) plus corn oil (2.5%). He concluded that the substitution of 2.5% of fish oil with corn oil or combination of both can be used as a supplemental lipid source for fingerling Nile tilapia. However, Gaber (1996) reported significant

effect of various dietary oil sources on chemical composition of flesh and whole fish body of Nile tilapia. El-Saidy *et al.* (1999) found a positive correlation between crude protein and fat contents of the fish, but El-Ebiary and Zaki (2003) and Abdelhamid *et al.* (2005-a & b) found a negative correlation between protein and fat contents of the fish. Abdelghany (1998) found that dietary vitamin C inclusion elevated fish protein percentage and reduced its fat percentage, similar to the present results. Also, El-Saidy and Gaber (1998) reported higher protein percentage in fish flesh by dietary ascorbic acid supplementation. Additionally, Abdelhamid *et al.* (2000) found that dietary vitamin C supplementation not only improved growth performance but also increased protein content of Nile tilapia.

Data of blood analyses for hematological and biochemical parameters are given in Tables 15–17. The brooder Nile tilapia fish fed T_1 had higher red blood cells (RBCs) count and packed cell volume (PCV%) or hematocrite (HT) percentage; yet, T_4 was responsible for higher hemoglobin (Hb), mean corpuscular hemoglobin (MCH) level and mean corpuscular hemoglobin concentration (MCHC) percentage than the other groups (Table 15). However, T_3 and T_4 were similar in Hb concentrations, T_2 and T_4 were similar also in MCH levels and platelets (Plt) count. T_2, T_3 and T_4 had values of MCHC% do not greatly differ from each other (Table 15). T_3 fish group showed the highest white blood cells (WBCs) count as well as lymphocytes, monocytes and neutrophils counts; yet its lymphocytic fraction as % was, to some extent, lower than those of T_1, T_2 and T_5 (Table 16). T_3 also reflected the lowest platelets (Plt) or thrompocytes count (Table 15).

Table (15): Hematological parameters of the experimented brood tilapia fish at the end of the experiment.

Treat-ments No.	RBC (10^6/µl)	HT (vol %)	Hb (g/dl)	MCV (μ^3)	MCH (Pg)	MCHC (%)	Plt (10^3/µl)
T_1	1.40	20.9	6.6	149	47	31.6	32
T_2	1.20	18.1	6.9	151	58	38.1	36
T_3	1.24	18.5	7.0	149	56	37.8	27
T_4	1.20	17.8	7.0	148	58	39.3	36
T_5	1.31	20.4	6.5	156	50	31.9	62

RBC: Red blood cells, HT: Hematocrite, Hb: Hemoglobin, MCV: Mean corpuscular volume, MCH:Mean corpuscular hemoglobin, Plt: Platelets (thrombocytes), MCHC: Mean corpuscular hemoglobin concentration.

Table (16): Leukocytes count and differentiation of the tested brood tilapia fish at the end of the experiment.

Treat-ments No.	WBC (10^3/µl)	Lymphocytes		Monocytes		Neutrophils	
		10^3/µl	%	10^3/µl	%	10^3/µl	%
T_1	81.8	77.8	96	2.9	3	1.1	1
T_2	92.2	88.1	96	3.1	3	1.0	1
T_3	94.0	88.5	95	4.1	4	1.4	1
T_4	82.4	77.3	95	3.7	4	1.4	1
T_5	65.0	61.3	96	2.5	3	1.2	1

All dietary additives used led to reduction of serum total protein, albumin and globulin concentrations, particularly the vitamins C and E supplementation (T_4), which reduced total protein and globulin concentrations (Table 17). However, the present blood analyses' data are similar to those given by Kobeisy and Hussein (1995) for Hb, total protein and globulin, but lower in RBCs and higher for HT and albumin than their results. Moreover, Abdelhamid *et al.* (2002) registered near values to those reported herein for hemoglobin concentration, packed cell volume % and serum total protein concentration in Nile tilapia fish. But WBCs were very lower and RBCs and Hb were higher in the study of Abdelhamid *et al.* (2004) than those found in the present study. Also, Abdelhamid *et al.* (2004) reported very higher Hb levels than in the present study.

Table (17): **Serum proteins of the tested brood tilapia fish at the end of the experiment.**

Treatments No.	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)
T_1	4.8	2.1	2.7
T_2	4.1	1.6	2.5
T_3	3.1	1.23	1.87
T_4	2.74	1.31	1.43
T_5	5.4	1.38	4.02

The present serum proteins' data disagreed with those given by El-Husseiny *et al.* (2001-b), who found that values of total serum protein and globulin significantly increased by dietary vitamin E addition. Also, El-Ebiary and Zaki (2003) reported similar values, i.e. HT (23.06 – 25.00%), Hb (6.36 – 8.39 g/dl), total protein (4.85 – 9.46 g/dl), albumin (2.26 – 2.64 g/dl), and globulin (2.59 – 6.82 g/dl). Chen *et al.* (2004) showed that vitamin E deficient fish reflected lower whole body protein, lipid, hematocrit, hemoglobin, lymphocyte (%) than those fed the vitamin E supplemented diets. They added that a sparing effect of vitamin E on vitamin C was evident.

REFERENCES

Abdelghany, A.E. (1998). Feed efficiency, nutrient retention and body composition of Nile tilapia, *Oreochromis niloticus* L., fed diets containing L-ascorbic acid, L-ascorbyl-2-sulphate or L-ascorbyl-2 polyphosphate. Aquaculture Research, 29: 503 – 510.

Abdelhamid, A.M. (2000). Fungi and Mycotoxins. Dar Al-Nashr for Universities, Cairo (I.S.B.N.977-5526180-9).

Abdelhamid, A.M. (2003). Scientific Fundamentals for Fish Production and Management. An Arabic Textbook, Mansoura University Press, 2nd Ed. (Deposit No.15733/2003).

Abdelhamid, A.M., El-Sadaney, H.H., El-Shinnawy, M.M. and Dorra, T.M. (1995). Effects of dietary graded levels of ascorbic acid on performance and chemical composition of tilapia fingerlings. J. Agric. Sci. Mansoura Univ., 20: 2731 – 2742.

Abdelhamid, A.M., Khalil, F.F.M., El-Barbary, M.I., Zaki, V.H. and Husien, H.S. (2002). Feeding Nile tilapia on Biogen® to detoxify aflatoxic diets. Proc. 1st Ann. Sc. Conf. Anim. & Fish Prod. Mansoura, 24 & 25 Sep., pp: 207 – 230.

Abdelhamid, A.M., Khalil, F.F. and El-Zareef, A.M. (2000). Studies on iron in Egyptian freshwater fishes, 2- Effect of vitamin C and/or vitamin A supplementation on iron utilization by tilapia (*Oreochromis niloticus*) and catfish (Clarias lazera). J. Agric. Sci., Mansoura Univ., 25(1): 113 – 131.

Abdelhamid, A.M., Khalil, F.F. and Essa, M.R. (1999-a). Effect of graded levels of vitamins C and/or E in diets of Nile tilapia broodstock fishes (*Oreochromis niloticus*) on: 1- Growth performance, chemical composition and feed utilization. Egypt. J. Nutr. & Feeds, 2: 823 – 838.

Abdelhamid, A.M., Khalil, F.F. and Essa, M.R. (1999-b). Effect of grading levels of vitamins C and/or E in diets on Nile tilapia brood stock fishes (*Oreochromis niloticus*) on: 2- Reproductive performance. J. Agric. Sci. Mansoura Univ., 24: 4655 – 4670.

Abdelhamid, A.M., Mehrim, A.I. and Khalil, F.F. (2004). Detoxification of aflatoxin-contaminated diet of tilapia fish using dietary supplementation with egg shell, Betafin®, clay or silica. J. Agric. Sci. Mansoura Univ., 29: 3163 – 3173.

Abdelhamid, A.M., Salem, M.F.I. and Tolan, A.E. (2005-a). Evaluation of linseed meal as feed ingredient in diets of growing Nile tilapia (*Oreochromis niloticus*). J.Agric. Res. Tanta Univ., 31(3): 385–402.

Abdelhamid, A.M., Salem, M.F.I. and Tolan, A.E. (2005-b). Utilization of black seed meal (Nigella sativa) in Nile tilapia (*Oreochromis niloticus*) diets. J. Agric. Res. Tanta Univ., 31(3): 403-419.

Association of Official Analytical Chemists, AOAC (1990). Official Methods of Analysis. 15th ed. Published by the North Nineteen St. Auite 210 Arlingth, Virginia 2220/USA.

Bardach, J.E.; Ryther, J.H. and McLarney, W.O. (1972). Aquactulture, The Farming and Husbandry of Freshwater and Marine Organisms. Wiley-Interscience, N.Y.

Behrends, L.L., Kingsley, J.B. and Bulls, M.J. (1990). Cold tolerance in maternal mouthbrooding tilapias: Phenotypic variation among species and hybrids. 3- Int. Symp. on Genetics in Aquaculture, Trondheim (Norwary), 20 – 24 Jun 1988. Genetics-in-Aquaculture-III, 85(1-4): 271 – 280.

Bhujel, R.C. (2000). A review of strategies for the management of Nile tilapia *Oreochromis niloticus* broodfish in seed production systems, especially hapa-based systems. Aquaculture, 181: 37-59.

Boyd, C.E. (1992). Water Quality in Warm Water Fish. Alabama Agric. Experiment Station, Auburn Univ., Alabama.

Chen, R., Lochmann, R., Goodwin, A., Praveen, K., Dabrowski, K. and Lee, K.J. (2004). Effects of dietary vitamins C and E on alternative complement activity, hematology, tissue composition, vitamin concentrations and response to heat stress in juvenile golden shiner (*Notemigonus crysoleucas*). Aquaculture, 242: 553 – 569.

Cruz, E.M. and Ridha, M. (1994). Overwintering tilapia, *Oreochromis spilurus* (Günther), fingerlings using warm underground seawater. Aquacult. Fish. Manage, 25(9): 865 – 871.

Delgado, C.L.; Wada, N.; Rosegrant, M.W.; Meijer, S. and Ahmed, M. (2003). Outlook for Fish to 2020. Meeting Global Demand: A 2020 Vision for Food, Agriculture, and the Environment Initiative. International Food Policy Research Institute Washington, D.C., U.S.A. and World Fish Center. Penang, Malaysia pp. 36. (ISBN 0-89629-647-4).

Duncan, D. (1955). Multiple range and multiple F-tests. Biometrics, 11: 1-42.

Eid, A. (1995). Evaluation of fish oil, corn oil, palm oil, olive oil and beef tallow singly and in combination as supplemental dietary lipid source for fingerling Nile tilapia diets. Zagazig J. Agric. Res., 22(4): 1015 – 1022.

El-Ebiary, E.H. and Zaki, M.A. (2003). Effect of supplementing active yeast to the diets on growth performance, nutrient utilization, whole body composition and blood constituents of mono-sex tilapia (*Oreochromis niloticus*). Egypt. J. Aquat. Biol. & Fish., 7(1): 127 – 139.

El-Husseiny, O.M., Abdul-Aziz, G.M., Goda, A.M.A., Ghoneim, S.M. and El-Haroun, E.R. (2001-a). Influence of dietary oil source and level of Nile tilapia (*Oreochromis niloticus*) performance. J. Egypt. Acad. Soc. Environ. Develop., (B-Aquaculture) 1(1): 83 – 102.

El-Husseiny, O.M., Goda, A.M.A., Abdul-Aziz, G.M., Ghoneim, S.M. and El-Haroun, E.R. (2001-b). Effect of vitamin E and selenium on the performance and humoral immune response of Nile tilapia *Oreochromis niloticus*. J. Egypt. Acad. Sco. Environ. Develop., (B-Aquaculture) 1(1): 103 – 117.

El-Kholy, Kh.F., Osman, M.F. and Abdel-Baset, A. (2003). Effect of oil sources on growth performance, feed utilization and body composition of tilapia hybrid fish (*Oreochromis niloticus x Oreochromis aureus*). Egypt. J. Nutr. Feeds, 6(special issue): 445 – 458.

El-Saidy, D.M.S.D. and Gaber, M.M.A. (1998). Effects of using ascorbate polyphosphate in practical diets on Nile tilapia (*Oreochromis niloticus*) fry performances. Menofiya J. Agric. Res., 23(2): 363 – 378.

El-Saidy, D.M.S.D., Gaber, M.M.A. and Magouz, F.I. (1999). Effect of dietary energy level on growth, feed conversion, body composition, digestibility coefficients and energy budget of Nile tilapia (*Oreochromis niloticus*). Egypt. J. Aquat. Biol. & Fish., 3(3): 159–178.

El-Sayed, A.F.M. and Teshima, S.I. (1991). Tilapia nutrition in aquaculture. Reviews in Aquatic Sciences, 5: 247 – 265.

FAO, Food and Agriculture Organization of the United Nations (1997). Aquaculture Production Statistics 1986–1995. FAO Fish. Circ. No. 815, Rev. 9, Rome, Italy. 179 pp.

Gaber, M.M.A. (1996). Effect of oil sources on growth, feed conversion and body composition of Nile tilapia (*Oreochromis niloticus*). Annals of Agric. Sci., Moshtohor, 34(4): 1549 – 1562.

GAFRD, General Authority of Fish Resources and Development (2005). "Fish Production Report for the Year 2004" Agriculture Ministry Press, Cairo.

Goda, A.M.A.S. (2002). The influences of n6/n3 dietary fatty acid ratio on growth performance and body fatty acid composition of Nile tilapia (*Oreochromis niloticus*). Vet. Med. J. Giza, 50(1): 33 – 45.

Hung, C.H., Chang, R.J., Huang, S.L. and Chen, W. (2003). Dietary vitamin E supplementation affects tissue lipid peroxidation of hybrid tilapia, *Oreochromis niloticus x O. aureus*. Comp. Biochem. Physiol., B 134: 265 – 270.

Izquierdo, M.S., Fernández-Palacios, H. and Tacon, A.G.J. (2001). Effect of broodstock nutrition on reproductive performance of fish. Aquaculture, 197: 25 – 42.

Khalil, F.F., Abdelhamid, A.M. and Mostafa, M.E.A. (2001). Nutritional influences on Nile tilapia broodstock fish (*Oreochromis niloticus*). 2- production and fecundity. Egypt. J. Nutr. and Feeds, 4 (special issue): 695 – 704.

Kobeisy, M.A. and Hussein, S.Y. (1995). Influence of dietary live yeast on growth performance and some blood constituents in *Oreochromis niloticus*. Proc. 5th Sci. Conf. Anim. Nut., Ismailia, 12 – 13 Dece., pp: 417 – 425.

Magouz, F.I., Mohsen, M.K. and Abd El-Moniem, N.M. (2002). Utilization of different sources and levels of lipids in the diet of Nile tilapia (*Oreochromis niloticus*). Proc. 2nd Conf. Foodborne Contamination and Egyptians' Health, 23–24 April, El-Mansoura, Egypt, pp:357–370.

Ng, W.K. and Chong, C.Y. (2004). An overview of lipid nutrition with emphasis on alternative lipid sources in tilapia feeds. Proc. 6th Int. Sym. Tilapia in Aquaculture, Manila, Philippines, Sept. 12 – 16, 1: 241.

SAS (1996). SAS procedure guide. "Version 6.12 Ed". SAS Institute Inc., Cary, NC, USA.

Shiau, S.Y. and Hsu, C.Y. (2002). Vitamin E sparing effect by dietary vitamin C in juvenile hybrid tilapia, *Oreochromis niloticus* x *O. aureus*. Aquaculture, 210: 335 – 342.

دراسة على أسماك البلطى أثناء التشتية بإضافات غذائية من الدهون أو الفيتامينات

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أجريت هذه الدراسة على أسماك البلطى النيلى، بإضافة الزيوت أو الفيتامينات لعلائق الأمهات لمقاومة مخاطر برد الشتاء. وتم تقييم النتائج على أساس وزن السمك، ونفوقه، وإنتاجه من البيض، وكفاءته الاقتصادية، وتركيب جسمه ودمه. من النتائج المتحصل عليها يثبت أن أمهات أسماك البلطى النيلى المستخدمة للتفريخ يمكن تغذيتها قبل الدخول فى توقيت وضع البيض على عليقة محتوية على ٢٥% بروتين خام مضافا إليها ٥% من مخلوط ١:١ من زيت السمك وزيت دوار الشمس، لأنها هى المعاملة التى أدت إلى أعلى نسبة أمهات تضع بيض، ومن ثم أعلى نسبة زريعة (لأنها أدت إلى أعلى زيادة فى وزن الأمهات ولوجود علاقة طردية بين وزن الأم وعدد البيض الذى تضعه)، وعليه فقد حققت أعلى أربحية من بيع الزريعة (التى تربى الأمهات من أجله) وأعلى كفاءة اقتصادية، وذلك نتيجة زيادة مناعتها بارتفاع عدد كرات الدم البيضاء ونسبة مكوناتها (الليمفاوية على الأخص).