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Hydroyeast Aquaculture® as a reproductive enhancer agent for the adult Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758)

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Abstract Tilapias are becoming increasingly popular culture fish because of their superior culture adaptability. In recent years, there has been a great interest in the use of probiotics in fish aquaculture. The objectives of the present study were to evaluate the effect of dietary graded levels (0, 5, 10, and 15 g/kg commercial diet, referred to treatments numbers T_1, T_2, T_3, and T_4, for males and T_5, T_6, T_7, and T_8 treatments for females) of a new probiotic Hydroyeast Aquaculture® on hematological and biochemical parameters, serum sex hormones, and the reproductive efficiency parameters of the adult Nile tilapia *Oreochromis niloticus* for 8 weeks. Results revealed that high levels of probiotics diet, 15 g (T_4, ♂) and 10 g (T_7, ♀) probiotic/kg diet, significantly ($P \leq 0.05$) enhanced the physiological responses (hematological as well as serum biochemical parameters) together with, reproductive performances (sex hormones, testes and sperm quality parameters, absolute and relative fecundity, and ovarian measurements). Therefore, it could be conclude that Hydroyeast Aquaculture® is useful at levels of 15 g (T_4) and 10 g (T_7)/kg diet in improving the reproductive efficiency of adult *O. niloticus* males and females, respectively. Thus, the use of Hydroyeast Aquaculture® may be economically important for fish hatcheries.

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Introduction

Tilapias are versatile species found in almost each tropical aquatic system. *Oreochromis niloticus* (*O. niloticus*) has become one of the most commonly farmed freshwater fish species throughout the world (Beveridge and McAndrew 2000). The global production of tilapia is expected to exceed 3.2 million metric tons in 2010 and estimated to increase to about 8.9 million metric tons by the year 2020, as well as tilapias are produced in more than 100 nations surpassing any other farmed fish (Fitzsimmons et al. 2011). Particularly, the latest fish production statistics in Egypt revealed that tilapias are considered as the major cultured species; they contributed about 85.5 % (870.9 metric tons) of the total aquaculture production (1,017.7 metric tons) (GAFRD 2012). The increasing demand for fish fry not only necessitates optimization of culture operations but also expansion of the operation to meet the requirements. Broodstock productivity, low fecundity and the asynchronous nature of tilapia spawning cycles, remains the most significant constraint on commercial production (Coward and Bromage 2000).

Food availability and favorable feeding might have important effects on the energy needed in somatic

growth and reproduction of fish. These factors may lead to early maturing of individuals, resulting in the production of more gametes because of the improved metabolism and surplus energy (Wootton 1990). Furthermore, fecundity and egg quality are affected by nutritional deficiency in broodstock diets (Fernández-Palacios et al. 1995). Food supply and quality improve both fecundity and egg size in *O. niloticus* females, (Trewevas 1983), as well as in *O. mossambicus* (Rana 1985). In addition, males generally utilize less energy for gonad maturation than females, because sperms represent a small cytoplasmic investment. Male fishes mature earlier than females (Wootton 1990).

Probiotics are usually live microorganisms, which confer health benefit on host when administered in adequate amounts. Nowadays, probiotics are considered as an integral part of the aquaculture practices for obtaining high production (Nayak 2010). With an increasing demand for environment friendly aquaculture, probiotics are widely accepted (Wang et al. 2008a). Thus, recent studies have greatly interested in examining the effect of probiotics on the growth performance (Abd El-Rhman et al. 2009; Khalil et al. 2012); physiological efficiency (Marzouk et al. 2008; Mehrim 2009) and immune responses and resistance of *O. niloticus* (Aly et al. 2008).

Few studies have been designed to evaluate the efficacy of probiotics on reproductive performance such as, livebearing *Poecilia sphenops* (Chitra and Krishnaveni 2013); female platy-fish, *Xiphophorus maculates* broodstocks (Abasali and Mohamad 2011); zebrafish, *Danio rerio* females (Gioacchini et al. 2011). Success of the spawning process depends on the health of the adult fishes; therefore, it would be interesting to examine the effect of probiotics on the adult health, especially on cultured finfish species, including tilapia. Many studies revealed the positive effects of probiotics on *O. niloticus* fry (Lara-Flores et al. 2010; Abdel-Tawwab 2012) and fingerlings (Mehrim 2009; Ghazala et al. 2010). Recently, Khalil et al. (2012) demonstrated that Hydroyeast Aquaculture® enhances the growth performance of adults of both sexes; hence, it may be effective in enhancing the reproductive performance in adult *O. niloticus*. Realizing the importance of probiotics in adult health, we made an effort to evaluate the effects of graded levels of a new dietary probiotic Hydroyeast Aquaculture® on the hematological, serum biochemical parameters, sex hormones and different reproductive parameters (such as gonado-somatic indices, sperm quality, ova weight, ovarian-specific gravity, egg numbers and diameters, absolute fecundity, and relative fecundity) of both male and female *O. niloticus* adults for 8 weeks.

Materials and methods

The experimental management

This study was conducted at Fish Research Unit, Faculty of Agriculture, Al-Mansoura University, Al-Dakahlia governorate, Egypt. Adult Nile tilapia (*O. niloticus*), with an average initial body weights of 83.4 ± 0.001 and 80.1 ± 0.002 g for males and females, respectively, were purchased from integrated fish farm at Al-Manzala (General Authority for Fish Resources Development (GAFRD), Ministry of Agriculture), Al-Dakhalia Governorate, Egypt. Fishes were stocked directly into rearing tanks for 2 weeks to adapt and fed a basal diet during this period of adaptation. A total of 240 fish (males and females each 120) were subjected to eight experimental treatments (as three replicates (tanks) per treatment, Table 1); male and female fishes are separately stocked with 10 fish per tank. Each tank (1 m^3 in volume) was supplied

Table 1 Details of the experimental treatments for the adult *O. niloticus* male and female

Treatment	Details
T_1, ♂	Basal ration (BR) + 0 g Hydroyeast Aquaculture®/kg diet (as a control)
T_2, ♂	Basal ration (BR) + 5 g Hydroyeast Aquaculture®/kg diet
T_3, ♂	Basal ration (BR) + 10 g Hydroyeast Aquaculture®/kg diet
T_4, ♂	Basal ration (BR) + 15 g Hydroyeast Aquaculture®/kg diet
T_5, ♀	Basal ration (BR) + 0 g Hydroyeast Aquaculture®/kg diet (as a control)
T_6, ♀	Basal ration (BR) + 5 g Hydroyeast Aquaculture®/kg diet
T_7, ♀	Basal ration (BR) + 10 g Hydroyeast Aquaculture®/kg diet
T_8, ♀	Basal ration (BR) + 15 g Hydroyeast Aquaculture®/kg diet

with an air stone connected to an electric compressor. Waste was removed from each tank by siphoning, followed by refilling one-third of the tank with fresh underground water every day.

The tested probiotic Hydroyeast Aquaculture® formula was comprised of oligosaccharides (50,000 ppm); enzymes (amylase 3.7×10^6, protease 5×10^5, cellulose 2×10^5, pectinase 1×10^5, xylanase 1×10^4, phytase 3×10^3 units/kg); live yeast (5×10^{12} colony forming units (CFU)/kg); and probiotics bacteria (*Lactobacillus acidophilus*, *Bifedobacterium longhum*, *B. thermophylu*, and *Streptococcus faecium* 22.5×10^8 CFU/kg for each). It was produced by Agranco corp., Gables, USA.

Commercial diet, as basal ration (BR), used in the present study contains 25 % crude protein. It was purchased from Al-Manzala factory for fish food, GAFRD, Al-Manzala, Al-Dakhalia Governorate, Egypt. Table 2 presents ingredients in commercial diet as per the factory's formula and proximate chemical analysis carried out according to AOAC (2004). The commercial diet was ground and the tested probiotic (Hydroyeast Aquaculture®) added at levels of 0, 5, 10, and 15 g/kg diet, referred to treatments numbers T_1, T_2, T_3, and T_4, for males, while T_5, T_6, T_7, and T_8 treatments for females (Table 1); all diets were repelleted. The experimental diets were manually introduced twice daily at 9.0 am and 3.0 pm into 3 % of the fish biomass. The fish were weighed every 2 weeks by a digital scale (accurate to ± 0.01 g) to adjust their feed quantity according to the actual body weight changes, the fish biomass present in each tank.

Table 2 Ingredients and proximate chemical analysis (amount in percent, on dry matter basis) of the experimental basal diet

Ingredient	%
Yellow corn	22.50
Rice bran	23.00
Soybean meal (44 % CP)	37.50
Fish meal (65 % CP)	6.00
Salts	0.50
Calcium carbonate	4.67
Vegetable oil	3.00
Vitamins and mineral premix[a]	0.30
Di-nitro bio (anti oxidant)	0.03
Bintonite (as banding agent)	2.50
Nutrient composition	
Dry matter (DM %)	88.18
Crude protein (CP %)	25.10
Ether extract (EE %)	7.90
Ash (%)	6.30
Crude fiber (%)	6.00
Nitrogen free extract (NFE %)	54.70
Total carbohydrates (%)	60.70
Gross energy (kcal/100 g DM) (GE)[b]	465.60
Protein/energy (*P*/*E*) ratio (mg CP/kcal GE)[c]	53.90

[a] Each 3 kg premix contains: Vit. A, 12,000,000 IU; Vit. D_3, 3,000,000 IU; Vit. E, 10,000 mg; Vit. K_3, 3,000 mg; Vit. B_1 200 mg; Vit. B_2, 5,000 mg; Vit. B_6, 3,000 mg; Vit. B_{12}, 15 mg; Biotin, 50 mg; Folic acid 1,000 mg; Nicotinic acid 35,000 mg; Pantothenic acid 10,000 mg; Mn 80 g; Cu 8.8 g; Zn 70 g; Fe 35 g; I 1 g; Co 0.15 g; and Se 0.3 g

[b] GE (kcal/100 g DM) = CP × 5.64 + EE × 9.44 + total carbohydrates × 4.11 calculated according to NRC (1993)

[c] *P*/*E* ratio (mg protein/kcal gross energy) = CP/GE × 1,000

Blood samples

For all treatments, five fishes from each tank were randomly taken and anaesthetized at the end of the experiment; fishes were transferred in a small plastic tank containing 10 L water supplemented with 3 mL pure clove oil (dissolved in 10 mL absolute ethanol) as a natural anesthetic material. For the hematological parameters analysis, blood samples (5 mL of whole blood at each collection) were collected from the fish by puncturing caudal venous with a syringe needle and the samples were kept in small plastic vials containing heparin-anticoagulant. Other blood samples were collected in dried plastic tubes and centrifuged for 20 min at 3,500 rpm to obtain the blood serum. Serum samples were kept in deep freezer (−20 °C) until the biochemical analysis was carried out.

Blood hematological parameters

Heparinized whole blood was used for the determination of hemoglobin (Hb) level using commercial colorimetric kits (Diamond Diagnostic, Egypt). In addition, red blood cells (RBCs × 10^6/mm^3), blood platelets, and white blood cells (WBCs × 10^3/mm^3) were counted (Dacie and Lewis 1995) on an Ao Bright-Line Hämocytometer model (Neubauer improved, Precicolor HBG, Germany). RBCs indices (mean corpuscular volume, MCV, mean corpuscular hemoglobin, MCH, and mean

corpuscular hemoglobin concentration, MCHC) were calculated according to Fischbach (2000), whereas packed cell volume (PCV %) was measured according to Stoskopf (1993).

Serum biochemical parameters

Serum biochemical constituents were determined using commercial kits (Diagnostic System Laboratories, Inc, USA). Total protein and albumin were determined according to the methods described by Tietz (1990) and Doumas et al. (1971), respectively. The concentration of serum globulin was obtained by subtracting the albumin from the serum total protein concentration (Doumas and Biggs 1972). Serum total cholesterol (Tchol) was assessed using the following method described by Trinder (1969). In addition, concentrations of blood serum hormones, progesterone, and testosterone were determined using commercial ELISA test kits catalog No. BC-1113 (BioCheck, Inc) and BC-1115 (BioCheck, Inc), respectively (Tietz 1995).

Measurements of reproductive performance

Gonado-somatic indices and condition factor (K)

Both adult male and female *O. niloticus* were anaesthetized at the end of the experiment, where fish weight (W) and the total body length (TBL) were individually measured, for calculating the condition factor (K) according to Ricker's (1975) mathematical equation: $K = 100\ [(W\ (\text{g})/\text{TBL}^3\ (\text{cm})]$. Next, the fish abdominal cavity was opened to remove the gonads, and they were individually weighed too. Gonado-somatic index (GSI) was calculated as = gonads weight (g) × 100/fish weight (g) (Tseng and Chan 1982).

Sperm quality parameters

At the end of the experiment, fresh semen was collected from five adult males from each tank for the measurement of the sperm quality parameters. Seminal fluid was collected from the five remaining adult males in each tank to evaluate sperm quality parameters. Sperm motility was assessed subjectively, using a light microscope at 400× magnification. A five (1–5) category classifications (0; 0–25; 25–50; 50–75; or >75 %) of sperm motility were used (Boussit 1994). While sperm concentration was estimated microscopically using a Neubauer® counting chamber, sperm viability was measured by the eosin nigrosin staining method as described elsewhere (Parente et al. 1994).

Female reproductive parameters

At the end of the experiment, five adult females from each tank were randomly chosen for the evaluation of the reproductive performance parameters, where the five remaining fishes were excluded. Fishes were killed and ovaries opened by a sharp scalpel to obtain the egg samples. Egg weights, numbers, and diameters per female were measured. Eggs number was counted per gram of eggs and then related to ovary weight or body weight of fish. Then, absolute fecundity (AF) and relative fecundity (RF) were calculated using the following equations proposed by Bhujel (2000): AF = total weight of eggs per female (g) × number of eggs per gram, and RF = absolute fecundity/body weight (g).

Statistical analysis

Results are presented as mean values and standard errors of mean (±SEM) in three replicates ($n = 3$) of each treatment. All data were subjected to one-way analysis of variance (ANOVA) using SAS (2001) software package (version 9.2) to detect the overall effects of treatments (T_1–T_8). All ratios and percentages were arcsine-transformed prior to statistical analyses. The differences between mean of treatments were compared using Tukey's post hoc significant test, and differences were considered statistically significant at $P \leq 0.05$.

Results

Blood hematological measurements

Table 3 presents hematological parameters for adult male and female *O. niloticus*. Among all the experimental treatments by feeding Hydroyeast Aquaculture®, adult male *O. niloticus* receiving at 15 g/kg diet (T_4) only had a significant ($P \leq 0.05$) increase in RBCs, PCV %, and WBCs compared with the control

Table 3 Effects of dietary Hydroyeast Aquaculture® probiotic on blood hematological parameters of the adult *O. niloticus* male and female

Treatment	Hb (g/dL)	RBCs ($\times 10^6$/mm^3)	PCV (%)	MCV (μ^3)	MCH (pg)	MCHC (%)	Platelets ($\times 10^3$/mm^3)	WBCs ($\times 10^3$/mm^3)
T_1 ♂	5.70 ± 1.27	1.25 b ± 0.14	19.50 b ± 2.59	155.00 ± 2.88	43.80 ± 5.08	28.10 ± 2.77	28.00 ± 1.73	88.65 b ± 0.77
T_2	6.15 ± 1.81	1.27 b ± 0.18	19.10 b ± 1.67	154.00 ± 9.52	58.33 ± 22.84	35.50 ± 12.64	28.50 ± 3.17	99.50 a ± 2.13
T_3	6.45 ± 1.58	1.40 b ± 0.05	24.75 a ± 0.25	177.90 ± 9.17	44.90 ± 9.46	26.30 ± 6.69	30.00 ± 1.73	103.25 a ± 1.47
T_4	7.35 ± 0.95	2.00 a ± 0.11	27.50 a ± 0.40	139.25 ± 10.07	37.95 ± 6.95	26.60 ± 3.05	32.00 ± 2.88	105.85 a ± 5.68
P value	0.871	0.013	0.013	0.066	0.735	0.799	0.674	0.021
T_5 ♀	4.25 b ± 0.14	0.75 b ± 0.02	13.40 b ± 0.34	179.00 ± 12.12	56.35 ± 0.43	31.90 ± 1.90	27.00 ± 6.35	36.50 b ± 1.44
T_6	4.45 b ± 0.20	0.82 b ± 0.10	13.30 b ± 1.82	163.96 ± 4.58	56.05 ± 4.64	34.05 ± 1.87	29.50 ± 7.21	36.00 b ± 2.30
T_7	6.75 a ± 0.72	1.25 a ± 0.08	20.80 a ± 1.38	166.50 ± 0.46	53.55 ± 2.04	32.20 ± 1.32	39.00 ± 4.04	95.00 a ± 5.54
T_8	4.55 b ± 0.14	0.92 b ± 0.04	15.05 b ± 1.24	162.80 ± 5.31	50.05 ± 4.07	31.10 ± 3.52	34.50 ± 5.48	40.50 b ± 1.84
P value	0.005	0.006	0.005	0.393	0.527	0.830	0.517	0.0001

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

(T_1). Likewise, among all other treatments, adult female fed 10 g Hydroyeast Aquaculture®/kg diet (T_7) only showed an increase in Hb level, RBCs count, PCV %, and WBCs count ($P \leq 0.05$). No significant effects ($P \geq 0.05$) were observed in the hematological parameters in adult fishes (both males and females) fed other levels of dietary Hydroyeast Aquaculture® probiotic.

Serum biochemical parameters

Results of serum total proteins of adult male *O. niloticus* showed that fishes received treatment no. 4 had significant increase ($P \leq 0.05$) in total protein, albumin, and globulin concentrations; they also had the lowest ($P \leq 0.05$) value of albumin/globulin ratio among all treatments (Table 4). However, no significant differences were found in all serum protein parameters among adult male fishes receiving other treatments, T_2 or T_3. On the other hand, adult female *O. niloticus* fed 10 g inclusion of Hydroyeast Aquaculture®/kg diet (T_7) had a significantly increased serum total protein and albumin concentrations ($P \leq 0.05$) compared with the fishes received control treatment (T_5). However, no significant ($P \geq 0.05$) effects of the tested probiotic on globulin and albumin/globulin ratio were detected in rest of the treatments; the lowest albumin/globulin ratio was detected in T_8 among all treatments.

Figure 1A, B shows significant decrease in serum Tchol and significant increase in total testosterone ($P \leq 0.05$) only in the adult male *O. niloticus* receiving 15 g Hydroyeast Aquaculture®/kg diet (T_4) among all treatments. On the other hand, adult female *O. niloticus* fed dietary Hydroyeast Aquaculture® at level of 10 g/kg diet (T_7) had a significant ($P \leq 0.05$) decrease in serum Tchol, while an increase in serum progesterone concentrations among all treatments (Fig. 1C, D). Fishes fed other levels of probiotic also had a significant decrease in Tchol, while an increase in sex hormones in both sexes.

Reproductive performance parameters

Results depicted in Table 5 reflect the positive ($P \leq 0.05$) effects of tested probiotic treatment at level of 15 g/kg diet (T_4) on testes weight, GSI, sperm quality parameters (count, motility, abnormalities, and

Table 4 Effects of dietary Hydroyeast Aquaculture® probiotic on serum protein parameters of the adult *O. niloticus* male and female

Treatment	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Albumin/globulin ratio
T_1 ♂	3.80 c ± 0.17	2.20 c ± 0.05	1.60 b ± 0.11	1.39 a ± 0.06
T_2	4.25 bc ± 0.14	2.45 bc ± 0.08	1.80 b ± 0.05	1.37 a ± 0.005
T_3	4.40 b ± 0.11	2.55 b ± 0.08	1.85 b ± 0.02	1.36 a ± 0.02
T_4	5.45 a ± 0.20	2.85 a ± 0.08	2.60 a ± 0.11	1.10 b ± 0.01
P value	0.0006	0.003	0.0002	0.001
T_5 ♀	4.25 b ± 0.14	2.35 b ± 0.08	1.90 ± 0.05	1.23 ± 0.005
T_6	4.35 b ± 0.14	2.70 ab ± 0.05	1.65 ± 0.20	1.63 ± 0.24
T_7	4.90 a ± 0.05	2.90 a ± 0.17	2.00 ± 0.23	1.45 ± 0.19
T_8	4.80 a ± 0.05	2.60 ab ± 0.05	2.20 ± 0.02	1.18 ± 0.03
P value	0.006	0.034	0.097	0.127

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

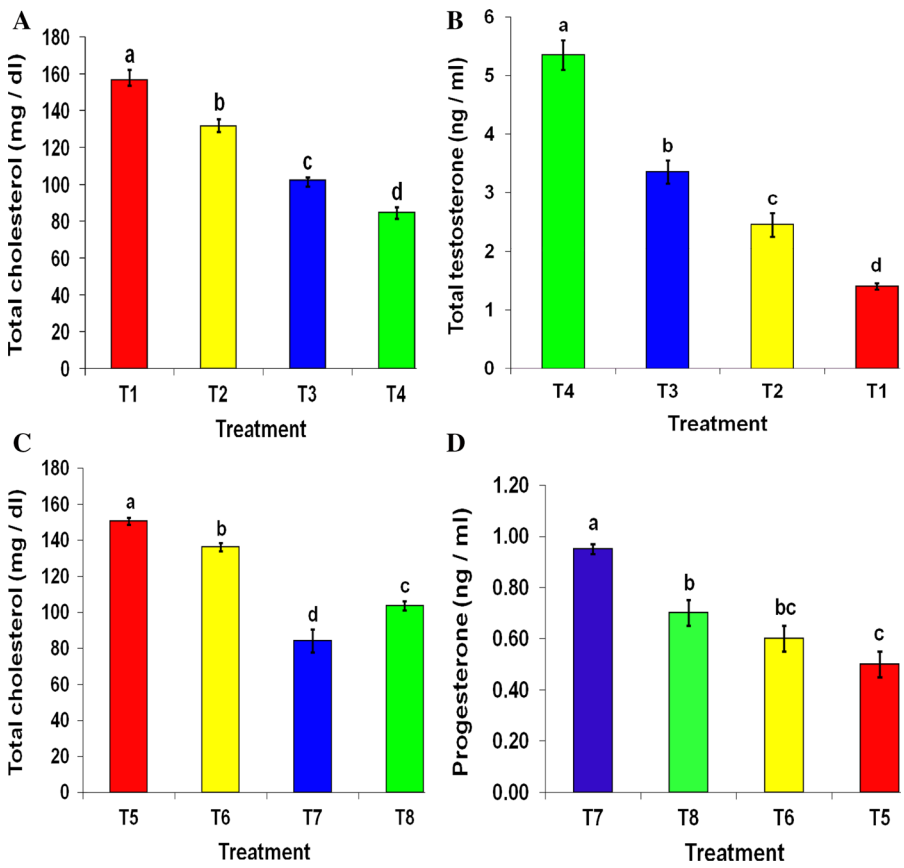

Fig. 1 Effects of dietary Hydroyeast Aquaculture® probiotic on serum biochemical parameters of the adult *O. niloticus* male (**A**, **B**) and female (**C**, **D**). Mean having *different small letters* are significantly different ($P \leq 0.05$)

dead) of the adult male *O. niloticus* among all treatments. However, no significant effects ($P \geq 0.05$) were observed in K-factor and sperm forward parameters among all treatments.

On the other hand, among all treatments, dietary inclusion of Hydroyeast Aquaculture® at level of 10 g/kg diet (T_7) in the adult female *O. niloticus* revealed significant ($P \leq 0.05$) values in K-factor, ova weight, ovarian-specific gravity, egg diameter, AF and RF parameters. However, no significant ($P \geq 0.05$) differences were observed in GSI and egg number/g in other treatments (Table 6).

Table 5 Effects of dietary Hydroyeast Aquaculture® probiotic on body, testes, and sperm quality of the adult *O. niloticus* male

Item	T_1	T_2	T_3	T_4
K-factor (%)	1.57 ± 0.02	1.56 ± 0.10	1.57 ± 0.07	1.65 ± 0.04
Testes weight (g)	1.20 b ± 0.11	2.13 b ± 0.57	2.10 b ± 0.50	4.03 a ± 0.40
GSI (%)	0.94 b ± 0.06	1.51 b ± 0.38	1.46 b ± 0.31	2.50 a ± 0.28
Count ($\times 10^6$/mL)	91.00 b ± 2.51	100.67 b ± 14.19	107.67 b ± 9.26	179.33 a ± 7.96
Motility (viability, %)	80.00 b ± 5.77	57.50 b ± 4.33	85.00 b ± 0.96	87.50 a ± 1.44
Forward (%)	3.50 ± 0.28	3.00 ± 0.57	4.00 ± 0.05	4.00 ± 0.07
Abnormalities (%)	23.50 a ± 0.86	18.00 b ± 1.15	10.50 c ± 0.86	6.50 d ± 0.86
Dead (%)	31.00 a ± 2.30	28.00 a ± 1.15	22.50 b ± 1.44	11.50 c ± 0.86

Mean in the same row having different small letters are significantly different ($P \leq 0.05$)

Discussion

In studying the immunopotentiators of fishes, hematological parameters are useful tools (Tukmechi et al. 2011). We found that increasing probiotic levels had positive effects on the hematological parameters in fishes of both sexes. While T_4 was the best treatment followed by T_3 concerning in *O. niloticus* males with significant increase in RBCs count, PCV %, and WBCs count, T_7 was considered the best treatment followed by T_8 in females. All these positive effects on the hematological parameters could be related to probiotic's role in improving the growth performance (Khalil et al. 2012). The significant increases in the different hematological parameters could also be due to beneficial physiological effects of the tested probiotic; by modulating specific functions in the gut and the immune system, the probiotics might contribute to the growth performance in addition to the nutritional impact of food (Isolauri 2004). In this context, Abdel-Tawwab et al. (2008) also reported higher RBCs, Hb, and Ht values in *O. niloticus*, fed diets containing 1.0–5.0 g yeast/kg. In a separate study by Rawling et al. (2009), erythrocytic counts, Hb content, and phagocytic activity were found to be higher in red tilapia fed diets containing probiotic bacteria *micrococcus species* than those of the control group. In a recent study, the number of monocytes has been found to be significantly elevated in channel catfish fed 0.2 % dietary yeast polysaccharides (Zhu et al. 2012); the increased number of blood monocytes might indicate an immunomodulatory effect. Others also reported in the same line (Welker et al. 2007; Hai and Fotedar 2009).

Hypoproteinemia is a condition resulting from abnormally low level of protein, decreased albumin, or increased globulin (Merck 1974). Interestingly, the present study found increasing levels of Hydroyeast Aquaculture® significantly increased serum total proteins but decreased albumin/globulin ratio in experimental fish of both sexes, along with the positive effects on hematological parameters; this could be explained in terms of dependence of growth factor on probiotic formula containing high levels of active live yeast (5×10^{12} CFU/kg min) and different beneficial bacteria species. Furthermore, yeasts contain various immunostimulating compounds, such as β-glucan, nucleic acids, mannan oligosaccharides, and chitin, which have been proved to enhance the immune response (Li and Gatlin III 2005). Our findings are in agreement with the previous studies of *O. niloticus* fed dietary probiotic (Mohamed 2007); *Labeo rohita* treated with different immunostimulants such as β-glucan and yeast RNA (Misra et al. 2006). In contrast, Diab et al. (2002) did not find any significant differences in serum total protein in *O. niloticus* fed diets containing 0.5, 1.0, and 1.5 % probiotic Biogen®. Wang et al. (2008b) also did not find any remarkable difference ($P > 0.05$) in serum total protein, albumin and globulin concentrations, and albumin/globulin ratio between the *O. niloticus* supplemented with the probiotic bacterium, *Enterococcus faecium* ZJ4 when compared to the control fishes fed the basal diet. While it has been suggested that increasing serum total proteins or decreasing ratio of albumin/globulin would be a good indicator of health condition of fish (Maita et al. 2002), the present study also shows that increasing probiotic levels positively impact on the immunity and health responses of fish.

Table 6 Effects of dietary Hydroyeast Aquaculture® probiotic on body, ovarian, and fecundity of the adult *O. niloticus* female

Item	T_5	T_6	T_7	T_8
K-factor (%)	1.65 b ± 0.03	1.74 b ± 0.06	2.04 a ± 0.23	1.73 b ± 0.12
Ova weight (g)	5.50 c ± 0.25	7.63 b ± 0.20	10.30 a ± 0.56	6.33 bc ± 0.49
GSI (%)	5.43 ± 0.27	5.74 ± 0.23	6.28 ± 0.80	5.85 ± 0.42
Ovarian-specific gravity (g/cm^3)	1.10 a ± 0.05	1.09 a ± 0.02	1.08 a ± 0.02	0.85 b ± 0.03
Egg number/g	270.00 ± 5.77	262.50 ± 21.65	325.00 ± 14.43	250.00 ± 28.86
Egg diameter (mm)	0.16 b ± 0.005	0.16 b ± 0.005	0.23 a ± 0.01	0.18 b ± 0.01
Absolute fecundity (AF)	1,117.5 c ± 93.90	2,046.8 b ± 190.00	3,416.6 a ± 297.95	1,411.8 bc ± 278.76
Relative fecundity (RF)	11.0 c ± 0.52	15.2 b ± 0.40	20.6 a ± 1.13	12.6 bc ± 0.98

Mean in the same row having different small letters are significantly different ($P \leq 0.05$)

With increasing levels of dietary probiotic in fish of both sexes, we found a significant decrease in serum Tchol. In this context, T_4 and T_7 were found to be the best treatments among all treatments for adult male and female *O. niloticus*, respectively. Probiotics influence blood cholesterol level either by inhibiting cholesterol synthesis or by decreasing its level directly through assimilation (Zacconi et al. 1992). Plasma Tchol levels were found to be elevated by the probiotic feeding in rainbow trout *Oncorhynchus mykiss* in contrast to most of the findings in endothermic animals (Panigrahi et al. 2010). Moreover, an increase in the plasma Tchol is correlated with a better health status in fish (Harikrishnan et al. 2003). However, in the present study, the decrease in the Tchol levels in both fish sexes may be because Tchol is a biosynthesis precursor of steroid hormones. Mechanistically, probiotic bacteria ferment food-derived indigestible carbohydrate to produce short chain fatty acids in the gut, which in turn decreases the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or redistributing cholesterol from plasma to the liver (Periera and Gibson 2002). Furthermore, some bacteria may interfere with cholesterol absorption from the gut, thereby affecting cholesterol metabolism or directly assimilating cholesterol. In addition, an elevation in the Tchol in response to viable probiotic feeding may be due to the influence of lactic acid bacteria on the cholesterol metabolism (Panigrahi et al. 2010).

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 a significant increase ($P \leq 0.05$) in serum total testosterone and progesterone of *O. niloticus* males and females, respectively. In this context, Yaron et al. (1983) found that estrogen (E_2) peaked on day 4 post-spawning in *O. aureus*. In fish, sperm quality may depend on several factors including the husbandry environment, feeding regime as well as quality of the feed, broodstock condition, genetic variability, and the methods utilized for artificial spawning (Rurangwa et al. 2004). In the present study, we observed positive effects of tested probiotic on sperm quality parameters, as reflected by significant increases ($P \leq 0.05$) in total testosterone (Fig. 1B), testes weight, and GSI ♂ (Table 6). Motility of sperm cells is used as an indication of sperm fitness and fertility (Fauvel et al. 1999). The observed sperm densities for tilapia species (3.59×10^9/ml) are within the magnitude (10^9) reported for other teleost species, Atlantic salmon (Aas et al. 1991), and sea bass, *Dicentrarchus labrax* (Fauvel et al. 1999). However, sperm density varied between 2.6×10^{10} and 3.5×10^{10}/ml in carp's species (Verma et al. 2009).

The gonado-somatic index (GSI) has been a useful index for monitoring the progression of gametogenesis in teleost fish (Guerrero et al. 2009). In the present study, adult *O. niloticus* female fed tested probiotic revealed high improvement in the ovarian and body measurements and fish fecundity (Table 6), which had a potential relationship with increasing the serum progesterone in these treatments compared with the control group (Fig. 1D). Tilapia species tend to sacrifice growth to maintain reproductive capacity (Coward and Bromage 1999) and have also been reported to exhibit tremendous plasticity in growth

and maturation (Turner and Robinson 2000). In physiological terms, early sexual maturity results in reduced growth and consequently poor feed conversion performances; this has economical impact on tilapia species enterprises in terms of higher feed costs and lower profitability.

Condition factor (*K*) is used for fish quality evaluation (Balcázar et al. 2006). The present findings revealed that *K* of *O. niloticus* females increased with increasing levels of probiotic up to 10 g/kg diet (T_7); however, no significant differences were found for *K* in case of *O. niloticus* males in all treatments. These differences could be due to differences in weights or lengths in males and females. In addition, *K* has been used as an index of growth and feeding intensity, as it decreases with greater fish lengths (Fagade 1979), as well as influences the reproductive cycle in fish (Welcome 1979). It has also been observed that *K* values in the same habitat and age groups have significant differences between both sexes, with higher *K* values for males than females. This probably indicates that males are generally heavier per unit length than females of the same age group. However, earlier studies indicated that during breeding period, female tilapia tend to starve for weeks while incubating eggs and protecting the fry in the mouth cavity; this lowers *K* values for females (Weatherly 1987). Furthermore, Kotos (1998) explained that sexual differences, age, change of season, gonad maturity stages, and nutritional levels of fish have an influence on *K* values.

Interestingly, in the present study, increasing levels of Hydroyeast Aquaculture® led to significant enhancement in reproductive efficiency of both fish sexes. In other words, the positive effects of the tested probiotic on fish reproductive parameters may be related with fish sex, maturation stage, probiotic level, exposure period, the probiotic components, and its positive effects on the physiological responses. Several studies have shown the importance of balancing the composition of dietary unsaturated fatty acids in fish to ensure optimized broodstock reproductive performances and enhanced larval quality; essential fatty acids also supply energy to sustain the spawning activities (Ling et al. 2006). In addition, probiotic bacteria also positively affect the production of the vitamins, particularly the B group vitamins (Ghosh et al. 2007).

Conclusions

The present study found the potential of feed additives, such as probiotics, as effective ingredients to improve the reproductive performance of fish. We found that Hydroyeast Aquaculture® probiotic is useful at levels of 15 g/kg diet (T_4) and 10 g/kg diet (T_7) for adult Nile tilapia *O. niloticus* males and females, respectively, in enhancing physiological responses together with reproductive efficiency. Therefore, the tested probiotic may also be beneficial from the economic point of view, especially for fish hatcheries.

However, further studies required to establish the efficacy on this new probiotic (Hydroyeast Aquaculture®) on Nile tilapia *O. niloticus* broodstock and other broodstock fish species in improving their reproductive efficiency on the commercial scale. In addition, more in depth studies are also required in establishing the appropriate dosage to achieve the highest benefits.

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