

MANIPULATING THE PRODUCTION OF MONO-SEX NILE TILAPIA, *OREOCHROMIS NILOTICUS*, FRY THROUGH WINTER SEASON

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SUMMARY

The influence of low temperature on survival rate and growth performance were compared on native fry of mono-sex *Oreochromis niloticus* to determine how winter season affects the final biomass and production of fry in Egypt. Six diets containing three protein (30, 35, and 40%) and two energy (370 and 470 kcal ME/100 g) levels were offered to fry (1.03g) to satiation, twice daily, for 6 months. At all protein levels, fry fed low-energy diets (370 kcal ME/100g) revealed poor growth and feed conversion efficiency, survival rates, compared to those fed high-energy (470 kcal ME/100g) diets. At dietary protein levels from 35 to 40% crude protein (CP), fish growth and survival rates were significantly improved by increasing dietary energy from 370 to 470 kcal (ME/100g diet). However, the best growth rate and feed utilization were obtained with 40% CP and 470 kcal (ME/100g diet). Body composition was significantly affected by both dietary protein and energy levels. As such, to overcome the low winter season temperatures the *O. niloticus* fry requires 40% CP and 470 kcal (ME/100g diet) for maximum growth and best survival rate when reared in earthen ponds in Egypt.

Keywords: *Mono-sex, Nile tilapia fry, cold tolerance, growth performance, temperature, winter season, production, Oreochromis niloticus.*

INTRODUCTION

Stress in farmed fish is of considerable significance to both health and productivity as it has been linked to reduction in growth, abnormal behavior, and immune suppression (Ashley 2007). Many exogenous factors have contributed in widespread of tilapia global growth due to its rapid compliant to wide range of environmental conditions (Mjoun *et al.*, 2010). Nile tilapia, *Oreochromis niloticus* (L.), is one of the most preferable cultured species due to its high growth rate, substantial tolerance to environmental stressors (Ballarin and Haller, 1982), spontaneity of reproduction, and high market demand (El-Sayed 2006a). Water temperature is one of several aspects that triggers the onset of gonadal maturation, spawning, and spawning frequency of tilapia reproduction (Philippart and Ruwet, 1982), and a major metabolic modifier (Mjoun *et al.*, 2010). Water temperature at which mortality begins should be highly considered in terms of cold tolerance in commercial tilapia production. However, optimal growing and spawning temperatures are typically between 22°C and 29°C (Caulton, 1982). Most tilapia species are unable to survive at temperatures below 10°C (Chervinski, 1982), and growth is poor below 20°C (Mires, 1995). Despite of water temperature, tilapia was found to tolerate an expandable range of environmental conditions including dissolved oxygen (Ross, 2000), salinity (El-Sayed 2006b), pH (Ross, 2000), and ammonia levels (El-Sherif *et al.*, 2008), compared to other cultured freshwater fishes. The low trophic level of tilapia and being an omnivorous type of fish tolerating high dietary fiber and carbohydrate concentration makes them inexpensive to feed (El-Sayed 2006a). Throughout its life stages, tilapia feed on a wide variety of dietary sources such as phytoplankton, periphyton, zooplanktons, larval fish, and detritus. A well balanced formulated feeds is required in tilapia culture to guarantee fast growth and high

yield. However, the nutritional requirements of tilapia varies among species and are mainly affected by age, size and stage (Al Hafedh, 1999). Fish uses dietary proteins for maintenance, growth, and reproduction. Jauncey (2000) suggested that to maximize protein utilization, dietary lipid concentration should be between 8 and 12% for tilapia up to 25 g, and 6 to 8% for larger fish. Therefore, the profile of dietary protein is important when formulating diets for tilapia. In Egypt, seasonal low temperatures creates a critical problem for tilapia culture and seed production. As water temperature drops between 9°C and 15°C causes reduction in breeding activities, and high mortality rates, especially in shallow ponds. Favorable temperature range for raising Nile tilapia are from 25°C to 35°C, which is between April and December (Dan and Little, 2000). However, natural spawning only begins in April, and seeds are not normally available until June-July, reducing the duration of the production season (Shamsuddin *et al.*, 2012). Besides, the lack of a reliable supply of fry has been an important constraint and a limiting factor in poorly established or limited financed infrastructure. Thus, self-sufficiency of fry production has been found to be necessary for the success of Nile tilapia culture between small-scale farmers. Overwintering of late-spawned tilapia fry may be a possible way to make fingerlings available early in the following grow-out season. Long winters and low temperature has been reported to have negative effects on tilapia production in China whereas the production have dropped by as much as 80% as estimated by observers (Globefish 2008). In many countries, overwintering of tilapia fry has been successfully conducted in heated facilities (Behrends *et al.*, 1990) or underground warm water (Cruz and Ridha, 1994) and in green houses (Jiazhao, 1991), but these resources are scarce and could be unavailable in Egypt. In general, production systems would probably not be economically sustainable if mortality rates because of low temperature exceed 10% (Patricio, 2004). The general consensus is that to decrease costs mortality should be reduced and growth of Nile tilapia should be prolonged, and thus enhancing the tolerance to low temperature is an important target (Kamel *et al.*, 2008). Therefore, the main objective of the present study is to enhance the survival and growth performance of *O. niloticus* fry through winter season using dietary protein and energy levels, to make fingerlings available early in the following grow-out season.

MATERIALS AND METHODS

The present study was conducted for six months, from October through March, on mono-sex *O. niloticus* fry with an average initial body weight of 1.03 g/fry. Fry were treated with 17 α -methyltestosterone after hatching for 21 day. Fry requirements for protein and energy was configured in the present study to attain the maximum production through the winter season. Fry were fed daily on formulated experimental diets (Table 1) to *ad-libitum* three times a day 7days/week. Three protein levels (30%, 35% and 40%, CP) were tested at two energy levels (370 and 470 kcal ME/100g diet) forming six practical diets embracing a (3 \times 2) factorial design experimental treatments (Table 1). Each treatment in the present study had two replicates. Water samples were taken monthly for water quality analysis, also fish samples were spared at the beginning and end of the experiment for carcass composition analysis.

Table (1). Ingredients composition and chemical analysis (dry matter) of experimental diets used for mono-sex *O. niloticus* in the present study.

Protein levels%	30%		35%		40%	
	370	470	370	470	370	470
Energy Kcal/100g						
Fish meal	150	150	200	200	250	250
Soybean	150	150	200	200	250	250
Shrimp meal	150	150	150	150	150	150
Wheat bran	400	400	320	300	250	200
Cod liver oil	10	60	10	60	10	60
Corn oil	10	60	10	60	10	60
Vitamin & mineral	30	30	30	30	30	30
Cellulose	100	0	80	0	50	0
Chemical analysis						
Crude protein	29.87	29.87	33.96	33.62	38.71	38.38
Lipids	6.92	16.32	5.47	15.89	5.64	15.56
NFE	34.74	35.73	31.15	31.54	26.36	26.12
Gross energy	370	470	370	470	370	470

Stocking density:

Twelve-indoor concrete tanks (water volume of 1m³/tank) with dimensions (0.75m × 1m × 2 m) were used in the first 45 days for stocking *O. niloticus* fry (1000 fry/tank). After the 45 days fry were transferred to earthen pond (200m³) with stocking rate of 500 fry/m³ bringing a total of 10,000 fry/pond.

Fish growth and feed utilization parameters:

Growth performance and feed utilization of Nile tilapia was determined, and was calculated as follows:

$$\text{Specific growth rate (SGR) (in percent per day)} = \frac{100(\text{Ln}W_2 - \text{Ln}W_1)}{T}$$

Where W1 and W2 are the initial and the final weights, respectively, and T is the number of days of the experiment;

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake (in grams)}}{\text{weight gain (in grams)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{weight gain (in grams)}}{\text{protein intake (in grams)}}$$

$$\text{Protein productive value (PPV) (in percent)} = 100 \left[\frac{\text{protein gain (in grams)}}{\text{protein intake (in grams)}} \right]$$

Proximate analysis:

Specimen of mono-sex *O. niloticus* fry from each treatment were spared frozen for final body composition analysis. Fish samples were randomly taken at the beginning and end of the experiments. Samples of fish diets were stored at (-18°C) until proximate analysis. All chemical analyses for fish and diets (Table 1) were determined according to standard methods of (AOAC, 2000).

Statistical analysis:

Results of growth parameters, feed utilization parameters, body carcasses composition, and survival rate (%) of 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. (2014) and EXCEL (windows 10, 2015). Graphs has been performed using SigmaPlot for windows (Systat Software, Inc., version 12.0, 2011).

RESULTS AND DISCUSSION

Growth of tilapia in Egypt is constrained by cold winter seasons, during which significant mortalities occurs in commercial fish farms leading up to great losses in the production and income. However, cold tolerance trait in some tilapia species could be of great economic importance in temperate countries, when sever cold winter seasons causes high mortalities (Hsieh *et al.*, 2007; Williams and Dasgupta, 2007). The most significant of the low temperature days in the study herein is during the night where temperatures drops critically sometimes below 8°C. Accordingly, the water temperatures of the experimental ponds in the present study was greatly influenced with the daily variation in weather temperature. Presumably, Likongwe *et al.* (1996) stated that temperature of shallow and small pieces of water bodies might follow air temperature. It has also been reported by Charo-Karisa *et al.* (2005) that tilapia reared under mid-summer conditions died between 13.6°C and 8.6°C, while those reared under autumn condition died between 11.7°C and 7.5°C. In the present study, the effect of low winter season temperature on fry of mono-sex *O. niloticus* on ADG and ADG% was observed from mid of December through end of January

even by supplying it with different protein and energy diets. In the study herein, the temperatures had reached as low as 8-9°C during some nights of the month of January. After six months of experimental trial the results demonstrated that the final body weight (FBW) (Table 2 and figure 1) and weight gain (WG) of fry significantly increased ($P<0.05$) with diets encompassed protein and energy levels of 35%CP; ME 470 Kcal/100g and 40% CP; ME 370 and 470 Kcal/100g (Table 2). The significantly least FBW and WG was observed on fry with diet contained 30% CP and ME 370 Kcal/100g. Dan and Little (2000) compared large (>1g) and small (<1g) tilapia fry to be over-wintered in deep hapas-in-ponds, and observed that survival rate of larger mono-sex tilapia fry was 54%, which was significantly higher than that of smaller fry (33.4%). This corresponds with the IBW of the experimented mono-sex fry of the present study (1.03g) to be overwintered for the next production season. However, fry that had the highest FBW and WG in the present study reached survival of (66%) compared to those lower in FBW and WG (41%). This coincides with the results reported by Charo-Karisa *et al.* (2005) who reported that fish body weight had a highly significant effect on cold tolerance. Moreover, Charo-Karisa *et al.* (2005) stated that the most appropriate way of enhancing cold tolerance of tilapia juveniles is by husbandry practices that increase pre-winter body weights. The average daily gain (ADG g/fish/day, and ADG %) fluctuated significantly ($P<0.05$) between all treatments from 0.09 to 0.15g/fry/day (Table 2). The highest was recorded for fry fed 35% CP; ME 470Kcal/100g diet, and the least ADG was for 30% CP; 370 Kcal ME/100g diet. In the same manner, Fayed (2014) implied that the growth rate of mono-sex *O. niloticus* represented by FBW, WG, and ADG was increased significantly ($P<0.01$) with the increase of dietary protein levels. However, Fayed *et al.* (2015) found that feeding *O. niloticus* broodstocks on diets containing not less than 30% CP during pre-spawning period enhanced weight gain and number of produced fry viability on tolerating low winter season temperature compared to broodstocks fed low dietary protein levels. Siddik *et al.* (2014) stated that culture of mono-sex tilapia during winter is more profitable due to its higher growth rate. Also, Siddik *et al.* (2014) implied that six months over-wintered culture of mixed sex and mono-sex tilapia showed that the growth of mono-sex tilapia was significantly 32% higher than of mixed-sex tilapia. Whereas, Lovshin *et al.* (1990) demonstrated that all male fish grew 121% and 69% larger than those in populations containing 2.5% and 5.0% females, respectively.

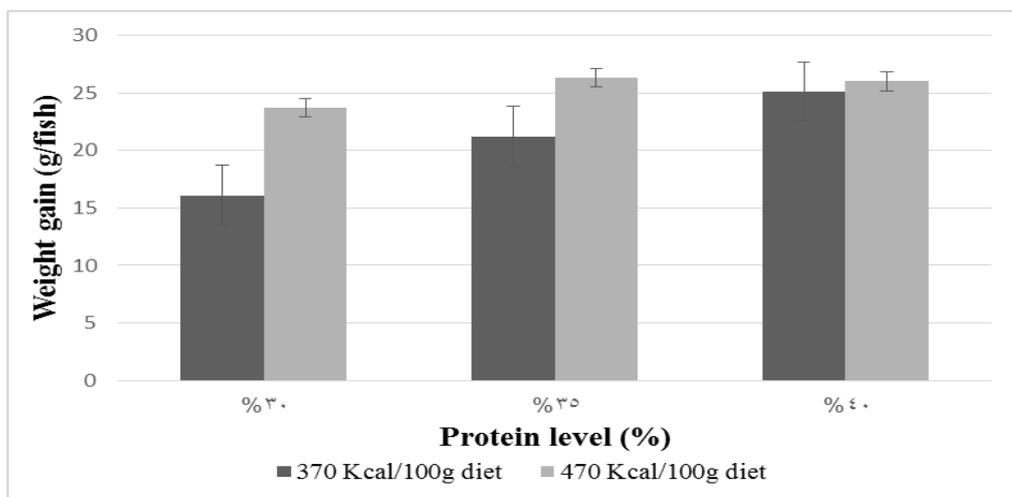


Fig. (1). Mean body weight gain of mono-sex *O. niloticus* fed three dietary protein and two energy levels.

The higher growth of mono-sex tilapia may have been due to the influence of methyl testosterone hormone used for sex-reversal, and has been shown to be a growth promoter in *O. mossambicus* (Kuwaye *et al.*, 1993). Also, Hernandez *et al.* (2014) indicated that the faster growth of mono-sex tilapia might be due to the hormone used for the sex conversion and lack of energy expenditure in gonad maturation. Meanwhile, the specific growth rate (SGR %) (Table 2) correspondingly revealed the same significant ($P<0.05$) fluctuated pattern as the FBW, WG, and ADG, as the highest SGR was observed for fry fed 35% CP; ME 470Kcal/100g diet, and the least was for 30% CP; 370Kcal ME/100g diet. Despite of fry body weight in the present study, the SGR (%) was significantly higher for fry fed high levels of protein

and energy than those fed low protein and energy levels. This corresponds with the findings of Hernandez *et al.* (2014) who found that the SGR (%) value of mono-sex tilapia was significantly higher than that of mixed-sex tilapia. Although, the dietary protein levels in the present study had two energy levels (low and high), but the significant highest growth performance observed was with the high dietary protein and energy levels. On the contrary, Soltan *et al.* (2002) studied the effect of dietary energy level on average body weight, body length and condition factor, and their results showed that the final body weight, body length and condition factor significantly decreased with increasing dietary energy level from 300 to 330 and 360 kcal ME/100g. In relation to high protein and energy levels, the fry survival (%) (Table 2 and figure 2) was triggered significantly ($P < 0.05$) and thus the 40% CP; ME 470Kcal/100g diet was reported to show the highest (66%) survival which was 4.5% to 25% higher than all other treatments. Consequently, the final biomass (FBM/pond and FBM/feddan) of fry maintained significantly ($P < 0.05$) the highest yield of fry (178kg/pond; 3560kg/feddan) for diet with 40% CP; ME 470Kcal/100g diet, and the lowest was for 30% CP; 370 Kcal ME/100g diet (Table 2 and figure 3). However, in consistence to growth parameters the whole body protein and lipid contents tend to increase (not significantly) between treatments as the dietary protein and energy levels increase (Table 3).

However, feed conversion rate (FCR) was significantly ($P < 0.05$) enhanced when fry were fed (40% CP; 470Kcal ME/100g diet) high protein and energy levels in winter. On the contrary, low protein and energy level (30% CP; 370 Kcal ME/100g diet) feed in low temperature season for fry of *O. niloticus* induces fish to consume more feed, therefore, high FCR (Table 3) will be maintained to compensate their growth performance in such conditions. In agreement with Ahmed *et al.* (2004) that the significant variation in growth with dietary protein level could be due to the fluctuation of fish size, age, protein quality, stocking density, and hygiene and environmental condition which conceals the optimization of the growth parameters. Consequently, Ahmed *et al.* (2004) stated that the best growth and FCR for *O. niloticus* fry exhibited with 45% crude protein diet, while fingerlings and adult grow the same with 35

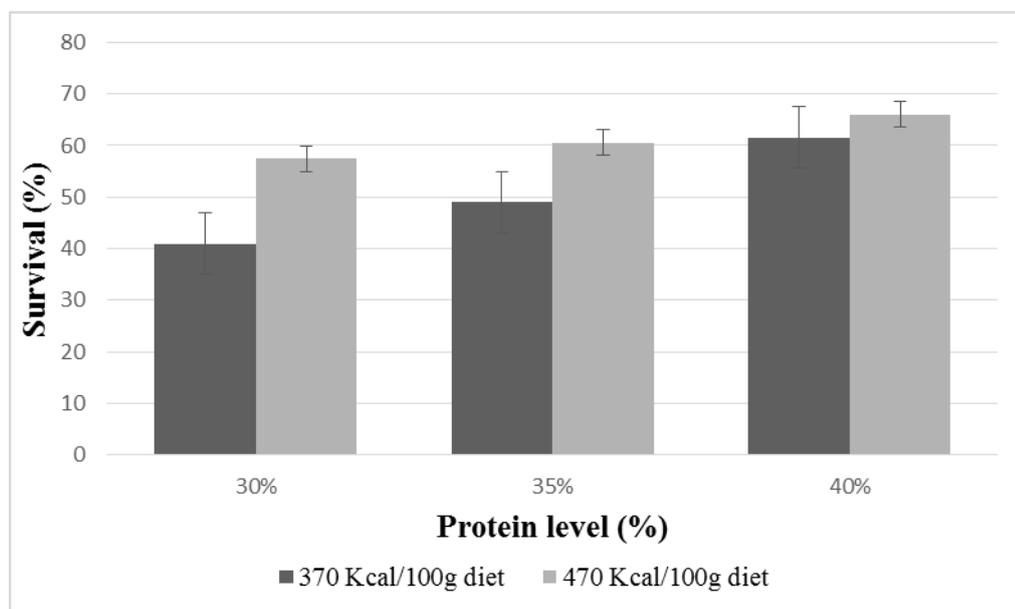


Fig. (2). Mean Survival of mono-sex *O. niloticus* fed three dietary protein and two energy levels.

Tables 2-3

and 45% protein diets. In spite of the low temperature, the assessment of the FCR in the study herein denoted that it was enhanced with the increase of dietary protein and energy levels. However, Bahnasawy (2009) indicated similar findings with respect of the FCR where it decreased significantly with increasing dietary protein levels and the best FCR was obtained from 35% protein diet, while the poorest FCR was obtained by the lowest protein diet. With respect to FCR, the protein productive value (PPV) increased significantly with the increase of dietary protein and energy levels, and being the highest in fry fed with 40% CP; 470Kcal ME/100g diet (Table 3). Although, the protein efficiency ratio (PER) was shown to increase with increasing dietary energy levels, but seems to decrease with the increase of dietary protein level (Table 3). With regard to the PER incurred in the present study that it significantly decreased with the increase of dietary protein and energy levels. The decrease of PER with increasing dietary protein level have also been reported by different authors for different tilapia species (Jauncey, 1982; Wee and Tuan, 1988; Shiau and Huang, 1989; Kheir, 1997; Ahmad *et al.*, 2004; Bahnasawy, 2009). In support to the findings of the present study, Shimeno *et al.* (1981) reported that increasing dietary carbohydrate and fat caused a reduction in the activities of amino acid-degrading enzymes in the hepato-pancreas and resulted in reduction of nitrogen excretion rate and a high protein efficiency ratio. Nevertheless, the PPV values in the present study increased with increasing dietary protein and energy levels. Since the interaction between the dietary protein and energy levels positively enhanced the PPV of *O. niloticus* fry. Therefore, the protein to energy ration was considered to be optimum for fry and in turns had induced the growth of fry and triggered the fish to tolerate the low winter temperature. In support to the present study, Kaushik (1980) indicated that the effect of water temperature on apparent digestibility is the result of an effect of feed intake rather than an effect of water temperature itself. However, Cho and Kaushik (1990) presented that fish oil and plant oils (rapeseed, soybean, and linseed) were well digested (80–95%) over a wide range of water temperatures (5 to 15°C). In the same manner, the constituent of the fish and plant oils in present study diets were 1:6 for the low energy and high energy diets, respectively. This explains the efficient utilization of the dietary protein with high dietary energy. In the present study a significant increase in muscle protein and decrease in lipid content of mono-sex fry of *O. niloticus* with increasing dietary protein level was detected. This relationship was also noticed by Dabrowski (1977) on grass carp, Jauncey (1982) on *Sarotherodon mossambicus*, Fah and Leng (1986) on guppy, *Poecilia reticulata*, Shiau and Huang (1989) on hybrid tilapia (*O. niloticus* x *O. aureus*), Wee and Tuan (1988), Kheir (1997), Al-Hafedh (1999) on Nile tilapia (*O. aureus*), and Bahnasawy (2009) on (*O. niloticus*). The increase in muscle protein and decrease in lipid content with increasing dietary protein levels could be attributed to the elevated carbohydrate level and low protein content in the diet of low protein level. The excess carbohydrate in the diet might trigger body fat storage (Fah and Leng, 1986). In accordance with the present study results, the high protein and energy levels appeared clearly to maintain the *O. niloticus* fry requirements to induce a better growth performance and survival rate throughout the winter in Egypt.

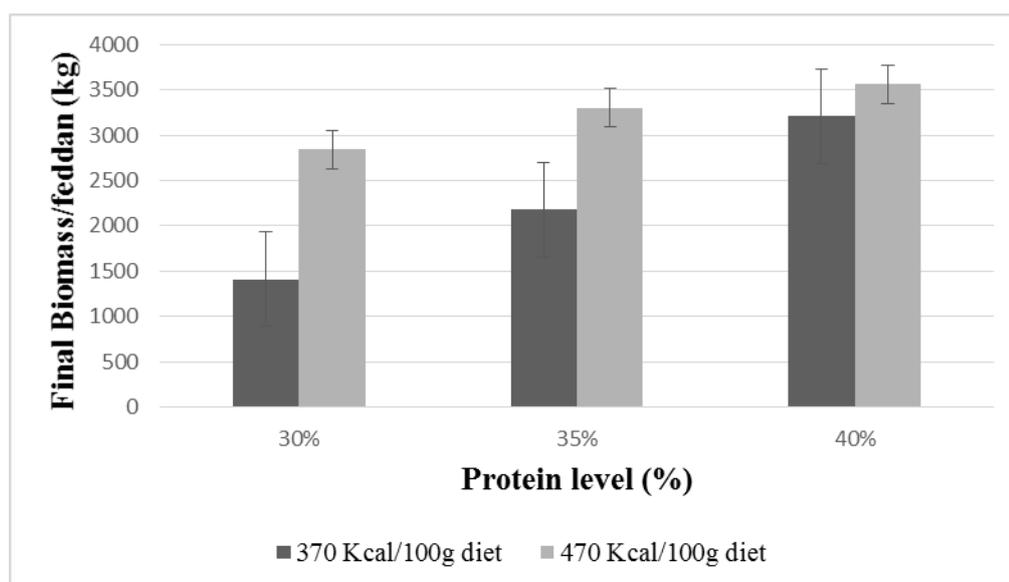


Fig. (3). Mean Final Biomass of mono-sex *O. niloticus* fed three dietary protein and two energy levels.

CONCLUSION

This study highlights the importance of protein and energy balance in diets offered to fry of *O. niloticus* in winter in order to tolerate the fluctuation of low temperatures and increase survival rate and growth performance throughout winter in Egypt.

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تحسين إنتاجية زريعة البلطي النيلي وحيد الجنس أثناء انخفاض درجات الحرارة في موسم الشتاء

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أجريت هذه التجربة بهدف الحصول على إصبعيات بلطي نيلي تكفي لبداية موسم إنتاج جديد تتمتع بحيويته ونمو جيد بعد تخزينها خلال فترة الشتاء، المنخفض والمتذبذب في درجات الحرارة في جمهورية مصر العربية، الأمر الذي يؤثر سلبا على حيويته ونمو أسماك البلطي خاصة الصغيره في الحجم. أجريت التجربة في إحدى مزارع القطاع الخاص بمدينة كفر الدوار محافظة البحيرة في الفترة من أكتوبر وحتى مارس 2011. استخدمت زريعة بلطي نيلي بعد الفقس مباشرة وتم وضعها في أحواض أسمنتيه سعة 1م³ بكثافة 1000 وحدة زريعة للمتر. وتم معاملتها بهورمون 17 الفا ميثيل تستستيرون لتحويلها إلى ذكور لمدة 21 يوم. واستمرت عملية تحضين الزريعة في الأحواض الأسمنتيه لمدة 45 يوم ثم تم نقلها إلى أحواض التششية الترابيه سعه 200م²/حوض بكثافته 1000 وحدة زريعة للحوض. تغذت الأسماك حتى الشبع مرتين يوميا، جميع أيام الاسبوع ما عدا الأيام الممطره وشديده الرياح. متوسط وزن الزريعة عند نقلها إلى الأحواض الترابيه 1.03 جم وتمت تغذيتها على مستويين للطاقة (370-470 كيلو كالورى طاقة ميتابوليزميه/100جم علف) وثلاث مستويات من البروتين (30-35-40%) وكانت مصادر البروتين المستخدمه من مسحوق السمك الجيد (نسبه البروتين 72%) ومسحوق الصويا وكانت مصادر الدهون من زيت كبد الحوت وزيت الذره. وكانت النتائج كالتالى. تحسن معدل أداء الأسماك وكذلك حيوتها بمعنويه كبيره مع ارتفاع كلا من نسبه البروتين والطاقة و كما تحسن معدل تحويل الغذاء (FCR) وأيضا محتوى الجسم من البروتين كما إنخفض محتوى الجسم من الدهون بزيادة بروتين العلف كما إرتفعت بناء على ذلك الناتج النهائى للفدان من الإصبعيات الجاهزه للتربيه خلال موسم التربيه الجديد. وعلى ذلك توصى الدراسه بضروره الإهتمام بتغذية زريعة البلطي النيلي خلال فترة التششية على علف لا تقل فيه نسبه الطاقه عن 470 كيلو كالورى/100 جم علف ونسبه بروتين 40% على ان تكون العليقه متوازنه فى مستوى البروتين والطاقة كما يجب الإهتمام بمصادر البروتين والطاقة للعليقه المقدمه خلال فترة التششية (حيث أن العلائق المستخدمه فى هذه التجربه كانت مكونه من مصادر عاليه الجوده من البروتين والطاقة) لتكمن من تحمل التذبذب الحادث فى درجة الحرارة خلال موسم الشتاء.

Table (2). Means (\pm Standard Error Mean) of final body weight (FBW, g/fish), weight gain (WG, g/fish), average daily gain (ADG, g/fish/day), average daily gain percentage (%), specific growth rate (SGR, %), final biomass (FBM, kg/pond and /feddan), and survival rate (SUR, %) for fry of mono-sex *O. niloticus* in the present study.

Variable	370 Kcal/100g diet			470 Kcal/100g diet			P-value		
	30%	35%	40%	30%	35%	40%	F1	F2	F1×F2 ¹
FBW	17.1 \pm 1.4 ^c	22.2 \pm 0.3 ^b	26.1 \pm 0.2 ^a	24.7 \pm 0.2 ^{ab}	27.3 \pm 0.4 ^a	27 \pm 0.5 ^a	<0.001	<0.001	0.006
WG	16.1 \pm 1.4 ^c	21.2 \pm 0.3 ^b	25.1 \pm 0.2 ^a	23.7 \pm 0.2 ^{ab}	26.3 \pm 0.4 ^a	26 \pm 0.5 ^a	<0.001	<0.001	0.006
ADG	0.09 \pm 0.01 ^c	0.12 \pm 0.002 ^b	0.14 \pm 0.001 ^a	0.13 \pm 0.001 ^{ab}	0.15 \pm 0.002 ^a	0.14 \pm 0.003 ^a	<0.001	<0.001	0.006
ADG%	8.67 \pm 0.76 ^c	11.4 \pm 0.16 ^b	13.5 \pm 0.11 ^a	12.8 \pm 0.11 ^{ab}	14.2 \pm 0.22 ^a	14 \pm 0.27 ^a	<0.001	<0.001	0.006
SGR%	1.56 \pm 0.05 ^c	1.71 \pm 0.01 ^b	1.8 \pm 0.004 ^{ab}	1.77 \pm 0.005 ^{ab}	1.82 \pm 0.01 ^a	1.81 \pm 0.01 ^{ab}	<0.001	0.001	0.009
FBM/pond	70.4 \pm 9.16 ^d	109 \pm 0.75 ^c	161 \pm 5.14 ^{ab}	142 \pm 7.33 ^b	165 \pm 1.67 ^{ab}	178 \pm 6 ^a	<0.001	<0.001	0.009
FBM/feddan	1410 \pm 183 ^d	2180 \pm 15 ^c	3210 \pm 103 ^{ab}	2840 \pm 146 ^b	3300 \pm 33.5 ^{ab}	3560 \pm 120 ^a	<0.001	<0.001	0.009
SUR %	41 \pm 2 ^c	49 \pm 1 ^{bc}	61.5 \pm 1.5 ^a	57.5 \pm 2.5 ^{ab}	60.5 \pm 1.5 ^a	66 \pm 1 ^a	<0.001	<0.001	0.031

Values are means \pm 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.

¹F1 \times F2 = Factor A \times Factor B interaction effect.

Table (3). Means (\pm Standard Error Mean) of body protein (g/fish), body lipid (g/fish), feed conversion ratio (FCR), protein productive value (PPV), and protein efficiency ratio (PER) for fry of mono-sex *O. niloticus* in the present study.

Variable	370 Kcal			470 Kcal			P-value		
	30%	35%	40%	30%	35%	40%	F1	F2	F1×F2 ¹
protein	15.6 \pm 0.5	17.6 \pm 0.5	17.8 \pm 0.5	15.7 \pm 0.5	17.8 \pm 0.5	18.4 \pm 0.5	0.529	0.006	0.87
lipid	7.71 \pm 0.04	6.45 \pm 0.5	6.54 \pm 0.5	7.95 \pm 0.04	7.91 \pm 0.04	6.49 \pm 0.04	0.058	0.011	0.084
FCR	1.77 \pm 0.03 ^a	1.47 \pm 0.03 ^b	1.33 \pm 0.002 ^{cd}	1.42 \pm 0.015 ^{bc}	1.25 \pm 0.02 ^d	1.23 \pm 0.02 ^d	<0.001	<0.001	0.003
PPV	8.5 \pm 0.5 ^c	19.9 \pm 0.5 ^b	21.6 \pm 0.5 ^b	7.38 \pm 0.5 ^c	24.7 \pm 0.5 ^a	26.7 \pm 0.5 ^a	<0.001	<0.001	0.001
PER	2.2 \pm 0.06 ^{ac}	2.15 \pm 0.06 ^{bc}	1.97 \pm 0.06 ^c	2.49 \pm 0.06 ^a	2.39 \pm 0.06 ^{ab}	2.1 \pm 0.06 ^{bc}	0.003	0.003	0.373

Values are means \pm 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.

¹F1 \times F2 = Factor A \times Factor B interaction effect.