The Effect of Dietary Probiotic (Bactocill, *Pediococcus acidilactici*) Supplementation on Reproductive Performance of *Oreochromis niloticus*

Abdelal, A. E.; A. M. El-Danasoury; E. K. Mohamed and Safaa M. Sharaf*
Animal Production & Fish Resources Dept., Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt

Received: *2/10/2021*

Abstract: This experiment was carried out in two spawning seasons, from August to September in 2019 and 2020 (56 days each season) to evaluate the effect of dietary probiotic Bactocill, *Pediococcus acidilactici* (1 x 10^{10} cell/g) supplementation on diets of broodstock Nile tilapia (*Oreochromis niloticus*) and its effect on reproductive performance. The experimental design were four treatments groups in four experimental diets in 12 hapas, each group was divided into 3 replicates (n=24: 6 females and 2 males per each hapa). The four groups (D1), (D2), (D3) and (D4) fed on basic diet supplemented with 0.0, 0.50, 1.0 and 1.50g probiotic/kg basic diet respectively. Nile tilapia [72 females with an average body weight (262.53±20.22 g) and twenty-four males with an average body weight (279.39±24.12 g) were distributed into 12 hapas. In the first season (2019), the highest values of the fry production were observed in the fish group fed on D4, while the highest values of the gonado-somatic index, fecundity and relative fecundity were observed in the fish group fed on D3. The results of the second season (2020), the highest values of the fry production were observed in the fish group fed on D4. The highest level of estrogen or testosterone for Nile tilapia broodstock was estimated when fish were fed on D2 containing 0.50 g probiotic/kg diet. The present study recommended the use of probiotic during reproduction spawning season of Nile tilapia broodstocks at a dose of 1.5 g kg^{-1} of diet.

Keywords: Probiotic, Bactocill, *Pediococcus acidilactici*, Nile tilapia, reproductive performance

INTRODUCTION

Aquaculture is the intervention in the rearing of aquatic species to enhance fish production, which accounts for nearly 50% of the world's food fish, is the fastest-growing food-producing sector. In this respect, it was argued that global production has increased from about 49.9 million tons in 2007 to 73.8 million tons in 2014, with most of this growth taking place in China (FAO, 2016). Nile tilapia is the main cultured fish species with a percentage of 65.15% from the total fish production in Egypt (Elsheshtawy *et al*., 2019).

Probiotics are live microbial cells which, when consumed in sufficient amounts lead to health benefits to the host by improving the quality of the host surrounding environment or by enhancing its resistance towards disease (Guarner and Schaafsma, 1998; Wang *et al.,* 2008; Hernandez *et al.,* 2010; Soccol *et al.,* 2010; Verschuere *et al.,* 2000). Salminen *et al*. (1999) stated that, probiotics are any preparation of microorganisms (but not necessarily living) or the components of microbial cells that have a positive impact on the health status of the host. Probiotic microorganisms have been shown to inhibit other pathogenic microbes both in-vitro and in-vivo through different mechanism. Several studies on probiotics were available during the last decade. However, the procedural and ethical restrictions of animal studies make it difficult to recognize the mechanisms of action of probiotics. Gram *et al.* (1999), noticed that *Pseudomonas fluorescens* AH2 inhibited the growth of *V. anguillarum* during co-culture, independent of the iron concentration. Irianto and Austin (2002), reported that the addition of probiotic bacteria to culture water or via feed supplementation during initial egg fertilization or pre-larval stages could have a distinct benefit through the mechanism of competitive exclusion for attachment sites on egg surfaces or in the digestive tract. Pirarat *et al*. (2006), recognized that *Lactobacillus rhamnosus* GG protecting against *Edwerdsiella tarda* via influencing the alternative complement system thereby increasing phagocytic cell aggregation and phagocytic activity.

To our knowledge, only a few studies Abasali and Mohamad (2011) have investigated the beneficial effects of probiotics dietary supplementation on the reproductive performance such as body weight (BW), total length (TL) of fry, fry production, fecundity and gonadosomatic index (GSI) of four live bearing ornamental species (*Poecilia reticulata*, *P. sphenops*, *Xiphophorus helleri*, *X. maculatus*). Their results have been complemented by the findings obtained by Gioacchini *et al.* (2010). Mehrim *et al*. (2015), tested the effect of Hydro yeast Aquaculture® as a reproductive hormone enhancer in the case of the adult Nile tilapia and concluded that increasing the levels of the dietary probiotic led to a significant increase in serum total testosterone and progesterone of *O. niloticus* males and females; respectively. Carnevali *et al*. (2017), reviewed the effects of probiotics on the integrated control of fish metabolism. In the last section of their assessment, condenses recent findings regarding to the positive effects of probiotic administration on fish gonads, which deeply support their role towards reproduction and gamete quality mainly achieved on zebrafish.

Nutrition is known to have a considerable effect upon gonadal development and reproductive performance of fish (Watanabe, 1995; Haroun *et al.,* 2006). In addition, a better ovulation detected in the broodstock feed with *L. rhamnosus* supplemented diet. In addition to environmental factors, the performance of hypothalamus-pituitary-gonadal axis (HPG) can be affected by nutrition and metabolism of fish (Lombardo *et al*., 2011), and there is a direct link between reproductive performance and nutrition of broodstock (Izquierdo *et al*., 2001).

*Corresponding author e-mail: safaa_sharaf@agr.suez.edu.eg

In this regard, proper nutrition can play a key role in improving the reproductive performance of broodstock fish (Bobe and Labbé, 2010). The additives modulate gastrointestinal microbial communities (Hoseinifar *et al.*, 2018) and can affect central nervous system function (Ringø *et al.*, 2018). The relation of these factors can influence reproductive performance in various processes, such as fertility, fertilization, hatching and development of larvae. Consequently, probiotics can affect the organism's energy metabolism and energy balance, leading to a higher growth rate (Kuebutornye *et al*., 2019) and improvement of the reproductive performance (Carnevali *et al.*, 2017). Hence, the proper level of probiotics can lead to the appropriate function of the (HPG) axis. It has been well documented that these additives can affect growth, and hematological and plasma biochemical parameters of finfish (Rodiles *et al.*, 2018).

Using Hydroyeast Aquaculture as a reproductive enhancer is useful at levels of 15 g and 10 g /kg diet in improving the reproductive efficiency of adult *O. niloticus* males and females, respectively (Mehrim *et al.,* 2015).

Pedicoccus acidilactici is a product from lactic acid bacteria (LAB) and it is a probiotic. It is a live harmless bacterium that helps the well-beings of the hostanimal and contributes, directly or indirectly to the host animal against harmful bacterial pathogens, when consumed in adequate amounts, production of antagonistic compounds against pathogens (Ferguson *et al*., 2010). The supplementation of bactocell (*Pedicoccus acidilactici*) at 1gm/kg diet of Nile tilapia and Common carp has been found to improve fish immunity, livability, body weight gain and decrease the mortality level with improvement the productive and economic efficiency of fish Farms (EL-Banna and Atallah, 2009).

Therefore, this study was conducted to investigate the effect of Bactocill food supplement, *Pediococcus acidilactici* (1×10^{10} cells/g) on *Oreochroims niloticus* diets and its effect on reproductive performance of Nile tilapia broodstocks.

MATERIALS AND METHODS

This experiment study was carried out in two spawning seasons, the first season (56 days) from August to September in 2019 and second season (56 days) from June to August in 2020 to evaluate the effect of the dietary probiotic Bactocill, *Pediococcus acidilactici* (1×10^{10} cell/g) supplementation in the diets of broodstock Nile tilapia *(Oreochromis niloticus*) on reproductive performance in the two seasons. Hematological and biochemical parameters were conducted in the second season in summer 2020.

Experimental design:

The experimental design was completely randomized design with four treatments groups (n=24: 6 females and 2 males each hapa) in 12 hapas each group was divided into 3 replicates. The first group was control fed on basic diet (D1) without addition of the probiotics, the second group fed on basic diet supplemented with 0.50 g probiotic/kg diet (D2), the third group fed on basic diet supplemented with 1.0 g probiotic/kg basic diet (D3) and the fourth group fed on basic diet supplemented with 1.50 g probiotic/kg basic diet (D 4).

Experimental system and tilapia fish.

During the two spawning seasons (2019 and 2020), with different fish broodstock for each season. Twelve hapas (1.5 × 3.00 × 1 m in each) submerged in fresh water earthen pond of total area 2400 m² (40 x 60 m) and 1.5 m of depth. Ninety-six healthy of Nile tilapia [seventy-two females with an average body weight (262.53±20.22 g) and twenty-four males with an average body weight (279.39±24.12 g) were distributed into 12 hapas (8 fishes in each hapa, 6 females and 2 males). Nile tilapia broodstock were obtained from experimental broodstock at the fish hatchery and acclimatized to the to the pond conditions for two weeks with diet which containing 35.12% crude Protein (CP)at a rate of 3% of total biomass.

Table(1): Feed ingredients (%) and proximate analysis (%) of the experimental diets used in the experiment

Item	Experimental diet[1]
Feed ingredients (g 100 g-1)	
Fish meal (65%)	6
Corn gluten meal	17
Soybean meal (44%)	40
Yellow corn	15.5
Rice bran	8
Wheat bran	8
Soybean oil	2.5
D-Calcium Phosphate	1
Min. premix[2]	1
Vitamin Mix[3]	1
Proximate chemical analysis (%) on DM basis:	
Dry matter	90.4
Crude protein	35.12
Lipids	7.39
Crude fiber	4.85
Ash	6.18
Nitrogen free extract	53.54
GE (Kcal/100 g) [4]	488.32
P/E ratio [5]	71.92

[1]Diet (control diet)
[2]Each Kg mineral mixture premix contained Mn, 22 g, Zn, 22 g: Fe, 12 g, Cu, 4 g; I, 0.4 g,
Selenium, 0.4 g and Co. 4.8 mg. Each Kg.
[3]Vitamin and contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; BI, 0.4 g;
Riboflavin 1.6 g, B6, 0.6 g. B12, 4 mg, Pantothenic acid, 4 g. Nicotine acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg - Thiamine (0.08)-Riboflavin (0.03)-Niacin (0.02).
[4]GE (Kcal/100g DM) = Gross energy was calculated using factors 5.64, 9.44 and 4.12 kcal per gram of protein, lipid and carbohydrate, respectively after (NRC, 1993).
[5]P/E ratio = Protein to energy ratio mg crude protein/Kcal gross energy.

Water quality parameters of the experiments:

Data of water quality parameters for this experiment were monitored weekly and determined according to the methods of APHA (1992). Thermometer used daily to determine the water temperature (°C). Dissolved oxygen (DO, mg L^{-1}), were determined weekly using digital dissolved oxygen meter (Model Crison model oxi 45p, Germany). The pH was monitored weekly throughout the experimental period using Waterproof Portable digital PH Meter (PH Meter L20 Me1-1 Ler Toledo, Switzerland).Total ammonia-nitrogen (TAN, mg L^{-1}) was measured by Hanna 2.The results of water quality were summarized as the follows: Temperature: 26–30°C; DO: 6.2 – 6.8 mg/l; ammonia: 0.05 – 0.06 mg/l and PH: 6.9 – 8.

Fish reproductive activity:

Foreach dietary treatment group, females were checked for mouth brooding activity; the spawners were identified and spawn date was recorded. The eggs in the mouth of females were easily observed by visual inspection from outside the hapas and their behavior was clear indicators of reproduction. Fry yield were collected by fine nets twice in the same season, and counted to calculate the productivity of the Nile tilapia fry.

Gonadosomatic index (GSI):

GSI was calculated after the spawning season of 2019 and 2020 according to Albertine-Berhaut (1973) as following equations: GSI = (gonad weight/body weight) × 100.

Fecundity: was calculated after the spawning season of 2019 and 2020 from the following equations according to Hunter, (1992):

F = (number of eggs in the sample× gonad weight)/ gonad weight of sample

Relative fecundity (RF): was calculated according to kjesbu (1989) as following equations:

RF= total number of eggs / fish weight.

Blood sampling for hematological and biochemical parameters:

At the end of the 2020 experiment, blood samples were collected immediately, from the dorsal vein or heart of random live fishes, by sterile syringe in vials for hormonal analysis in serum.

Steroid Hormones: Blood samples were centrifuged at 4000g for 15 min, serum was collected and stored at -20°C for radioimmuno assay **(Rinchard *et al*., 1993).** 17β- Estradiol and testosterone, were measured using ELISA KITS for direct radioimmunoassay determination.

Statistical Analysis:

Data were subjected to one-way ANOVA analysis. All the statistical analyses were calculated using SPSS program version 20, (IBM SPSS2011, Statistics for Windows, Version 20.0. Armonk, NY: USA).Values are given as Mean ± SE. Mean was tested for significant differences at P-values ≤ 0.05 (Duncan, 1955).

RESULTS

This study investigated the effect of dietary probiotic Bactocill, *Pediococcus acidilactici* (1 x 10^{10} cell/g) supplementation in the diets of Nile tilapia broodstock (*Oreocromis niloticus*) and its effect on reproductive performance, hematological and biochemical parameters in two seasons.

The first season (summer of 2019)

Fry production:

Number of spawned, non-spawned and mortality of female Nile tilapia (*O. niloticus*) broodstock during summer 2019 are shown in Table (2). In the first season of the study, fry production (%) obtained from control diet (D1) without probiotic; 0.50 g probiotic/kg diet (D2); 1.00 g probiotic/kg diet (D3) and 1.50 g probiotic/kg diet (D4) are shown in Table (3). According to the results of the first season; it can be said that the highest values of the fry production (%) were observed for fish group fed on diet D3 (1.00 g probiotic/kg diet), followed by the fish group fed on the following diets (D4, D1 and D2, respectively) and there were no significant differences (P>0.05) between all diets. The lowest fry production was obtained in fish group fed on control diet D1 (without probiotic addition) in the first and second spawning were (38.89 and 33.33 %) respectively. Significant differences (P≤0.05) were observed in the second spawning season, the highest fry production were observed in fish group fed on diets D4 (50%), while the lowest value were recorded in diets D1 (33.33%).

Table (2): Number of spawned, non-spawned and mortality of female Nile tilapia (*O. niloticus*) broodstock during summer of 2019.

Season 2019				
Diet	**Female (N)**	**spawned female (N)**	**Non-spawned (N)**	**Mortality (%)**
D1	18	7	10	1
D2	18	7	10	1
D3	18	9	9	0
D4	18	8	10	0

Table (3): Effects of dietary probiotics supplementations on fry production from tilapia (*O. niloticus*) broodstock during summer 2019

Diet*	Second spawning			
	Fry production (N)	**Fry production (%)**	**Fry production (N**	**Fry production (%)**
D1	1326.00±44.86	38.89±5.56	1500.00±237.29	33.33^{b}±0.00
D2	993.33±263.28	38.89±5.56	2066.67 ±285.03	44.44^{ab}±5.55
D3	1402.00 ±212.23	49.99 ±9.62	2078.33 ±69.48	44.44^{ab}±5.56
D4	1062.00±270.35	44.44±25.46	1978.33 ±139.67	50.00^{a}±0.00

Means followed by different letters in the same column differ significantly (P<0.05).
[1]Diet 1(control diet without probiotic); diet 2,3and 4 were containing 0.50, 1.00 and 1.50 g probiotic/kg diet, respectively.

Gonadosomatic index:

In the first season (2019), gonado-somatic index value obtained from control diet (D1) without probiotic; D2, D3 and D14are shown in Table (4). The results shows that the highest values of the gonado-somatic index were for those fish fed on diet D3 (3.59), followed by the fish fed on the following diets (D2, D1 and D4, 3.23, 2.91 and 2.76 respectively). Significant differences (P≤0.05) were observed between all diets. The lowest gonado-somatic index was obtained for fish group fed on diet D4 (2.76).

Fecundity:

Fecundity results obtained from control diet D1, D2, D3 and D4 are in Table 4.Significant differences (P≤0.05) were observed between all treatments. The results showed that the highest values of the fecundity were for fish group fed on diet D3 (16947), followed by fish group fed on diets D2 and D1 4 (1404, 1229, respectively). The lowest Fecundity was obtained for fish group fed on diet D4 (1051.33).

Relative fecundity:

There are significant differences (P≤0.05) in the relative fecundity (Table 4). According to the results of the first season (2019); the highest values of the relative fecundity were obtained for fish group fed on diet D3 (5.80), followed by fish group fed on the following diets D2, D1 and D4 (5.02, 4.61 and 3.79, respectively). The lowest relative fecundity was obtained for fish group fed on diet D4 (3.79).

Survival rate (%)

Survival rate obtained is shown in Table (4). The highest values of the survival rate were estimated for fish group fed on dietsD3and D4 (100%), followed by the fish group fed on diets D2 and D1 (94.44%) and there were no significant difference (P>0.05) between all treatments. The lowest survival rates were obtained for fish group fed on dietsD2 and D1 (94.44%).

Table (4): Effects of dietary probiotics supplementations on gonado-somatic index, fecundity, relative fecundity and survival rate of Nile tilapia (*O. niloticus*) broodstock during summer of 2019

Diet*	Gonado-somatic index	Fecundity	Relative fecundity	Survival rate (%)
D1	2.91^{ab}±0.19	1229.00^{bc}±46.18	4.61^{b}±0.13	94.44±5.55
D2	3.23^{ab}±0.33	1404.00^{b}±29.51	5.02^{ab}±0.27	94.44±5.55
D3	3.59^{a}±0.15	1697.00^{a}±112.17	5.80^{a}±0.71	100.00±0.00
D4	2.76^{b}±0.08	1051.33^{c}±101.95	3.79^{c}±0.16	100.00±0.00

Means followed by different letters in the same column differ significantly (P<0.05).
[1]Diet 1(control diet without probiotic); diet 2, 3, and 4 were containing 0.50, 1.00 and 1.50 g probiotic/kg diet, respectively.

The second season in the summer during 2020

Fry production:

The number of spawned, non-spawned and mortality of female Nile tilapia (*O. niloticus*) broodstock during summer of 2020 are shown in (Table 5). There were significant differences in fry production (%) (P≤0.05) between all treatments (Table 6). The highest values of the fry production (%) were obtained in the second spawning for fish group fed on diet D4, followed by fish group fed on diets D1, D2 and D3, respectively. The lowest fry production (%) was obtained for fish group fed on diet D2, D3, and D4 in the first spawning (27.77 %). There were no significant differences (P>0.05) in fry production at the second spawning between all treatments.

Table (5): Number of spawned, non-spawned and mortality of female Nile tilapia (*O. niloticus*) broodstock during summer 2020.

	Season 2020			
Diet	**Female (N)**	**spawned female (N)**	**Non-spawned (N)**	**Mortality (%)**
D1	18	7	10	1
D2	18	7	9	2
D3	18	8	10	0
D4	18	10	7	1

Table (6): Effects of dietary probiotics supplementations on fry production (%) from Nile tilapia (*O. niloticus*) broodstock during summer of 2020

Diet*	First spawning		Second spawning	
	Fry production (N	Fry production (%)	Fry production (N	Fry production (%)
D1	1250.67^{b}±189.39	44.44±5.56	2469.00±465.01	41.11±4.84
D2	735.00^{bc}±105.63	27.77±5.56	1265.00±205.37	47.22±13.89
D3	296.00^{c}±36.66	27.77±11.11	1559.00±823.60	44.44±5.56
D4	1733.00^{a}±345.05	27.77±5.56	793.67±179.17	58.89±4.84

Means followed by different letters in the same column differ significantly (P<0.05).
[1]Diet 1(control diet without probiotic); diet 2,3and 4 were containing 0.50, 1.00 and 1.50 g probiotic/kg diet, respectively.

Gonado-Somatic index: In the second season (2020), gonado-somatic index results are shown in Table (7). The highest values of the gonado-somatic index were for fish group fed on diet D2 (4.28), followed by fish group fed on diets D1, D4 and D3, (2.98, 2.81 and 2.30 respectively) and there were significant difference between all diets (P ≤ 0.05).

Fecundity: Fecundity results obtained are shown in Table (7). The highest values of the fecundity were obtained for fish group fed on diet D2 (2034.33), followed by the fish fed on diets D3, D1 and D4, respectively. There were significant differences between all diets (P ≤ 0.05). The lowest Fecundity was obtained by for fish group fed on diet D4 (779.33).

Relative fecundity: There were significant differences (P≤0.05) in the relative fecundity between different diets (Table 7). The highest value of the relative fecundity were for fish group fed on diet D2 (7.02), followed by fish fed on diets D3, D1 and D4 (4.87, 4.47 and 3.36, respectively). The lowest relative fecundity was obtained for fish fed on diet D4 (3.36).

Survival rate (%): There were no significant differences (P>0.05) in survival rate (Table 7). There were no mortality were recorded for fish group fed on diet D4 (100%), followed by fish group fed on diets D1, D2 and D3 (94.44, 88.89 and 94.44%, respectively).

Table (7): Effects of dietary probiotics supplementations on gonado-somatic index, fecundity, relative fecundity and survival rate of Nile tilapia (*O. niloticus*) broodstock during summer 2020

Diet*	Gonado-somatic index	Fecundity	Relative Fecundity	Survival rate (%)
D1	2.98^{b}±0.06	1210.67^{b}±61.53	4.47^{b}±0.146	94.44±5.56
D2	4.28^{a}±0.31	2034.33^{a}±31.22	7.02^{a}±0.333	88.89±11.11
D3	2.30^{c}±0.04	1248.33^{b}±60.98	4.87^{b}±0.19	94.44±5.56
D4	2.81^{bc}±0.03	779.33^{c}±10.35	3.36^{c}±0.09	100.00±0.00

Means followed by different letters in the same column differ significantly (P<0.05).
[1]Diet 1(control diet without probiotic); diet 2, 3, and 4 were containing 0.50, 1.00 and 1.50 g probiotic/kg diet, respectively.

Estrogens and Testosterone Hormones: In the first (2019) and second season (2020), estrogens and testosterone hormones results obtained from control diet D1, D2, D3 and D4are shown in Figure (1). The highest level of estrogen or testosterone for Nile tilapia broodstock was obtained for fish group fed on diet D2, while the lowest level was obtained for fish group fed on diet D4 which containing 1.50 g probiotic/kg diet.

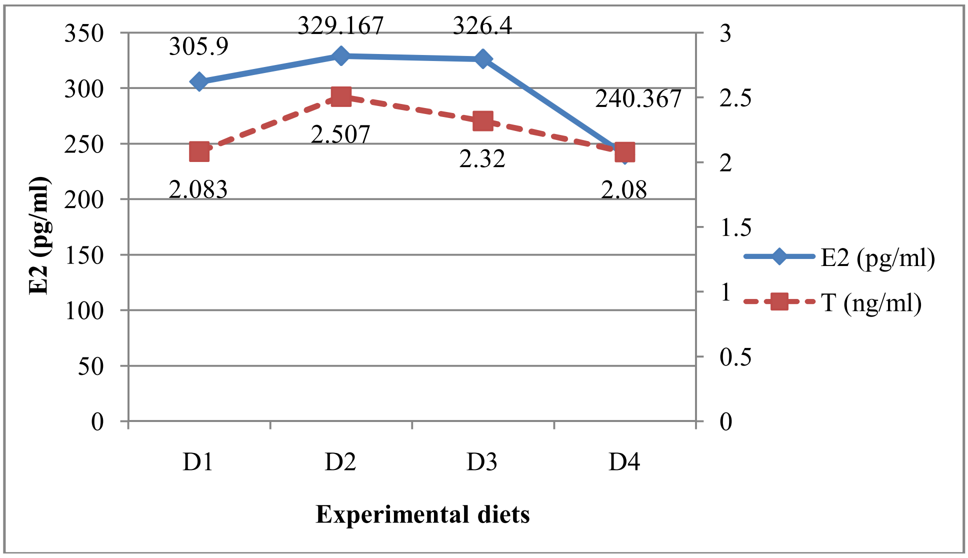

Fig. (1): Effects of dietary probiotics supplementations on estrogen of female and testosterone of male Nile tilapia (*O. niloticus*) broodstock

DISCUSSION

Probiotic effects on Nile tilapia (*Oreochromis niloticus*) reproduction:

Probotic addition in the diet consequently supports good oocyte development and maturation as well as higher vitellogenesis rate (Ghosh *et al*., 2007; Shim *et al*., 1989; Carnevali *et al*., 1993).

After, 56 days of feeding of dietary probiotic improved Nile tilapia (*Oreochromis niloticus*) reproductive performance, increasing GSI, fecundity, and fry production. This increase may be due to the concentration effect of probiotic to increase vitellogenesis and subsequently oocyte maturation, as well as metabolic signals both at central nervous system (CNS) and at peripheral level, as recently demonstrated in the zebrafish *D. rerio* (Gioacchini *et al*., 2010). Thus, the stimulatory role of probiotic on Nile tilapia reproductive performance may be due to both the activation of the neuroendocrine system that regulates reproduction and to local factors that control oocyte development and maturation. Gioacchini *et al.* (2010) investigated the effects of the probiotic *Lactobacillus rhamnosus*on zebrafish fecundity as a feed supplement. The higher number of ovulated eggs in vivo demonstrated the increase in fecundity and by the higher germinal vesicle breakdown rate was obtained with the in vitro maturation assay. In the present study, adult *O. niloticus* female fed tested probiotic revealed high improvement in the ovarian and fish GSI, fecundity, relative fecundity and fry production.

The results demonstrated the beneficial effects of probiotics on the reproduction of butter catfish, (*Ompok apabda*) the reproductive performance; the GSI, fecundity, and larval survival were significantly enhanced by probiotic administration (Rahman *et al*., 2018). Further studies have emphasized probiotics ability to stimulate gonadal development, maturation, gamete quality. Carnevali *et al*. (2017), reviewed the effects of probiotics on the integrated control of fish metabolism. In the last section of their assessment, condenses recent findings regarding to the positive effects of probiotic administration on fish gonads, which deeply support their role towards reproduction on Nile tilapia.

Supplementation of feed with probiotic primalac significantly increased the Gonadosomatic indices, fecundity and larval quality of female broodstock. (Abasali and Mohamad, 2010). Carnevali *et al.* (2013) investigated the effects of *L. rhamnosus* on the reproductive performance of zebrafish as a biomedical fish model. They recorded that long-term administration of *L. rhamnosus* has a positive effect on the physiology of reproductive system. In addition, better ovulation detected in broodstock feed with *L. rhamnosus* supplemented diet.

Sex steroids are implicated in many important physiological processes in all vertebrates, and measurement of blood sexual hormones seems to be a valuable tool for assessing the reproductive cycle of fish (Guerrero *et al.,* 2009). In the present study, increasing levels of the dietary probiotic led to increase in serum total testosterone and estradiol of *O. niloticus* males and females, respectively, these results were in agreement with Mehrim *et al.* (2015).

CONCLUSION

This study concluded that probiotics addition to fish diets optimizes the reproductive performance of Nile tilapia broodstocks, resulting in healthy and economic advantages. The present study recommended regular supplementation of Bactocill, *Pediococcus acidilactici* (1 x 10^{10} cell/g) to Nile tilapia broodstock diets with at a dose of 1.5 g kg^{-1} of diet during reproductive season. Therefore, the tested probiotic in the present study could serve beneficial role for increasing the production of Nile tilapia hatcheries. In the future, it is necessary to conduct more research to assess the effects of some other probiotics for Nile tilapia and other commercially important fish species.

REFERENCES

Abasali, H. and S. Mohamad (2010). Effect of dietary supplementation with probiotic on reproductive performance of female live bearing ornamental fish. Research Journal of Animal Sciences, 4(4): 103-107.

Abasali, H. and S. Mohamad (2011). Effect of dietary probiotic level on the reproductive performance of female platy *Xiphophorus maculatus*. Agricultural Journal, 6(3): 119-123.

Albertine-Berhaut, J. (1973). Biologie des stades juveniles de téleostéens Mugilidae *Mugil auratus* Risso 1810, *Mugil capito* Cuvier 1829 et *Mugil saliens* Risso 1810: I. Régime alimentaire. Aquaculture, 2: 251-266.

APHA (1992). Standard Methods for the Examination of Water and Wastewater. American Public Health Association. Washington, DC., USA.

Bobe, J. and C. Labbé (2010). Egg and sperm quality in fish. General and Comparative Endocrinology, 165: 535-548.

Carnevali, O., G. Mosconi and A. Roncarati (1993). Yolk protein changes dying oocyte growth in European sea bass *Dicentrarchus labrax*. Journal of Applied Ichthyology, 9(3-4): 175-184.

Carnevali, O., M. A. Avella and G. Gioacchini (2013). Effect of probiotic administration on zebrafish development and reproduction. General and Comparative Endocrinology, 188: 297–302.

Carnevali, O., F. Maradonna and G. Gioacchini (2017). Integrated control of fish metabolism, wellbeing and reproduction: the role of probiotic. Aquaculture, 472: 144–155.

Duncan, D. B. (1955). Multiple range and multiple F test. Biometrics, 11: 344-358.

El-Banna. S. A. and S. T. Atallah (2009). Study the Role of Feed Additives in Prevention of Fish Diseases Incidence in *Oreochromis niloticus* and Common Carp Fish and Its Economic Importance. J. Arabian Aquaculture Society. December Vol. 4 No 2.

Elsheshtawy, A., N. Yehia, M. Elkemary and H. Soliman (2019). Investigation of Nile tilapia summer mortality in Kafr El-Sheikh governorate, Egypt. Genetics of Aquatic Organisms, 3 (1), pp. 17-25, 10.4194/2459-1831-v3-1-03.

FAO (2016). Fishery and aquaculture country profiles - The Republic of Kenya. Rome, Italy.

Ferguson, R. M. W., D. L. Merrifield, G. M. Harper, M. D. Rawling, S. Mustafa, S. Picchietti, J. L. Balcazar and S. J. Davies (2010). The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). J Applied microbiology, 109(3): 851- 862.

Ghosh, S., A. Sinha and C. Sahu (2007). Effect of probiotic on reproductive performance in female live bearing ornamental fish. Aquaculture Research, 38: 518-526.

Gioacchini, G., F. Maradonna, F. Lombardo, D. Bizzaro, I. Olivotto and O. Carnevali (2010). Increase of fecundity by probiotic administration in zebrafish (*Danio rerio*). Reproduction, 140: 953–959.

Gram, L., M. Jette, S. Bettina, H. Ingrid and F. N. Torben (1999).“Inhibition of *Vibrio anguillarum* By *Pseudomonas fluorescens* AH2, a Possible Probiotic Treatment of Fish.” Applied and Environmental Microbiology, 65(3): 969-973.

Guarner, F. and G. J. Schaafsma (1998).“Probiotics”. International Journal of Food Microbiology, 39(3): 237-238.

Guerrero, H. Y., E. Cardillo, G. Poleo and D. Marcano (2009). Reproductive biology of freshwater fishes from the Venezulan floodplains. Fish Physiol. Biochem., 35: 189-196.

Haroun, E., A. Goda and M. Kabir (2006). Effect of dietary probiotic Biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis iloticus* (L.). Aquaculture Research, 37(14): 1473–1480.

Hernandez, L. H. H., T. C. Barrera, J. C. Mejia, *et al.* (2010). Effects of the commercial probiotic *Lactobacillus casei* on the growth, protein content of skin mucus and stress resistance of juveniles of the Porthole livebearer *Poecilopsis gracilis* (Poecilidae). Aquaculture Nutrition,16(4):407–411.

Hoseinifar, S. H., Y. Sun, A. Wang and Z. Zhou (2018). Probiotics as means of diseases control in aquaculture, A review of current knowledge and future perspectives. Frontiers in Microbiology, 9: 24-29.

Hunter, J. R. (1992). Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. Fish. Bull. (Wash. DC), 90: 101-128.

Irianto, A. and B. Austin (2002). Probiotics in aquaculture: Review. Journal of Fish Diseases, 25: 633-642.

Izquierdo, M., P. H. Fernandez and A. Tacon (2001). Effect of broodstock nutrition on reproductive performance of fish. Aquaculture, 197: 25–42.

Kjesbu, O. S. (1989). The spawning activity of cod, Gadus morhua L. Journal of Fish Biology, 34(2): 195-206.

Kuebutornye, F. K., E. D. Abarike and Y. Lu (2019). A review on the application of *Bacillus* as probiotics in aquaculture. Fish and Shellfish Immunology, 87: 820–828.

Lombardo, F., G. Gioacchini and O. Carnevali (2011). Probiotic-based nutritional effects on killifish reproduction. Fish. Aquac. J., 2011: FAJ-33-44.

Mehrim, A. I., F. K. Fathy and E. H. Montaha (2015).“Hydroyeast Aquaculture® as a Reproductive Enhancer Agent for the Adult

Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758).” Fish Physiology and Biochemistry, 41(2): 371–381.

NRC (1993). (National Research Council), Nutrient Requirements of Fish. National Academy Press, Washington, D.C., 114 pp.

Pirarat, N., K. Takeshi, K. Takayuki, M. Masashi and E. Makoto (2006).“Protective Effects and Mechanisms of a Probiotic Bacterium *Lactobacillus rhamnosus* against Experimental *Edward siellatarda* Infection in Tilapia (*Oreochromis niloticus*).”Veterinary Immunology and Immunopathology, 113(3): 339–47.

Rahman, M. L., S. Akhter, M. D. K. M. Mallik and I. Rashid (2018). Probiotic enrich dietary effect on the reproduction of butter catfish, *Ompok pabda* (Hamilton, 1872). International Journal of Current Research in Life Sciences, 7(2): 866-873. ISSN: 2319-9490.

Rinchard, J., P. Kestemont, E. Kühn and A. Fostier (1993). Seasonal changes in plasma levels of steroid hormones in an asynchronous fish the gudgeon *Gobio gobio* L. (Teleostei, Cyprinidae). General and Comparative Endocrinology, 92: 168-178.

Ringø, E., S. H. Hoseinifar, K. Ghosh, H. V. Doan, B. R. Beck and S. Song (2018). Lactic acid bacteria in finfish–An update. Frontiers in Microbiology, 9: 1–37.

Rodiles, A., M. D. Rawling, D. L. Peggs, G. do Vale Pereira, S. Voller, R. Yomla and D. L. Merrifield (2018). Probiotic applications for finfish aquaculture. In D. Di Gioia and B. Biavati (Eds.), Probiotics and prebiotics in animal health and food safety, (pp. 197–217). Cham, Switzerland: Springer.

Salminen, S. O. A., Y. Benno and Y. K. Lee (1999). “Probiotics: How Should They Be Defined?” Trends in Food Science and Technology, 10(3): 107–110.

Shim, K. F., L. Landesman and T. J. Lam (1989). Effect of dietary protein on growth ovarian development and fecundity in the dwarf gourami *Colisalalia* (Hamilton). Journal of Aquaculture in the Tropics, 4: 111-123.

Spss, I. B. M. (2011). IBM SPSS statistics for Windows, version 20.0. New York: IBM Corp, 440, 394.

Soccol, C., L. Porto, M. Rigon, *et al.* (2010). The potential of probiotics: a review. Food Technology and Biotechnology, 48(4):413–434.

Verschuere, L., R. Geert, S. Patrick and V. Willy (2000).“Probiotic Bacteria as Biological Control Agents in Aquaculture.”Microbiology and Molecular Biology Reviews, 64(4): 655–671.

Wang, Y. B., J. R. Li and J. Lin (2008). Probiotics in aquaculture: challenges and outlook. Aquaculture, 281(1–4): 1–4.

Watanabe, T. (1995). Broodstock management and nutritional approaches for quality off springs in the Red Sea Bream. In: Brom age, N. R., Roberts, R. J. Eds., Broodstock Management and Egg and Larval Quality. (pp. 154-195). Cambridge Univ. Press, Cambridge, First Publication.

تأثير إضافة البروبيوتيك الغذائي باكتوسيل (بديوكوكس اسيدولاكتيسي) في العليقة على أداء التكاثر لأسماك البلطي النيلي

السيد عبد الله محمد عبد العال، محمد عبد الحميد الدناصورى، خالد أحمد محمد السيد، صفاء محمود شرف

قسم الإنتاج الحيواني والثروة السمكية، كلية الزراعة، الإسماعيلية، جامعة قناة السويس

أجريت هذه الدراسة التجريبية خلال موسمي تفريخ، من أغسطس إلى سبتمبر في عام ٢٠١٩ وفي عام ٢٠٢٠ (٥٦ يومًا في كل موسم) لتقييم تأثير مكملات البروبيوتيك الغذائية Bactocill، *Pediococcus acidilactici* (١×١٠`` خلية/جم) على الوجبات الغذائية التي تحتوي على أمهات البلطي النيلي وتأثيره على الأداء التناسلي في موسمين. كان التصميم التجريبي عبارة عن أربع مجموعات في أربع علائق تجريبية في ١٢ هابا ، تم تقسيم كل مجموعة إلى ٣ مكررات (ن = 42: ٦ إناث + ٢ ذكور). تغذت المجموعات الأربع (D1) و (D2) و (D3) و (D4) على نظام غذائي أساسي مكمل بـ ٠.٠ و ٠.٥٠ و ١.٠ و ١.٥٠ جم من البروبيوتيك/كجم غذاء أساسي على التوالي. البلطي النيلي (٧٢ أنثى و ٢٤ ذكر تم توزيعها على ١٢ هابا (٦ إناث و ٢ ذكور لكل هابا). يمكن القول في الموسم الأول من عام ٢٠١٩ أن أعلى قيم لإنتاج الزريعة كانت في التفريخ الثاني لتلك الأسماك التي تغذت علىD4 ، وكانت أعلى قيم للدليل المنسلي، الخصوبة والخصوبة النسبية لتلك الأسماك التي تتغذى على .D3 نتائج الموسم الثاني ٢٠٢٠ يمكن القول أن أعلى قيم إنتاج الزريعة كانت في التبويض الثاني لتلك الأسماك التي تغذت على D4أعلى مستوى من الأستروجين أو التستوستيرون في أمهات البلطي النيلي عند تغذية الأسماك على D2 والتي تحتوي على ٠.٥٠ جم من البروبيوتيك/كجم علف. أوصت الدراسة الحالية باستمرار استخدام البروبيوتيك أثناء مرحلة الأداء التناسلي في موسم التفريخ لأمهات أسماك البلطي النيلي بجرعة ١.٥ جرام/كجم من العلف.