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Original Article

Influence of Protein Levels and Pond Fertilization during Broodstock Pre-Spawning Period on the Tolerance of Nile Tilapia, *Oreochromis niloticus*, Fry to Winter Season Temperature

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Abstract

Nile tilapia, *Oreochromis niloticus* females were fed three commercial diets (20, 25 and 30% crude protein) in 200 m^3 earthen ponds fertilized with compost at rate of 25 kg/pond/week to evaluate their growth performance, seed production and survival rate of produced fry throughout winter season. Average initial body weight of females (19.30±1.05g) and males (23.45±2.1g) were stocked separately with density of 1000 fish/pond, and fed to satiation twice daily for 120 days as a pre-spawning period. Both sexes were then transferred to concrete ponds in hothouses with a ratio (6:2 females: male) per tank. During spawning period, broodstocks were fed a diet (25% crude protein) at 1% of their live body weight for 90 days. Last batch of produced fry were nursed in concrete tanks in hothouses, and fed on 30% crude protein diet for 45 days. Fry from each treatment was then transferred to wintering ponds, stocked at 500 fry/m^3, and fed twice daily to satiation for 120 days. Results indicated that final body weight, gain and average daily gain of broodstocks increased significantly ($P<0.05$) with increasing dietary protein level in fertilized ponds. Feed conversion rate and protein efficiency ratio improved, but insignificantly with increasing dietary protein level and with adding fertilizers. However, number of progeny increased significantly ($P<0.05$) by increasing protein level in fertilized ponds. Correspondingly, initial and final body weight of the offspring was significantly ($P<0.05$) triggered with increasing protein level and fertilizers. Also, feed conversion rate of fry improved significantly ($P<0.05$) with increasing dietary protein level and fertilized treatments. Moreover, survival of fry increased significantly ($P<0.05$) with fertilization treatments and increasing protein level. Therefore, the study highlights that by feeding *O. niloticus* broodstocks diets containing 25% crude protein supplemented with compost fertilizers during pre-spawning period, could be sufficient to trigger the production of viable fry that might tolerate low winter season temperatures.

Keywords: *Oreochromis niloticus, growth performance, pre-spawning, winter season, fry production, survival rate.*

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Introduction

Tilapia can rely totally on the availability of natural food in ponds by means of fertilization up to a certain size, beyond it; growth will slow down due to insufficient natural food to satisfy the fish requirements. Although, pond fertilization in semi-intensive tilapia culture is often associated with supplemental feeding, especially during the later grow-out stages (Ntengwe and Edema 2008), but adding fertilizers to fish ponds boosts the production of beneficial phytoplankton, microscopic free-floating algae. In the matter of fact, increasing the phytoplankton density in ponds, more food substances will be available for smaller fish (McNabb *et al*. 1990). Thereby, productivity increases, which in turns increases the amount of harvestable fish. The harvested yield of a fertilized pond can be triple that of an unfertilized pond (Diana and Lin 1998; Brunson *et al.* 2001). Furthermore, fertilization requirements may vary between earthen ponds and experimental cement tanks attributable to the more leaching capacity of bottom soil in earthen ponds than in cement tanks (Bhakta *et al.* 2004). In order to sustain fish growth, where supplemental feed are becomes necessary. However, growth of Nile tilapia fry reaches a preferable size when formulated diets contain 40% crude protein (Siddiqui *et al.* 1988). Increasing dietary protein levels increases significantly weight gain of cultured Nile tilapia and specific growth rate (Loum *et al.* 2013). However, El-Dahhar (1994) reported that best growth was obtained when the dietary crude protein level was 26% for Nile tilapia fingerlings weighing (9.6 g) with feeding rate 5% of their body weight. Al-Hafedh (1999) stated that tilapia fry and fingerlings fed on a diet containing 45% crude protein had better growth, survival and food conversion than those fed lower protein levels. Besides, Ogunji and Wirth (2000) reported that diet containing 33.3% dietary protein appeared optimal for the protein requirement of *O. niloticus* fingerlings. However, experimental trials revealed that protein content of the body increases significantly with increasing protein levels in the diets for fry, fingerlings and adult fish of Nile tilapia (Sallam 2010). Moreover, fish fed diets of higher protein levels had higher growth and feed conversion ratio than those on lower protein levels. The relative fecundity for *O. niloticus* fluctuated significantly with dietary protein levels (Al-Hafedh *et al*. 1999). Even though, fast-growing fish matured earlier, thus maturation rate is affected by the dietary protein levels (Al-Hafedh *et al.* 1999). Correspondingly, reproductive output could be maintained at the expense of somatic growth. While, supplying inadequate dietary protein for long period results in slow ovarian renaissance (Gunasekera and Lam 1997), prolonged intervals between spawning **(**Gunasekera *et al.*1996) and a complete halt to reproduction. Therefore, brood fish should be provided with optimum levels of protein in the diet from a young age up to the egg producing stage. In a study by Gunasekera *et al*, (1995) Nile tilapia fingerlings fed a low protein diet 17% did not show oocyte maturation, while females fed 25% protein showed slow oocyte growth, whereas, the females fed 32% protein level had early oocyte growth and maturation. Moreover, the protein level required for the normal growth of Nile tilapia seems to be sufficient to support broodstock maturation.

Dietary protein plays a crucial role in supplying protein content of eggs, number of eggs per spawning and the spawning interval in *O. niloticus* (El-Sayed and Kawanna 2008). In fact, reproduction performance of *O. niloticus* can be improved by exposing Nile tilapia broodstocks to water temperature and dietary protein levels. Thus, temperature is one of the major environmental factors involved in cueing reproductive activity (Emit *et al.* 1989). In tanks with semi-purified isocaloric diets, *O. niloticus* females fed with 35% protein diet produced eggs with significantly higher protein than females fed with 10% and 20% protein diets (Gunasekera *et al***.** 1996). In the same study, females fed on 20% and 35% diets produced more number of eggs per spawning than those fed on 10% protein diet. According to Santiago et al. (1985**)** diets with 40% crude protein induced higher fry production of *O. niloticus* than a 20% protein diet under outdoor tank conditions. In spite of Nile tilapia, red tilapia brood fish fed for only 32 day with 45% protein gave higher seed production compared to 25% protein diets (Chang *et al.* 1988). Thus, the main purposes of the present study were to estimate the effect of dietary protein levels on growth performance and maturation of *O. niloticus* fingerlings, vitality and tolerance of produced larvae to low winter season temperature.

Materials and methods

The study herein was conducted for 12 months (from mid of March until the end of March of the following year) in a private fish farm at the city of Edku, El-Beheira governorate, Egypt. Nile tilapia females were supplemented with three dietary protein levels (20%, 25% and 30% crude protein, CP) during pre-spawning period in ponds fertilized (25 kg/pond/week) with organic fertilizer (compost) for 120 days (Table 1 and Table 2). Females with average body weight of (19.30±1.05g) were stocked in 200 m^2 ponds filled to 1.2 m depth with density of 1000 female/pond. Males with the average body weight (23.45±2.1g) were stocked separately in two fertilized ponds and fed commercial diet of 25% CP during pre-spawning period. During this period, water samples were analyzed and fish specimens were weighed monthly. Fish were fed twice daily to satiation throughout the whole experimental duration period. Treatments were configured as follows: (T_1) 20% dietary protein level with organic fertilizer, (T_2) 25% dietary protein level with organic fertilizer, (T_3) 30% dietary protein level with organic fertilizer, (T_4) 20% dietary protein level without organic fertilizer, (T_5) 25% dietary protein level without organic fertilizer, and (T_6) 30% dietary protein level without organic fertilizer. Subsequently, females were transferred to concrete ponds (4×6×1.5m) with males (at a sex ratio of 3:1, females: male) in a hothouse. Broodstocks were fed a 25% crude protein diet during the spawning season at the rate of 1% of their live body weight twice daily for an additional 3 months. After one week of the spawning period, broodstocks were checked daily for spawning activity, and every 10 days the mean body weight, survival rate was recorded. When brooders started to spawn, the offspring were collected from brooders mouth every 10 days, and then weighted, counted, nursed and the last batch was stocked (500 fry/m^3) in concert ponds for 45 days in hothouses. Fry were fed on 30% CP diet to satiation, then transferred to earthen ponds for wintering for 4 months. Growth performance, and survival rate of fry during winter season was determined. Females were stocked in 6 fertilized and 6 unfertilized ponds with dissolved oxygen concentration ranged between 5.29 and 6.11mg/L throughout the experimental period. Water temperature throughout the pre-spawning and spawning periods of the broodstocks ranged from 24.12 to 34.31C˚ with no significant difference among treatments. However, in fry stage the temperature ranged from 26 to 5C˚. The 3 dietary protein levels (20, 25, and 30%) were introduced as a pre-spawning period (stage I) feeds.

Analytical methods

Ten fingerlings and 50 larvae were spared for proximate analysis. Feed samples were kept frozen for chemical analysis and were determined according to AOAC (2000). All chemical analysis for fish and feed were determined according to the AOAC (2000). Water temperatures (C˚) were measured daily using standard thermometer.

Table 1. Proximate composition of the three experimental diets (dry weight basis).

Diet contents(%	Dietary protein levels (%)		
	20	25	30
Moisture	9.20	9.16	9.00
Crude protein	18.88	24.81	29.73
Crude fat	8.98	8.21	7.60
Ash	7.62	8.50	8.00

Growth performance and feed utilization

Average body weight (ABW, g), average weight gain (AWG, g), average daily gain (AVD, g/day), specific growth rate (SGR%, /day) for brood stocks and offspring were calculated as follows:

Weight gain (WG) = final weight – initial weight.

Average daily gain (ADG) = (final weight – initial weight)/number of days.

Fish body moisture, crude protein and crude fat contents were determined according to AOAC (1990). Feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value, and (PPV %), were estimated for brood stocks and fry as follows:

FCR = dry matter feed intake/ gain.

PER= gain/protein intake.

PPV%= protein gain in fish (g) x 100/protein intake in food (g).

Specific growth rate (SGR%/day) = (ln final weight - ln initial weight) 100/number of days.

Table 2. Proximate analysis of organic fertilizer (compost) used in experimental earthen ponds.

Compost contents	Value (%)
Moisture content	9.80
Nitrogen	1.74
Phosphorus	0.85
Organic matter(carbon)	63.55

Statistical analysis

Results of growth parameters, feed utilization parameters, and survival rate (%) of broodstock and fry of the experimental treatments were treated using the ANOVA test (analysis of variance) and Tuckey test to a minimum significance (P<0.05). The results are expressed as means ± SEM. Statistical analysis was performed using two-way ANOVA according to Assaad *et al.* (2015) and EXCEL (windows 10 2015). Graphs have been performed using SigmaPlot for windows (Systat Software, Inc., version 12.0 2011).

Results

Growth performance and feed utilization

Pre-spawning period (stage I)

Average finial body weight (FBW, g), weight gain (WG, g), average daily gain (ADG, g) and specific growth rate (SGR, %) of fish are demonstrated in (Table 3). A positive relationship between fertilization and dietary protein levels on FBW, WG, ADG, and SGR% were detected. FBW, WG, ADG, and SGR% increased significantly (P<0.05) in fertilized treatments and with increasing protein levels. The highest FBW, WG, ADG, and SGR% was observed at (T3) followed by (T2) with a significantly difference between them, then (T6) (T5) and (T1) and the lowest FBW was for (T4). The average value of feed intake (g/fish), feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish are shown in Table (3). Moreover, FCR was not significantly affected with dietary protein level. Consequently, a positive relationship between fertilization and FCR was established, as it decreased significantly (P<0.05) with fertilized treatments. The best FCR obtained was with (T2), (T6) and (T3) with no significant differences, followed by (T1) then (T5) and the least with a significant difference was (T4). Similarly, PER was the same trend as FCR in the present study.

Spawning period (stage II)

However, weight gain was the indicating parameter for this phase, where the results reveal that the fluctuation was due to significant difference in their initial body weight at this stage. Broodstocks during spawning season under the hothouse condition had no significant differences between treatments in their WG, ADG and SGR for *O. niloticus*. Although, the PER increased significantly with the increase of protein level from 20% to 25% CP, but was not affected with neither protein levels nor pond fertilization from 25% to 30% CP. whereas for the total number of fry/female increased significantly (P<0.05) with pond fertilization and increased protein levels (Table 4). Therefore, pond fertilization increased significantly the potency of dietary protein utilization.

Fry production

Meanwhile, the number of fry produced from broodstocks fed on different dietary protein level with or without organic fertilizer during pre-spawning period (stage I) had a positive correlation between both of dietary protein level, fertilization treatments. By increasing dietary protein from 20 to 25%, number of fry produced increased significantly ($P<0.05$) from 1800.25±520 to 2410±325 fry/female, then increased insignificantly to 2517±103 fry/female at 30% dietary protein level. Also, with fertilized treatment number of fry produced increased significantly ($P<0.05$) from 2067.5±586.9 to 2417±237.13 fry/female. Result reveled that (T_2) had the highest number of fry followed by (T_6) and (T_3) with no significant difference (Table 4).

Fry tolerance for low temperature (stage III)

Throughout this stage the evaluation of produced fry influenced by different levels of dietary protein and fertilization treatments of Nile tilapia broodstocks during stage (I). DO during this stage was maintained as that for previous stages. Differences between ponds in water temperature were very slight at the sampling times, but variations among the experimental intervals (November to March) were high. Temperature had gradually declined from the first week of the experiment until week 11, and then it started to increase until the end of the experimental period. The highest water temperature 24^{o}C was recorded at the first week of the fry stage on the 17th of November, while the lowest 5^{o}C was recorded at dawn of the week 10 in January and week 11 in February. However, IBW, FBW, final density, survival and SGR (%) of produced fry indicated a positive correlation between both of dietary protein level, fertilization treatments and IBW. With increasing dietary protein levels in stage (I) IBW increased significantly ($P<0.05$). Also, the IBW increased significantly ($P<0.05$) with fertilized treatments. Similar results were disclosed for FBW; however, SGR (%) showed insignificant differences among treatments for dietary protein levels or fertilization. In addition, final density and survival rate of produced fry revealed a significant differences ($P<0.05$) for dietary protein and fertilization treatments. The results indicated that the higher protein fed to broodstock through stage (I), the better survival and final density obtained. The results concerning WG, and ADG followed the same trend observed for FBW. Also, FCR was affected significantly with fertilized treatments ($P<0.05$) and dietary protein levels; it was1.4±0.03 in fertilized ponds, however, it was 1.7±0.35 in unfertilized ponds 1.76 ± 0.04, 1.59±0.21 and 1.43±0.10 for diet containing 20, 25 and 30% protein level, respectively. The best FCR was obtained with T_3 1.35±0.07 and T_2 and T_1 1.44±0.05 with no significant, followed by T_6 and T_5 1.74±0.0 and the least significant treatment was T_4 2.07±0.09. With respect to PER, data in Table (5) showed no significant differences between dietary protein levels. It was found to be 2.46±0.54, 2.55±0.34 and 2.80±0.11 for diets containing 20, 25 and 30% protein level, respectively. Also, data indicated that there was an insignificant different between pond fertilization and PER with value range between 2.37±0.32 and 2.84±0.04. The highest was 2.87±0.01 observed for T_3, and the lowest was 2.08±0.10 for T_4.

Discussion

Data collected along the period of the study indicated that average DO, pH, and water temperature were within the range considered for fish growth. Neill and Bryan (1991) reported that optimal temperature for tilapias is 20-30C˚. However, water temperatures generally are quite predictable by season and location. This explains the reason for the variations observed in water temperature throughout the experimental period in the present study and that it was not a limiting factor. Popma *et al.* (1993) mentioned that both growth and feed conversion efficiency of tilapia improves as optimum water temperature approached (28 C˚). Although in stage (III) herein, fry were reared in winter, water temperatures declined gradually

Table 3. Means (±) Standard error of growth performance, survival rate (SUR %), feed conversion ratio (FCR, g/g), protein efficiency ratio (PER, g/g) and protein productive value (PPV, %) of *O. niloticus* broodstock reared in fertilized (FR) and unfertilized (UF) ponds fed three dietary protein levels (20, 25 and 30% CP) throughout pre-spawning period (Stage I).

Variable	**FR**			**UF**			***P*-value**		
	20% (T_1)	**25%(T_2)**	**30% (T_3)**	**20% (T_4)**	**25% (T_5)**	**30% (T_6)**	**Col1**	**Group**	**C×G[1]**
FBW g/fish	111 ± 2.5^{b}	130 ± 0^{a}	132 ± 3.1^{a}	89.8 ± 3^{c}	117 ± 3.9^{ab}	128 ± 2.8^{a}	0.001	<0.001	0.065
WG g/fish	92 ± 2.5^{b}	110 ± 0^{a}	113 ± 3.1^{a}	70.6 ± 3^{c}	97.4 ± 3.9^{ab}	108 ± 2.8^{a}	0.001	<0.001	0.065
ADG g/fish	1.06 ± 0.03^{b}	1.27 ± 0^{a}	1.3 ± 0.04^{a}	0.81 ± 0.04^{c}	1.12 ± 0.05^{ab}	1.24 ± 0.03^{a}	0.001	<0.001	0.065
SGR %/day	1.81 ± 0.02^{b}	1.96 ± 0^{a}	1.98 ± 0.024^{a}	1.6 ± 0.034^{c}	1.85 ± 0.04^{ab}	1.95 ± 0.023^{ab}	0.001	<0.001	0.032
SUR %	92 ± 1^{ab}	95 ± 1^{ab}	95.5 ± 0.5^{a}	87.5 ± 0.5^{c}	91.5 ± 0.5^{b}	94.5 ± 0.5^{ab}	0.002	0.001	0.111
FCR g/g	1.75 ± 0.23	1.18 ± 0.09	1.16 ± 0.11	2.42 ± 0.6	2.1 ± 0.6	1.67 ± 0.33	0.057	0.252	0.855
PER g/g	2.91 ± 0.4	3.41 ± 0.3	2.9 ± 0.28	2.18 ± 0.51	2.05 ± 0.54	2.08 ± 0.41	0.027	0.834	0.714
PPV %	20.9 ± 1.6^{ab}	24.8 ± 1.63^{a}	25.5 ± 0.73^{a}	19.3 ± 0.9^{ab}	17.4 ± 0.14^{b}	21.2 ± 1.5^{ab}	0.004	0.083	0.13

Values are means ± SEM, n = 2 per treatment group.

[a-c] Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

[1]C × G = Col1 × group interaction effect.

Table 4. Means (±) Standard error for growth performance, initial body weight (IBW, g/fish), final body weight (FBW, g/fish), weight gain (WG, g/fish), average daily gain (ADG, g/fish/day), specific growth rate (SGR,%/day), protein efficiency ratio (PER, g/g) and total number of produced fry (TF, fry/female) of *O. niloticus* broodstock reared concrete tanks throughout spawning period (Stage II).

Variable	**FR**			**UF**			***P*-value**		
	20% (T$_1$)	**25%(T$_2$)**	**30% (T$_3$)**	**20% (T$_4$)**	**25% (T$_5$)**	**30% (T$_6$)**	**Col1**	**Group**	**C×G[1]**
IBW g/fish	111 ± 2.5^b	130 ± 0^a	132 ± 3.1^a	89.8 ± 3^c	117 ± 3.9^{ab}	128 ± 2.8^a	0.001	<0.001	0.065
FBW g/fish	197 ± 3^{ab}	216 ± 6^a	219 ± 3.5^a	174 ± 6^b	201 ± 7^{ab}	212 ± 4.5^a	0.012	0.003	0.339
WG g/fish	86 ± 5.5	87 ± 6	87 ± 0.4	84.2 ± 9	84 ± 10.9	85 ± 7.3	0.715	0.992	0.996
ADG g/fish	0.94 ± 0.06	0.95 ± 0.07	0.95 ± 0.004	0.92 ± 0.1	0.91 ± 0.12	0.92 ± 0.08	0.715	0.992	0.996
SGR %/day	0.6 ± 0.04	0.6 ± 0.03	0.6 ± 0.01	0.73 ± 0.074	0.6 ± 0.07	0.6 ± 0.05	0.335	0.126	0.678
PER	4 ± 0.1^a	3.31 ± 0.11^b	3.31 ± 0.06^b	4.4 ± 0.15^a	3.34 ± 0.14^b	3.31 ± 0.06^b	0.151	<0.001	0.194
TF	1770 ± 43^c	2240 ± 14^a	2040 ± 19^b	1030 ± 33^d	1780 ± 23^c	2140 ± 11^{ab}	<0.001	<0.001	<0.001

Values are means ± SEM, n = 2 per treatment group.

[a-d]Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

[1]C × G = Col1 × group interaction effect.

Table 5. Means (±) Standard error for growth performance, initial body weight (IBW, g/fish), final body weight (FBW, g/fish), weight gain (WG, g/fish), average daily gain (ADG, g/fish/day), specific growth rate (SGR,%/day), feed conversion ratio (FCR, g/g), survival rate (SUR,%), protein efficiency ratio (PER, g/g), and final fry density (FD) fry/m^3 of *O. niloticus* fry descendant from broodstock reared in earthen ponds throughout pre-spawning period.

Variable	**FR**			**UF**			***P*-value**		
	20% (T$_1$)	**25%(T$_2$)**	**30% (T$_3$)**	**20% (T$_4$)**	**25% (T$_5$)**	**30% (T$_6$)**	**Col1**	**group**	**C×G[1]**
IBW g/fish	1.4 ± 0.04^{c}	1.95 ± 0.05^{a}	1.8 ± 0.06^{ab}	0.95 ± 0.02^{d}	1.45 ± 0.03^{c}	1.65 ± 0.06^{bc}	<0.001	<0.001	0.018
FBW g/fish	13.8 ± 0.04^{d}	15.7 ± 0.05^{b}	16 ± 0.06^{a}	8.2 ± 0.02^{f}	13 ± 0.03^{e}	15.3 ± 0.06^{c}	<0.001	<0.001	<0.001
WG g/fish	12.4 ± 0.08^{c}	13.8 ± 0.1^{ab}	14.2 ± 0.12^{a}	7.25 ± 0.04^{e}	11.6 ± 0.06^{d}	13.7 ± 0.12^{b}	<0.001	<0.001	<0.001
ADG g/fish	0.1 ± 0.001^{c}	0.12 ± 0.01^{ab}	0.12 ± 0.01^{a}	0.06 ± 0.003^{e}	0.1 ± 5e-04^{d}	0.114 ± 0.01^{b}	<0.001	<0.001	<0.001
SGR %/day	2.34 ± 0.03^{a}	2.2 ± 0.03^{ab}	2.28 ± 0.03^{ab}	2.15 ± 0.02^{b}	2.26 ± 0.02^{ab}	2.31 ± 0.034^{a}	0.149	0.085	0.006
SUR %	46.5 ± 2^{b}	65 ± 2^{a}	66 ± 1^{a}	33.5 ± 1.6^{c}	47.5 ± 2.3^{b}	65.5 ± 1.5^{a}	<0.001	<0.001	0.008
FCR g/g	1.44 ± 0.06^{b}	1.44 ± 0.12^{b}	1.35 ± 0.04^{b}	2.07 ± 0.09^{a}	1.74 ± 0.11^{ab}	1.5 ± 0.05^{b}	0.002	0.022	0.07
PER g/g	2.85 ± 0.16^{a}	2.79 ± 0.12^{a}	2.87 ± 0.13^{a}	2.08 ± 0.08^{b}	2.31 ± 0.11^{ab}	2.72 ± 0.09^{ab}	0.003	0.072	0.1
FD	232 ± 10^{b}	325 ± 10^{a}	330 ± 5^{a}	168 ± 8^{c}	238 ± 11.5^{b}	328 ± 7.5^{a}	<0.001	<0.001	0.008

Values are means ± SEM, n = 2 per treatment group.

[a-f]Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

[1]C × G = Col1 × group interaction effect.

and mortality rates increased. Likewise, El-Tawil (2006) observed low survival rates for Nile tilapia fry in winter season fed different protein levels in fertilized and unfertilized ponds treatments. The author attributed high mortality rates to low winter season temperatures. DO levels in the present study were above the normal tolerance of tilapia appears capable of tolerating short-term conditions near 0 mg/L of oxygen without lethal effects (Diana *et al.* 1997) but their growth appears to decline when conditions reach such levels. Moreover, Diana *et al.* (1994) reported that events of low DO (<1 mg/L) were not correlated to total inputs in ponds (fertilizers and feed) or to phytoplankton biomass, but rather occurred sporadically in ponds spanning the range of lowest to highest feeding rates. Whereas, the pH values varied according to type of fertilizers added which ranged between 7.52 and 7.95 in the present study. Close results were obtained by Moustafa (2005) who mentioned that pH ranged between 8.05-8.6 with chicken litter treatment, while Abis fertilizer treatment had a pH range of 7.9-8.9. Kurten *et al.* (1999) reported that pH values recorded in only organic fertilizers treatment were significantly lower than those recorded in ponds treated with both organic and mineral fertilizers.

Growth performance

FBW increased significantly in fertilized pond all over the present study. Meanwhile, El-Tawil (2006) indicated that there were significant differences between fertilized treatments and control treatment in final body weight, weight gain, average daily gain and total yield. The author suggested that natural production in fertilized ponds partially compensates the insufficient feeding during this period. On the other hand, FBW differed significantly among treatments in the stage (I) by increasing dietary protein levels. Consequently, Al-Hafedh *et al.* (1999) showed that tilapia fry, weighing 0.5 grams, had the best growth and matured earlier when fed protein levels around 40%. As tilapia increased in age (100 – 200 g), optimal dietary protein level was shown to decrease to 30%, resulting in higher relative fecundity for females compared with higher dietary protein levels. However, results for FBW of fry collected from broodstock fed on dietary protein levels (20, 25 and 30%) with or without fertilization during stage (I) showed that there were significantly differences among treatments. Therefore, unfavorable bloodstock's nutritional conditions may cause poor fecundity and fertility, deformed embryos, week larvae, where females produce imperfect eggs and males may produce low-grade quality of milt (Mabroke, 2012). These results may be attributed to the feeding habits of tilapia fish which is an efficient converter phytoplankton and can utilize a wide variety of food especially formulated feeds. Similarly, Liti *et al.* (2006) stated that fish in ponds received fertilizer and supplemental feed had higher weight gain than others. Size of female is more important than age in terms of fecundity and total number of eggs produced (Rana 1986, 1988). In tilapia, females maturing earlier at smaller size produces smaller eggs but relatively more eggs than a larger fish per unit body weight **(**Rana, 1988**)**. Some authors have indicated that number of eggs produced is related to body length (De Silva, 1986; Rana 1986) while others have claimed that it is more related to the body weight of the female (Rana 1988). However, relative fecundity decreases with maternal age, weight and length (Rana 1986). Nile tilapia females of larger size were found to produce more and bigger eggs (Rana 1986) and more fry per female, but smaller females spawn more frequently **(**Guerrero and Guerrero 1985**)**. In the present study, the number of fry produced significantly increased with increasing dietary protein level from 20 to 25 or to 30%. Gunasekera *et al.* (1997) studied the effect of increasing dietary protein level (10, 20, and 35%) on *O. niloticus* females during pre-spawning period. Their result indicated that the egg collected from females fed on 35%

protein diet was significantly higher than those fed diets of 10% and 20% protein. Mabroke (2012) found that relative fecundity increase significantly with increasing dietary protein level. This corresponds with the results of the present study where broodstocks reared in fertilized ponds produced higher number of fry than those reared in unfertilized ponds. With respect to fish survival rate, data obtained in the present study revealed that there were no significant differences in survival rate with fertilization treatments of stage (I). Generally, the survival rate in the present study is considerably higher than 65% except for stage (III). This result agrees with Brown et al. (2001) which reported that genetically improved farmed Nile tilapia (GIFT) had similar survival rates as of sex reversed *O. niloticus* fed experimental diets in fertilized ponds. Although, stage (III) (fry during winter season), had low survival rates for all treatments in this stage 33.5%, 46.5%, 47.5%, 65%, 65.5% and 66.0% for T_4, T_1, T_5, T_2, T_6 and T_3, respectively. These results could be attributed to low water temperature (5-15°C) in winter season. Concurrently, Tidwell *et al.* (2003) stated that growth and feed conversion efficiency are reduced when temperature decreased. Additionally, De Wet and Takle (2004) found that at sub-optimal water temperature, poor production performance was obtained for tilapia. Moreover, Dan and Little (2000) reported that large fry were higher in survival rates than small one. Meanwhile, FCR herein indicate that, there were significant differences among treatments. FCR significantly improved with fertilizer treatments and had better conversion rate than many previous studies. Thus, enhancing natural food by fertilization resulted in higher contribution in fish nutrition at all experimental period and reduces the amount of supplemental feed required. McNabb et al. (1990) reported that fertilization of fish pond actually increases the production of phytoplankton in pond and more food items are available for fish. However, Ogunji and Wirth (2000) obtained FCR of 0.83 and 1.28 when sex reversed Nile tilapia fed on 30% crude protein diet in fertilized ponds. In the present study, PER was significantly affected by increasing natural food and with fertilization treatments. With increasing natural food depending on fertilization PER increased significantly from 1.99 to 3.04 in fertilized ponds. These results agree with the results of previous studies by Green and Boyd (1995) they reported that fish grows faster in ponds received both fertilizers and supplemental feed than those in ponds received fertilizers only or formulated feeds only. Also, the present results agree with that El-Tawil (2006) who found that PER increased significantly from 2.35 in unfertilized ponds to 3.24 in fertilized ponds. He suggested that fertilized treatments spared dietary protein for growth and increases the efficiency of protein utilization. On the other hand, Kim and Lee (2005) found that with increasing dietary protein level from 22 to 32% for catfish fingerlings, PER decreased from 3.56 to 3.43 but without any significant differences. Therefore, this study concluded that increasing the dietary protein levels of *O. niloticus* broodstocks to 30% CP in pre spawning period increased significantly weight gain, number of produced fry, and alleviated survival and the tolerance of unfavorable conditions of fry. Thus, quality of dietary protein and presence of fertilization is preferred more to be offered in pre spawning period than in spawning period. However, fish fed on high dietary protein levels in spawning period enhanced weight gain and number of produced fry compared to broodstocks fed low dietary protein levels. Consequently, feeding broodstocks during spawning period neither had any significant effect on tolerating unfavorable conditions, nor on survival and initial and final weight of produced fry. Therefore, the study highlights that feeding *O. niloticus* broodstocks on diets containing not less than 30% CP without fertilization or 25% CP with fertilization during pre-spawning period is preferred to produce viable fry that could tolerate stressful unfavorable conditions.

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