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An attempt to improve the reproductive efficiency of Nile tilapia brood stock fish

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Abstract A field study was conducted on brood stock Nile tilapia to increase the propagation. Both sexes were individually stocked into Habas (enclosures) in an earthen pond and fed for 19 days on a basal diet supplemented with different additives at graded levels of each (0.5, 1.0 and 2.0 g Therigon®; 1.0, 2.0, and 3.0 g Nuvisol Hatch P®; 20, 40 and 60 mg Gibberellic acid and 700, 900 and 1,100 mg L-carnitine/Kg diet). The obtained results were evaluated, and the best treatment for each sex was chosen for mating. Results indicated that all pretreatments for male and female brood stocks of Nile tilapia positively affected the total count of the offspring produced. Yet, the Haba, in which the females were pretreated with 0.5 g Therigon®/Kg diet and the males pretreated with 700 mg L-carnitine/Kg diet, gave the highest total count of the offspring comparing with the other Habas. But, because of the high feed cost due to the additives cost, 0.5 g Therigon®/kg diet as pretreatment for ♀ only (3rd Haba), 2 g Nuvisol Hatch P®/Kg diet as pretreatment for ♀ only (5th Haba), followed by 0.5 g Therigon® and 700 mg L-carnitine/Kg diet for ♀ and ♂, respectively (4th Haba), respectively were the best economically.

Keywords Nile tilapia · Brood stock · Feed additives · Reproductive performance

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Introduction

Tilapia aquaculture is and will continue to be important, particularly for the lesser-developed countries in the tropics (FAO 2001). Nile tilapia *Oreochromis niloticus* are considered as the most common and popular fish in Egypt. Egypt, a country where, arguably, the farming of tilapia has its roots (Stickney 2006), where tilapia culture is believed to have originated some 4,000 years ago. Tilapia consist 36% of the Egyptian production from fish culture (Sadek 2000) and occupy the 10th order concerning the world production from aquaculture (Van Hauwaert et al. 2000). Hence, Egypt produces 20% of the world tilapia capture and 12% of the world farmed tilapia (El-Sayed 2006). The culture of *O. niloticus* in Egypt has recently developed into a major industry. This industry, however, is still growing in a remarkable way with apparent intend toward intensification that pressurize the need of enormous number of seeds. Many limitations associated with tilapia fry production under the prevailing

Egyptian conditions were described by El-Gamal (2002). Also, brood stock husbandry and spawning techniques are constantly upgraded as Egyptian hatcheries require a high number of good quality eggs to satisfy the needs for aquaculture, so rigorous management of large numbers of brood stock is necessary for mass production of eggs and fry due to the complex reproductive biology and asynchronously spawning with relatively small number of eggs produced per spawning. Accordingly, the development of more elaborated forms of brood stock management is crucial to improve fry yield and system efficiency. Today, it is widely accepted that effective seed production demands a thorough understanding of the special husbandry and particular nutritional requirements of brood stock fish which significantly affect fecundity, survival, egg size and egg and larval quality (Bromage 1998). The objective of the present research was to study the possibility of improving reproductive performance of Nile tilapia fish using some feed additives.

Materials and methods

The present study was carried out during Nile tilapia hatching season of 2008 (June and July) in two phases, the first was to study the effects of three commercial feed additives on females (Therigon®, Nuvisol Hatch P® and Gibberellic acid) and fourth one (L-carnitine) on males brood stock, concerning their gonads characteristics and some reproductive traits. The second phase was to select treatments of both 0.5 g Therigon® and 2 g Nuvisol Hatch P®/Kg diet (for females) and 700 mg L-carnitine/Kg diet (for males) to apply in a mating trial to be evaluated via hatchability.

Experimental management of the 1st phase

A field study was conducted in a private earthen pond fish farm located at Alabhar belonging to Alhamol, Kafr Alshiekh governorate. Fourteen Habas (each 3 m width × 6 m length × 0.5 m depth and 1 m total depth) were constructed in a two-Feddan (one Feddan equal 4,200 m^2) earthen pond. The first ten Habas were stocked with female brood stock of 1 year (yearlings) Nile tilapia fish (average body weight of 150 g) from the same farm. The other four Habas were stocked with male brood stock of (the same age as the females, but of an average body weight of 200 g) Nile tilapia fish from the same farm also. Each Haba was stocked with twenty fish. This study was a feeding trial to test the effects of some commercial dietary supplements on Nile tilapia (*O. niloticus*) propagation. The experimental feeding began on the 20th June till the 8th of July, where the feed was offered to fish twice daily, at a daily feeding rate of 3% of the fish biomass in each Haba. The feed additives were purchased from the local market and added directly to a mash diet, which was purchased also from the local market (contained 90.31% dry matter, 80.88% organic matter, 23.81% crude protein, 5.47% ether extract, and 9.43% ash) after the proximate analysis according to AOAC (2004) and moistened to be pelleted via a hand mincer. The feeding continued for males and females before mating. Water of fish rearing in each individual Haba was tested daily for water quality parameters including water temperature, pH value, and dissolved oxygen concentration.

Dietary treatments during the 1st phase

Fish were fed on a basal ration (BR) with or without (control) the tested feed additives (as illustrated in Table 1) which were:

1. Therigon® powder for veterinary use, manufactured by Adwia Co., S. A. E. 10th of Ramadan

Table 1 Explanation of the experimental diets during the 1st phase

Haba's No. & sex	Diets
1, ♀	Basal ration (control females)
2, ♀	Basal ration + 0.5 g Therigon®/kg diet
3, ♀	Basal ration + 1.0 g Therigon®/kg diet
4, ♀	Basal ration + 2.0 g Therigon®/kg diet
5, ♀	Basal ration + 1 g Nuvisol Hatch P®/Kg diet
6, ♀	Basal ration + 2 g Nuvisol Hatch P®/Kg diet
7, ♀	Basal ration + 3 g Nuvisol Hatch P®/Kg diet
8, ♀	Basal ration + 20 mg Gibberellic acid/Kg diet
9, ♀	Basal ration + 40 mg Gibberellic acid/Kg diet
10, ♀	Basal ration + 60 mg Gibberellic acid/Kg diet
11, ♂	Basal ration (control males)
12, ♂	Basal ration + 700 mg L-carnitine/Kg diet
13, ♂	Basal ration + 900 mg L-carnitine/Kg diet
14, ♂	Basal ration + 1,100 mg L-carnitine/Kg diet

city, Egypt. Each 1 g contains alpha-amino-*p*-hydroxyhydrocynnamic acid, 1,000 g package as GnRH stimulant (Batch No. 0601116).

2. Nuvisol Hatch P®, imported by Khirat Alnile Co., 27 Alferdos Buildings, Flat 43, Nasr city, Egypt from Newtrix Co., Belgium, in 500 g package. Each 1 kg contains the following vitamins (in mg): B_1 4,000, B_2 5,000, B_3 4,000, B_6 2,000, B_9 1,000, B_{12} 20, PP 10,000, Biotin 50, and L-carnitine 30,000.
3. Gibberellic acid (C_{19} H_{22} O_6), type analysis, Art. 3,930, M. W. 346.38, M. P. 225°C, GA_3 content 90%, 1 g package, Batch No. 43124, imported from Lobal Chemie, Pvt. LTD, 2042 Bombay, India.
4. L-carnitine powder from Mebaco, Egypt.

Criteria measured at the end of the 1st phase

After the 19 days feeding trial of the separate sexes of brood stock, three fish from each experimental Haba were catched to collect blood, eggs and milt for different measurements and determinations. Tri sodium citrate was used as an anticoagulant for blood plasma collection. Blood plasma determinations for the glycoprotein hormones follicle stimulating-OO-like hormone (FSH) and luteinizing-OO-like hormone (LH), as well as the sex steroid hormones progesterone hormone and testosterone hormone were done using commercial ELISA test kits catalog No. E-115 (Immunospec corporation), E1-118 (Immunospec corporation), BC-1113 (BioCheck, Inc), and BC-1115 (BioCheck, Inc), respectively according to Tietz (1995) and milt analyses (count, motility, forward, sluggish and dead percents) were done too.

Experimental management during the 2nd phase

Six Habas (similar to those used in phase one, in the same earthen pond, at the same private farm) were used in the second phase (beginning from the 9th of July) of this study. The Habas were stocked with nine females and three males each (sex ratio 3 ♀:1 ♂) to test the best treatments from phase one as following in Table 2.

The level 0.5 g Therigon®/Kg diet was chosen for its high value of FSH (Table 7), 2 g Nuvisol Hatch P®/Kg diet was chosen for its high value of progesterone (Table 7). On the 22nd July, the fry were collected and counted. Throughout this phase also, water quality parameters were measured daily as in phase one, at 9–11 am.

Statistical analysis

Data collected were statistically analyzed using SAS (2001), when ANOVA-test was significant ($P \leq 0.05$), least significant difference was calculated (Duncan 1955) to differentiate between means.

Table 2 Explanation of the experimental reproductive trait during the 2nd phase

Haba's No.	♀ × ♂ from the 1st phase
1	♀ fed on Basal ration (BR) × ♂ fed on BR
2	♀ fed on BR × ♂ fed on BR + 700 mg L-carnitine/Kg diet
3	♀ fed on BR + 0.5 g Therigon®/kg diet × ♂ fed on BR
4	♀ fed on BR + 0.5 g Therigon®/kg diet × ♂ fed on BR + 700 mg L-carnitine/Kg diet
5	♀ fed on BR + 2 g Nuvisol Hatch P®/Kg diet × ♂ fed on BR
6	♀ fed on BR + 2 g Nuvisol Hatch P®/Kg diet × ♂ fed on BR + 700 mg L-carnitine/Kg diet

Table 3 Mean values of some quality parameters of the Habas' waters used for rearing Nile tilapia brood stock in an earthen pond throughout the experimental course

Item	Temperature (°C)	The pH values	Dissolved oxygen (mg/l)
Mean ± SE	29.4 ± 0.19	7.50 ± 0.06	5.70 ± 0.11

Results

As shown from Table 3, there were no significant differences among all Habas concerning the tested water quality parameters. Therefore, the data were presented as overall means ± standard errors (SE). The tested parameters showed very suitable water conditions for rearing tilapia.

Data of the studied females' reproductive traits are illustrated in Table 4. Except GSI, the other tested parameters showed significant ($P \leq 0.05$) differences among treatments. The significantly heavier body weight (288.9 ± 23.8 g) after the 19 day-study was realized by the fish in the 8th Haba (20 mg gibberellic acid/Kg diet) followed by Haba No. 6 (2 g Nuvisol Hatch P®/Kg diet) without significant difference between both Habas (6 and 8). The best ($P \leq 0.05$) ovaries weight (11.5 ± 0.07 g) was recorded in fish of the 4th Haba (2 g Therigon®/Kg diet); yet, there were no significant differences among all treatments and the control. Egg number per fish was the highest (935.5 ± 120.2 and 926.0 ± 12.7) significantly ($P \leq 0.05$) by the 5th and 3rd Habas' fish (treated with 1 g Nuvisol Hatch P®/Kg diet and 1 g Therigon®/kg diet), respectively. Yet, the egg number/Kg fish weight (fecundity) of these fish groups in Habas No. 5 and 3 did not differ significantly ($P \geq 0.05$) with the control, which was better ($P \leq 0.05$) than all the other treatments. Moreover, the lowest ($P \leq 0.05$) egg diameter was reflected by the fish groups of Habas No. 6 and 5, being 0.96 ± 0.06 and 1.25 ± 0.00 mm. Otherwise, all other treatments were significantly similar to the control.

Tables 5 and 6 present data of male reproductive traits tested including testes weight and GSI as well as milt quality parameters. Although there were no significant differences among treatments and the control; yet, the control was more pronounced in fish weight, testes weight, GSI, and sperms count than the treatments. But the motility and dead percentages were better in fish group of Haba No. 12 (700 mg L-carnitine/Kg diet) followed by Haba No. 13 (900 mg L-carnitine/Kg diet) concerning motility, forward, sluggish and dead percentages.

Data of plasma hormones of the experimental fish are presented in Table 7. Females fish of the 3rd and

Table 5 Males' gonado-somatic index of brood stock Nile tilapia as affected by the dietary supplementations for 19 days feeding trial in Habas in an earthen pond (means ± SE)

Haba No.	Fish weight (g)	Testes weight (g)	GSI
11	283.05 ± 0.85	4.050 ± 0.05	1.428 ± 0.01
12	236.10 ± 22.3	2.350 ± 0.95	0.995 ± 0.32
13	250.00 ± 33.0	2.550 ± 0.05	1.020 ± 0.12
14	263.55 ± 14.0	2.450 ± 0.75	0.929 ± 0.24

Means do not differ significantly ($P \geq 0.05$)

GSI gonado-somatic index = gonads weight (g) × 100/fish weight (g)

Table 4 Females' sexual parameters of brood stock Nile tilapia as affected by the dietary supplementations for 19 days feeding trial in Habas in an earthen pond (means ± SE)

Haba No.	Fish weight (g)	Ovaries weight (g)	Egg number/fish	Egg no./Kg BW	Egg diameter (mm)	GSI
1	178.1^{b} ± 17.1	8.45^{ab} ± 1.35	769.3^{b} ± 34.4	4341^{a} ± 224.8	1.55^{a} ± 0.12	4.74^{a} ± 0.31
2	211.7^{b} ± 5.70	9.00^{ab} ± 0.90	689.0^{bc} ± 99.0	3267^{bcd} ± 491.1	1.55^{a} ± 0.12	4.25^{a} ± 0.76
3	227.0^{b} ± 22.1	9.25^{ab} ± 1.75	926.0^{a} ± 9.00	4114^{ab} ± 360.1	1.55^{a} ± 0.12	4.07^{a} ± 0.38
4	227.0^{b} ± 32.1	11.5^{a} ± 0.05	698.3^{bc} ± 14.3	3037^{cde} ± 286.8	1.67^{a} ± 0.00	5.06^{a} ± 0.70
5	224.5^{b} ± 3.50	9.00^{ab} ± 1.20	935.5^{a} ± 85.2	4171^{ab} ± 445.0	1.25^{b} ± 0.00	4.01^{a} ± 0.59
6	239.0^{ab} ± 19.1	10.7^{ab} ± 1.75	519.0^{de} ± 74.2	2161^{ef} ± 138.2	0.96^{c} ± 0.04	4.47^{a} ± 0.38
7	223.2^{b} ± 20.3	7.5^{ab} ± 2.50	537.5^{d} ± 12.6	2423^{def} ± 163.7	1.67^{a} ± 0.00	3.36^{a} ± 1.44
8	288.9^{a} ± 16.9	7.90^{ab} ± 0.40	630.0^{bcd} ± 14.1	2191^{ef} ± 176.8	1.67^{a} ± 0.00	2.73^{a} ± 0.83
9	178.5^{b} ± 4.96	5.95^{b} ± 2.35	602.0^{cd} ± 0.00	3376^{bc} ± 93.9	1.67^{a} ± 0.00	3.33^{a} ± 1.22
10	223.9^{b} ± 15.9	10.1^{ab} ± 0.05	391.5^{e} ± 12.6	1754^{f} ± 68.5	1.67^{a} ± 0.00	4.51^{a} ± 0.40

Means with the same letter within the same column do not differ significantly ($P \geq 0.05$)

BW body weight, *GSI* gonado-somatic index = gonads weight (g) × 100/fish weight (g)

Table 6 Data of some quality parameters of milt (using Hämocytometer slide) collected from the tested male brood stock Nile tilapia as affected by the dietary supplementations for 19 days feeding trial in Habas in an earthen pond

Haba No.	Count ($\times 10^6$/ml)	Motility (viability) (%)	Forward (%)	Sluggish (%)	Dead (%)
11	660	55	20	45	35
12	450	75	35	40	25
13	350	60	50	40	30
14	390	45	35	45	30

Table 7 Data of blood plasma analysis for both glycoprotein as well as sex steroid hormones of the tested Nile tilapia brood stock fish as affected by the dietary treatments for 19 days during rearing in Habas stocked in an earthen pond

Haba's No. & sex	FSH-OO-like hormone, mIU/ml	LH-OO-like hormone, mIU/ml	Progesterone (♀)/ testosterone (♂), ng/ml
1, ♀	32	19	0.014
2, ♀	91	18	0.110
3, ♀	13	19	0.080
4, ♀	83	61	0.060
5, ♀	14	52	0.130
6, ♀	63	48	0.140
7, ♀	16	27	0.009
8, ♀	72	84	0.006
9, ♀	14	26	0.003
10, ♀	84	28	0.017
11, ♂	48	59	2.780
12, ♂	89	76	4.310
13, ♂	78	89	5.140
14, ♂	24	38	1.010

5th Habas reflected lower concentrations of (FSH-OO-like hormone) but higher concentrations of either (LH-OO-like hormone) or progesterone hormone, comparing with the other Habas' fish. This is in good accordance with the fecundity which was given in Table 4. Also, male fish of the 12th and 13th Habas (Table 7) had plasma with higher levels of FSH-OO-like hormone, LH-OO-like hormone, and testosterone comparing with the other treatments. This also confirms the previous results of the milt analysis for its quality parameters (Table 6).

The following Table 8 shows that all pretreatments for male and female brood stocks of Nile tilapia positively affected propagation (2nd phase of the experiment, mating), i.e., total count of the offspring produced. Yet, the 4th Haba (in which the females were pretreated with 0.5 g Therigon®/Kg diet and the males were pretreated with 700 mg L-carnitine/Kg diet, for 19 days before mating or fertilization) gave the highest total count of the offspring comparing with the other Habas. But, the 3rd, 5th, and 4th Habas were the best economically, since they were responsible for 43.5, 31.7, and 25.3% superiority than the control (1st Haba).

Discussion

The quality of fish rearing water was not influenced by the experimental treatments and was suitable for rearing Nile tilapia brood stock according to Abdelhamid (2009).

Reproductive performance of fish is influenced by many factors, e.g., feeding regime including dietary protein (Gunasekera and Lam 1997; Abdelhamid et al. 2003) and vitamin (Abdelhamid et al. 1999a, b) levels, feeding rate (Abou-Zied 2006), and hatchery management (Abou-Zied and Ali 2007), as well as endocrine regulation (Melamed et al. 1998; Sharaf 2005). Other environmental conditions may also affect, including photoperiod and water temperature (El-Nady et al. 1999; Kamanga et al. 2004) as well as water depth (Salem et al. 2005).

The fish farming industry has been more focused on the quality of eggs or larvae rather than that of sperm, even though the quality of both gametes may affect fertilization success and larval survival. Sperm quality in farmed fish may be affected by different components of brood stock husbandry, during collection and storage of sperm prior to fertilization or the fertilization procedure. Motility is most commonly used, since high motility is a prerequisite for fertilization and correlates strongly with fertilization success (Rurangwa et al. 2004).

Gibberellins (GAs) are involved in a wide range of plant developmental processes. Of all the plant

Table 8 Total count of the offspring produced at the end of phase two as affected by the dietary pretreatment of the brood stock in phase one and economic efficiency

Haba's No.	Fry count	Fry price, LE/1,000	Consumed feed price, LE	Economic efficiency	
				Absolute	Relative
1	3,400	119.0	2.44	48.77	100.0
2	3,500	122.5	3.19	38.40	78.74
3	4,000	140.0	2.00	70.00	143.5
4	4,800	168.0	2.75	61.09	125.3
5	4,000	140.0	2.18	64.22	131.7
6	4,100	143.5	2.94	48.80	100.1

Economic efficiency = Income from buying the produced fry in LE/feed costs of the brood stock during the pretreatment in LE, where the local price of 1,000 fry was 35 LE and for 1 kg diet without additives was 2.2 LE

hormones, the GAs represent perhaps the most diverse group, with currently 126 different structures known (Croker and Hedden 2001). Gibberellins are tetracyclic diterpenes that are found in plants and fungi. A few of the identified to date are known to be active hormones that are involved in seed germination, seedling emergence, stem elongation, fertility, and flower and fruit development. The gibberellin receptor has not yet been conclusively identified. GAs act in stem growth via an enhancement of both cell division and cell elongation. Gibberellins get their unusual name from the fungus *Gibberella fujikuroi*, from which they were first isolated (Sponsel 2001). GA_3 is naturally widespread than the other gibberellins, which have sexual influences (Hifny 1974). GA_3 has a pesticide effect (WHO 1990), and also carcinogenic effect on rectal protozoan (El-Mofty 1974) and Egyptian toads' liver (El-Mofty and Sakr 1988). But, Macgregor (1988) clearly demonstrated that it was essentially nontoxic by various routs of applications for different animal species.

Yet, it promotes growth of rats, poultry, pigs, and calves (Alkhiat et al. 1981; Abdelhamid et al. 1993) as well as it improves laying hens' production, concerning egg production, egg mass and hatchability (Anderson et al. 1982; Abdel-Fattah et al. 2007). Gibberellins have an estrogenic effect on animals (Marasas et al. 1984). It increased blood protein significantly, but affected different organs weight and their histology in chickens (Abdelhamid et al. 1994). In fish, GA_3 at low levels improved Nile tilapia growth and gonado-somatic index (Abdelhamid et al. 1998), since it is a nitrogenous compound (Alkhiat et al. 1981) with estrogenic effect; where it increased the percent of egg production, hatchability and ovary and oviduct relative weight significantly (El-Sebai et al. 2003). So using natural GA_3 instead of the synthetic estrogen is safer and environmentally friend and therefore should be considered.

L-carnitine is a naturally occurring amino acid derivative (dipeptide amino acid), synthesized from methionine and lysine. L-carmitin, a betaine derivative of β-hydroxybutyrate, could be biosynthesized in plant and animal cells via lysine, methionine, and some vitamins like B_6, C, nicotinic acid and folate (Zeyner and Hameyer 1999). It is an essential cofactor of fatty acid metabolism (it provides energy by transporting long and medium chain fatty acids to mitochondria to act as fuel). Deficiency in carnitine is associated with male infertility. Since L-carnitine provides an energetic substrate for the spermatozoa in the epididymis, it contributes directly to sperm motility and may be involved in the successful maturation of the sperm. Carnitine lowers triglycerides and raises the high density lipoprotein levels. L-isomer of carnitine is more effective than DL-isomer (Chatzifotis et al. 1995).

Cellular parameters of the seminogram have been previously shown to correlate with L-carnitine concentration in the seminal fluid. Carnitine is involved in maintaining an active oxidative phosphorylation (OXPHOS). Therefore, it was strongly suggested that relationship between carnitine secretions, seminal quality and OXPHOS activities could be because of a parallel response to the same regulatory event (Ruiz-Pesini et al. 2001; Agarwal and Said 2004). L-carnitine improved semen quality and histological characteristics of the testes (El-Damrawy 2007). Generally, a low level of L-carnitine enrichment

provides several protective effects in fish reared under intensive pond-culture conditions (Schlechtriem et al. 2004; Harpaz 2005).

Pituitary homogenate induced artificially maturing and increased both serum testosterone and estradiol (Matsubara et al. 2005). Gonadotropin-releasing hormones (GnRHs) bind to the specific receptor on the gonadotrophs to activate the synthesis and release of gonadotropins (follicle stimulating hormone or FSH and luteinizing hormone or LH), which in turn control gonadal maturation, gametogenesis and gamete release (Alok et al. 2000; Hu et al. 2007). Not only do teleosts exhibit the highest variety of GnRH variants, but recent data and whole genome analyses indicate that they may also possess multiple GnRH receptor (Lethimonier et al. 2004). Synthetic analog of gonadotropin-releasing hormone was used for inducing ovulation and enhancing spermiation in brood fish (Mylonas et al. 1995). Moreover, territorial males of African cichlid, *Haplochromis burtoni*, characterized by aggressive and reproductive activity, have significantly larger hypothalamic gonadotropin-releasing hormone (GnRH)-containing neurons and larger testes than nonterritorial males (Soma et al. 1996).

Dietary inclusion of 1 g Nuvisol Hatch P®/Kg diet and 1 g Therigon®/kg diet before mating realized good female reproduction performance. Also, dietary supplementation with 700 and 900 mg L-carnitine/Kg diet before mating gave better results of males' reproductive performance. But, because of the high feed cost due to the additives cost, 0.5 g Therigon®/kg diet as pretreatment for ♀ only (3rd Haba of the 2nd phase, mating), 2 g Nuvisol Hatch P®/Kg diet as pretreatment for ♀ only (5th Haba of the 2nd phase, mating), followed by 0.5 g Therigon® and 700 mg L-carnitine/Kg diet for ♀ and ♂, respectively (4th Haba of the 2nd phase, mating), respectively were the best economically.

Conclusion

It could be recommended to use such commercial feed additives for improving reproductive performance of Nile tilapia brood stocks to offer enough seeds for fish farms. It is recommend also to make other trials on different other additives at economical levels.

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