Influence of dietary commercial Beaker's yeast, *Saccharomyces cerevisae* on growth performance, survival and immunostimulation of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*.

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Abstract:
Eight weeks feeding trials were conducted to examine the effect of dietary commercial brewer's yeast, (Beaker's yeast), *Saccharomyces cerevisiae* on growth performance, survival and immunostimulation of Nile tilapia, *Oreochromis niloticus*. Brewer's yeast supplemented at 0, 1, 2, 3 and 6 gm/kg diet A, B, C, D and E respectively. Each diet was fed to triplicate group of *O. niloticus* with initial body weight at 77.39 ± 5.33 g at 8 weeks feeding period. Control group fed non supplemented diet at total period of experiment. Final weight, weight gain, specific growth rate (SGR), condition factor (CF) were recorded, and the optimum growth performance were obtained with 3.0 g yeast/kg diet. Physiological and biochemical parameters (RBCs count, Hb concentration, HCT value, glucose and lipids of fish), cellular immune parameters (total leucocytic count, phagocytic activity) and hormonal immune parameters (Total protein, albumin, globulin and lysozyme concentration) were significantly elevated than the control group(fed on A diet) and improved in *O. niloticus* fed brewer's yeast up to 3.0 g/kg diet. After experimental period (8 weeks) fish from each group were challenged by pathogenic *Aeromonas hydrophila* IP, kept under observation for 7 days, total fish mortality, clinical signs were recorded, and mortality percent decreased with the increase of yeast level in fish diets. [Nature and Science 2010;8(3):96-103]. (ISSN: 1545-0740).

Keywords: *Oreochromis niloticus* ; brewer's yeast ; growth performance ; immuno-stimulation ; condition factor ; immune promoters ; *Aeromonas hydrophila*.

1. Introduction

With the worldwide fish production and intensive cultivation system, fish are subjected to a wide spectrum of diseases which lead to great losses and decrease in fish production. The lack of effective disease control has the potential of being the chief limiting factor of realization of highly stable fish production (Phillip *et al*., 2000). Improving fish performance and disease resistance of cultured organisms are major challenges facing fish culturists, moreover bacterial diseases are one of the limiting factors for fish culture including Nile tilapia in particular, *Aeromonas hydrophila*, cause mass mortalities in several species and is the etiological agent of several diseases (Rahman *et al.,* 2001; Li *et al.,* 2006 and Abdel-Tawwab *et al*., 2008). Most of chemicals and antibiotics are ineffective in cleaning an infective cultivation system also their uses are major expenses that significantly reduce the profitability for fish production so, prevention is better than cure (Clark *et al*., 2000 and Phillip *et al*., 2000). Therefore, several alternative strategies to use of antimicrobials have been proposed such as immunotherapy like probiotics and another immunostimulants such alginic acid, mannon oligosaccharides B- glucan and live yeast *Saccharomyces cerevisae* which that may serve as dietary supplements to improve fish growth and immune responses (Irianto and Austin, 2002). Brewer's yeast, *Saccharomyces cerevisae* contains various immunostimulating compounds such as B-glucans, nucleic acids as well as mannon oligosaccharides which has the capability to enhance immune responses (Ortuno *et al*., 2002 and Abdel-Tawwab *et al*., 2008) as well as growth performance (Olivo-Teles and Goncalves, 2001; Lara-Flores *et al*., 2003; Li and Gatlin 2003, 2004, 2005 and Abdel-Tawwab *et al*., 2008) of various fish species, however, the administration of yeast has been recognized to have important effect as immunostimulants agent (Sakai, 1999). Recent investigations have showed that live brewer's yeast, *Saccharomyces cerevisae* and B (1, 3) glucans long chain polysaccharides containing repetitive glucopyronosyl which extracted from the yeast cell wall have the ability to stimulate non-specific defense mechanisms in vivo and vitro (Nayar, *et al.,* 1998). Therefore, the aim of present study was a trial to evaluate the efficacy of brewer's yeast, *Saccharomyces cerevisae* on growth performance, survival, immunostimulation for *O. niloticus* challenged with *A. hydrophila*

2. Materials and Methods

2.1 Experimental diets:

The formulation of the diet to give 45% crude protein, 19.9% lipid, 13.2% ash and 1.89% fiber and 8.7% moisture.

Five diets were prepared, A, B, C, D, and E, each supplemented with 0, 1, 2, 3 and 6 g/kg diet respectively dry brewer's yeast was added to diet. The diets were prepared by thoroughly mixing the dry ingredients with oil then adding cold water until stiff dough resulted. The dough was placed into a grinder for through mixing and extruded through 2.0 mm diameter strand. Diet was stored at 4 °C until used, control diet (0) brewer's yeast; diet (A) was prepared in the same way without the addition of brewer's yeast.

2.2 Experimental design:

100 *O. niloticus* fish with mean body weight of 70 ± 5 g obtained from Kafr El- Sheikh Governorate (private fish farm). They were randomly distributed in 4 aquaria at a density of 20 fish per each. The experimental diet was fed at feeding rate of 3% fish weight per day. Feeding rate was adjusted to actual fish biomass in each treatment.

The fish were fed two times per day, 7 days per week for 8 weeks. The water of the experimental aquaria replaced every 24 hr to prevent accumulation of ammonia and other toxic metabolites and uneaten diet were removed from the bottom. Continuous aeration was also provided to maintain dissolved oxygen level near saturation.

2.3 Water quality analysis:

Water samples were collected weekly at 20 cm depth from each aquarium, dissolved oxygen; temperature and unionized ammonia were measured in all treatments.

2.4 Growth performance:

Growth performance was determined and feed utilization was calculated as following:

-Weight gain= final weight(g)–initial weight (g).

-Condition factor (CF) = $\frac{\text{Weight (gm)}}{\text{Length (cm)}} \times 100$

-Specific growth rate (SGR) =
(Ln Wt– Ln Wo) X 100 / n

Lin: is the natural logarithm

-Total gain (g/ fish) = Wt – Wo

Wt: is the final fish weight (gm) at the end of the experiment.

Wo: is the initial fish weight (gm) at the start of the experiment.

n: is the duration period of the experiment in days.

-Feed conversion ratio (FCR) = Feed intake (g) / weight gain (g).

-Survival = $N_t \times 100 / N_o$.

Where N_t and N_o are the final and initial numbers of fish in each replicate.

2.5 Analysis and measurement:

At the initiation of the experiment, the fish were fasted for 24 hr and weight after being anesthetized with eugenol 1:10,000. Fish were not fed for 24 hr prior to blood sampling; fish blood was collected with disposable syringe from the caudal vein. The collected blood was divided into two sets of eppendorf tubes. One set contained 500µ sodium heparinate/ ml, used as an anticoagulant for hematology (hemoglobin, hematocrite and red blood cells counting). The second set was left to clot at 4 °C and centrifuged at 5000 rpm for 5 min. at room temperature; the collected serum was stored at -20 °C for further assays.

Red Blood Cells (RBCs) were counted under light microscope using Neubauer haemocytometer after dilution with phosphate- buffered saline, Hemoglobin (Hb) level was determined colorimetrically by measuring the formation of cyanomthaemoglobin using a commercial kit. Total protein content of serum was determined colorimetrically according to Henry (1964). Albumin and globulin were determined colorimetrically according to Wotten and Freeman (1982). The determination of lysozyme level in serum was determined as described by Ellis (1990). Results are expressed in units of lysozyme / ml serum. One unit is defined as amount of sample causing a decrease in absorbance of 0.001 min^{-1} at 530 nm compared to the control (*Micrococcus lysodeiktics* solution without serum). Phagocytosis was determined according to Kawahara *et al*., (1991).

Breifly fifty µ g *Candida albicans* culture were added to 1 ml of whole blood collected from treated and control fish then shacked in water bath at 23-25 °C for 3-5 hr, air dried blood smears were then stained with Giemsa stain.

Phagocytic cells which contain intracellular yeast cells in random count of 100 phagocytic cells and expressed as percentage of phagocytic activity (PA).

Phagocytic activity (PA) = Percentage of Phagocytic cells containing yeast cells.

Challenge test:

At the end of the study fish in each group were divided into two subgroups.

The first subgroup was challenged with pathogenic *Aeromonas hydrophila* at 0.2 ml dose of 24 hr. Saline from virulent bacterial broth of *Aeromonas hydrophila* 1 X 10^7 cells / ml was injected interperitoneal (IP) according to Schaperclaus *et al*., (1992).

The second subgroup was IP injected with 0.2 ml of saline solution as control. All groups were kept under observation for 7 days to record clinical signs and daily mortality rates.

3. Results and Discussion:

3.1 Clinical picture and postmortem findings:

Clinical picture and postmortem findings of experimentally inoculated fish of all treatments and control were nearly similar but varied in severity of developed lesions; they include poor appetite, loss of equilibrium with erratic movement of some fish, swimming with head down due to abdominal distension and loss of all reflexes and death. These beside presence of congestion and hemorrhage of the body and all fins, dorsal and caudal peduncle, Fig.1 (A), protruded anal opening Fig.1 (C), internally, congestion of all internal organs, Fig.1 (B) with serous yellowish fluid in abdominal cavity, the liver enlarged with hemorrhagic patches, distended gall bladder, enlarged spleen, while gills varied from pale anemic in some cases to congested in other cases Fig.1 (D).

Fish mortality after IP injection of *A. hydrophila* increased in the 1st day after injection till the 3rd day then decreased, table (1) and, moreover, total fish mortality 7 days after IP injection with *A. hydrophila* decreased with the increase of yeast supplementation.

3.2 Growth performance and survival:

Final fish weight, weight gain and specific growth rate increased significantly ($P<0.05$) with increase in dietary yeast level (table 2).

The optimum growth was obtained at 1.0 – 3.0 g yeast / kg diet while the control diet produced the lowest growth.

There were significant change in survival among the different treatment where there was significant survival rate was start from 2.0 to 6.0 g / kg diet. This means that the brewer's yeast affects survival of fish also.

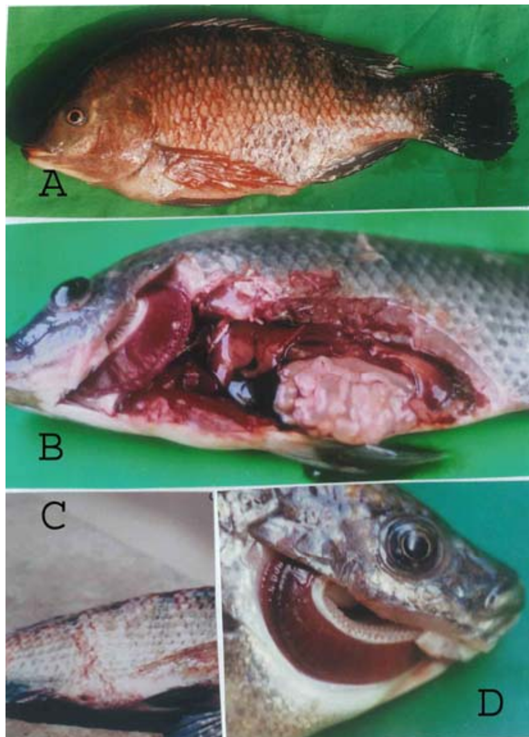

Fig. 1 *Oreochromis niloticus* Showing, A. congestion and hemorrhage of the body and all fins, B. congestion of all internal organs, C. protruded anal opening, D. Congested Gills.

3.3 Hematological and immune parameters:

Fish fed diets containing 1.0 – 3.0 g brewer's yeast / kg diet exhibited higher RBCs, Hb, HCt, total protein, albumin, globulin, lysozyme concentration, phagocytic activity and phagocytic index. Values up to 3.0 g/ kg diet after which those parameters decreased but not reached to those of control group, table (3).

Brewer's yeast, *Saccharomyces cerevisiae* have been used in aquaculture as a probiotic due to their fast growth, low cost, high stability and fact that they are not common constituents of feed (Irianto and Austin, 2002).

In spite of all these advantages, there is little information on the use of whole yeast in fish diets concerning the hypothesis that in vivo administration of whole yeast could enhance the fish immune system (Ortuno *et al.,* 2002).

Brewer's yeast, *S. cerevisae* have been recognized to have potential as a substitute for live food in the production of certain fish (Nayar *et al.,* 1998) or as a potential replacement for fish meal (Olivo **-** Teles and Goncalves, 2001).

In the present study, the supplementation of commercial live yeast, *S. cerevisae* improved growth and fed utilization. These results agree with that obtained with Catla carp (Mohanty *et al.,* 1996), mrigal Carp (Swain *et al.,* 1996), hybrid striped bass (Li and Galtin, 2003, 2004 and 2005) and Japanese flounder (Toaka *et al.,* 2006a). Similar results were obtained when *S. cerevisae* was added to fish diet of Nile tilapia (Lara – flores *et al.,* 2003 and Abdel-Tawwab *et al*., 2008). The effect of yeast incorporation in diet of digestive enzymes activity of sea bass D. labrax and reported that it lead to great improvement of the survival and growth rates.

Tovar *et al.,* (2002) in addition Irianto and Austin, (2002) reported that yeast was capable of adherence to the gut when supplied with diet led to enhance amylase secretion and stimulation of brush border membrane enzyme. The improved fish growth and feed utilization may possibly be due to improved nutrient digestibility. Tovar *et al.* (2002), Lara – Flores *et al*. (2003), Wache *et al*. (2006) and Abdel – Tawwab *et al.* (2008) found that the addition of live yeast improved diet and protein digestibility which may explain the better growth and feed efficiency with yeast supplements.

Brewer's yeast supplementation significantly affect the whole fish body composition. These results suggest that yeast supplementation plays a role in enhancing feed intake with subsequent enhancement of fish body performance.

The better feed intake in yeast supplemented diets in this study was recorded from 1.0 up to 3.0 g / kg diet; this may have been due to increased fish appetite resulting in a higher feed intake and therefore improved growth performance, physiological and biochemical analysis often provide vital information for health status about cultured fish (Cnaani *et al*., 2004; Rehulka *et al.,* 2004 and Abdel – Tawwab *et al*., 2008).

In the present study fish fed diets containing 1.0 g yeast to diets containing 3.0 g yeast / kg diet revealed higher RBCs, Hb and HCt values, also, protein, albumin and globulin values were increased up to 3.0 g yeast / kg diet after those the parameters were decreased. These results suggest an improvement of fish health when fed yeast supplement, measurement of albumin, globulin and total protein in serum is of considerable diagnostic value in Nile tilapia because it relates to general nutritional status as well as the integrity of vascular system and liver function (Abdel – Tawwab *et al*., 2008). These results nearly agree with the result obtained by Oliva – Teles and Goncalves (2001); Li et al., (2003) & (2005) and Abdel – Tawwab *et al.,* (2006) & (2008**).**

In regard to the immunological parameters, TLC, lysozyme concentration, phagocytic activity and phagocytic index that there is significant increase than that of the control group (A diet) from up to diet

contain 3.0 g / kg diet then decreased to the level not reached to the control group. These results nearly similar to that obtained by Ortuno et al., (2002) who reported that lyophilized whole yeast *S. cerevisae* in the diet of sea bream activates phagocytic activity and phagocytic index also Nevien (2005) reported that *O. niloticus* fed on *S. cerevisae* supplemented diet showed increased TLC, neutrophil count, phagocytic activity (P.A) and phagocytic index (P.I). Similar results were obtained by (Siwicki *et al.,* 1994) who observed an increase of the same cellular activities in rainbow trout after feeding *S. cerevisae*. This enhanced cellular activity could be attributed to many factors, first the presence of glucon receptors on the cell surface of blood monocytes, macrophages and neutrophils (Esteban *et al.,* 2001).

Nucleic acids especially yeast – RNA could act as immune activators and essential immune activity in mammals not only growth and reproduction (Rudolph *et al*., 1990 and Cerra *et al*., 1991). The administration of yeast B- glucans by injection strongly activates serum lysozyme activities in fish (Engstad *et al*., 1992; Santarem *et al*., 1997 and Paulsen *et al*., 2001).

Concerning resistance against diseases, mortality rate of *O. niloticus* fed on yeast supplemented diet for 8 weeks after I/ P injection of *A. hydrophila* was 50, 30, 0 and 10% respectively comparing to control +ve , 90% and control –ve, 0%. The mortality rate was decreased with increase of the yeast supplementation; this may be due to increased phagocytic activity, phagocytic index and serum lysozyme concentration. These results agree with Abdel – Tawwab *et al.,* (2008) who suggest that the yeast supplementation could increase the non-specific immune system of Nile tilapia resulting in resistance to *A. hydrophila*. Also, Toaka *et al.,* (2006b) investigated the effect of live and dead probiotic cells on the non – specific immune system of Nile tilapia such as lysozyme activity, migration of neutrophils and plasma bactericidal activity resulting in improved resistance to *Edwardsiella tarda* infection. Also, Abdel – Tawwab *et al*., (2008) proved that the cumulative mortality of Nile tilapia, ten days after I/P injection of *A. hydrophila* decreased significantly with the increased dose of yeast supplementation. Brewer's yeast is a source of nucleic acids and B 1,3- glucans which have been recognized to effectively enhance immune functions of African catfish (Yoshida et al., 1995), Atlantic salmon (Engstad *et al*., 1992), rainbow trout (Jorgensen *et al.,* 1993 and Siwicki *et al.,* 1994) and shrimp, *Penaeus nonadon* (Thanardkit *et al*.,2002). Moreover, Sakai *et al*., (2001) mentioned that the nucleotides from brewer's yeast RNA were capable of enhancing the phagocytic oxidative activities of kidney phagocytic cells, serum lysozyme in common carp as well as resistance to *A. hydrophila*.

In conclusion, brewer's yeast is promising as an alternative method to antibiotics for disease prevention in tilapia aquaculture and enhanced growth performance and the optimum level of dietary live brewer's yeast is about 3.0 g / kg diet for *O. niloticus*.

Table (1): The effect of dietary Brewer's yeast supplementation for 7 days in *O. niloticus*.

Item / Gp.(*)	No. of fish	Type Of inocu-lation	Days after challenge							No. of dead fish	No. of survi-ved fish	Mort-ality %	Surv-ival %
			1	2	3	4	5	6	7				
Contr-ol -ve	10	PBS*	0	0	0	0	0	0	0	0	10	0	100
Contr-ol +ve	10	A. hyd.**	4	1	1	1	2	1	0	9	1	90	10
Dose 1g/kg	10	A. hyd.	2	2	1	0	0	0	0	5	5	50	50
Dose 2g/kg	10	A. hyd.	2	1	0	0	0	0	0	3	7	30	70
Dose 3g/kg	10	A. hyd.	0	0	0	0	0	0	0	0	10	0	100
Dose 6g/kg	10	A. hyd.	0	1	0	0	0	0	0	1	9	10	90

(*)Gp.: group. ***PBS:** Phosphate buffer saline. ****A.hyd.:** *Aeromonas hydrophila*.

Table (2): The growth performance, condition factor and survival of *O. niloticus* fed on brewer's yeast for 7 days.

Items	Yeast levels, g / kg diet				
	A, control (0)	**B (1.0)**	**C (2.0)**	**D (3.0)**	**E (6.0)**
Initial weight (g)	77.39±15.46	76.93±15.38	77.20±15.44	77.10±15.42	77.30±15.46
Final weight	82.79±4.14	86.23±4.31	86.58±4.33	87.78±4.39	86.80±4.34
Weight gain (g)	5.4±0.27A	8.1±0.40aB	9.3±0.47abC	10.68±0.53abc	9.5±0.48ab
SGR %	0.17±0.017A	0.29±0.015a	0.26±0.013a	0.29±0.015a	0.26±0.013a
C.F	3.90±0.20A	4.70±0.24aB	5.00±0.50a	5.74±0.29ab	5.41±0.27a
Survival rate (%)	96.7±4.83	95.0±4.75	100±5.0	98.2±4.91	99.1±4.95

Small letters (a), (b),(c) and(d) represent a significant change to capital letters, A, B,C and D respectively (by LSD using ANOVA at $p \leq 0.05$).

Table (3): Showing (RBCs, Hb and HCt) and immunological parameters of *O. niloticus* fed on Brewer's yeast for 8 weeks.

Items	Yeast levels, g / kg diet				
	A, control (0)	**B (1.0)**	**C (2.0)**	**D (3.0)**	**E (6.0)**
RBCs	1.43±0.15A	1.89±0.09Ba	1.85±0.11aC	2.39±0.15ab	2.00±0.14a
Hb	4.30±0.15A	5.43±0.15Ba	5.43±0.15a	6.95±0.26ab	6.51±0.24ab
HCt	12.45±0.75A	17.51±0.90Ba	21.62±0.90abC	23.23±0.93ab	21.45±1.03ab
Total protein	3.62±0.13A	4.02±0.10Ba	4.68±0.14abC	5.14±0.13ab	4.54±0.12ab
Albumin	1.94±0.12A	2.30±0.10B	2.85±0.20abC	2.30±0.16c	2.56±0.13a
Globulin	1.68±0.08A	1.72±0.09B	1.83±0.09C	2.84±0.14abc	1.98±0.01a
Lysozyme	360.93±14.16A	401.43±14.66B	426.40±13.36a	466.75±15.52ab	408.51±13.35
Phagocytic activity	21.65±1.06A	26.40±1.56Ba	28.53±1.23aC	37.11±1.21abc	30.68±1.25a
Phagocytic index	3.09±0.19A	4.23±0.20Ba	6.25±0.25abC	9.50±0.40abc	8.64±0.31abc

Small letters (a), (b),(c) and(d) represent a significant change to capital letters A, B,C and D respectively (by LSD using ANOVA at $p \leq 0.05$).

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