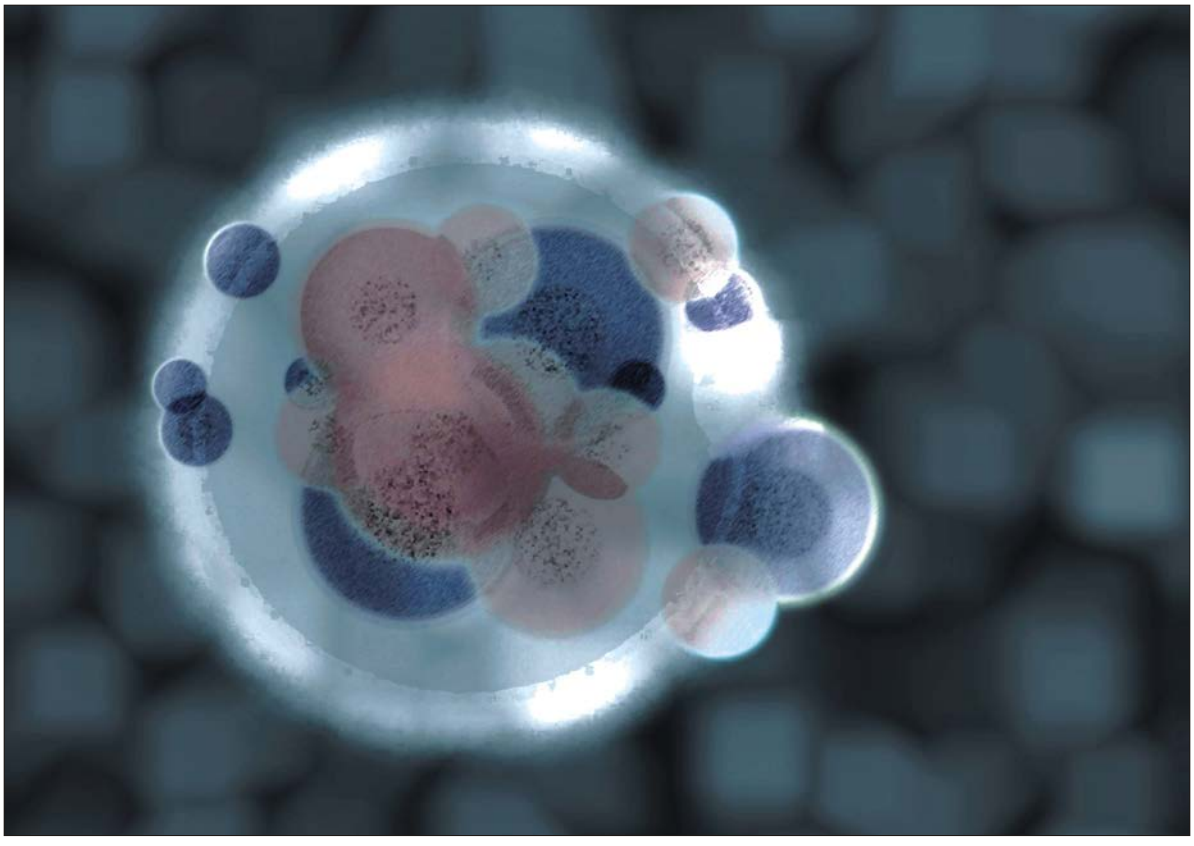

Journal of Biological Sciences

ISSN 1727-3048

science alert

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2017.305.311

Research Article
Enhancement of Reproductive Performance of Nile Tilapia *Oreochromis niloticus* using Phytobiotic *Spirulina platensis*

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Abstract

Background and Objective: Phytobiotics as additives in aquaculture feed are increasingly recognized as feed for young and adult fish. *Spirulina* improves digestion and direct fats in the fish to growth. The beneficial effect of inclusion of *Spirulina platensis* as feed additives in tilapia diets during their development was studied to estimate its effect on tilapia reproductive performance. **Materials and Methods:** A phytobiotic *Spirulina platensis* was introduced to tilapia feed for 19 weeks at concentrations of (0.5, 7.5, 10 and 20 g kg^{-1} *Spirulina platensis* diet named: S_0, S_5, $S_{7.5}$, S_{10} and S_{20}, respectively). One hundred fish were divided to 5 groups and fed diets supplemented with algae at variable levels as well as control. The gonadosomatic index (GSI), fecundity, percentage of spawned eggs, egg and fry qualities were evaluated. Data were analyzed using one-way analysis of variance (ANOVA) at significance of ($p<0.05$). **Results:** Fish weight, GSI, spawning activity and spawned eggs number showed significant increase ($p<0.05$) on higher supplementation of phytobiotics and also fry length and weight increased in all the groups tested compared to control. Fry mortality and deformations percentage showed significant decrease ($p<0.05$) in phytobiotic fed fish. Group supplemented with algal level of (20 g kg^{-1} *Spirulina platensis* diet) in their feed spawned earlier at 45.3±7.8 days compared to 49.5, 51.3, 54.1 and 66.7 days for fish fed the 10, 7.5, 5 g kg^{-1} *Spirulina platensis* diet and control, respectively. Also, percentage of spawned eggs, total number of eggs/female recorded significant increase ($p<0.05$) in groups of fish supplied with the 20 and 10 g kg^{-1} *Spirulina platensis* diet. Eggs diameter, volume as well as weight showed insignificant change, while hatchability percentage increase in *Spirulina* fed fish compared to fish receiving control diet. **Conclusion:** Inclusion of *Spirulina* in fish feeds improve reproduction performance of tilapia fish, suggesting that *Spirulina* is an appropriate reproduction-stimulating additive in Nile tilapia culture.

Key words: *Spirulina platensis*, feed additives, reproductive parameters, eggs and larval quality, tilapia

Citation: Olfat Malak Wahbi and Yasser Sangak, 2017. Enhancement of reproductive performance of Nile tilapia *Oreochromis niloticus* using phytobiotic *Spirulina platensis*. J. Biol. Sci., 17: 305-311.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is one of the most important economic freshwater fish of Egypt. Tilapia is considered suitable for culture because of their relatively fast growth, good utilization of artificial diets, high tolerance to adverse environmental conditions, high diseases resistance and easy breeding[1].

The importance of phytobiotics as additives in aquaculture feed are increasingly recognized as a feed for young and adults fish. *Spirulina* improves digestion through production of extra cellular enzymes (protease and lipase) and able to direct fats in the fish for growth rather than storage[2]. *Spirulina platensis*, contain 60-70% by weight protein, vitamins *B*-12, carotene, minerals, essential amino acids (62%) and fatty acids[3]. Its lipid contents are similar to that of vegetable oils[4], rich in linoleic 18:2*n*6 and α-linolenic 18:3*n*3 acids and their C20 derivatives[5,6]. Some of the cyanobacteria tend to contain large quantities of the total fatty acids, polyunsaturated fatty acids (PUFA) (20-60%), Eicosapentaenoic acids 20:5*n*3 and Arachidonic acids 20:4*n*6 that influence reproductive performance in fish[7]. Olvera-Novoa *et al.*[8] indicated the usefulness of *Spirulina* for partial replacement upto 40% of the fish meal protein in Nile tilapia diets. El-Sheekh *et al.*[9] suggests the possibility of using *Spirulina* as commercial nutrient source for large scale culture of fish in general and tilapia in particular. Takeuchi *et al.*[10] and Elsayed *et al.*[11] concluded that *Spirulina* is an appropriate growth-stimulating additive in tilapia culture. Lu *et al.*[12] indicated that *Spirulina* is an effective uni-feed for larval tilapia at a feeding rate of 30% of body weight. Ibrahem *et al.*[13] and Ungsethaphand *et al.*[14] documented the use of *Spirulina* for enhancing growth parameters and disease resistance in fish. Research on the effect of feeding phytobiotics on the reproductive performance of tilapia fish is limited. Using *Spirulina* alga as the main feed to Nile tilapia increase, egg quality and survival rates, compared with standard fish feed[15]. Studies have reported the effect of *Spirulina* on fish reproduction. *Spirulina* enhanced seed production in yellow tail cichlid, *Pseudotropheus acei*[3], in goldfish, *Carassius auratus*[16], swordtail[2] and bassa fish, *Pangasius bocourti*[17].

The present study aimed to discover the beneficial effect of *Spirulina platensis* as additive in diets of tilapia, *Oreochromis niloticus* on their reproductive performance and to assess possibility of using *Spirulina* as reproduction-stimulating.

MATERIALS AND METHODS

Fish breeding: The study was carried out at Aquaculture laboratory of the Arab Academy for Sciences and Technology, Alexandria, Egypt at the period from 15/3/2016-8/8/2016. The experiment was carried out using hundred Nile tilapia *Oreochromis niloticus* (body weight 55±2.5 g and total length 12.2±0.40 cm) which were obtained from the same parental breed stock pair held at Barseek fish farm at Behera Governorate and transported alive to the Aquaculture laboratory of the Arab Academy for Sciences and Technology, Alexandria, Egypt and left acclimated for 2 weeks prior to the experiments. Before the start of the experiment females were tagged using threading needle and a surgical thread. The females were pierced dorsally and a different colored tag was tied to identify females during spawning. They were divided into 5 equal groups each consisting of 2 replicates [10 fish/replicate (7♀, 3♂)]. Fish in each replicate were reared in aquaria (0.5 ×0.5×1 m, each) supplied with chlorine free tap water which was partially renewed daily.

They were fed twice daily on a basal diet (~34% protein and ~8.2% lipid) at 5% of body weight/day. Fish were weighed every 15 day and amounts of feed were adjusted according to the new weight. The water was cleaned regularly to remove unused feed and fecal materials and water were partially renewed daily. The temperature was maintained at 28±0.5°C using electric heaters, dissolved oxygen was measured using dissolved oxygen meter (Jenuay Model 9070 water proof meter) and continuous aeration was provided by air pumps. Dissolved oxygen ranged from 7-8 mg L^{-1} during experiments period.

Reproductive parameters: Reproductive performance of the females fed the different *Spirulina platensis* diets were observed all over the period of experiment (19 weeks). Females were observed daily to check whether it was about to spawn (with red and protruding genital papilla) or spawned. When eggs were seen in the mouth of females, all eggs in the buccal cavity were removed completely using a plastic dropper and the number of eggs from each female was counted. Eggs from females spawned naturally were used for periodicity (mean number of days taken for an individual female to complete one spawning cycle) and fecundity estimations. Post-spawning females were weighed. Absolute fecundity and relative fecundity were determined as mean number of freshly spawned eggs at each spawning/female or/gram body weight respectively, for determination of percentage of spawned eggs; after each spawning one

freshly spawned female from each treatment was sacrificed and a segment of middle region of ovary (1 cm) was fixed, dehydrated, cleared, imbedded, sectioned, stained and microscopically examined. The number of post ovulatory follicle to total oocytes number was determined.

Eggs were artificially stripped from the ovarian cavity of females with red and protruding genital papilla by gentle pressing female belly. Ovulated eggs were fertilized by milt from males of same experimental group. Artificial spawns were used to determine eggs and fries quality. A subsample of 20 eggs from each replicate were measured (long, short axis) microscopically using eyepiece micrometer. Artificially fertilized eggs were placed to hatch in bottomless cylindrical container with a mesh at base supplied with a gentle flow of dechlorinated tap water to keep eggs rolling (mimic maternal incubation).

Two groups of fertilized eggs (20 eggs/treatment) were incubated till hatching to determined hatch ability rates, time to hatch and yolk-sac absorption, survival rate and the larvae length and weight. Unhatched eggs were counted. Deformity was determined as the ratio of normal larvae to larvae with curved body or tailless ones. At the end of experiment, fish were weighed, sacrificed and their ovaries weighed and their gonad index was calculated:

$$GSI = \frac{\text{Weight of ovary}}{\text{Total body weight}} \times 100$$

***Spirulina platensis*:** *Spirulina platensis* was obtained as a fresh powder reared in the Aquaculture Research Laboratory in Arab Academy for Science, Technology and Maritime Transport, Alexandria, Egypt.

Rations: A standard commercial ration formulated to fulfill dietary requirements of Nile tilapia were used containing crude protein, crude lipid, vitamins and minerals (Table 1). Five experimental rations were formulated containing feed additives for Nile tilapia: One as a control diet without supplements and a supplements at 5, 7.5, 10 and 20 g kg^{-1} *Spirulina platensis* diet where used. The basal and tested diets were formulated from the commercial feed ingredients. All the dry ingredients were grounded through a feed grinder. Experimental diets were formulated to be isocaloric (~8.2% lipid) and isonitrogenous (~34.5% protein) (Table 1). The ingredients were mixed with water in meat mincer with a 1mm diameter and the pellets were left to dry at room temperature. The required diet was prepared weekly and stored in plastic bags, kept in refrigerator at 4°C for daily use.

Statistical analysis: The data presented as Mean±SD, analyzed using one way analysis of variance ANOVA, followed by Duncan's test using the SPSS package, version 17 (Chicago, IL,USA). Data were statistically significant at $p<0.05$. Homogeneity of data was confirmed before test.

RESULTS

Reproductive performance of fish

Spawning parameters: The reproductive parameters of female Nile tilapia fed the experimental feeds all over experiment period (19 week) is illustrated at Table 2. Fish weight decreased in fish feed *Spirulina* supplemented diets recording significant decrease ($p<0.05$) in males and females fish fed the S_{20} diet. No fish mortality was recorded in all tested groups during the period of experiment.

Tilapia fed on *Spirulina* supplemented diets showed better spawning performance compared to fish fed the controldiet (Table 2).

The percentage of spawned eggs increased ($p<0.05$) significantly in fish fed S_{20}, S_{10} and $S_{7.5}$ diets. The highest percentage of spawned eggs observed in fish fed the S_{20} diet (6.5±1.6) compared to (3.8±0.7) at S_0 diet. Fish fed on *Spirulina* supplemented diet at level of 20 g kg^{-1} *Spirulina platensis* diet (S_{20}) diet were the first to spawn for the first time (45.3 days) compared with 66.7 days in females fed the standard diet 0 g kg^{-1} *Spirulina platensis* diet (S_0). Females fed S_{20} or S_{10} diets had the highest total eggs number ($p<0.05$) with respect to fish or tank (Table 2). No significant difference in absolute or relative fecundity values was recorded among fish fed the various diets. Fish fed the two diets S_{20} and S_{10} have the shortest inter-spawning interval that differ significantly ($p<0.05$) with fish fed the standard (S_0) or *Spirulina* supplemented diet (S_5).

Weight, diameter and volume of eggs from the four successive spawns did not show significant differences between fish fed the different *Spirulina* diets and control (Table 3). Fish fed the *Spirulina* diets have egg hatchability rates higher than eggs of fish fed the control diet (Table 4). *Oreochromis niloticus* females that fed *Spirulina* diets at all levels, showed significant increase in egg hatch ability ($p<0.05$). The incubation time of eggs to hatch (72-78 h) as well as the time for larval yolk sac to be absorbed (6-7days) did not differ among treatment at the fourth spawn. All *Spirulina* fed fish produced better larval quality with significant increases in fry weight and length ($p<0.05$). Percentage of dead fry significantly decreased in larvae of fish feed the *spirulina* supplemented diets compared to those fed the S_0

Table 1: Composition and proximate analysis of the experimental diets on dry weight basis

	Diets				
Compositions	S_0	S_{20}	S_{10}	$S_{7.5}$	S_5
Feed ingredients (%)					
Fish meal	20	20	20	20.00	20.0
Soybean meal	30	30	30	30.00	30.0
Yellow corn	15	15	15	15.00	15.0
Wheat milling	13	13	13	13.00	13.0
Wheat bran	15	13	14	14.20	14.5
Sunflower oil	5	5	5	5.00	5.0
Vitamins and minerals premix	2	2	2	2.00	2.0
Spirulina platensis	0	2	1	0.75	0.5
Chemical analysis					
Dry matter	89.5	88.89	89.1	89.34	89.4
Crude protein	34.7	34.50	34.5	34.60	35.2
Ether extract	8.5	8.10	8.0	8.00	8.3
Crude fiber	4.0	3.85	3.8	3.85	3.6
Ash	5.5	4.10	4.4	4.85	5.5

Vitamins and minerals premix kg^{-1} diet:75000IU Vit. A: 9000IU Vit. D3: 150 mg Vit. E: 30 mg Vit. K3: 26.7 mg Vit. B1: 30 mg Vit. B2: 24.7 mg Vit. B6: 75 mg Vit. B12: 225 mg Nicotinic acid, 69 mg Pantothenic acid: 7.5 mg Folic acid: 150 mg Vit. C: 150 mg Biotein: 500 mg Choline chloride: 300 mg DL-methionine: 93 mg Fe: 11.25mg Cu: 210 mg Zn: 204 mg Mn: 5 mg Se and Co 5 mg. Diets analyzed according to AOAC[18]

Table 2: Reproductive parameters of female tilapia fed *Spirulina* supplemented diets all over period of 19 weeks

	Spirulina level (g kg^{-1} diet)				
Parameters	S_0	S_{20}	S_{10}	$S_{7.5}$	S_5
Final weight (g)					
♀ weight	235.02±12.31^a	208.61±8.11^b	225.34±10.4^a	229.10±7.7^a	230.64±13.19^a
♂ weight	249.61±10.11^a	224.15±7.27^b	238.15±3.57^a	240.22±8.9^a	243.18±12.46^a
Gonadosomatic index (GSI)	6.8±0.5^a	7.7±0.5^b	7.7±0.2^b	7.4±0.3^b	6.9±0.3^a
Percentage of spawned eggs/spawning♀	3.8±0.7^a	6.5±1.6^b	6.0±1.1^b	5.2±0.6^b	3.9±0.6^a
Time to first spawning (days)	66.7±11.3^a	45.3±7.8^c	49.5±9.2^c	51.3±4.9^b	54.1±13.7^b
Mean total eggs spawned/fish	3194±193^a	4511±218^c	4319±291^c	3797±946^b	3250±315^a
Mean absolute fecundity[1]	730±73	831±23	793±23	781±16	723±60
Mean relative fecundity[2]	6.5±0.2	6.7±0	6.7±0	6.6±0.1	6.2±1
Inter-spawning interval (days between two successive spawns)	20.8±0.6^a	16.5±0.7^b	16.5±0.5^b	18.0±0.4^{ab}	19.2±1.2^a

Mean±SD of two groups of four ♀ tilapia/treatment, different superscripts in same row were significantly different($p<0.05$), [1](No. of eggs/fish/spawning), [2](No. of eggs g^{-1} b.wt/spawning)

Table 3: Mean eggs diameter, volume and weight from four consecutive spawns of♀tilapia fed *Spirulina* supplemented diets

	Number of spawns			
Diets	First	Second	Third	Fourth
Egg diameter (mm)				
S_0	2.32±0.03	2.30±0.04	2.27±0.06	2.30±0.01
S_{20}	2.38±0.01	2.35±0.03	2.11±0.09	2.10±0.01
S_{10}	2.37±0.05	2.36±0.01	2.28±0.01	2.34±0.05
$S_{7.5}$	2.32±0.06	2.33±0.02	2.27±0.09	2.34±0.03
S_5	2.34±0.01	2.31±0.03	2.29±0.08	2.26±0.10
Egg volume (mm^3)				
S_0	6.28±0.39	6.08±0.08	5.78±0.05	5.39±0.03
S_{20}	6.60±0.55	6.40±0.10	6.00±0.22	6.11±0.17
S_{10}	6.48±0.36	6.30±0.02	6.08±0.14	6.00±0.14
$S_{7.5}$	6.27±0.70	6.25±0.31	5.72±0.16	6.03±0.14
S_5	6.31±0.10	6.09±0.24	5.90±0.47	5.68±0.33
Egg weight (mg)				
S_0	5.56±0.04	5.30±0. 10	5.26±0.05	5.00±0.19
S_{20}	6.20±0.10	5.85±0.32	5.56±0.14	5.40±0.30
S_{10}	5.97±0.05	5.70±0.08	5.48±0.07	5.31±0.12
$S_{7.5}$	5.67±0.38	5.58±0.57	5.24±0.22	5.13±0.14
S_5	5.59±0.10	5.43±0.02	5.30±0.10	5.02±0.23

Mean±SD of two groups of ♀ tilapia/treatment, Egg diameter= (Long axis×short axis)/2 and Egg volume= $\pi/6\times$(Long axis×short axis)

Table 4: Egg hatchability and larval quality from the fourth spawning of tilapia fed diets supplemented with *Spirulina* levels

	Diets				
Parameters	S_0	S_{20}	S_{10}	$S_{7.5}$	S_5
Egg					
Hatchability (%)	50.0±3.0[a]	68.0±1.5[b]	63.0±2.5[b]	65.0±3.5[b]	58.0±2.0[b]
Yolk-free larvae					
Weight (mg)	13.9±0.1[a]	14.9±0.0[b]	14.5±0.11[b]	14.6±0.2[b]	14.8±0.3[b]
Length (mm)	8.0±0.1[a]	9.0±0.5[b]	9.0±0.2[b]	9.0±0.4[b]	8.5±0.4[ab]
Fry survival (%)	65.0±4.5[a]	80.0±10.5[c]	81.0±6.5[c]	73.0±7.5[b]	66.0±8.0[a]
Dead fry (%)	4.0±2.0[a]	3.0±1.0[b]	3.0±2.0[b]	3.0±2.0[b]	4.0±0.0[a]
Deformity (%)	3.0±2.0[a]	2.0±1.0[b]	2.0±1.0[b]	2.0±1.0[b]	2.0±2.0[b]

Mean±SD of two groups per treatment of ♀ tilapia fed diet supplemented with different levels of *Spirulina*. Different superscripts in same row were significantly different (p<0.05)

Table 5: Mean percentage(%) of spawning female tilapia over 4 successive spawns

	Number of spawn			
Diets	First	Second	Third	Forth
S_0	53.3±8.3[a]	42.0±16.7[b]	33.3±16.7[b]	30.0±7.3[b]
S_{20}	70.6±4.7a	65.5±10.1[a]	55.1±9.1[b]	48.2±8.8[b]
S_{10}	60.7±16.7[a]	60.7±16.7[a]	58.7±6.2[a]	53.3±9.3[b]
$S_{7.5}$	73.3±0.0[a]	65.0±8.3[b]	56.7±0.0[c]	48.3±8.0[c]
S_5	53.4±8.1[a]	45.3±6.8[b]	39.8±6.3[c]	38.0±5.2[c]

Mean±S.D. of two groups of four ♀ tilapia per treatment. Different superscripts in the same column were significantly different (p<0.05)

diet (Table 4). Also, percentage of deformed fry decreased significantly (p<0.05) in fish fed phytobiotic feeds with respect to fish fed standard diet, having maximum values (4 and 3% for dead and deformed fries) in fish fed the standard feed, minimum values (3 and 2% for dead and deformed fries) in fish fed the phytobiotic feed. Regardless of diet the number of spawning females showed decreased values by time. After four successive spawning, 53% of the brood fish fed the S_{10} diet were still fecund compared to only 30 and 38% in fish fed the S_0 or S_5 diets, respectively (Table 5).

DISCUSSION

The incorporation of *Spirulina* in tilapia feed favorably influenced, fecundity, GSI, fry production and survival. *Spirulina* contain significant quantities of protein, lipids and fatty acids which are the main constituents of egg yolk. Also, its essential fatty acids content provide energy for spawning activities. Dahlgren[19] demonstrate that lipids, essential fatty acids and proteins of fish diet influenced rate of vitellogenesis, development and maturation of oocytes.

The improvement in reproduction efficiency of *Spirulina* feed fed fish reported in this work may bealso due to its content of micronutrients (B group vitamins), this agree with the finding of Coves *et al.*[20] who stated the important of B vitamins supply in elevating reproductive performance of fish.

In present study the incorporation of *Spirulina* in tilapia feed, reduced fry mortality and deformity in *Spirulina* feed-fed fish compared to control ones. The reduction in numbers of dead and deformed fry in fish fed the phytobiotic diet may be due to the presence of B group vitamins specially (vitamin B1 and B12) in *Spirulina*. Wooster *et al.*[21] demonstrated that the reduction in the mortality of Atlantic salmon friesis due to the beneficial effect of thiamin (vitamin B1). In the present study, the weight and length of produced fries were greater in the *Spirulina*-fed fish could be attributed to the fatty acids contain of *Spirulina*. Similarly Mazorra *et al.*[22] concluded that, *Spirulina* inclusion in fish feed, enhance the spawning success and quality of fries produced due to the lipid content of *Spirulina* specially n-6 fatty acids. Santiago and Reyes[23]also stated the important of n-6 fatty acids group specially ARA acid in improving spawning efficiency and fry morphometric characteristics. Through the experimental period, fish fed the *Spirulina* supplemented diets produced, greater number of eggs compared to fish feed on standard diet which was therefore due to increased percentage of spawned eggs, shorter inters pawning intervals, rather than absolute and relative fish fecundity, that did not showed significant changes among all treatments used. The supplementation of, *Spirulina* to fish feed in this study lead to a higher percentage of spawned eggs, higher hatch ability rate and fries survival percentage than those fed on control feed. Similarly Guroy *et al.*[3], James *et al.*[24], Vasudhevan and James[16], James *et al.*[2] and Meng-Umphan[17] have reported that, *Spirulina* enhanced seed production in yellow tail cichlid, *Pseudotropheus acei*, in goldfish, *Carassius auratus*, in swordtail, *Xiphophorus helleri* and bassa fish, *Pangasius*

bocourti respectively. Although there were no detected differences in egg morphometric characters, the quality (length and weight) of fries from fish fed on the *Spirulina* supplemented diets was higher compared to control that may be attributed to high *Spirulina* content of essential amino acids, fatty acids and vitamins that influence the growth performance of fish. Growth of the red swordtail, *Xiphophorus helleri* and gold fish was enhanced on addition of *Spirulina* at level of 8% in fish feed[2,24].

CONCLUSION

Incorporation of *Spirulina* in diets of tilapia during their gonadal development improve reproduction performance of tilapia fish leading to higher fecundity and GSI values, better quality of eggs and larvae. Eliciting higher egg hatchability and larval survival, suggesting that *Spirulina* is an appropriate reproduction-stimulating additive in Nile tilapia.

SIGNIFICANCE STATEMENTS

This study discovers the possibility of improving fish breeding via studying the impact of *Spirulina* in fish feed on reproductive parameters of Nile tilapia. This study will help the researcher to uncover the critical areas of possibility of using *Spirulina* as reproduction-stimulating additive in Nile tilapia feeds.

ACKNOWLEDGMENTS

Authors are indebted to Assistant Associate Prof. Essam Abdel maula, Head of Aquaculture Research Lab. Arab Academy for Science and Technology, Alexandria, Egypt for supporting the present study and supplying the present study with *Spirulina.*

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