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Combined Effect of Pond, Lipid Source and Level in Survival and Growth Performance of Over-Wintered Nile Tilapia, *Oreochromis niloticus*

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Abstract: This study aimed to investigate the effect of lipid source (Sunflower or fish oil and mixture of 1:1 sunflower and fish oil). For each lipid source, two lipd level were tested (2.5 or 5%). Therefore, six experimental diets were formulate and fed to over-wintered mono sex males, *Oreochromis niloticus* reared in two pond types (Concrete ponds or earthen ponds, hapas). The experiment was carried out during the period from 4th of December 2014 to the 12th of August 2015. Twelve treatments of two replicates were fed a diet containing 32% crude protein. While T1 contained 2.5% sunflower oil, T2 2.5% fish oil, T3 2.5% of a mixture between sunflower and fish oil, T4 5% sunflower oil, T5 5% fish oil and T6 5% mixture of both sunflower and fish oil. Fish of the first six treatments (1-6) were reared in concrete ponds and repeated in hapas to represents additional six treatments (7-12). Samples from each treatment were taken during cool and warm months for the evaluation of using different concentrations and types of lipids on the biochemical parameters, growth performance and health status. Results showed that T4 gave the most improvement concerning biochemical parameters. T6 gave the best growth performance parameters. On the other hand, the first three treatments containing the lower lipid level (2.5%) suffered some fungal infection (Saprolegniosis). Moreovere, T7 showed the highest biochemical parameters when samples were taken in the summer season and T9 showed the better growth performance. Treatments containing the high lipids level (5%) either single or mixed suffered from mixed bacterial infection especially in warmer months. Generally we can concluded that, fish diets contained the higher lepid level (5%) of mixed oils are of great importance for over-wintered stocks while lower lipd level (2.%) is sutible for warm seasons.

Key words: Lipid Rations · Nile Tilapia · Over-Wintering · Growth Performance

INTRODUCTION

Tilapias are warm water fish and their natural geographical evidence tolerating a relatively wide range of temperatures (8-42°C). No growth at temperatures 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 [1-3].

There are various culture methods practiced by fish farmers to raise fishes either for sale or for family consumption. Fish may be raised in big bowls, in unused canoes and in depressions that can hold water either in constructed tanks or in earthen ponds. In Egypt, the majority of fish farmers do not feed fish during winter season when water temperature varies from 6-20°C. Many ectotherm suffer from their sensitivity of low ambient temperature in which extreme cold can cause mass mortalities [6]. Over-wintering fish therefore presents a serious problem.

Fish oil is a major dietary lipid source used in tilapia feeds. Other than providing a source of energy and essential fatty acids, it is commonly used to coat the extruded pellets to improve the palatability and appearance of the feed. Other important functions of lipids include aiding in absorption of fat-soluble vitamins, precursors of hormones and prostaglandins and as

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building blocks of cellular and membrane structures. Therefore, in order to sustain the rapid growth of the tilapia industry, many developing countries where tilapia is farmed will have to partially or totally replace fish oil with cheaper and sustainable sources of dietary lipid.

Plant oils rich in 18:2n-6, such as soybean oil, corn oil, sunflower oil, canola/rapeseed oil and various palm oil products are equally good lipid sources for tilapia. Linseed or flaxseed oil rich in 18:3n-3 provided growth performance similar to that of fish fed plant oils high in n-6 fatty acids, but at the beginning of the study, fish reluctantly consumed the linseed oil diet [8].

Dietary lipids are the main source of energy for the development of fish fry, especially essential fatty acids in sufficient amounts are critical for rapid growth [9]. Fatty acid requirements have been extensively studied in fish fry using live prey enriched with different oils. During the last two decades, the substitution of live food by artificial diets for the early stages of fish has been a major concern in aquaculture research in order to reduce costs and increase predictability of juvenile production. However, there is still a lack of information on optimal dietary lipid level in artificial diets for fish fry. The ability of fry to assimilate the required nutrients is dependent on the composition of the diet and on their capacity of modulating their digestive enzymes and metabolic processes [10].

The aim of this study is to investigate the effect of lipid source and level in Nile tilapia diets during winter period and its impact on growth performance and health status under Kafr El-Sheikh culture conditions.

MATERIALS AND METHODS

Experimental Design

Experimental Design and Management: The present study was carried out in a private farm at Tollumbat No. 7 in Riyad City, Kafr El-Sheikh governorate, Egypt to study the effect of oil supplementation to rations of over wintered tilapia. Twelve treatments of two replicates (Ponds: 3×7 m in diameter with a water depth of 1m) were fed a diet containing 32% crude protein. T_1 contained 2.5% sunflower oil, T_2 2.5 % fish oil, T_3 2.5 % of a mixture between sunflower and fish oil (1:1), T_4 5% sunflower oil, T_5 5% fish oil and T_6 5% mixture of both sunflower and fish oil (1:1). The six experimental diets (T_1 to T_6). Were fed to Nile tilapia reared in 12 concrete ponds (Two replicates for each diet) and repeated in another 12 in hapas (T_7 to T_{12}). Nile tilapia monosex males (1.6±0.04g initial weight) were stocked in both rearing systems at a density of 30 fry/m^3. Fish were fed on commercial floating diets to keep the diets available for fish containing 32% crude protein. The experimental period expanded almost 36 weeks during the period from 4th December 2014 to 12th August 2015. During over-wintering period, artificial feeding was given in sunny days at a feeding rate of 2% of total biomass and the diets were offered daily once at 11am. During the growing period, the experimental diets were offered in two equal parts daily at 9 am and 1 pm. And feeding rate increased to 3% of the total biomass.

Diet Preparation: Six diets were prepared by thoroughly mixing the ingredients of fish meal, soybean meal, yellow corn, rice bran, gluten with different percentage of sunflower oil and fish oil (Table 1). All diets were formulated to be isonitrogenous (32% protein) and digestible energy (About 3300 kcal ME/kg diet). Feed quantity was adjusted according to the average body weight of the sample in each pond. In order to determine the average weight of fish, biweekly samples were taken by seining 30 fish from each pond (Replicate) for individual measuring of both weight and length then returned back into ponds.

Growth Parameters: Initial and final weight and length, average daily gain (DWG), specific growth rate (SGR) were calculated according to Jauncey and Rose [11] and the condition factor (K) was calculated according to the equations used by Schreck and Moyle [12] using the following equations:

$$\text{Fish condition factor (K)} = (W_t/L^3) \times 100$$

where, W_t is the total gutted weight of the fish (g) and L is the total length (cm)

- Specific growth rate (SGR) was calculated according to (19) as:

$$SGR = (\ln W_2 - \ln W_1 \times 100) / t$$

where, w_1 = first fish weight in grams, w_2 = final fish weight in grams, t = period in day.

- Daily weight gain (DWG) was calculated using the formula:

$$DWG = [\text{Average } W_2\,(g) - \text{Average } W_1\,(g)] / t$$

Table 1: Composition and chemical analysis of the experimental diets

Feed ingredients	Experimental diets Diet1	Diet2	Diet3	Diet4	Diet5	Diet6
Fish meal (72%)	20	20	20	20	20	20
Yellow corn	27.5	27.5	27.5	25	25	25
Soybean meal (44%)	30	30	30	30	30	30
Glutine	5	5	5	5	5	5
Rice bran	12	12	12	12	12	12
Sunflower oil	2.5	-	1.25	5	-	2.5
Fish oil	-	2.5	1.25	-	5	2.5
Vit. & Min. mixture1	3	3	3	3	3	3
Sum	100	100	100	100	100	100
Proximate analysis (dry matter basis)						
Crude protein (CP)	31.29	31.17	31.22	31.30	31.11	31.09
Ether extract (EE)	6.36	6.58	6.76	8.20	8.13	8.48
Crude fiber (CF)	9.33	10.22	10.10	10.24	9.82	9.57
Ash	10.12	10.14	10.33	10.45	10.09	10.01
Digestible energy (Kcal/kg)	3302	3312	3305	3547	3356	3349

Biochemical Parameters: Blood samples were taken from fish at the end of over-wintering and growing periods. Samples were sent to the laboratory for estimating the values of blood glucose, serum cholesterol, Triglycerides, Total lipids, Hb and liver enzymes according to Joseph *et al.* [13] and Dacie and Lewis [14].

Clinical, Postmortem and Laboratory Examinations: Freshly dead and moribund fish were subjected to full clinical and postmortem inspection as described by Schaperclaus *et al.* [15] microbiologically [16] and Parasitologically [17].

Statistical Analysis: The statistical analysis for collected data was analyzed by applying the computer program [18]. Differences among means were tested for significance according to Duncan's multiple range tests [19].

RESULTS AND DISSCUSSION

Over-Wintering Period

Biochemical Parameters: In the present study fish reared in concrete ponds showed a significant increase in blood glucose, cholesterol, triglycerides and total lipids compared with the hapas during the over-wintering period (Table, 2). Also, the sunflower oil and fish oil mixture (1:1) at the higher level (5%) in fish significantly (P<0.05) increased the levels of these parameters. The interaction between ponds and lipid source and level in the present study showed that, fish fed the 5% mixture between sunflower oil and fish oil in concrete ponds showed the highest blood glucose, cholesterol, triglycerides and total lipids compared with the low dietary lipid content (2.5%). Analysis of variance showed that, blood glucose, serum cholesterol, serum triglycerides and serum total lipids were significantly (P<0.05) affected by over-wintering and feeding regimes used in the present study. Chen *et al.* [22] studied the comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus* or *Streptococcus iniae* or exposed to carbon tetrachloride, gentamicin, or copper sulphate. He found that, The haematological parameters are an important tool of diagnosis that reveals the state of health of fish. For example, decreased red blood cells and hematocrit were found in Nile tilapia experimentally infected with *Streptococcus iniae*.

Growth Parameters: The major goal of over-wintering of tilapia is to supply production ponds with fingerlings of good conoditin and high survival rate [2, 3, 23]. Table (3) shows that eventhough all fish had almost similar body weights (BW) and body lengths (BL) at the begining of the experiment, fish reared in concrete ponds showed the highest BW and BL compared to fish reared in earthern ponds (Hpas). This diference may be atributed to temperature loss from earthen ponds [24, 25].

Especially the average body weight after feeding a 5% mixture between sunflower oil and fish oil was higher than other treatments and the differences among treatments were significant (P<0.05). This result somewhat met the findings of Thiaw [26] who mentioned that the type of dietary lipid significantly affected the growth performance of *O. niloticus*. Moreover, the best growth rate from feeding diets containing a mixture of fish oil and vegetable oil in equal proportions. Degani [25] concluded from his studies on glass eel (*Anguilla anguilla*) fed

Table 2: Effect of different lipid sources and levels on blood components, Hemoglobin (Hb) of *O. niloticus*

Variable	No.	Glucose	Cholesterol	Triglycerides	Total lipid	Hb%
Effect of ponds (P)						
Concrete (P1)	24	78.17±0.35a	84.33±0.36a	248.67±0.96a	587.66±1.18a	7.65±0.12
Hapa (P2)	24	66.67±0.41b	77.83±0.32b	202.76±0.89b	461.17±1.06b	7.34±0.11
Effect of lipid levels (L)						
2.5% sunflower oil (L1)	8	63.50±0.61d	77.50±0.58b	207.51±0.92cd	506.12±1.18c	7.35±0.20
2.5% fish oil (L2)	8	66.54±0.63c	77.60±0.60b	208.47±0.89c	486.77±1.11d	7.44±0.21
2.5% Mixture (L3)	8	67.48±0.67c	76.89±0.56b	205.46±0.94d	479.51±1.10d	7.60±0.21
5% sunflower oil (L4)	8	76.39±0.59b	85.13±0.52a	247.04±0.90a	567.03±1.15a	7.41±0.22
5% fish oil (L5)	8	79.18±0.60a	85.16±0.61a	243.49±0.82b	556.23±1.20b	7.55±0.20
5% Mixture (L6)	8	81.06±0.68a	85.09±0.54a	242.50±0.96b	552.79±1.17b	7.65±0.21
Interaction between ponds level and lipid levels (P×L)						
T1 (P1×L1)	4	68.02±0.87d	79.14±0.76c	216.07±0.96c	536.11±1.84c	7.62±0.20
T2 (P1×L2)	4	73.11±0.86c	79.17±0.79c	214.22±0.90cd	539.02±1.72c	7.61±0.21
T3 (P1×L3)	4	71.09±0.84cd	77.23±0.75cd	210.16±0.92d	540.06±1.69c	7.81±0.21
T4 (P1×L4)	4	82.22±0.91b	91.07±0.82a	289.01±0.95a	648.11±1.75a	7.59±0.22
T5 (P1×L5)	4	87.04±0.86a	90.52±0.70a	282.10±0.99b	633.19±1.89b	7.73±0.20
T6 (P1×L6)	4	88.10±0.85a	90.57±0.74a	281.89±0.86b	630.88±1.96b	7.72±0.19
T7 (P2×L1)	4	59.56±0.89e	76.00±0.71d	199.65±0.92f	476.22±1.66d	7.10±0.22
T8 (P2×L2)	4	60.16±0.90e	75.84±0.77e	203.07±0.96ef	433.79±1.89e	7.33±0.22
T9 (P2×L3)	4	64.32±0.88de	76.03±0.76d	200.22±0.90f	419.19±2.03f	7.42±0.21
T10 (P2×L4)	4	71.32±0.87cd	79.11±0.78c	205.18±0.98e	486.04±1.59d	7.32±0.20
T11 (P2×L5)	4	72.08±0.84cd	81.06±0.71b	205.14±0.96e	479.76±1.80d	7.41±0.20
T12 (P2×L6)	4	74.19±0.86c	80.14±0.70bc	204.96±0.94ef	474.99±1.67d	7.61±0.18

Values are means±SE of two replications. Means within each column having different letters were significantly different (P<0.05).

Table 3: Effect of different lipid sources and levels on the initial and final weight (gm) and length (cm)

Variable	No.	Initial W	Final W	Initial L	Final L
Effect of ponds (P)					
Concrete (P1)	120	1.62±0.04a	23.68±1.14a	4.42±0.11b	11.39±0.55a
Hapa (P2)	120	1.64±0.05a	20.62±1.23b	4.48±0.09a	10.95±0.34b
Effect of lipid level (L)					
2.5% sunflower oil (L1)	40	1.63±0.06a	18.21±1.06f	4.45±0.12b	10.65±0.45d
2.5% fish oil (L2)	40	1.63±0.07a	20.56±1.13e	4.40±0.13bc	10.88±0.63c
2.5% Mixture (L3)	40	1.67±0.05a	21.64±1.09d	4.50±0.12ab	10.94±0.74c
5% sunflower oil (L4)	40	1.63±0.06a	22.80±1.11c	4.55±0.14a	11.51±0.42b
5% fish oil (L5)	40	1.60±0.10a	24.29±1.02b	4.45±0.10b	11.40±0.68b
5% Mixture (L6)	40	1.62±0.08a	25.39±1.15a	4.35±0.09c	11.64±0.76a
Interaction between ponds (P) and lipid level (L)					
T1 (P1×L1)	20	1.62±0.11a	19.28±1.03i	4.40±0.13c	10.74±0.63f
T2 (P1×L2)	20	1.63±0.08a	22.14±1.10f	4.50±0.14b	11.07±0.52e
T3 (P1×L3)	20	1.64±0.13a	22.92±1.08e	4.40±0.10c	11.15±0.48de
T4 (P1×L4)	20	1.61±0.09a	24.39±1.14c	4.50±0.15b	11.80±0.80b
T5 (P1×L5)	20	1.57±0.06a	25.40±1.20b	4.40±0.12c	11.54±0.71c
T6 (P1×L6)	20	1.65±0.08a	27.94±1.09a	4.30±0.11d	12.05±0.60a
T7 (P2×L1)	20	1.63±0.07a	17.14±1.15k	4.50±0.09b	10.65±0.72fg
T8 (P2×L2)	20	1.63±0.10a	18.98±1.06j	4.30±0.11d	10.68±0.39g
T9 (P2×L3)	20	1.70±0.07a	20.36±1.03h	4.60±0.13a	10.74±0.55f
T10 (P2×L4)	20	1.65±0.09a	21.21±1.11g	4.60±0.10a	11.21±0.84de
T11 (P2×L5)	20	1.62±0.12a	23.18±1.19d	4.50±0.08b	11.26±0.90d
T12 (P2×L6)	20	1.59±0.05a	22.85±1.14e	4.40±0.07c	11.24±0.68de

Values are means±SE of two replications. Means within each column having different letters were significantly different (P<0.05).

different fat levels within variable temperatures that not only higher body weights were obtained from diets of more energy contents, but also the source of fat (Poultry fat 5-10% was more efficient than the same levels from soya bean.

Concrete ponds showed the highest DWG and SGR of mono sex *O. niloticus* and the effect of interaction between ponds acted dependently on each other and also each of them had its own significant effect (Table, 4). More precisely, fish fed on diet containing 5% mixture between sunflower oil and fish oil in concrete ponds (T_6) significantly gave the highest DWG and SGR. The differences among treatments were significant (P<0.05). Thiaw [26] also, found that the best growth rate, DWG and SGR were observed in fish fed on a diet containing a mixture of fish oil and vegetable oil in equal proportions. This cold be interpritted by the fact that mixing between animal and plant lipids supply fish with mor fatty acid composition [27, 28].

Growing Period

Biochemical Parameters: Analysis of variance showed that, blood glucose, serum cholesterol, serum triglycerides, serum total lipids and Hb (Table, 5) were significantly (P<0.05) affected by over-wintering and feeding regimes used in the present study. Ochang *et al.* [30] showed that, hematological parameters of Nile tilapia, *Oreochromis niloticus* increased with increasing substitution level of fish oil by vegetables oil in the diets.

Growth Parameters: Results of Table (6) showed that fish reared in earthen ponds showed the highest BW and BL Fish fed T9 (2.5% Mixture between sunflower oil and fish oil) showed the hidhest significant BW and BL compared with the other treatments. Eventhough, Zheng *et al.* [31] found that, there were no significant differences in wet weight fed high dietary lipid. On the other hand, Gallagher *et al.* [32], Dave *et al*. [33] and Dave *et al*. [34] with the European eel, *Anguilla Anguilla* reached better growth in eels and Nile tilapia [35]. These differences may be due to the variation in the nutritional requirements of the different fish studied. Table (7) shows that, the average (K) for concerete ponds was of higher significance than earthen ponds. Regarding the interaction between ponds it was obvious that both factors act dependently on each other and also each of them expressed insignificant interaction. But results indicated that, earthen ponds gave the highest DWG and SGR of fish. Pond acted dependently on each other and each of them had its own significant effect. Results indicated that fish fed on diet containing 2.5% mixture between sunflower oil and fish oil in earthen ponds significantly released the highest DWG and SGR. While, fish fed 2.5% sunflower oil in earthen ponds significantly relaesd the highest SGR. The differences among treatments were significant (P<0.05).

Lim *et al.* [8], Thiaw [26] and Teshima *et al.* [36] mentioned that, 1% vegetable oils are enough for tilapia (Since vegetable oils contain high levels of n-6 and also huge quantities of n-3 fatty acids, they can be efficiently used in Tilapia diets. Concerning the source of oil, Takeuchi *et al.* [35] reported that, *O. niloticus* fingerlings fed diets containing either corn oil or soybean oil, attained the best weight gain and feed efficiencies as compared to those given beef tallow or cod liver oil. Moreover, Yingst and Stickney [37] reported that the channel catfish fry showed the best growth rate when reared on practical diets containing menhaden oil followed by soybean oil and the poorest on beef tallow. While, Santiago and Reyes [38] observed the highest weight gains in *O. niloticus* (L) brood stock fed cod liver oil. Many authors obtained conflicting results from their studies on the replacement of fish oil with vegetable oils [39-41]. In contrast, some studies have recomended partial replacement (at least 50 % up to 80 %) of dietary fish oil by vegetable oil [42].

Survival Rate (%) and Total Yield

During Over-Wintering Period: Survival rate of fish reared in concerete ponds showed the highest rate compared with fish reared in earthen ponds (Table 8). Fish in T_6 (5% Mixture between sunflower oil and fish oil) was most viable. While the interaction between ponds got best survivability from 5% lipid addition regardless the type of lipid. This may be supported by warmer water in covered concrete ponds [23]. These remarks came cloose to the studies of Siddiqui and Howlader [43] on response of Nile tilapia (Stocked in concrete tanks) to winter feeding (From November to March where temperature ranged from 11.5 to 23.7°C in Saudi Arabia) no mortality recorded during winter when minimum water temperature during feeding was 10°C for 8 days and 11°C for 9 days.

During Growing Period: As shown from Table (8), survival rates in earthen ponds were higher than concrete ponds and best viability came from feeding 2.5% mixture between sunflower oil and fish oil in the third group. The interaction between ponds got highest survival rate in T_9 (2.5% mixture between sunflower oil and fish oil for earthen ponds). Concerning the requirements for fish in that period the results were more cloose to the recomendations of Lim *et al.* [8].

Table 4: Effect of different lipid sources and levels on condition factor (K), Daily Weight Gain (DWG) and Specific Growth Rate (SGR).

Variable	No.	DWG	SGR	Initial K	Final K
Effect of ponds (P)					
Concrete (P1)	120	0.18±0.003a	2.12±0.23a	1.89±0.18a	1.60±0.14a
Hapa (P2)	120	0.15±0.002b	2.01±0.19b	1.83±0.15b	1.57±0.11b
Effect of lipid levels (L)					
2.5% sunflower oil (L1)	40	0.13±0.004f	1.92±0.19f	1.86±0.12bc	1.51±0.13c
2.5% fish oil (L2)	40	0.15±0.002e	2.01±0.22e	1.93±0.17ab	1.60±0.10b
2.5% Mixture (L3)	40	0.16±0.003d	2.03±0.18d	1.85±0.16c	1.65±0.12a
5% sunflower oil (L4)	40	0.17±0.002c	2.09±0.17c	1.74±0.20d	1.50±0.16c
5% fish oil (L5)	40	0.18±0.002b	2.16±0.20b	1.81±0.14cd	1.64±0.12a
5% Mixture (L6)	40	0.18±0.003a	2.18±0.22a	1.98±0.17a	1.61±0.15ab
Interaction between ponds level and lipid levels					
T1 (P1×L1)	20	0.14±0.008i	1.96±0.19hi	1.91±0.19cd	1.56±0.14bc
T2 (P1×L2)	20	0.16±0.007f	2.07±0.12f	1.80±0.13de	1.64±0.19a
T3 (P1×L3)	20	0.17±0.003e	2.09±0.12e	1.94±0.15bc	1.66±0.23a
T4 (P1×L4)	20	0.18±0.007c	2.16±0.09c	1.78±0.12e	1.49±0.17cd
T5 (P1×L5)	20	0.19±0.006b	2.21±0.14b	1.84±0.14cd	1.66±0.15a
T6 (P1×L6)	20	0.21±0.007a	2.25±0.16a	2.09±0.20a	1.60±0.18ab
T7 (P2×L1)	20	0.12±0.008k	1.87±0.20j	1.79±0.16cd	1.46±0.14d
T8 (P2×L2)	20	0.13±0.005j	1.95±0.17i	2.06±0.15ab	1.56±0.17b
T9 (P2×L3)	20	0.15±0.009h	1.97±0.19h	1.76±0.15ef	1.65±0.17a
T10 (P2×L4)	20	0.16±0.009g	2.03±0.16g	1.71±0.13f	1.51±0.14c
T11 (P2×L5)	20	0.17±0.008d	2.11±0.16de	1.78±0.19e	1.63±0.19ab
T12 (P2×L6)	20	0.17±0.008e	2.12±0.18d	1.88±0.17cd	1.62±0.21ab

Values are means±SE of two replications. Means within each column having different letters were significantly different (P<0.05).

Table 5: Effect of different lipid sources and levels on blood components, Hemoglobin (Hb) of *O. niloticus*

Variable	No.	Glucose	Cholesterol	Triglycerides	Total lipid	Hb%
Effect of ponds (P)						
Concrete (P1)	24	103.39±1.26a	77.33±1.36a	235.67±2.79a	469.69±3.06a	8.15±0.13
Hapa (P2)	24	95.83±1.01b	69.99±0.89b	190.51±2.04b	353.17±2.23b	7.85±0.08
Effect of lipid levels (L)						
2.5% sunflower oil (L1)	8	92.50±1.16de	70.48±1.15b	194.39±1.55cd	398.14±2.37c	7.85±0.14
2.5% fish oil (L2)	8	91.35±0.99e	70.51±1.06b	195.11±1.17c	378.55±1.97d	7.95±0.10
2.5% Mixture (L3)	8	94.46±1.03d	68.97±0.94b	192.07±1.68d	371.84±2.08d	8.10±0.07
5% sunflower oil (L4)	8	103.41±1.14c	77.99±0.89a	235.22±1.44a	459.12±1.88a	7.92±0.11
5% fish oil (L5)	8	106.12±0.93b	77.47±1.08a	231.38±1.51b	448.23±1.37b	8.05±0.12
5% Mixture (L6)	8	110.51±1.07a	77.02±1.17a	230.47±1.22b	444.61±2.11b	8.14±0.08
Interaction between ponds level and lipid levels						
T1 (P1×L1)	4	96.08±1.66ef	72.01±1.19b	203.33±1.58c	428.16±1.89c	8.11±0.17
T2 (P1×L2)	4	94.12±1.19f	72.06±1.60b	201.26±1.42cd	431.22±1.56c	8.12±0.18
T3 (P1×L3)	4	99.02±1.23e	70.16±1.09b	197.79±1.39d	432.19±2.09c	8.31±0.21
T4 (P1×L4)	4	106.17±1.49c	84.11±1.13a	276.11±1.53a	540.02±2.14a	8.02±0.16
T5 (P1×L5)	4	111.22±1.34b	83.81±1.21a	269.18±1.42b	525.11±1.46b	8.21±0.19
T6 (P1×L6)	4	117.09±1.38a	83.92±1.18a	268.12±1.38b	522.09±1.13b	8.20±0.20
T7 (P2×L1)	4	89.43±1.61g	69.03±1.08b	186.31±1.51ef	368.19±1.51d	7.61±0.18
T8 (P2×L2)	4	89.37±1.59g	68.19±1.51b	190.19±1.29e	325.30±1.30e	7.83±0.17
T9 (P2×L3)	4	90.12±1.50fg	68.26±1.29b	187.88±1.41ef	311.82±1.37f	7.90±0.16
T10 (P2×L4)	4	101.10±1.41de	72.12±1.36b	194.09±1.49de	378.12±1.43d	7.80±0.21
T11 (P2×L5)	4	102.26±1.39d	72.08±1.47b	193.87±1.30de	371.29±1.58d	7.91±0.16
T12 (P2×L6)	4	104.01±1.41cd	71.94±1.68b	193.79±1.19de	366.69±1.28d	8.13±0.19

Values are means±SE of two replications. Means within each column having different letters were significantly different (P<0.05).

Table 6: Effect of different lipid sources and levels on the final weight (gm) and length (cm) of Nile tilapia

Variable	No.	Initial W	Final W	Initial L	Final L
Effect of ponds (P)					
Concrete (P1)	120	23.68±1.14a	237.75±2.89b	11.39±0.55a	25.28±1.38b
Hapa (P2)	120	20.62±1.23b	245.50±3.16a	10.95±0.34b	26.22±1.16a
Effect of lipid levels (L)					
2.5% sunflower oil (L1)	40	18.21±1.06f	235.75±2.38d	10.65±0.45d	25.25±1.17a
2.5% fish oil (L2)	40	20.56±1.13e	244.80±2.12b	10.88±0.63c	26.00±1.09a
2.5% Mixture (L3)	40	21.64±1.09d	250.41±2.24a	10.94±0.74c	26.30±1.10a
5% sun flower oil (L4)	40	22.80±1.11c	231.63±2.18e	11.51±0.42b	25.10±1.21a
5% fish oil (L5)	40	24.29±1.02b	241.92±2.30c	11.40±0.68b	25.80±1.19a
5% Mixture (L6)	40	25.39±1.15a	245.34±2.21b	11.64±0.76a	26.05±1.26a
Interaction between ponds level and lipid levels					
T1 (P1×L1)	20	19.28±1.03i	232.62±2.55f	10.74±0.63f	25.11±1.19a
T2 (P1×L2)	20	22.14±1.10f	239.84±2.14d	11.07±0.52e	25.42±1.22a
T3 (P1×L3)	20	22.92±1.08e	247.52±2.08bc	11.15±0.48de	25.71±1.15a
T4 (P1×L4)	20	24.39±1.14c	228.51±2.21g	11.80±0.80b	24.90±1.14a
T5 (P1×L5)	20	25.40±1.20b	236.90±1.89e	11.54±0.71c	25.22±1.19a
T6 (P1×L6)	20	27.94±1.09a	241.22±2.03d	12.05±0.60a	25.43±1.16a
T7 (P2×L1)	20	17.14±1.15k	238.93±2.19de	10.65±0.72fg	25.41±1.21a
T8 (P2×L2)	20	18.98±1.06j	249.80±2.50b	10.68±0.39g	26.63±1.14a
T9 (P2×L3)	20	20.36±1.03h	253.33±2.42a	10.74±0.55f	26.90±1.23a
T10 (P2×L4)	20	21.21±1.11g	234.72±2.48e	11.21±0.84de	25.30±1.15a
T11 (P2×L5)	20	23.18±1.19d	246.91±2.36c	11.26±0.90d	26.44±1.28a
T12 (P2×L6)	20	22.85±1.14e	249.41±1.88bc	11.24±0.68de	26.71±1.23a

Values are means±SE of two replications. Means within each column having different letters were significantly different (P<0.05).

Table 7: Effect of different lipid sources and levels on condition factor (K), Daily Weight Gain (DWG) and Specific Growth Rate (SGR)

Variable	No.	DWG	SGR	Initial K	Final K
Effect of ponds (P)					
Concrete (P1)	120	1.70±0.06b	1.84±0.07b	1.60±0.14a	1.64±0.12a
Hapa (P2)	120	1.79±0.05a	1.97±0.08a	1.57±0.11b	1.51±0.10b
Effect of lipid levels (L)					
2.5% sunflower oil (L1)	40	1.73±0.04d	2.03±0.07a	1.51±0.13c	1.62±0.16a
2.5% fish oil (L2)	40	1.78±0.03b	1.97±0.06b	1.60±0.10b	1.55±0.12a
2.5% Mixture (L3)	40	1.82±0.04a	1.95±0.09c	1.65±0.12a	1.53±0.09a
5% sunflower oil (L4)	40	1.66±0.04e	1.84±0.06d	1.50±0.16c	1.62±0.11a
5% fish oil (L5)	40	1.73±0.07d	1.83±0.05e	1.64±0.12a	1.57±0.12a
5% Mixture (L6)	40	1.75±0.05c	1.80±0.06f	1.61±0.15ab	1.54±0.07a
Interaction between ponds level and lipid levels					
T1 (P1×L1)	20	1.69±0.09e	1.98±0.08c	1.56±0.14bc	1.63±0.09a
T2 (P1×L2)	20	1.73±0.06d	1.89±0.07f	1.64±0.19a	1.63±0.11a
T3 (P1×L3)	20	1.78±0.10bc	1.89±0.08f	1.66±0.23a	1.62±0.08a
T4 (P1×L4)	20	1.62±0.06f	1.78±0.10h	1.49±0.17cd	1.64±0.08a
T5 (P1×L5)	20	1.68±0.05e	1.77±0.09h	1.66±0.15a	1.65±0.10a
T6 (P1×L6)	20	1.69±0.11e	1.71±0.06i	1.60±0.18ab	1.64±0.07a
T7 (P2×L1)	20	1.76±0.08cd	2.09±0.04a	1.46±0.14d	1.61±0.06a
T8 (P2×L2)	20	1.83±0.09a	2.04±0.05b	1.56±0.17b	1.47±0.12a
T9 (P2×L3)	20	1.84±0.07a	2.08±0.08a	1.65±0.17a	1.43±0.10a
T10 (P2×L4)	20	1.69±0.10e	1.91±0.07f	1.51±0.14c	1.61±0.08a
T11 (P2×L5)	20	1.78±0.08c	1.88±0.04g	1.63±0.19ab	1.48±0.12a
T12 (P2×L6)	20	1.80±0.06b	1.90±0.09f	1.62±0.21ab	1.45±0.011a

Values are means±SE of two replications. Means within each column having different letters were significantly different (P<0.05).

Table 8: Survival rate and total yield of of ponds kind and lipid levels.

Variable	Survival rate% After over-wintring	Survival rate% After growing period	% of the total production
Effect of ponds (P)			
Concrete (P1)	91.65	96.41	48.91%
Hapa (P2)	89.29	97.34	51.09%
Effect of lipid levels (L)			
2.5% sunflower oil (L1)	88.84	95.97	16.11%
2.5% fish oil (L2)	89.14	96.75	16.87%
2.5% Mixture (L3)	89.42	97.31	17.31%
5% sunflower oil (L4)	91.375	97.07	16.01%
5% fish oil (L5)	91.69	96.88	16.68%
5% Mixture (L6)	92.37	97.28	17.02%
Interaction between pond (P) and lipid level (L)			
T1 (P1×L1)	89.64	95.76	7.93%
T2 (P1×L2)	90.09	96.23	8.22%
T3 (P1×L3)	89.97	96.69	8.45%
T4 (P1×L4)	93.16	96.35	7.84%
T5 (P1×L5)	93.22	96.44	8.13%
T6 (P1×L6)	93.83	96.97	8.33%
T7 (P2×L1)	88.04	96.17	8.18%
T8 (P2×L2)	88.19	97.26	8.65%
T9 (P2×L3)	88.87	97.93	8.84%
T10 (P2×L4)	89.59	97.78	8.17%
T11 (P2×L5)	90.16	97.31	8.55%
T12 (P2×L6)	90.90	97.59	8.69%

Clinical, Postmortem and Laboratory Examinations

Over-Wintering: Some fingerlings fed lowest lipid level (T_1, T_2, T_3, T_7, T_8 and T_9) either in concrete or hapas (Figure 1) suffered lethargy, imbalance, poor appetite, hemorrhage near the base of fins. Sometimes, the hemorrhages may be extended to cover wider body surface . There are an external, cotton-like lesions with grayish dull discolouration and in advanced cases showed severe dermatitis with rayed fins. Bacterial infections, poor husbandry including poor water quality, adverse water temperature, all of these factors increased occurrence of saprolegnia infections [15, 44]. On the other side, Fingerlings fed higher concentrations of lipid (T_4, T_5, T_6, T_{10}, T_{11} and T_{12}) were more healthier. Dietary lipids are the main source of energy for the development of fish fry, the supply of essential fatty acids in sufficient amounts is critical for survivability and rapid growth [43].

Growing Period: Clinically examined morbid fish fed higher concentrations of lipids (T_4, T_5, T_6, T_{10}, T_{11} and T_{12}) of both types of ponds showed dullness, loss of equilibrium, loss of appetite, sluggish movements, gathering near the surface water, abnormal swimming. Roughness and easily detached scales, dark pigmented skin, hemorrhage at the base of fins, frayed tail and fins, off scales, wide ulcers, exophthalmia, opaqueness of the eyes with hemorrhage and abdominal distention, cutaneous grayish patches beside the slight hemorrhage at the base of the fins, (Figure 2 and 3). Some developed anal protrusion with petechial hemorrhages around the vent. Similar signs were reported by Lavilla [45]. Moreover, postmortem examination of infected fish showed congestion of the gills and internal organs, hepatosplenomegaly, distended gall bladder, Figure, 4.

Accurate diagnosis showed mixed bacterial infection (*Aeromonas spp., Pseudomonas fluorescens Flavobacterium columnare, Edwardsiella tarda and Streptococcus spp.*) which are commonly found in the facilities [46]. The previously described clinical signs, post mortum inspection and microbiological findings were more or less mentioned by Abou El-Atta and El.Tantawy [47] who emphasized the pathogenesis of such lesions. The predisposing causes of diseases may be due to rearing fish in high densities in semi-intensive fish farms, high temperature, poor water quality and stress.

Lower concentrations of lipid (T_1, T_2, T_3, T_7, T_8 and T_9) either in concrete or earthen ponds were more disease resistant [26, 36].

Fig. 1: Severe haemoragic dermatitis with rayed fins

Fig. 2: Hemorrhage at the base of fins, frayed tail and fins, off scales and wide ulcers

Fig. 3: Abdominal distention, cutaneous grayish patches, anal protrusion with petechial hemorrhages around the vent

Fig. 4: Congestion of internal organs, hepatosplenomegaly and distended gall bladder

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