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Effects of Algal Diets Supplementation on Reproductive Performance Parameters of Nile Tilapia Broodstock

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Abstract

Four isonitrogenous (34.6 g kg^{-1} of crude protein) and isoenergetic (19.5MJ kg^{-1} gross energy) were formulated. Each diet was supplemented with 0 (Control), 0.5 g/ 100 g (T1), 1 g/ 100 g (T2) and 1.5 g/ 100 g (T3) dried *Cyclotella* spp powder. Nile tilapia broodstock were used in the present experiment 32 broodstocks (24 females + 8 males). Broodstock were sexed and transferred to conditioning 8 concrete ponds (3×1×1 m), and kept separately where, males stocked in 4 concrete ponds at stocking of 2 males in each pond also, the same was conducted for females while, stocked at 6 females in each pond in two replicate. No significant differences were found in growth performance and feed utilization for male and female fed diets supplemented with *Cyclotella* spp for 30 days before mating. The best condition factor and gonadosmoatic index were recorded in T3, which supplemented with 1.5% *Cyclotella* spp. The higher pH and sperm motility value were detected in male of T3 and T2 with insignificant differences between treatments, while the lowest one was observed in control. The highest value of absolute fecundity, relative fecundity and system productivity were obtained in T3.

Keywords: Broodstock, Nile tilapia, *Cyclotella* spp, reproductive, semen

Introduction

Aquaculture is becoming increasingly important as a potential means for the sustainable production and supply of seafood throughout the world. The reproduction from broodstock fish is one of the reasons to achieve the sustainable of aquaculture industry **(Soaudy *et al.*, 2021).** Nevertheless, yields of production from broodstock is low due to the using nonspecific diets for brooders **(Khanzadeh *et al.*, 2016).** Due to the role of breeder's nutrition on reproductive performance, the use of more fitting feed enriched by combining bioactive natural sources can play a key role in the enhancement of the reproductive performances of broodstock. Therefore, the main characteristics of reproductive such as; fecundity, egg diameter, fertilization, and hatchability rates can be developed through the supply of the main requirements of broodstock **(Chong *et al.*, 2004; Kumaraguru vasagam *et al.*, 2007)**. Artificial diets with naturally functional properties that can improve the immune response of fish subsequently increased the reproductive performance **(Hoseinifar *et al.*, 2018**; **Wan *et al.*, 2018**; **Saeed *et al.*, 2021)**.

Microalgae has a high content of crude protein as well as polyunsaturated fatty acids (PUFA), particularly the n-3 series, which can be used a natural source of bioactive compounds **(Christaki *et al.*, 2011; Abd El-Hack *et al.*, 2019)**. Microalgae are an important source of oils and other molecules that can be used as feed stocks to produce biofuels and high-value products, having the potential of becoming a significant renewable energy source **(Moreno-Garcia *et al.*, 2017).** Microalgae can grow effectively on various wastewaters, including those generated in aquaculture as a result of operation of hatcheries and farming systems **(Malibari *et al.*, 2018; Dourou *et al.*, 2018 2020).**

The diatom *Cyclotella* spp are nontoxic, suitably sized, and is capable of heterotrophic growth and has been recommended as a species worthy of further investigation **(Gladue and Maxey 1994; Pahl *et al.*, 2010a; Wood *et al.*, 1999).** *Cyclotella* spp. have been used as a food source of aquatic animals **(De Pauw and Persoone 1988; Webb and Chu 1983),** but did not focused in reproductive performance. To the authors knowledge, reports of the effects of *Cyclotella* spp as feed additives for broodstock is lack, thus this study aimed to display this effects. Therefore, this study aimed to study the effect of dried *Cyclotella* spp supplementation on reproductive performance of Nile tilapia broodstock as well as effects on performance and survival of their fries.

2. Materials and methods

2.1. Source and chemical *Cyclotella* spp

Cyclotella spp was obtained from Elqanater Elkhyria farm of National Institute of Oceanography and Fisheries (NIOF), Egypt. The chemical analysis were conducted according to **(AOAC 1995),** and stored at –20 ºC for subsequent analysis **(Table 1)**. Proximate analysis was conducted on *Cyclotella* spp which obtained from Elqanater Elkhyria farm of National Institute of Oceanography and Fisheries (NIOF), Egypt.

Table 1: Chemical analysis of the *Cyclotella* spp.

Item	%
Dry matter	89.17
Crude protein	11.70
Crude fat	8.20
Ash	13.41
Total carbohydrate	66.96

2.2. Experimental diets

Diet of the present study were formulated at El-Qanater Elkyriya Fish Research Station, Qaliubiya Governorate, National Institute of Oceanography and Fisheries (NIOF). Four isonitrogeous (34.6% crude protein) and isoenergetic (19.5 MJ kg^{-1} gross energy) were formulated. Each diet was supplemented with 0 (Control), 0.5 g/ 100 g (T1), 1 g/ 100 g (T2) and 1.5 g/ 100 g (T3) dried *Cyclotella* spp powder (Table 2). The ingredients such as fishmeal, soybean meal, yellow corn, corn gluten and bran were blended for 5 min in a feed mixer use homogenous mixture grinder. Dried *Cyclotella* spp were mixed with the ingredients then added to the blend followed by homogenization. Then, mixed with fish oil, vitamin and minerals followed by homogenization. The formulated diet was made by dry pellets using a laboratory pellet mill (California Pellet Mill, San Francisco, CA, USA). The pellets (2-mm die) were dried for 4 h at 60°C and stored at −20 °C until use.

Table 2. Formulation and proximate composition of the experiment diets (g/ 100 g diet)

	Experimental diets			
Ingredients	control	T1	T2	T3
Fish Meal	10	10	10	10
Soybean Meal	45	45	45	45
Corn Gluten	8	8	8	8
Yellow Corn	20	20	20	20
Bran	13	12.5	12	11.5
Fish Oil	4	4	4	4
Cyclotella spp	0	0.5	1	1.5
Vitamin &Minerals	2	2	2	2
Proximate analysis				
Dry matter	89.16	89.02	88.68	88.87
Crude protein	34.26	34.38	34.69	34.56
Ether extract	7.62	7.69	7.68	7.81
Ash	6.56	6.65	6.49	6.89
Total carbohydrate[2]	51.56	51.28	51.14	50.74
Gross energy (Mj kg^{-1})[3]	199.4	19.95	20.01	19.95

Vitamin and mineral mixture kg^{-1} of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B_{12}, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 65mg; manganese sulfate ($MnSO_4$, 36% Mn), 89 mg; zinc sulfate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I),
Total carbohydrate =100-(CP + EE+ Ash).
Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kj/g for protein, fat and carbohydrate, respectively according to **Brett (1973)**.

2.3. Experimental broodstock fish condition for spawning

Nile tilapia, *Oreochromis niloticus* broodstock were obtained from Fish hatchery of General Authority for Fisheries resource Development, Sahary, Aswan, Egypt. All number of broodstock was used in the present experiment 32 broodstocks (24 females + 8 males). Broodstock were sexed and transferred to conditioning 8 concrete ponds (3×1×1 m), and kept separately where, males stocked in 4 concrete ponds at stocking of 2 males in each pond also, the same was conducted for females while, stocked at 6 females in each pond in two replicate. Prior to mating all ponds feed on experimental diets for two weeks for adaptation to new environment and prepare the broodstock. All ponds were supplied with well–aerated. Air was compressed to each pond via air stones by air pumps during the experimental period. Water volume (20 %) in each pond was daily replaced by new freshwater after removing of the accumulated excreta.

2.4. Growth and reproductive performance of broodstocks

After adaptation period, males and females were weighted the average body weights of male was 440 g and female 286 g, then stocked at a rate of 4 fish m^{-3}(4 broodstock $pond^{-1}$) with a female: male sex ratio of 3:1 and fed on experimental diets for one month . Broodstocks were fed twice daily at 10:00 am and 4:00 pm to 2% from body weight. Two batches of fry were collected from each pond and the time between two batches was one month. The same condition of the experiment continued for two weeks for egg collection from broodstocks. After spawning period, fish checked for spawning activity (eggs in buccal cavity). Growth performance, feed utilization parameters of reproductive performance was estimated as following:

Weight gain (WG) = final body weight (FBW g) - initial body weight (IBW g).

Specific growth rate (SGR %, day $fish^{-1}$) = (*ln* FBW-*ln* IBW)/t×100; where: FBW is final body weight (g); IBW is initial body weight (g); *ln*= natural logarithmic; t = time in days.

Feed conversion ratio (FCR) = Feed intake (g)/WG (g).

Protein efficiency ratio (PER) = WG (g)/protein intake (g).

Condition factor = Weight (g)/ L^3; where, L is length of fish cm

Gonadosomatic index = weight of gonad/ weight of fish × 100

Absolute fecundity for hatching = Mean number of seeds at each spawning per female.

Relative fecundity for hatching = Mean number of seeds at each spawning per female body weight (g).

System productivity for hatching = Mean number of seeds per day/pond size

Egg diameter (mm), Egg volume (mm^3) by Hemacytometer.

3.8. Semen quality

For stripping and collecting milt, the genital area was dried and handily pressure was applied to the fish abdomen, mid- way between the pectoral and pelvic fins, moving posteriorly down to the urogenital papilla **(Van der waal, 1985)** as an attempt for milt stripping, this process be repeated 10-15 times for each male. To assessment semen indices such as sperm pH were evaluated immediately using the first squeezed drop of milt and measured by pH tapes according method by **Amer *et al.* (2005)**. Sperm motility was classified subjected, according to the percentage of motile spermatozoa **Viveiros *et al.* (2001).** Spermatozoa concentration was evaluated using a 10µl subsample of semen with a dilution ratio of 1:20 (one volume of sperm and 20 volume of physiological saline solution) **(Billard, Gillet, 1981).** The concentration of sperm in the seminal fluid was estimated according to **Ruranguwa *et al.* (2004).** The standard methods for determining sperm density (sperm cells/ ml milt) in fish was used to count spermatozoa generally using haemocytometer counting chamber **(Buyukhatipoglu and Holtz, 1984).**

2.5. Statistical analysis

All data were presented as means ± standard error (SE). Growth, hematology, blood chemistry and hormones data were analyzed using one way ANOVA, followed by Duncan's multiple range tests which was used to compare differences among individual means, with statistical software SAS ANOVA procedure **(Statistical Analysis System 1993)**. A probability of 0.05 was utilized to account for the statistical difference between the means. Before the analysis, percentage data were normalized by arcsine-transformation.

Results

3.1. Growth performance of broodstock of Nile tilapia as affected by *Cyclotella* spp supplementation

Results presented in Table 3 showed that the differences in fish initial body weight for male and female among different treatments were insignificant ($P > 0.05$) indicating that the experimental groups at the start of the experiment were randomly distributed. Also, Table 3 presents the effects of *Cyclotella* spp on growth performance; final body weight, weight gain (WG) and Specific growth rate (SGR) for 30 days before mating. No significant differences were found in final body weight, WG and SGR for male and female fed diets supplemented with *Cyclotella* spp.

Table 3: Growth performance of male and female Nile tilapia fed experimental diets for 30 days

Items	Experimental diets				±SE	P-value
	Control	T1	T2	T3		
Male						
Initial body weight (g $fish^{-1}$)	441.50	428.50	453.00	440.00	8.7986	0.4179
Initial body length (cm $fish^{-1}$)	28.25	28.00	28.75	28.00	0.1443	0.0876
Final body weight (g fish-1)	468.50	491.00	505.00	496.50	7.8845	0.1459
Final body length (cm $fish^{-1}$)	28.75	29.50	30.50	30.00	0.6884	0.4486
Weight gain (g $fish^{-1}$)	27.00[b]	62.50	52.00	56.50	5.5151	0.0607
Specific growth rate (% day)	0.21[b]	0.47	0.37	0.42	0.0409	0.0629
Female						
Initial body weight (g $fish^{-1}$)	283.08	281.58	289.63	285.25	0.738	0.063
Initial body length (cm $fish^{-1)}$	24.50	24.50	25.00	24.50	0.250	0.500

Final body weight (g fish^{-1})	300.08	304.25	314.25	312.08	0.079	0.091
Final body length (cm fish$^{-1)}$	25.80	25.50	25.50	26.33	0.396	0.861
Weight gain (g fish^{-1})	17.00	21.16	24.66	26.83	0.067	0.083
Specific growth rate (% day)	0.19	0.25	0.28	0.30	0.003	0.071

- Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05).

3.2. Feed intake and feed utilization of broodstock of Nile tilapia as affected by *Cyclotella* spp supplementation

Table 4 showed feed intake (FI) and feed utilization of male and female Nile tilapia fed diets supplemented with different levels of *Cyclotella* spp. Feed intake (FI) did not affected by *Cyclotella* spp supplementation with different levels, while the highest FI was noted in T2 that supplemented with 1 % *Cyclotella* spp followed by T3 and T2, respectively without significant difference (P > 0.05). The same trend was observed in feed conversion ratio (FCR) and protein efficiency (PER) for broodstock fed diet supplemented with different levels of *Cyclotella* spp. Whereas, the best FCR and PER were observed in male of T3, which supplemented with 1.5 % *Cyclotella* spp.

Table 4: Feed intake and feed utilization of male and female Nile tilapia fed experimental diets for 30 days

Items	Experimental diets				±SE	P-value
	Control	T1	T2	T3		
Male						
Feed intake g kg^{-1}	271.73	284.78	292.90	287.97	4.5730	0.1459
Feed conversion	10.44	4.55	5.76	5.09	1.2382	0.1163
Protein efficiency ratio	0.59	0.51	0.49	0.43	0.0005	0.650
Female						
Feed intake g kg^{-1}	164.11	164.11	168.00	159.37^{c}	0.1587	0.082
Feed conversion	5.61	7.64	6.78	6.48	0.0301	0.092
Protein efficiency ratio	0.59	0.43	0.49	0.52	0.012	0.071

- Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05).

3.3. Condition factor and gonadosomatic index of male and female broodstock Nile tilapia as affected by *Cyclotella* spp supplementation

Table 5 showed condition factor (K) and gonadosomatic index (GSI) of broodstock Nile tilapia fed diets supplemented with different levels of *Cyclotella* spp. Condition factor (K) of male and female Nile tilapia significantly improved in fish fed diets supplemented with different levels of *Cyclotella* spp compared with control diet. The best K was recorded in T3, which supplemented with 1.5% *Cyclotella* spp, while no significant differences between T2 and T1 which supplemented with 1 % and 0.5%, respectively. With respect of gonadosomatic index (GSI) was significantly higher in female Nile tilapia fed diets supplemented with different levels of *Cyclotella* spp. The highest value of GSI for female Nile tilapia was noted in T3 that supplemented with 1.5% *Cyclotella* spp.

Table 5: Condition factor and gonadosomatic index of male and female Nile tilapia fed experimental diets for 30 days

Items	Experimental diets				±SE	P-value
	Control	T1	T2	T3		
Male						
Condition factor	0.11^{b}	0.24^{a}	0.18^{ab}	0.21^{a}	0.0198	0.0440
Female						
Condition factor	0.16^{a}	0.12^{b}	0.15^{ab}	0.15^{ab}	0.006	0.077
Gonadosomatic index (GSI)	3.02^{d}	3.21^{c}	3.69^{b}	3.98^{a}	0.056	0.002

- Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05).

3.4. Semen quality

3.4.1. pH of semen

pH values of semen value of male fed diet supplemented with different levels of dried *Cyclotella* spp are showed in Table 6. Values of pH ranged between (7.0 to 8.1). The higher pH value was detected in male of T3 and T2 with insignificant differences between treatments, while the lowest one was observed in control.

3.4.2. Sperm motility (%)

Table 6 showed the data of sperm motility (%) as affected by different levels of dried *Cyclotella* spp. Sperm motility of male female fed the experimental diets ranged from (50 to 75 %). Addition of dried *Cyclotella* spp significantly improved the sperm motility for male. Sperm motility level was significantly higher in male fed diet of T3 (75.0 %) compared with other treatments diets.

3.4.3. Sperm concentration

Sperm concentration of male fed diet supplemented with different levels of dried *Cyclotella* spp are showed in Table 6 whereas, dried *Cyclotella* spp supplementation improved the sperm concentration of male. Values of Sperm concentration ranged between (2.88×10^{9} to 4.43×10^{9}). The higher sperm concentration was detected in male of T3, while the lowest one was observed in control.

Table 6: Semen quality of male of Nile tilapia fed experimental diets for 30 days

Items	Experimental diets				±SE	P-value
	Control	T1	T2	T3		
pH of semen	7.0^{b}	7.01^{b}	8.1^{a}	8.0^{a}	0.06	0.0459
Sperm motility (%)	50.0	60.0	70.0	75.0	2.33	0.0163
Sperm concentration	2.88×10^{9d}	2.98×10^{9c}	3.21×10^{9b}	4.43×10^{9a}	0.135	<.0001

- Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05).

3.4. Reproductive performance of broodstock Nile tilapia as affected by *Cyclotella pp* supplementation

The reproductive performance such as absolute fecundity, relative fecundity and system productivity of broodstock fed diet supplemented with different levels of dried *Cyclotella* spp are showed in Table 7. Values of absolute fecundity ranged from (475.16 to 1042.83), Absolute fecundity ranged from (4.68 to 10.42) and system productivity ranged from (31.68 to 69.52). Absolute fecundity, relative fecundity and system productivity for broodstock fed diet supplemented with were significantly (P < 0.05) improved dried *Cyclotella* spp compared with control. The highest value of absolute fecundity (1042.83), relative fecundity (10.42) and system productivity (69.52) were obtained in T3.

Table 7: Absolute fecundity, Relative fecundity and System productivity of male and female Nile tilapia fed experimental diets for 30 days

Items	Fecundity 1				±SE	P-Value
	Control	T1	T2	T3		
Absolute fecundity	609.67^{cb}	475.16^{c}	709.00^{b}	1042.83^{a}	51.7247	0.015
Relative fecundity	5.86^{b}	4.68^{b}	6.76^{b}	10.42^{a}	0.5091	0.013
System productivity	40.64^{cb}	31.68^{c}	47.26^{b}	69.52^{a}	3.4495	0.015

- Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05).

4. Discussion

This is the first study to evaluate the inclusion of dietary *Cyclotella* spp on reproductive performance of Nile tilapia. Under condition of this study, the growth performance; FBW, WG and SGR for broodstock the conditioning period lasted for four weeks before the operation of spawning. While, growth performance was detected by the end of feeding to showed the effect of *Cyclotella* spp supplementation on the male and female of Nile tilapia performance in these short time of feeding. As expected, no significant (P > 0.05) differences were recorded among different treatments regarding fish growth performance after 30 day. Microalgae are promising aquafeed ingredients, as they present a good nutritional composition and promote improvements in the fish immune response **(Reyes-Becerril *et al.*, 2013; Sarker *et al.*, 2018).** In addition, *Cyclotella* spp have a high lipid content, contain a good amino acid and fatty acid profile and have compounds with antioxidant and immunostimulating action, such as β-carotene and vitamin A. The main reason of masked or absence of improved the growth may be due to the short

duration of feeding, also, the main target in this feeding period do more developing of gonad of broodstock of Nile tilapia.

Condition factor (K) and gonadosomatic index (GSI) indices are a good indicator of fish gonad maturation, which liver organ have an important role for vitellogenin synthesis at the reproduction period of fish where it can be expressed the estradiol receptors determination **(Orlando *et al* 2017)**. In the present study, the best K was recorded in T3, which supplemented with 1.5 % *Cyclotella* spp. Also, gonadosomatic index (GSI) was significantly higher in female Nile tilapia fed diets supplemented with different levels of *Cyclotella* spp Females fed high level of dried *Cyclotella* spp had significantly ($P < 0.05$) higher Gondosomatic index (GSI) than those the rest of diet. The importance of conditioning period for tilapia broods was reported by many authors with different suggestions for the optimal conditioning period. Short resting period (5-15 days) for Nile tilapia broods was suggested for its improvement effect on the reproductive performance in comparison with non-rested broods **(El-Sayed, 2006 and Abou-Zied, 2015)**.

Obtaining quality gametes and success in the initial husbandry of larvae obtained is markedly dependent on the adequate nutrition of breeders **(Fernandez-Palacios *et al*., 2011)**. **Putri and Budi (2020)** showed that supplementation of *S. platensis* in the diet at 3 % was improved the sperm performance of silver rasbora (*Rasbora argyrotaenia*) such as milt volume, sperm motility, sperm concentration, and sperm viability ($P < 0.05$). The high nutrient founded in *Cyclotella* spp such as vitamin A beta-carotene and amino acid may be the reason of improved semen quality and reproductive performance in the present study.

In another study, **James *et al*. (2006)** reported that Spirulina inclusion of 8% promoted an increase in the reproductive performance of swordtail (*Xiphophorus helleri*). Reports have shown that the profile of carotenoids, ascorbic acid and PUFA content can influence egg production and quality **(Scabini *et al*., 2011)**. **Watanabe and Vassallo-Agius (2003)** observed that dietary supplementation with carotenoids enhanced broodstock performance in yellowtail fish (*Seriola quinqueradiata*). Recently, **Carneiro *et al*. (2020)** indicated that egg production per female and hatching rate were obtained from Zebra fish fed diet containing 40 g *Cyclotella* spp kg^{-1} compared to control diet, while, no significant differences in fertilization rate in any of the dietary groups.

Conclusion

Broodstock of Nile tilapia fed diets supplemented with 1% or 1.5 % dried *Cyclotella* spp improved condition factor, gonadosomatic index, semen quality and absolute fecundity, relative fecundity.

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تأثير المكملات الغذائية الطحلبية على معايير الأداء التناسلي لقطيع تفريخ البلطي النيلي

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تم تكوين اربع علائق متساوية فى محتواها من البروتين (34.6 جرام بروتين/كجم) والطاقة (19.5 ميجاجول/كجم). تم استكمال كل عليقة ب صفر (كنترول), 0.5 جرام /100 جرام (T1), 1 جرام/100جرام (T2), 1.5 جرام/100جرام (T3) من مسحوق *Cyclotella* spp المجففة. استخدمت امهات البلطي النيلي في التجربة الحالية 32 سمكة (24 أنثى + 8 ذكور). تم تجنيس امهات التفريخ ونقلها إلى 8 أحواض خرسانية (3 × 1 × 1 م). تم الاحتفاظ بها بشكل منفصل حيث تم تخزين الذكور فى اربع احواض خرسانية بمعدل ذكرين بكل حوض، كما تم إجراء نفس الشيء للإناث حيث تم تخزينها بمعدل 6 إناث بكل حوض فى نسختين متماثلتين. اوضحت نتائج التجربة انه لا توجد فروق معنوية في أداء النمو ومعاملات التغذية لدى الذكور والإناث التى تم تغذيتها على العلائق المضاف اليها Cyclotella spp لمدة 30 يومًا قبل التزاوج. تم تسجيل افضل عامل حالة ومؤشر كتلة الغدد التناسلية في الامهات التى تغذت على المعاملة T3 , المضاف اليها 1.5% من Cyclotella spp. كما لوحظ ارتفاع قيمة الأس الهيدروجيني والنسبة المئوية لحركة الحيوانات المنوية في ذكور T3 و T2 (1.5− 1 جرام / 100 جرام) مع وجود فروق معنوية بين المعاملات، فى حين لوحظ ان أقل قيمة لهذه القيم وجدت بالذكور التى تغذت على المعاملة الكنترول. تم الحصول على أعلى قيمة للخصوبة المطلقة والخصوبة النسبية وإنتاجية النظام وجدت في الذكور التى تغذت على المعاملة T3.