EFFECT OF DIETARY PROTEIN LEVELS AND SOURCES ON REPRODUCTIVE PERFORMANCE AND SEED QUALITY OF NILE *Oreochromis niloticus* (L.) BROODSTOCK.

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Abstract

The present work was undertaken to investigate the effects of using different dietary protein levels and sources on reproductive performance of Nile tilapia *Oreochromis niloticus* broodstock. Two dietary protein levels (25 and 40% CP) were tested using three main dietary protein sources [fish meal (FM) 65% CP, soybean meal (SBM), 44% CP and a mixture of FM and SBM proteins]. Six experimental treatments were assigned according to 2×3 factorial design with two replicates/ treatment giving total number of 12 spawning hapas. The highest (P≤0.05) seed/ female (672.08± 0.417), seed/g female (23.62± 0.09), seed/ female/ day (6.93± 0.14) and seed/ m2/ day (41.59± 0.83) were found in T6 (40% CP level from animal protein source) while T1 recorded the lowest corresponding values for the above-mentioned parameters, they were 462.00± 3.67, 17.19± 0.43, 4.74± 0.16 and 28.45± 0.95, respectively. The fecundity of females increased significantly (P≤0.05) with increasing the level of protein regardless of the source of protein. The fecundity of females increased significantly (P≤0.05) with increasing the level of animal protein inclusion in the experimental diets. The highest fry AIW, AFW, AWG and ADG were found in T6 while, the lowest values were recorded for T1. Fry survival rates were also significantly (P≤0.05) affected by the level and the source of dietary protein of broodstock diets. The results revealed that, in order to have efficient reproductive performance, high seed out-put and high fry growth and survival rate, it is recommended to use diets containing high level (up to 40%CP) of good quality protein (animal source).

INTRODUCTION

Mass production of quality tilapia fry remains seriously constrained by low fecundity and the asynchronous nature of tilapia spawning cycles. Seed production is an essential component in the successful production of any organism. Seed should be available in a reliable quality and quantity to enable the producer to begin production in anticipation of resource availability, seasonal changes and market demand (Al-Hafedh *et al.*, 1999 and Phelps and Bart, 2001). There are many possible reasons for the low production of tilapia fry. These include too low density of broodstock, inappropriate sex ratios, inadequate spawning techniques, broodstock nutrition and high fry mortality (Salama, 1996). Increased understanding of the specific factors involved in the regulation of fecundity and spawning frequency in multiple-spawning tilapia would be of immense benefit to practical culture and the continued success of tilapia culture. Due to differences in biological processes, the nutrient requirements of broodstock may be different from growing juvenile animals. However, a full and comprehensive understanding of the reproduction mechanisms such as gonadal maturation, fertilization success and larval quality is far from complete as these coordinated processes are very complex. Broodstock nutrition studies offer to provide knowledge by determining if reproductive performance of a particular fish species can be improved by maternal dietary intake. However, broodstock nutrition is still poorly understood due to difficulties in conducting studies involving proper feeding and reproduction of broodstock. Izquierdo *et al.* (2001) outlined protein, lipid, fatty acids, vitamins E and C and carotenoids as major nutrients influencing various reproduction processes such as fecundity, fertilization, hatching and larval development (Chong *et al.*, 2004). Problems of seed supply together with the quality of the eggs and fry produced constitute one of the most constraints on current and future aquaculture developments. Improvements of our understanding about the appropriate culture conditions and management procedure for the brood-fish are essential if we are programming reproductive development to produce reliably the numbers of eggs and fry required by grow-out farm. Therefore, this study was carried out in an attempt to investigate the effects of dietary protein levels and sources on reproductive performance and seed quality of Nile tilapia broodstock.

MATERIALS AND METHODS

The present work was carried out during spawning season of the year 2004 to investigate the effects of using different dietary protein levels and sources on broodstock growth performance, feed efficiency, protein utilization and reproductive performance and seed output of Nile tilapia broodstock. The present work was conducted under the conditions of hapa - in pond - based hatchery system. All spawning hapas, each measuring 2×1×1 m^3 (length× width× highest) was suspended in an earthen pond of 10.000 m^2. The water depth in each hapa was maintained at a bout of 0.5 m to attain a total water volume of about 1 m^3 per hapa.

Experimental design

Two dietary protein levels (25 and 40% CP) were tested using three main dietary protein sources [fishmeal (FM) protein, 65% CP, soybean meal (SBM), 44% CP and a mixture of FM and SBM proteins]. The six protein level/ source combinations (treatments) were assigned according to 2×3 factorial design with two replicates/ treatment giving total number of 12 spawning hapas. The experimental treatments were subjected to be studied as follows: -

-Protein levels and sources in each of the six treatments.

Symbol	Protein level (% CP)	Protein source
T1	25	Soybean meal (44% CP).
T2	25	Fish meal (65% CP) + Soybean meal (44% CP).
T3	25	Fish meal (65% CP).
T4	40	Soybean meal (44% CP).
T5	40	Fish meal (65% CP) + Soybean meal (44% CP).
T6	40	Fish meal (65% CP).

Experimental fish

Broodstock trial (110 days)

An over-wintered Nile tilapia, *Oreochromis niloticus* broodstock were obtained from commercial fish farm located in Kafr El-Sheikh Governorate. Broodstock were netted from earthen ponds, manually selected, sexed and transferred to conditioning hapas, where they were held and kept separately for 25 days for adaptation to the new environment until starting the experiment (8th of June, 2004). 24 and 72 Nile tilapia males and females broodstock, respectively were stocked at a sex ratio of three females to one male with a stocking density of eight (six females: two males) fish/ m^2 in each experimental hapa ($1m^3$). The mature broodstock selected for experiment ranged in weights from 136.500± 2.00 to 140.500± 2.500 g for females and from 144.0± 2.00 to 152.0± 2.00 g for males. Random samples of females and males tilapia broodstock were taken, individually weighed, immediately killed and frozen at 18°C until proximate analysis at the end of the experiment. Body weight was recorded at the beginning, subsequent spawnings and at the end of experiment, which lasted for 110 days.

Fry nursing trial (70 days)

One hundred free-swimming fry were selected from the first seed collection from each broodstock spawning hapa and stocked in twelve fry rearing happas (2X1X1 m) at a density of 100 fry/ m^2 with two replicates/ treatment in order to asses the effect of different dietary protein levels and sources (the six experimental treatments) on the fry growth performance feeding efficiency and fry **survival rates.**

Experimental diets

Three different protein sources and two protein levels (25 and 40%CP) were used to formulate six experimental diets for feeding broodstock (Table 1). The dietary protein sources were:-

-Plant protein [soybean meal (SM), 44% CP].

-A mixture from both animal (FM, 65% CP) and plant (SM, 44% CP) proteins.

-Animal protein [fish meal (FM), 65% CP].

Protein energy ratio ranged between 69.40 to 70.43 mg protein/ Kcal ME for diets containing 25%CP, while diets containing 40% CP, protein energy ratio ranged between 103–106 mg protein/ Kcal ME. Broodstock were fed the experimental diets at a feeding rate of 3 % of fresh biomass in each hapa (six days/ week). Broodstock were fed two times daily at 9.00 am and 4 pm with feed amounts adjusted at approximately 15–20 days intervals in response to weight gain.The feed was introduced to fry at feeding frequency of 10 times daily in equal portions. The rate of feeding was 20% of total fry biomass at the first 20 days and reduced to 10 % in the second growth interval. Fry were fed diet no. 7 (containing 35%CP and 3834 K Cal/ Kg ME) with a protein to energy ratio of 103.02 (Table 1). The nursing trial was lasted for 70 days.

Table1.Composition and proximate analysis of the experimental Nile tilapia *Oreochromis niloticus* broodstock and fry diets.

Experimental Diets	1	2	3	4	5	6	Diet 7
Ingredients:- Fish meal (65 % CP)	----	4.0	32.0	----	7.0	60.0	35.0
Soybean meal (44 % CP)	50.0	41.0	----	91.0	79.0	----	35.0
Corn grain	43.0	23.0	61.0	2.0	7.0	33.0	23.0
Wheat bran	----	25	----	----	----	----	----
Corn Oil	3.0	3.0	3.0	3.0	3.0	3.0	5.0
Molasses	2.0	2.0	2.0	2.0	2.0	2.0	----
Minerals[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamins[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Proximate analysis							
Dry matter (%)	91.5	92.0	91.0	91.4	90.0	92.0	92.00
Crude protein (%)	25.4	25.4	25.7	40.2	39.9	40.0	39.50
Ether extract (%)	5.84	5.21	6.00	5.94	5.51	7.25	6.80
Crude fiber (%)	3.28	3.95	2.97	4.19	3.09	3.49	2.65
Ash (%)	7.35	6.81	7.62	5.95	5.51	7.85	8.00
Nitrogen free extract (%)	58.1	59.6	58.6	43.7	43.2	41.4	43.05
Metabolizable energy (K Cal / Kg)[3]	3666	3614	3688	3788	3752	3881	3834
p/ E ratio (mg protein/K Cal)	69.4	70.4	69.6	106.3	106.3	103.1	103.02

1- *Minerals (g/Kg): -* Ca CO_3 :314 g, KH_3PO_4 :469.2, Mg SO_4 .7 H_2O :147.4, Na Cl, :49.8, Trace elements 19.6 g consists of Fe^{+2} – gluconate, Mn $SO_4.H_2O$:10900 mg, Zn SO_4 .7 H_2O :3120, Cu SO_4 .5 H_2O, 620 mg, KI:160, Cl CO_3 .6 H_2O, Ammonium molybdate : 60, Na_2SeO_3 (Sodium selenate):20 mg.
2- *Vitamins: -* Vit. A: 1,000,000 IU, Vit. D3: 85.714 IU, Vit. E: 100,000 IU, Vit. B1: 5,000 mg, Vit. B2: 10,000 mg, Vit. B6: 10,000 mg, Vit. B12: 10,000 mg, Vit.C: 20,000 mg, Biotin: 500 mg, folic acid: 2,000 mg and pantothenic acid 20.000 mg.Vit. K_3 2,000
3-*Metabolizable energy (ME):-)* calculated using values of 4.50, 8.1 and 3.49 K Cal for protein, fat and carbohydrate, respectively according to Pantha (1982).

Amino acid determination

Amino acids content of each experimental broodstock and fry diets were determined using a high performance amino acid analyzer as described by Moor *et al.* (1958). Amino acids content of experimental diets are shown in table (2).

Table 2. Amino acid contents of the Nile tilapia, *Oreocromis niloticus* (L.) broodstock diets.

Amino acid	Diet 1 (25% CP)	Diet 2 (25% CP)	Diet 3 (25% CP)	Diet 4 (40% CP)	Diet 5 (40% CP)	Diet 6 (40% CP)	Diet 7 Fry diet	Requirements*
Arginine	1.94	1.89	1..99	1.87	2.2	2.05	2.00	1.18
Histidine	0.75	0.72	0.76	0.90	0.92	0.74	0.86	0.48
Isoleucine	1.49	1.54	2.15	2.65	2.18	2.75	2.55	0.87
Leucine	2.15	2.00	1.46	1.60	1.55	1.50	1.62	0.95
Lysine	2.00	1.90	2.16	2.85	2.50	2.90	2.56	1.43
Methionin	0.87	0.85	0.88	1.01	1.00	0.95	1.08	0.75
Phenylalanine	1.30	1.45	1.65	1.75	1.60	1.85	1.72	1.05
Threonine	1.15	1.25	1.43	1.89	1.75	1.65	1.47	1.05
Tryptophan	0.45	0.49	0.50	0.40	0.33	0.45	0.42	0.28
Valine	1.25	1.44	1.80	1.32	1.56	1.85	1.75	0.78

*Source, Santiago and Lovell (1988).

Analytical methods

At the end of the experiment, all broodstock and fry in each hapa were netted, weighed and finally frozen for final body composition analysis. Representative samples of the experimental fish were randomly taken at the beginning and at the end of the experiment. Fish samples were killed and kept frozen (-18 °C) until performing the body chemical analysis. Samples of the experimental fish diets were taken, ground and stored in a deep freezer at -18°C until proximate analysis. All of chemical analyses of fish and fish diets were determined according to A.O.A.C. (1990). Initial analyses were carried out on a pooled sample of fish, which were weighed and frozen prior to the experiment.

Growth performance parameters

The growth performance parameters are calculated as follows:-

Average Weight Gain (AWG) = Average final weight (g) – Average initial weight (g)

Average Daily Gain (ADG) = [AFW (g) – AIW (g)]/ time (days).

Specific Growth Rate (SGR%/ day) = 100 [Ln Wt1 – Ln Wt 0/ t]

Where: Wt 0: initial weight (g), Wt 1: final weight (g) and T: time of days.

Feed and protein utilization parameters

Feed and protein utilization parameters are calculated as follows

Feed intake = Total feed consumption (g).

Feed Conversion Ratio (FCR) = Total feed consumption/ weight gain.

Statistical analysis

Statistical analysis of the experiment was done using SAS Version 9 (SAS Institute, 2002) statistical package. Data were statistically analysed in a factorial design procedure. Mean of treatments were compared by Duncan (1955) multiple range test. Duncan test ($p<0.05$) was used to compare means and ($F \leq 0.05$) was considered for the variance analyses.

Spawning performance and seed output

Variables were estimated (according to Mair *et al.*, 2004) from the data included:-

-Absolute fecundity: - the number of seeds per spawning per female.

-Relative fecundity: - the number of seeds per unit weight of female.

-Absolute spawning frequency: - number of spawnings of the experimental period.

RESULTS AND DISCUSSIONS

Seed production

As shown in table (3), the highest seed/ female (672.08± 0.417), seed/ g female (23.62± 0.09), seed/ female/ day (6.93± 0.14) and seed/ m^2/ day (41.59± 0.83) were found in T6 (40% CP level from animal protein source) while, T1 recorded the lowest corresponding values for the above-mentioned parameters. The effects of different protein levels regardless of protein sources on fecundity of female tilapia broodstock presented in table (4). The dietary protein level significantly ($P \leq 0.05$) affected the seed/ female, seed/g female, seed/ female/ day and seed/ m2/ day. The fecundity of females increased significantly ($P \leq 0.05$) with increasing the level of protein from 25 to 40% CP. Duray *et al.* (1994) found that, elevation of dietary lipid levels from 12 to 18% in broodstock diets for rabbitfish resulted in an increase in fecundity and hatching. Moreover, Izquierdo *et al.* (2001) found that an improvement in broodstock nutrition and feeding has been shown to greatly improve not only seed production but also egg and sperm quality. Gonadal development and fecundity are affected by certain essential dietary nutrients especially in continuous spawners with short vitellogenic periods. They added that, fecundity is the total number of seed produced by each fish expressed either in terms of seeds/ spawn or seeds/ body weight. Reduced fecundity, reported in several marine fish species, could be either by the influence of a nutrient imbalance on the brain pituitary gonad system or by the restriction in the availability of biochemical components for egg formation.

The reproductive performance of Nile tilapia broodstock as affected by different dietary protein levels has been widely investigated from different parts of the globe. The supply of high quality feed for the breeders is believed to be essential for the successive spawning of tilapia. There are many reports of the role of dietary protein levels on gonad maturation, spawning and fecundity of tilapia (Santiago *et al.*, 1983, Santiago *et al.*, 1985, Chang *et al.*, 1988, Cisse, 1988, Wee and Tuan, 1988, El-Ebiary, 1994, Gunasekera *et al.*, 1996a, Gunasekera *et al.*, 1996 b and El-Sayed *et al.*, 2003 and Mohamed, 2006).

Table 3. Effect of dietary protein levels and protein sources on seed production of Nile tilapia, *Oreocromis niloticus* (L.) broodstock (Mean ± SE).

Treatments	AIW	Female AFW	Mean weight	Total seed	Seed/ female	S/ g female	S/ F/ day	S/ m²/ day
T1	136.50 a± 3.50	186.0 b± 2.50	161.25 a± 2.75	2772 d± 22.0	462 d± 3.67	20.17 e± 0.43	4.74 d± 0.1٦	28.45 d± 0.9٦
T2	136.50. a± 0.50	190.5 ab± 1.50	163.50 a± 1.00	323٢ c± 101.50	539 c± 16.9٢	19.76 d± 0.50	5.4٧ c± 0.20	32.81 c± ٢٠١
T3	137.0a ± 3.00	190.٠ ab± 6.00	163.50 a± 4.50	3499.0 bc± 85.5	538 bc± 14.25	21.4٠ c± 0.0٧	6.04 bc± 0.05	36.26 bc± 0.32
T4	140.50 a± 50.2	187.5 ab± 3.50	164.0 a± 3.00	3630 b± 45.0	605 b± 7.50	22.1٥ bc± 0.68	6.27 b± 0.1٨	37.63 b± 1.05
T5	137.0٠ a± 5.00	188.5 ab± 5.50	162.75 a± 5.25	3798 ab± 173.0	633 ab± 28.83	23.3٢ ab± 0.31	6.33 ab± 0.2٩	37.98 ab± 1.73
T6	140.50 a± 0.50	201.0 a± 1.00	170.75 a± 0.75	4032 a± 2.5	672 a± 0.4٢	23.6٢ a± 0.0٩	6.93 a± 0.1٤	41.5٩ a± 0.83

Means in the same column having different letters are significantly ($P \leq 0.05$) different.

T1:- Broodstock fed 25% CP diet from plant protein source.

T2:- Broodstock fed 25% CP die from a mix. of plant and animal protein

T3:- Broodstock fed 25% diet CP from animal protein source.

T4:- Broodstock fed 40% CP from plant protein source.

T5:- Broodstock fed 40% CP from a mix. of plant and animal protein

T6:- Broodstock fed 40% CP diet from animal protein source.

Table 4. Effect of dietary protein levels regardless of protein sources on seed production of Nile tilapia, *Oreocromis niloticus* (L.) broodstock (Mean ± SE).

Treatme	Female	Female	Mean	Total	S/ F	S/ g	S/ F/	S/ m²/
T1	136.66 ±a	188.83 ±b	162.75 ±b	3167.7 ±b	527.94 ±b	19.45±b 0.79	5.42 ±b	32.51 ±b
T2	139.33 ±a	192.33 a	165.83 ±a	a 3820.0 ±	636.69 ±a	±a 23.03 0.34	a 6.51 ±	39.07 ±a

Means in the same column having different letters are significantly (P≤0.05) different.

T1:- Broodstock fed 25% CP diet. T2:- Broodstock fed 40% CP diet.

Table 5. Effect of dietary protein sources regardless of protein levels on seed production of Nile tilapia, *Oreocromis niloticus* (L.) broodstock (Mean ± SE).

Treat.	Female AIW	Female AFW	Mean weight	Total seed number	S/ F	S/ g female	S/ F/ day	S/ m²/ day
T1	138.50 a± 2.10	186.75 b± 1.70	162.63 b± 1.84	533.50 c± 41.42	3201 c± 248.53	19.67 b± 1.47	5.51 b± 0.45	33.04 b± 2.71
T2	136.75 a± 2.06	189.50 b± 2.38	163.13 b ± 2.19	585.79 b ± 30.48	3514 b ± 182.89	21.54 a ± 1.06	5.90 b± 29.0	35.40 b ± 1.72
T3	138.75 a± 1.60	195.50 a ± 4.03	167.13 a ± 2.80	627.67 a ± 26.27	3766 a ± 157.78	22.51 a ± 0.64	6.49 a ± 0.26	38.92 a± 1.58

Means in the same column having different letters are significantly (P≤0.05) different.

T1:- Broodstock fed dietary protein from plant source.

T2:- Broodstock fed dietary protein froma mixture of plant and animal source.

T3:- Broodstock fed dietary protein from animal protein source.

However, the results of these studies are inconsistent and no clear picture emerges regarding the dietary protein requirement of tilapia broodfish. This may be due to the fact that, information came from experimental studies conducted in small aquaria where the optimum growth potential of the fish was not fully realized and, thus, the results of these studies cannot be fully applied to tilapia culture practiced in outdoor tanks and ponds (Al-Hafedh *et al.*, 1999). Far more attention has been focused upon the effect of dietary protein level, Wee and Tuan (1988), for example, fecundity was much higher in *O. niloticus* fed diets containing high levels (42.5% and 50%) of protein than those fed lower-protein (20–35%) diets exhibited much lower fecundity and reduced spawning intensity. Cisse (1988), however, found no differences in terms of fecundity or spawning frequency amongst groups of *Sarotherodon melanotheron* fed various diets containing 20–50% protein. Other studies have reported that reduced dietary protein content can (1) significantly ($P \leq 0.05$) reduce the proportion of post-vitellogenic oocytes in the *O. niloticus* ovary and (2) increase the inter-spawning interval (Gunasekera *et al.*, 1996 a). Although fecundity was higher in *O. niloticus* fed protein diets containing high (20–35%) levels of protein, relative fecundity and egg size remained unchanged. The influence of dietary protein level upon larval quality and amino acid distribution amongst various gonadal and somatic tissues are given in Gunaskera *et al.* (1996 b) and Gunasekera *et al.* (1997) respectively. Most recently, a study by Siddiqui *et al.* (1998) failed to observe a definite trend in fecundity amongst groups of *O. niloticus* fed diets containing differing levels of protein. Gunasekera *et al.* (1996 a) reported that Nile tilapia females fed 20 and 35% protein diets produced a higher number of eggs per spawn than those fed 10% especially at later spawnings. On the other hand, Santiago *et al.* (1983) reported that *O. niloticus* females fed varying protein diets of 20 to 50% did not show significant differences in the mean number of eggs per one spawn. Our findings support that seed production per spawn per female varied significantly ($P \leq 0.05$) between the tested levels of crude protein. Our results are in a partial agreement with those of Al- Hafedh *et al.* (1999) who tested the effects of different protein levels (25, 30, 40 and 45% by dry weight) on reproductive performance of Nile tilapia broodstock and found that, the number of eggs produced per female increased with the increasing dietary protein level, but it was only significantly ($P \leq 0.05$) different between fish fed 40–45% and 25–35% protein diets, whereas the relative fecundity (egg/g female) was significantly ($P \leq 0.05$) higher for 25–35% dietary protein fed fish than those fed 40–45% protein diets. The increase in fish fecundity with increasing dietary protein in the present study is also in agreement with the results reported by Gunasekera *et al.* (1996 a). On the contrary, Santiago *et al.* (1983) reported that the fecundity of *O. niloticus* females did not affected by dietary protein levels (20–50%). Our results also indicated that dietary protein level might have been associated with feed consumption. Higher protein diet might be more palatable, resulting in greater consumption that affected reproductive performance. Several authors have found that dietary protein level affect reproductive performance in tilapia. Wee and Tuan (1988) found that broodstock that were fed with 20 % CP diet produced less seed compared to fish fed medium protein 27.6 % and 35 % CP diets. It is important to consider that mouth-brooding tilapia deprive themselves of food throughout oral incubation. Days between incubation periods lasting up to 15 days because females produce several clutches in succession, they may ingest food for only 4–5 days between non-feeding short feeding periods lasting for 10-13 days (Macintosh, 1985). The relationship between nutrition and reproductive physiology in these fish is therefore highly complex and requires far more detailed research. In support, El-Sayed *et al.* (2003) experimented on Nile tilapia and found that the lowest fecundity (both total number of eggs produced and number of eggs per spawning) was obtained in fish fed 25% protein, and increased with increasing protein levels, Fish fecundity increased linearly with increasing dietary protein up to 40%. The authors concluded that 40% dietary protein is required for optimum spawning performance of Nile tilapia broodstock reared at 0, 7 and 14‰ salinity in clear water and threw the lights on the significant practical implication of their findings in tilapia nutrition as it attracts the attention for more research on broodstock nutrition, because the existing information on nutrient requirements of tilapia broodstock under different culture conditions is limited. Furthermore, the 30% protein diet group exhibited the lowest relative fecundity. In contrast, significant increases were observed in weight gain and fecundity in fish fed the 35% protein diet. In tilapia and swordtail, provision of inadequate dietary protein resulted in inferior growth and fecundity (Al-Hafedh *et al.* 1999, Chong *et al.*, 2004). An important contribution of dietary protein level toward broodstock performance relates to the effect on body size, with several studies reporting maturation of gonads and eggs occur earlier in larger broodstock (El-Sayed *et al.*, 2003, Chong *et al.* 2004 and Abidin *et al.*, 2006).

The effects of different protein sources regardless of protein levels on fecundity of female tilapia broodstock are presented in table (5). It is clear that, the dietary protein sources significantly ($P \leq 0.05$) affected the seed/ female, seed/g female, seed/ female/ day and seed/ day. The highest female fecundity was found in T3 (animal protein source followed in descending order by T2 and T1. The fecundity of females increased significantly ($P \leq 0.05$) with increasing the level of animal protein inclusion in the experimental diets. The increase in fatty acids may played an important structural role as components of phospholipids in fish bio-membranes and are associated with the membrane fluidity and correct physiological functions for membrane enzymes and cell function (Bell *et al.* 1986 and Izquierdo *et al.* 2001). Little work has considered the effect of dietary protein sources on spawning frequency of tilapia (Chang *et al.*, 1988, Santiago *et al.*, 1988 and Cumaratunga and Thabrew, 1989). Santiago *et al.* (1988) have reported that inclusion of *Leucaena leucocephala* leaf meal at more than 40% to replace fishmeal decreased weight of female Nile tilapia resulting in low fry production. The underlying reason might be an imbalance of amino acids in the vegetable protein. Cumaratunga and Thabrew (1989) have also found that Nile tilapia females fed with a diet containing fishmeal, instead of legume meal, had better ovarian growth and larger oocytes. Cuttlefish meal has been found to be more beneficial for egg viability, hatchability and condition of larvae. Chang *et al.* (1988) have also collected more seed from red tilapia when fed with eel diet 44% CP as compared to tilapia diet 24% CP and trash fish 21.7% CP. El-Ebiary (1994) concluded that, the use of high levels of *Leucaena* leaves (15 or 25%) in formulated diets for tilapia *O. aureus* broodfish negatively affected the total larvae production. The low level of soaked *Leucaena* leaves (5% of the diet) as an alternative protein source without affecting fry production can be used. These studies clearly indicate that quality of protein is also very important in brood-fish performance. The protein quality or the assumed low level of fishmeal of the herbivorous diet in our study might be the main reason for the low seed output. As no systematic work has been carried out, so far, to determine the optimal level of amino acids for reproduction, the levels optimal for growth are considered to be optimal for reproduction as well (De Silva and Anderson, 1995). Siddiqui *et al.* (1998) concluded that the use of 30% protein diet, based on both fishmeal and other protein sources, is cost- effective for tilapia seed production under the condition of outdoor concrete tank hatchery system. Rinchard *et al.* (2002) found that, the substitution of fish meal (animal protein) with cotton seed meal (plant protein) at any level did not affect the reproductive parameters examined, but cannot exclude the possibility that cotton seed meal and more specifically, gossypol, can modify directly or indirectly other reproductive parameters. Further studies are needed to elucidate the effect of gossypol on concentrations, motility and the fertilizing ability of spermatozoa in males. The fecundity, quality of eggs produced, such as fertilization and hatching rate, and juvenile survival need to be analyzed in females. Bhujel *et al.* (2001) found that, Nile tilapia brood-fish fed near to satiation twice daily with large and small catfish pellets produced 27% and 30% more seed, respectively, as compared to those females that were fed a herbivorous diet. The number of seed per gram of feed was significantly ($P \leq 0.05$) higher which resulted in the lowest production cost when the females were fed with the large catfish pellets as compared to the females that received the herbivorous feed. The herbivorous diet resulted in lower seed production and was less profitable compared to the two catfish pellets in terms of seed output of Nile tilapia in a green water system.

Fry nursing trial

As shown in table (6). it is clear that, the dietary protein levels and sources significantly (P≤0.05) affected fry average initial weight (AIW), AWG and ADG while the SGR did not reflected any significant differences among fry groups. The highest AIW (0.19± 0.01 g), AFW (6.65± 0.15 g), AWG (6.47± 0.15 g) and ADG (92.36± 2.07 mg/ day) were found in T6 while, the lowest values of the above parameters were 0.12±0.00, 4.20± 0.10 g, 4.09± 0.11 g and 58.38± 1.50 mg/ day, respectively were recorded for T1. Fry survival rates were significantly (P≤0.05) affected by the level and the source of dietary protein of broodstock diets. They ranged between 96.67± 0.88 % (T6) and 90.0± 0.58 % (T1). Concerning the effects of different dietary protein levels of broodstock diets regardless of dietary protein source on fry growth performance measurements (Table 7), it can note that, there were significant effects observed in fry growth performance measurements (AFW, AWG, ADG and SGR) and survival rates. The 40%CP significantly (P≤0.05) recorded the better growth performance parameters, FCR and survival rates (%). Table (8) demonstrates the effects of different protein sources in broodstock diets regardless of the dietary protein level on fry growth performance and survival rates. Without consideration of the level of dietary protein, it can note that, the AIW, AFW, AWG, ADG, SGR, feed intake, FCR and survival rate were significantly (P≤0.05) affected by the source of dietary protein in the broodstock diets. With regard to the present results on the effect of broodstock nutrition as affected by protein levels and sources it can say that, the quality of the broodstock diets influenced the initial weight and the growth and quality of seed produced in terms of fry growth, feeding efficiency and survival rates. In this context, Izquierdo *et al.* (2001) stated that, lipids and fatty acid content of the broodstock diet have been identified as major dietary factors that determine successful reproduction and survival of offspring. As in other animals, it is clear that many of the deficiencies and problems encountered during the early rearing phases of newly hatched finfish larvae are directly related to feeding regime (including nutrient level and duration) of the broodstock.

Table 6. Effect of Nile tilapia *Oreocromis niloticus* (L.) broodstock fed different protein levels and sources on fry growth performance and survival rates (Mean ± SE).

Treatments	AIW	AFW	AWG	ADG (mg/ day)	SGR (%/ day)	Feed intake (g)	FCR	Survival rate (%)
T1	0.12 d± 0.00	4.20 e± 0.10	4.09 e± 0.11	58.38 e± 1.50	5.80 a± 0.11	7.35 e± 0.02	1.80 a± 0.05	90.00 d± 0.58
T2	0.14 c± 0.00	04.70 d± 0.05	4.57 d± 0.06	65.21 d± 0.79	5.73 a± 0.08	7.76 e± 0.13	1.70 ab± 0.05	93.00 c± 0.58
T3	0.15 b± 0.00	5.50 b± 0.10	5.35 b± 0.11	76.36 b± 1.50	5.76 a± 0.08	8.42 c± 0.03	1.58 cd± 0.03	95.33 ab± 0.33
T4	0.15 bc± 0.00	5.10 c± 0.10	4.95 c± 0.10	70.71 c± 1.43	5.69 a± 0.03	8.32 cd± 0.22	1.68 bc± 0.10	93.00 bc± 0.88
T5	0.17 b± 0.00	5.80 b± 0.10	5.64 b± 0.01	80.50 b± 1.38	5.74 a± 0.02	9.27 b± 0.18	1.65 bcd± 0.01	95.00 abc± 0.58
T6	0.19 a± 0.01	6.65 a± 0.15	6.47 a± 0.15	92.36 a± 2.07	5.78 a± 0.01	10.09 a± 0.29	1.56 d± 0.01	96.67 a± 0.88

Means in the same column having different letters are significantly different ($P \leq 0.05$).

T1:- Broodstock fed 25% CP diet from plant protein source.

T2:- Broodstock fed 25% CP die from a mix. of plant and animal protein

T3:- Broodstock fed 25% diet CP from animal protein source.

T4:- Broodstock fed 40% CP from plant protein source.

T5:- Broodstock fed 40% CP from a mix. of plant and animal protein

T6:- Broodstock fed 40% CP diet from animal protein source.

Table 7. Fry growth performance of Nile tilapia, *Oreocromis niloticus* (L.) broodstock fed different protein levels regardless of protein sources (Mean ± SE).

Treat.	AIW	AFW	AWG	ADG	SGR	Feed intake	FCR	Survival rate (%)
T1	0.14 b± 0.01	4.8 b± 0.24	4.67b± 0.24	66.6 b± 3.69	5.76 b± 0.04	7.8b± 0.19	1.7a± 0.05	92.8b± 0.81
T2	0.17a± 0.01	5.85a± 0.29	5.68a± 0.28	81.2a ± 4.03	5.84 a± 0.02	9.2a± 0.34	1.6b± 0.02	95.a ± 0.62

Means in the same column having different letters are significantly (P≤0.05) different.

T1:- Broodstock fed 25% CP diet. T2:- Broodstock fed 40% CP diet.

Table 8. Fry growth performance of Nile tilapia, *Oreocromis niloticus* (L.) broodstock fed different protein sources regardless of protein levels (Mean ± SE).

Treat.	AIW	AFW	AWG	ADG	SGR	Feed intake	FCR	Survival Rate (%)
T1	0.13c± 0.01	65.4c± 0.27	4.52c± 0.26	64.5c± 3.67	5.75b± 0.06	7.83c± 0.29	1.74a± 0.04	91.6c± 0.88
T2	0.15b± 0,01	5.3 b ± 0.32	5.1b± 0.31	72.9b± 4.46	5.73b± 0.03	8.51b± 0.44	1.67b± 0.03	94 b± 0.58
T3	0.17a± 0.01	6.08a± 0.34	5.91a± 0.33	84.4a± 4.74	5.77a± 0.03	9.25a± 0.50	1.57c± 0.01	96.0a± 0.52

Means in the same column having different letters are significantly different (P≤0.05).

T1:- Broodstock fed dietary protein from plant source.

T2:- Broodstock fed dietary protein from a mix. of plant and animal source.

T3:- Broodstock fed dietary protein protein from animal protein source.

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دراسة تأثير مستويات و مصادر مختلفة من البروتين علي الأداء التناسلي وإنتاج قطيع أسماك البلطي النيلي من الزريعة

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أجريت هذه التجربة في مفرخ ومزرعة تجارية بمحافظة الإسماعيلية في موسم تفريخ ٢٠٠٤ بهدف دراسة تأثير استخدام مستويات و مصادر مختلفة من البروتين علي كل من أداء النمو وكفاءة الاستفادة من الغذاء والأداء التناسلي. تم اختبار مستويين مختلفين من البروتين(٢٥% و ٤٠% بروتين خام) من ٣ مصادر بروتينية رئيسية (مسحوق سمك ٦٥% بروتين خام؛ كسب فول الصويا ٤٤ % بروتين خام و خليط من مسحوق سمك ٦٥% + كسب فول الصويا ٤٤%) في تجربة عامليه (3X2) في مكررتين لكل معاملة (١٢ وحدة تجريبية) وتم دراسة المعاملات كما يلي:-

المعاملة الأولي:-أسماك غذيت علي علائق ٢٥% بروتين خام من مصدر بروتين نباتي.

المعاملة الثانية:-أسماك غذيت علي علائق ٢٥% بروتين خام من مصدر بروتين نباتي وحيواني.

المعاملة الثالثة:-أسماك غذيت علي علائق ٢٥% بروتين خام من مصدر بروتين حيواني.

المعاملة الرابعة:-أسماك غذيت علي علائق ٤٠%بروتين خام من مصدر بروتين نباتي.

المعاملة الخامسة:-أسماك غذيت علي علائق ٤٠%بروتين خام من مصدر بروتين نباتي وحيواني. المعاملة السادسة:-أسماك غذيت علي علائق ٤٠% بروتين خام من مصدر بروتين حيواني.

بينت نتائج التجربة وجود تأثير معنوي لكل من مستوي ومصدر البروتين الغذائي المستخدمة في علائق قطعان التفريخ فيأسماك البلطي النيلى على معايير خصوبة القطيع حيث سجلت المعاملة السادسة أعلى قيمة لعدد الزريعة/أنثى 0.42 ± 672 وعدد الزريعة/جم/أنثى

0.09 ± 23.62 وعدد الزريعة/أنثى /يوم 0.14 ± 6.93 وعـــدد الزريعـــة/ يـــوم/م٢ ± 41.59 0.83بينما سجلت المعاملة الأولى أقل قيمة للمعايير السابقة والتي كانت 3.67 ± 462 زريعة/أنثى و0.43 ± 17.2 زريعة/جم/ أنثى و 0.16 ± 4.74 زريعة/أنثى/يوم و 0.96 ± 28.45 زريعة/م٢/ يوم .

أوضحت نتائج التجربة وجود تأثير معنوي لمستوى البروتين الغـــذائي علـــى خصـــوبة القطيع(بغض النظرعن مصادر البروتين المستخدمة في علائق قطعان التفريخ) حيـــث تفوقـــت مجموعات الأسماك المغذاة على مستوى البروتين الأعلى (٤٠% بروتين خام) على مثيلاتهـــا المغذاة على مستوى البروتين الأقل (٢٥% بروتين خام) في قيم كل من متوسط عدد الزريعـــة الناتجة/أنثى ومتوسط عدد الزريعة/جم/أنثى، عدد الزريعة/أنثى/يوم وعدد الزريعـــة/ م٢/ يـــوم وأظهرت نتائج التجربة أن مصادر البروتين الغذائي(بغض النظر عن مستوى البروتين الغذائي) كان لها تأثير على عدد الزريعة/أنثى ، عدد الزريعة/ جم/ أنثى ، عدد الزريعة/ أنثى/ يوم و عدد الزريعة/م٢/يوم .

بينت نتائج تجربة تحضين ورعاية الزريعة الناتجة أن الزريعة الناتجة منأسماك المجموعة السادسة قد تفوقت على باقي المجموعات الأخرى في متوسط الوزن الابتـــدائي AIW والنهـــائي AFW والزيادة في وزن الجسمAWG ومعدل النمو اليومي بينما لم تسجل أية فروق معنوية بـــين مجموعات الزريعة الناتجة عن المعاملات المختلفة في متوسط المعدل النوعي للنمو SGR كمـــا دلت النتائج أيضاً على تفوق زريعةأسماك المعاملة السادسة (تمت تغذيتها علـــى علائـــق ٤٠% بروتين خام من مصدر حيواني) في متوسط الغذاء المأكول ومعامل تحول الغذاء وأعلى نســـبة حياة بين مجموعات الزريعة الأخرى .