

# Effect of Some Over-Wintering Regimes on Survival and Growth Performance of Nile Tilapia, *Oreochromis Niloticus*

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**Abstract:** The present study aimed to study the effectiveness of some over-wintering regimes (covering the tanks with polyethylene sheets, supplementation the fish diets with L-carnitine and using the bio-floc technology) on survival rate, growth performance and proximate composition of Nile tilapia (*Oreochromis niloticus*). The experiment was carried out during winter season (1<sup>st</sup> February to 1<sup>st</sup> April 2013) and the most important results can be summarize as follows:

At the experiment end (after 90 days), the three regimes, plastic sheet, L-carnitine and bio-floc technology, BFT significantly improved survival rate of *O. niloticus* and the most effective over-wintering regime (based on survival rate) was recorded by BFT followed by supplementation the diets with L-carnitine and by covering ponds with plastic sheets. Fish in the BFT regime showed the highest body weight (BW), the longest body length (BL), the best weight gain (WG) and specific growth rate (SGR) and consumed the highest amount of feed and showed the best feed conversion ratio (FCR) and protein efficiency ratio (PER) compared to the other over-wintering regimes and control, and the differences between the other feeding regimes (L-carnitine, covering with plastic sheets and control groups) for feed intake, FCR and PER are not significant

Fish group of the bio-floc (BFT) gained the highest significant crude protein and ash and the lowest fat and dry matter content compared with control group.

**Keywords:** Over-wintering, survival rate, growth, Nile tilapia

## 1. Introduction

The global success of tilapia production due to, their tolerance to poor water quality and the fact that they can feed on a wide range of food includes planktonic, algae (Lutz *et al.*, 2003). Tilapia is an aquatic animal that can be farmed as easily and economically, and with the same broad market appeal, as chickens (Pullin, 1984). In addition, it reaches the marketable size of 500 to 600g within six months therefore, it is considered the most

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important and cheapest source of animal protein in tropical and sub tropical countries that suffer from lack of animal protein.

Tilapias are warm water fish and their natural geographical evidence tolerating a relatively wide range of temperatures (8-42°C). *O. niloticus* L. does not grow at temperature below 16°C and does not survive at temperature below 10°C for more than a few days. Activity and feeding become reduced when the temperature falls below 20°C and stops completely at around 16°C. Reproductive activities occur above 22°C (Chervinski, 1982).

Different strains of *O. niloticus* have variable cold tolerance, which has been correlated to geographical origins mean low lethal temperatures of Egyptian, Ghanaian and Ivory Coast strains of *O. niloticus* have been reported as 10°C, 14.1°C and 12.2°C, respectively (Khater and Smitherman, 1988).

During winter months (December, January, February and March) in Egypt water temperature in aquaculture ponds dropped to 10°C and lower, which is not only suitable to tilapia growth but also tilapia survival. Most of fish farms in Egypt experienced a significant fish kill. The exception of this high mortality was in some farms that used house plastic to protect their fish against low temperature (Abdel-Aal, 2008).

Noor El Deen and Zaki (2010) indicated that, growth of tilapia has been affected by pond coverage as well as by water depth. On the other hand, un-covered ponds by plastic sheets showed increasing fish mortality which coincided with the decrease of water temperatures. These results indicate that un-covered ponds failed to protect during this particular winter. The low temperature with more space in pond not covered may make more air circulation inside pond and the hot air went out pond, which is not saving for temperature inside pond.

So, the aim of the present study was to evaluate the effect of some over-wintering regimes' on growth performance and survival rate of tilapia fish under cool season condition in Egypt.

## **2. Materials and Methods**

The present study was carried out at the Laboratory of Fish Nutrition, Faculty of Agriculture , Benha University. The aim of this study was to investigate the effect of some over-wintering regimes on growth performance and survival rate of Nile tilapia (*Oreochromis niloticus*).

The experiment was conducted during the winter period from the 1<sup>st</sup> of January 2013 till the 1<sup>st</sup> of April of the same year (90 days). Twelve plastic tanks (500 liter for each) were used in the study and each tank was filled by 400 liter fresh water to represent four treatments (3 replicates for each treatment) and each tank was stocked with 40 fish (6.34±0.01 g) therefore, 480 fingerlings were used in the study. The experimental fish were obtained from Abbassa hatchery, Abbassa, Abu-Hammad district, Sharkya Governorate, Egypt. After acclimatization period (two weeks), the experimental fish were randomly distributed into the tanks. At stocking, body weight and length of fingerlings per tank were recorded individually and feed amount was calculated according to live body weight.

The tanks were cleaned and water was replaced at a daily rate of 20% of total water in three treatments (control & L-carnitine and covering with plastic sheets) while it was replaced at a daily rate of 2% of total water in BFT group. Dissolved oxygen was maintained at 5-6 mg/l by continuous aeration (estimated by using dissolved oxygen meter) and water temperature was daily measured at 9 am.

#### ***Diet Preparation:***

Ingredients (table 1) of the basal diet were thoroughly mixed, blended and divided into two parts, the first was used as control diet and the second part was supplemented by 1000 mg L-carnitine/kg diet. The ingredients were thoroughly mixed with addition of 200 ml of water per kg diet. The pastes were separately passed through a grinder, and pelletized (3-mm diameter). The diets were air-dried for 48 hours and stored in plastic bags in a refrigerator (-2°C) until use.

Fish were given the diets at a daily rate of 1% of total biomass 6 day/week during the experimental period (at 10 .00 am). Starch was added in BFT tanks at a daily rate of 48% of pelleted feed to activate the bio-floc. Every two weeks, all fish were taken from each tank then weighed and the amount of feed was adjusted according to the changes in body weight throughout the experimental period.

Growth performance parameters, feed utilization and survival rate were measured by using the following equation:

**Condition factor (K)** =  $(W/L^3) \times 100$ , Where, W=weight of fish in grams and L=total length of fish in “cm”.

**Weight gain (WG)** = final weight(g)-initial weight(g)

**Specific growth rate (SGR)** =  $\frac{\ln W_2 - \ln W_1}{t}$  (days), Where, Ln=the natural log;  $W_1$ =first fish weight,  $W_2$ =the following fish weight in grams and t =period in days.

**Feed conversion ratio (FCR)** = Feed intake (g)/weight gain (g)

**Protein efficiency ratio (PER)**= Weight gain (g)/protein ingested (g)

**Survival rate (SR %)** = [(total fish number – dead fish)/total fish number]×100

At the end of the experiment, three fish were randomly sampled from each tank and subjected to the chemical analysis of whole fish body according to the methods described in **AOAC (1990)**: dry matter after drying in an oven at 105°C until constant weight; ash content by incineration in a muffle furnace at 600°C for 12 hrs; crude protein (N×6.25) by the kjeldhal method after acid digestion; and ether extract by petroleum ether (60-80°C) extraction.

Statistical analysis of the obtained data was analyzed according to **SAS (1996)**. Differences between means were tested for significance according to Duncan's multiple rang test as described by **Duncan (1955)**.

### 3. Results and Discussion

#### 3.1 Survival Rate

At the experiment termination (after 90 days), the average survival rates found to be 61, 66, 73 and 84% for control, covering the tanks with plastic sheets, supplementation the diet with L-carnitine and BFT regimes, respectively and the differences among the different treatments were significant.

From the obtained results (table 2) it could be observed that, the three regimes, plastic sheet, L-carnitine and BFT significantly improved survival rate of *O. niloticus* compared with control group and the most effective over-wintering regime (based on survival rate) was recorded by BFT followed by supplementation the diets with L-carnitine and by covering ponds with plastic sheets. **Crab et al., (2009)** concluded that BFT emerge as an alternative to overcome over-wintering problems, particularly mass mortality of tilapia due to low temperatures.

The major goal in the over-wintering of tilapia fingerlings is to obtain a high survival of the fish and to keep the fish in good condition for future growth in the production ponds. **Crab et al., (2009)** demonstrated that, temperature in the covered ponds with polyethylene sheets could easily be controlled and was 0.4-4.9°C higher than the influent water and hybrid tilapia fingerlings (*O. niloticus* × *O. aureus*) survival levels were excellent being 97± 6% for 100 g fish and 80±4% for 50 g fish and these findings can help to overcome over-wintering

problems, particularly mass mortality of fish due to low temperatures in the ponds.

**Abdel-Aal (2008)**. The author divided *O. niloticus* L. fry into 3 groups with different covering systems of polyethylene sheet as 100% covered, 75% covered and use dried plant, then each group was divided into three water depths. The best survival rate are showed in group that totally covered by polyethylene sheet, water depth had significant effects on survival rate.

The obtained results in the present study indicated that L-carnitine supplementation to the diets of *O. niloticus* during the over-wintering rearing period significantly improved survival rate of *O. niloticus* compared with control group. In the same trend, **Soltan et al., (2004)** showed a significant high survival rate of all *O. niloticus* groups received the diets supplemented with L-carnitine (300, 600, 900 or 1200 mg/kg diet) compared to control group (without L-carnitine) and fish group received L-carnitine at a level of 900 or 1200 mg/kg diet exhibited the best survival rate.

Similar results were also obtained by **Harpaz et al., (1999)**. They found that, addition of L-carnitine to diets of *Pelvicachromis pulcher* at a level of 900 or 1000 mg/kg diet seems to yield the best protection against exposure to cold.

Carnitine, through its involvement in lipid metabolism, has an important role in the process of temperature acclimation and can provide some protection to fish kept under aquaculture conditions with rapid environmental changes resulting in high temperature fluctuations (**Harpaz, 2005**).

Ammonia toxicity is known to be one of the common stressors in fish culture. Carnitine has been shown to provide protection of fish against acute ammonia toxicity (**Tremblay and Bradley 1992**). **Abou-Seif, (2012)** indicated that dietary administration of L-carnitine (1000 mg/kg diet) can improve stress tolerance to both high stocking density and cold tolerance of *O. niloticus*.

### **3.2 Growth Performance**

All fish has almost similar body weight (BW) and body length (BL) at the start of the experiment (table 3). At experiment termination (after 90 days) results showed that, fish in BFT showed the highest BW (10.79 g), the longest BL (8.40 cm), the best WG (4.48 g) and SGR (0.32%) while control group showed the lowest BW (9.11 g), the lowest WG (2.79 g) and SGR (0.22%). Although the BFT significantly improved the final BW, WG and SGR of *O. niloticus* compared with both, covering with plastic sheets, supplementation with

L-carnitine and the control groups, results also indicated that no significant differences were observed between covering with plastic, supplementation with L-carnitine and the control groups.

At the experimental start average values of K (table 3) ranged between 2.44 to 2.87 and the differences between these means were insignificant. At the end of the experiment, K values decreased and ranged between 1.60 to 1.86 with significant differences between the tested over-wintering regimes of the present study.

In recent study, **Souady (2013)** indicated that, growth parameters improved under bio-floc system. It could assume that starch addition to bioflok tanks activate growth of bacterial floc and algae which in turn act as secondary protein source for fish under those treatment. Carbohydrate addition leads to the C/N ratio which helps to convert inorganic nitrogen into organic nitrogen as dense floc that cause doubling of protein utilization and supply of essential lipids and vitamins (**Avimelech et al., 2008**). **Azim et al., (2008)** concluded that growth performance for Nile tilapia in bio-floc system improved and it contributed 43% of growth compared with system without BFT.

**Emerenciano et al., (2011)** observed that presence of bio-flocs resulted in increases of 50% in weight and almost 80% in final biomass in *F. paulensis* early post larval stage when compared to conventional clear-water system. This trend was observed even when post larvae were not fed with a commercial feed (bio-floc without commercial feed). Also, **Emerenciano et al., (2012)** found that *F. brasiliensis* postlarvae grow similarly with or without pelletized feed in bio-floc conditions during 30-d of nursery phase, which was 40% more than conventional clear-water continuous exchange system.

Covering ponds with plastic sheets as an over-wintering regimes did not significantly improved SGR of *O. niloticus* compared to control group. **Abdel-Aal (2008)** found that, the best growth performance of *O. niloticus* L are showed in group that totally covered by polyethylene sheet.

L-carnitine supplementation as an overwintering regimes did not significantly improved growth performance of *O. niloticus* compared to control group (table 3). **Soltan et al., (2004)** came to similar results. The authors found that, the increased levels of L-carnitine (0, 300, 600, 900 or 1200 mg/kg) did not significantly affected BW or BL of *O. niloticus* reared under over-wintering regime (L-carnitine) for three months (water temperature ranged between 9.35 and 11.50°C). In the same trend, **Harpaz, et al., (1999)** found that addition of L-carnitine to the diet of *Pelvicachromis pulcher* at a level of 1000 mg/kg seems to yield the best protection against exposure to cold shock while growth differences among the treated fish were not significant.

The improved energy production in mitochondria through B-oxidation of fatty acids may suggest that, exogenous administration of L-carnitine could enhance the performance of fish by improving energy utilization efficiency from lipid oxidation (**Torreale *et al.*, 1993**).

Generally the obtained results of the present study indicated that, over-wintering of *O. niloticus* by BFT significantly improved BW, BL, K, WG and SGR (table, 3). Therefore, BFT considered (based on our results) the most suitable regime compared to covering with plastic sheets or L-carnitine supplementation which did not differ significantly from control group.

### **3.3 Feed Intake and Feed Utilization**

For the whole experimental period (90 days) fish in the BFT regime consumed the highest feed and showed the best FCR and PER compared to the other over-wintering regimes and control, and the differences between the other feeding regimes (L-carnitine, covering with plastic sheets and control groups) for feed intake, FCR and PER are not significant (table 4). Therefore, the present study refers to the beneficial effect of BFT as an over-wintering regime showed the highest significant feed utilization of *O. niloticus* compared with the other over-wintering regimes and control group. Same trend was noticed for tilapia feed utilization under bio-floc system by **Azim and Little (2008) and Souady (2013)**. **Avnimelech, (2007)** observed that bio-floc consumed by tilapia fish may represent a very significant feed source, constituting about 50% of the regular feed ration of fish (assuming daily feeding of 2% body weight). **Burford *et al.*, (2004)** estimated that more than 29% of the daily food intake of *L. vannamei* consisted of microbial flocs, improving FCR and reducing costs in feed.

In another study, **Crab *et al.*, (2009)** found that, the feed conversion ratio (FCR) of hybrid tilapia (*O. niloticus* × *O. aureus*) did not significantly differ between the two fish size (50 or 100g) reared under BFT over-wintering regime and the overall FCR was 1.9±0.4 kg feed/kg fish produced.

L-carnitine supplementation improves FCR compared to control group (table 4). Improvement of FCR when using L-carnitine in the diet had been observed in other studies. **Abou-Seif (2012)** and **Azab *et al.*, (2002)** reported that an enhancement in growth which was not due to an increase in feed uptake but results from better FCR when L-carnitine supplemented diet was fed to the tilapia fish. **Hari *et al.*, (2004)** demonstrated that carbohydrate addition in extensive shrimp ponds improved the nitrogen retention efficiency and had a positive effect on production. Low toxic inorganic nitrogen levels and utilization of microbial cells are demonstrated to be an effective potential food source for tilapia and shrimp (**Burford *et al.*, 2004 and Avnimelech, 2007**).

Another reason for the improvement of feed utilization under biofoc system is that the increased activities of digestive proteinases indicated enhanced digestive capabilities of the feed (Avnimelech, 2007). As a massive number of live microorganisms existed in the bio-flocs, they could transit through the stomach into the intestine and interfere with resident intestinal micro flora balance which plays an important role in the production or secretion of digestive enzymes (Hari *et al.*, 2006).

### 3.4 Proximate Analysis of Whole Fish

Results of table 5 showed that, BFT gained the highest significant crude protein and ash and the lowest fat and dry matter content compared with control group.

Previous results suggest that bio-floc conditions stimulate accumulation of both fat and ash in tilapia carcass especially with the elevation of dietary protein level. Bio-floc chemical analysis confirmed this hypothesis as with increase of dietary protein bio-floc content of lipid and ash elevated. Azim and Little (2008) revealed that no significant difference between clear and bio-floc system in chemical composition of Nile tilapia were recognized. In the same context, no significant difference between bio-floc and clear system in shrimp dry matter and crud protein content but bio-floc shows superiority for ether extract and ash content (Azim *et al.*, 2008).

It was hypothesized that under bio-floc system shrimp (*L. vannamei*) have better nutrient assimilation when compared to those fed only the formulated feed, because of the greater amount of essential amino acids, fatty acids (PUFA and HUFA) and other nutritional elements supplied by the bio-flocs (Ray, 2010). This finding support our results as increase in bio-floc protein and ether extract content recorded with the elevation of dietary protein. Wasielesky *et al.*, (2006) reported that the increased whole body ash content of the shrimp might be explained by continuous availability of abundant minerals and trace elements from the bio-flocs as indicated by high ash content. Our results recorded the same phenomena where, tilapia under bio-floc system noticed to have highest ash content. Meanwhile, chemical analysis of bio-floc samples showed increase in ash content with increasing dietary protein. Same results were suggested by Azim *et al.*, (2008) they observed significant difference in bio-floc composition in terms of protein and ash content between different protein levels.



The obtained results indicated that, L-carnitine group showed the highest protein content (70.28% and the lowest fat content (12.81%). These results are in agreement with those obtained by **Azab *et al.*, (2002)** who found no significant effect of dietary L-carnitine on tissue composition at low dietary fat level (5%), while in high level of dietary fat (10%), carnitine caused a significant increase in tissue protein.

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**Table 1:** Composition and chemical analysis of the basal diet.

Feed ingredients	%
Fish meal (65%)	16
Yellow corn	28
Soybean meal (40%)	40
Wheat bran	10.5
Vegetable oil	2.5
Vit.& Min. mixture <sup>1</sup>	3
Sum	100
<i>Chemical analysisi (%) of the basal diet on dry matter basis</i>	
Dry matter (DM)	92.56
Crude protein (CP)	30.18
Ether extract (EE)	4.44
Crude fiber (CF)	9.33
Ash	10.12
NFE <sup>2</sup>	45.93
ME (Kcal/kg diet) <sup>3</sup>	2610
P/E ratio <sup>4</sup>	115.63

<sup>1</sup> vitamin & mineral mixture/ kg premix : vitamin D3, 0.8 million IU; A, 4.8million IU; E, 4g; K, 0.8g; BI, 0.4g; Riboflavin, 1.6g; B6,0.6g; B12, 4mg; pantothenic acid, 4g; Nicotinic acid, 8g; Folic acid, 0.4g Biotin, 20 mg, Mn, 22g; Zn, 22g; Fe, 12g; Cu, 4g; I, 0.4g; Selenium, 0.4g and Co, 4.8 mg.

<sup>2</sup> Nitrogen free extract (NFE) = 100 – (CP+EE+CF+Ash).

<sup>3</sup> Metabolizable energy was calculated from ingredients based on **NRC (1993)** values for tilapia

<sup>4</sup> Protein to energy ratio in mg protein /Kcal ME.

**Table 2:** Effect of different over-wintering regimes on survival rate (%) of *O. niloticus*

Treatments	At stocking	After 2 weeks	After 4 weeks	After 6 weeks	After 8 weeks	After 10 weeks	After 12 weeks
Control	100	87 c	78 c	72 c	68 c	66 c	61 c
Plastic sheets	100	92 b	82 ab	76 c	73 c	70 c	66 b
L-carnitine	100	92 b	88 b	82 b	79 b	77 b	73 b
Bio-flocs	100	100 a	97 a	96 a	89 a	87 a	84 a
Standard error (SE)		0.390	1.100	1.410	1.450	1.230	0.690

Means followed by different letters in each column are significantly ( $P < 0.05$ ) different.

**Table 3:** Effect of different over-wintering regimes on growth performance of *O. niloticus*

Treatments	Body weight (BW, gm)		Body length (BL, cm)		Condition factor (K)		WG (gm)	SGR (%)
	Initial	Final	Initial	Final	Initial	Final		
Control	6.32	9.11 b	6.19	8.23	2.66	1.63 b	2.79 b	0.22 b
Plastic sheets	6.33	9.15 b	6.04	8.30	2.87	1.60 ab	2.82 b	0.22 b
L-carnitine	6.39	9.51 b	6.40	8.00	2.44	1.86 a	3.12 b	0.24 b
Bio-flocs	6.31	10.79a	6.13	8.40	2.74	1.82 a	4.48 a	0.32 a
Standard error(SE)	0.003	0.063	0.003	0.057	0.023	0.05 1	0.050	0.001

Means followed by different letters in each column are significantly ( $P < 0.05$ ) different.

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**Table 4:** Effect of different over-wintering regimes on feed intake (g/fish) of *O. niloticus*

Treatments	Feed intake (g/fish)	FCR	PER
Control	5.36 b	1.92 a	1.74 b
Plastic sheets	5.34 b	1.89 a	1.76 b
L-carnitine	5.42 b	1.74 a	1.92 b
Bio-flocs	5.63 a	1.26 b	2.65 a
Standard error (SE)	0.006	0.0140	0.017

Means followed by different letters in each column are significantly ( $P < 0.05$ ) different.

**Table 5:** Effect of different over-wintering regimes on proximate analysis of *O. niloticus*.

Treatments	Dry matter (%)	Crude protein (%)	Fat (%)	Ash (%)
Control	25.29 a	68.90 b	14.00 a	15.18 c
Plastic sheets	25.10 a	68.48 b	14.08 a	15.66 ab
L-carnitine	24.70 b	70.28 a	12.81 c	15.52 b
Bio-flocs	24.84 b	69.75 a	13.57 b	15.79 a
Standard error (SE)	0.015	0.098	0.045	0.010

Means followed by different letters in each column are significantly ( $P < 0.05$ ) different.