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EVALUATION OF LIVE YEAST, *SACCAROMYCES CASTELLI* AS A PROBIOTIC IN NILE TILAPIA, (*OREOCHROMIS NILOTICUS*)

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ABSTRACT

The purpose of this study was to evaluate the effect of dietary administration of the live yeast *Saccaromyces castelli* in *Oreochromis niloticus* as immunostimulant, growth promoter and increase fish resistance to the bacterial diseases. *Saccaromyces castelli* was isolated from intestine of black carp *(Mylopharyngodon piceus)*. One hundred and thirty five apparently healthy *O. niloticus* (20 ± 3 g average body weight) were assigned randomly to three groups; each group in three replicates (15 fish per each replica). Commercial basal diet (crude protein 30%) was crushed, and then divided into three parts. The first part was basal diet mixed with sterile saline as a control (T_1). The second (T_2) and third (T_3) parts were basal diet mixed with (5 and 10 g of live yeast / kg diet) respectively. Fish were fed twice daily until satiation for 28 days. At the end of experimental period, fish was challenged i.p. with *Aeromonas sobria* and *Pseudomonase fluorescens* and kept under observation for 14 days.

Saccaromyces castelli had antibacterial activity against *Psudomonas anguilliseptica, Ps. fluorescens, Aeromonas veronii* and *A. sobria* in vitro with inhibition zones 38, 40, 13 and 42 mm in diameters respectively. The best growth rate, specific growth rate and feed conversion ratio were obtained with T_2 and T_3 compared to T_1. Significance increased in the heamatocrite value, respiratory burst, lysozyme activity and serum bactericidal activities among fish fed with diet contained live yeast compared to control fish. *Saccaromyces castelli* supplemented diet had positive effect on the total bacterial viable count in the intestine by decreasing its count than control group. Also it increased antibody titer of *A. sobria* and *Ps. fluorescence* than control group. Fish of group T_2 and T_3 had resistance against *A. sobria* and *Ps. fluorescence* while, fish of T_3 gave relative level of protection (RLP) higher than T_2 against *Pseudomonase fluorescence*. So, *Saccaromyces castelli* enhanced growth, immune response and resistance of *O. niloticus* against *A. sobria* and *Ps. fluorescens.*

INTRODUCTION

Aquaculture is still the fastest growing food producing sector, compared to other food commodities with an annual increase of approximately 12% (FAO, 2004 and FAO, 2009). Nile tilapia, *Oreochromis niloticus* (L.) is an important species for freshwater

aquaculture and the most widely cultured in tropical and subtropical countries. Consumers like tilapia's firm flesh and mild flavor, so markets have expanded rapidly (FAO, 2005).

Disease outbreaks are a serious constraint to the development of intensive aquaculture systems and can have a major impact on production due to mortality and decreased growth. Fish disease, especially bacterial infections, is a major problem facing the fish farming industry, which is currently growing fast (Peinado-Guevara and Lopez-Meyer, 2006).

The use of antibiotics in aquaculture as disease prevention and growth promotion may introduce potential hazard to public health and to the environment by the emergence of drug-resistant microorganisms and antibiotic residues. Furthermore, the normal microbial flora in the digestive tract, which is beneficial to fish, is also killed or inhibited by oral chemotherapy (Sugita *et al.*, 1991 and FAO/WHO/OIE 2006). With increasing demand for environment friendly aquaculture, the use of probiotics in aquaculture is now widely used instead of chemotherapy and antibiotics to increase safety protein production for human.

The positive effects of probiotic uses include the production of inhibitory compounds against pathogens, competition for nutrients and adhesion sites and the stimulation of both local and systemic immune responses (McCraken and Gaskins 1999). Probiotics include bacteria and yeasts, the beneficial role of yeasts being of particular interest because they represent an important source for nonspecific immuostimulants as β-glucans (Sahoo and Mukherjee 2002), chitin (Vecchiarelli 2000), nucleic acids as well as mannan oligosaccharides (Li *et al.*, 2004) and acts as well as growth promoters (Lara-Flores et al., 2003; Li and Gatlin, 2003, 2004, 2005) of various fish species.

Yeasts are a rich source of protein and B-complex vitamins. They have been used successfully as a complementary protein source in fish diet. Also, they have been used as a supplement in animals feed to compensate for the amino acid and vitamin deficiencies of cereals, and are recommended as substitute soyabean oil in diets for fowl (Gohi, 1991). In addition they are considered a cheaper dietary supplement as they are easily produced on an industrial level from a number of carbon-rich substrate by product (Lee and Kim, 2001).

The present study was carried out to evaluate the antibacterial effect of live yeast (*Saccaromyces castelli*) and study its use as growth promoters, mmunostimulant, and increase fish resistance to the bacterial diseases in cultured *Oreochromis niloticus*.

MATERIALS AND METHODS

This study was carried out in Central Laboratory for Aquaculture Research at Abbassa, Agriculture Research Center, Ministry of Agriculture, Egypt.

Antibacterial activity of yeasts in vitro:

Saccaromyces castelli was previously isolated from intestine of black carp and identified according to physical and biochemical tests. The antibacterial activity of the isolated yeast was carried out using disc diffusion method to study the antibacterial activity of yeast against harmful bacteria in fish, according to Gonsales, etal., (2006). The bacterial strains were obtained from Fish Diseases Department, Central Lab for Aquaculture Research in Abbassa. These bacterial strains were isolated previously from diseased Nile tilapia (*O. niloticus*). The pathogenicity of these bacterial strains was tested and the results confirmed that some of bacterial strains were pathogenic for *O. niloticus*. These bacterial isolates were *Aeromonas hydrophila, A. veronii, A. sobria, A. jandaei, Pseudomonase anguilliseptica, Ps. fluorescence, Citrobacter frundii, Enterobacter sp., Aerobacter sp., Moraxalla kingil, Actinobacter anitratus* and *Haemophilus aphrophilus.* The inhibition zones were determined after incubation at 30°C for 24 hours by mm.

Yeasts growths curve

One colony of *Saccaromyces castelli* at 24 hrs live was inoculated in 10 ml corn-meal medium and incubated at 25°C for 48hrs. The ten ml culture yeast was inoculated in 100 ml corn-meal medium and incubated at 25°C. Medium turbidity was increased by increasing yeast growth. The optical density of growth culture was measured every 24 hrs by using a spectrophotometer at wave length 620 nm according to Polonelli *et al.* (1997).

Yeast biomass Production:

A yeast culture at 48 hrs live in 400 ml corn-meal medium was inoculated in 4 liters corn-meal medium and incubated at 25°C. It was harvested at 4th day according to the growth curve by centrifugation at 3000 rpm for 15 minutes according to Peter *et al.* (1994).

Yeast dry weight:

10 ml of saline containing yeast cells was put on filter paper (three replicates) with known its weight and dried in oven at 60°C. The filter paper with resulting powder was weight until gave five fixed weight respectively. The difference between the filter paper weight alone and the filter paper weight with yeast powder was gave the amount of dry yeast in 10 ml saline contained the yeast cells (Leganes *et al.* 1987).

Yeasts probiotic activities:

Diet preparation:

Commercial basal diet (crude protein 30%) was crushed, and then divided into three parts. The first part was basal diet mixed with sterile saline as a control group. The second and third parts were basal diet mixed with 5 and 10 g of yeast / kg diet. The diets were reformed into pellets, air dry and stored at 4°C for the feeding experiment.

Feeding experiment:

One hundred and thirty five *Oreochromis niloticus* (20±2 g/fish) were randomly collected from earth ponds of Abbassa Fish Farm. The fish were acclimatized for two weeks, under laboratory condations and then divided into three equal groups. Twelve glasses aquaria (60x50x70 cm) were supplied with chlorine free water and continuous aeration using air pumping compressors. Each group, in three replicates, each contained 15 fish. The first group (T_1) fish was fed with the control diet (not treated basal diet). The second and third groups were fed with basal diet contained 5 and 10 g yeast/kg diet (T_2 and T_3) respectively. The fish were hand-fed for 28 days until satiation. The water of the aquaria was changed to third daily. The fish were weighted at beginning and the end of the feeding experiment. Fish and blood samples were taken at 14^{th} and 28^{th} day of the experiment.

Growth performance

The average weight-gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency ratio (FER) were calculated according to the following equations:

AWG (g/fish) = Average final weight (g) - Average initial weight (g)/experimental period (day)

SGR (%/day) = 100(ln final body weight (g) - ln initial body weight (g))/experimental period (day)

FCR = Feed intake (g) /weight gain (g)

FER = Body weight gain (g)/Feed intake (g)

Organ-somatic index:

The fish were killed by rapid cervical chopping, and then weighted. The liver and spleen were removed and weighed. Moreover, the hepatosomatic and splenosomatic indices were calculated according to (Fox et al., 1997). Organ-somatic index = (organ-weight (g) / bodyweight (g)) x 100.

Blood and serum sampling:

At the 14th and 28th day of the feeding experiment and after challenge test with two weeks, the fish were anaesthetized by immersing the fish in water containing 0.1 ppm tricaine methane sulphonate (MS-222). Blood-samples were collected from the caudal vein of fish, by using needles previously rinsed in heparin (15unit/ml) for the evaluation of hematocrit value and respiratory burst activity. For serum separation the non-heparinzed blood was centrifuged at 3000 rpm for 15 minutes. The serum was stored at -20°C in screw cap glass vials until used for lysozyme, serum bactericidal activities and to determine antibody titre.

Hematocrit level:

Hematocrite capillary tubes previously rinsed in heparin (15unit/ml) were filled 2/3 with whole blood, tubes were centrifuged in hematocrite centrifuge for 5 minute, after centrifugation. The percentage of erythrocyte volume is measured by hematocrite tube reader (Schaperclaus *et al.* 1992).

Respiratory burst activity by measuring Nitro Blue Titrazolium (NBT):

0.1 ml blood was placed into microtiter plate then equal amount of 0.2% NBT solution was added and incubated for 30 min at room temperature, 0.1 ml of NBT blood cell suspension was taken and added to a glass tube contain 1 ml N, N- dimethyl formamide and centrifuged for 5 minutes at 3000 rpm, the supernatant fluids was read in spectrophotometer at 620 nm in 1 ml cuvettes (Siwicki *et al.* 1985).

Serum Lysozyme activity:

The lysozyme activity was measured using photoelectric colorimeter with attachment for turbidity measurement. A series of dilution was prepared by diluting the standard lysozyme from hen egg-white (Fluka, Switzerland) and mixed with *Micrococcus lysodeikticus* (ATCC No. 1698 Sigma) suspension for establishing the calibration curve. Ten ml of standard solution or serum were added to 200 ml of *Micrococcus* suspension (35 mg of *Micrococcus* dry powder/95 ml of 1/15 M phosphate buffer and 5.0 ml of NaCl solution). The changes in the extinction were measured at 546 nm by measuring the extinction immediately after adding the solution which contained the lysozyme (start of the reaction) and after a 20 min incubation of the preparation under investigation at 40°C (end of the reaction). The lysozyme content is determined on the basis of the calibration curve and the extinction measured (Schaperclaus *et al.* 1992).

Serum bactericidal activity (SBA):

Bacterial cultures of *A. sobria* and *Ps. fluorescens* were centrifuged and the pellet was washed and suspended in phosphate buffer saline. The optical density of the suspension was adjusted to 0.5 (10^5) at 546 nm. This suspension was serially diluted (1:10) with PBS five times. Serum bactericidal activity was determined by incubating 2 µl of this diluted bacterial suspension with 20 µl of serum in a micro-vial for 1 h at 37°C. In the bacterial control group, phosphate buffer saline replaced the serum. After incubation, the number of viable bacteria was determined by counting the colonies after culturing on tryptic soya broth plates for 24 hrs at 37°C.

Total bacterial count of fish intestine:

Three fish samples from each replicate were collected randomly and under complete aseptic condition the fish samples were dissected, weighted one gram of intestine and grinding with 9.0 ml sterile saline. Six-fold serial dilutions of this suspension prepared in saline and 0.1 ml of each dilution was spread onto Tryptone-glucose yeast agar medium as recommended by APHA (1995). The colonies were counted after incubation at 30°C for 48 hours.

Challenge test:

At the end of the feeding experiment, the fish of each group were divided into three subgroups (distributed in 3 aquaria). The fish was challenged intraperitoneally with 0.5 ml 10^7 cells of 24 h cultures of live *A. sobria, and Ps. fluorescens*. The challenged fish were kept under observation for 14 days. The moribund fish was used for bacterial re-isolation (Abd El-Rhman, 2009). The mortalities were recorded and the relative level of protection (RLP) among the challenged fish was determined according the following question:

RLP =1 -[percentage of treated mortality/ percentage of control mortality] x100.

Antibody titer by using hemagglutination inhibition:

A dilution sequence from the antigen extract under consideration was prepared in buffer physiological saline and an equal quantity of erythrocytes suspension was added to each dilution stage. The preparation was shaken and kept for 1-2 hours in the water bath at 37°C. Dilution were then prepared from the serum samples. An equal amount of erythrocyte suspension and antigen were pipeted into each tube. The preparations were incubated in water bath at 37°C for 30-120 minutes. The maximum serum dilution showing hemagglutination had a high titer (Schaperclaus *et al.* 1992).

Statistical analysis:

Statistical analysis was performed using the one way analysis of variance (ANOVA). It was performed with SPSS statistical software (version 10.0, SPSS). The data were subjected for test of homogeneity of variances and Duncan post-hoc test. Data were considered significantly different when $P < 0.05$.

RESULTS

Antibacterial activity of yeast in vitro

Data in Table (1) and Fig (1) illustrated that *Saccaromyces castelli* had antibacterial activity against *Aeromonas hydrophila, A. veronii, A. sobria, A. jandaei, Pseudomonase anguilliseptica and Ps. fluorescens.* Where; it had no effect against *Citrobacter frundii, Enterobacter sp., Aerobacter sp., Moraxalla kingil, Actinobacter anitratus* or *Haemophilus aphrophilus.* The inhibition zones of *Saccaromyces castelli* against *Ps. anguilliseptica, Ps. fluorescens, A. veronii* and *A. sobria* were 38, 40, 13 and 42 mm in diameters respectively.

The growth curve of *S. castelli*:

Fig (2) showed the value of optical density due to the growth of *Saccaromyces castelli* per day. The growth of yeast was stopped at the 5th and 6th day of growth. Yeast was harvested at 4th day of growth.

Growth performance:

The growth rate was significantly increased with (T_2), and (T_3) when compared with T_1 as showed in (Fig., 3) while no significant difference between T_2 and T_3. The average weight gains (AWG), specific growth rate (SGR) and feed efficiency ratio (FER) have significant increased with T_2 and T_3 compared with T_1 (Fig 3). On the other hand, the best feed conversion ratio (FCR) was obtained with T_3 (1.126).

Organ-somatic index:

The hepatosomatic and splenosomatic indices had no difference among the three treatments.

Heamatocrite value:

The results in Fig (4) indicated that the initial heamatocrite value was 22.7±6.08 at zero time and increased significantly at the 14th and 28th day of feeding experiment. It was 34.7±0.47 and 43.1±2.12, for T_2 and T_3, respectively at 14th day while, it was 45.5±2.63 and 49±4.3 for T_2 and T_3, respectively at 28th day in compared with T_1 (22.8±6.08).

Respiratory burst activity by measuring Nitro Blue Titrazolium activity (NBT):

The result from Fig (5) showed that the initial value of NBT was (1.47±0.02 mg/ml) at zero time of feeding experiment and increased significantly at 14th day to 1.85 ± 0.05 and 1.96 ± 0.02 mg/ml in T_2 and T_3 respectively. At the 28th day of feeding experiment, NBT assay was significantly increased with groups received diet supplemented with *Saccaromyces castelli* to 1.96 ± 0.05 and 2.58 ± 0.1 mg/ml for T_2 and T_3 respectively compared with T_1 (1.47±0.02). There was a significant difference of NBT assay between the T_2 and T_3 at the 14th and 28th day of feeding experiment.

Serum lysozyme activity:

The result from Fig (6) illustrated that the initial value of serum lysozyme activity was (1.29± 0.01 µg/ml) at zero time and increased significantly at the 14th day of feeding experiment to 1.73 ± 0.04 and 1.79 ± 0.07, µg/ml for T_2 and T_3 respectively in comparison with T_1 (1.29± 0.01 µg/ml).

At the 28th day of feeding experiment, serum lysozyme activity was significantly increased in T_3 (2.08 ±0.11 µg/ml) and decreased significantly in T_2 (1.50 ± 0.07 µg/ml). There was a significance difference of serum lysozyme activity of T_2 and T_3 between the 14th and 28th day of feeding experiment.

Serum bactericidal activity (SBA):

1- *Aeromonas sobria*:

The result from Fig (7) illustrated that at the 14th day of feeding experiment, the viable bacterial counts of *A. sobria* were significantly lower in T_2 and T_3 with values 5.8 x 10^5 ± 0.08 and 5.4 x 10^5 ± 0.03 cfu/ml serum respectively when compared with T_1 (6 x 10^6 ± 0.57 cfu/ml). At the 28th day, the viable bacterial counts of *A. sobria* were significantly lower in T_2 and T_3 (4 x 10^5 ± 0.03 and 2.9 x 10^5 ± 0.01, cfu/ml respectively) when compared with control group (3.9 x 10^6 ± 0.5 cfu/ml).

2- *Pseudomonase fluorescens*:

At the 14th day of feeding experiment, serum bactericidal activity against *Ps. fluorescens* due to *Saccaromyces castelli* supplemented diet was lowest in T_1 and highest in T_3. The viable bacterial counts were significantly lower in T_2 and T_3 with values 2.5 x 10^6 ± 0.5 and 6 x 10^4 ± 0.05, respectively when compared with T_1 6.5 x 10^6 ± 0.5 cfu/ml.

At the 28th day of feeding, serum bactericidal activity against *Ps. fluorescens* was lowest in the control group than other treatment. The viable bacterial counts, of *Ps. fluorescens* were significantly lower in T_2 and T_3 5.6 x 10^5 ± 0.2 and 2.7 x 10^4 ± 0.008, cfu/ml respectively than T_1 5.2 x 10^6 ± 0.5 cfu/ml (Fig 8).

Total bacterial count of fish intestine:

Fig (9) showed that the total bacterial count of fish intestine at the 14th day was $3.2 \times 10^6 \pm 37.11$, $1.6 \times 10^6 \pm 185$ and $3.8 \times 10^5 \pm 2.17$ cfu/g in T_1, T_2 and T_3, respectively and significantly decreased to $6.6 \times 10^4 \pm 12.01$ and $8.6 \times 10^3 \pm 2.4$ cfu/g in T_2, T_3 at the 28th day of feeding experiment.

Challenge test:

Results of Fig (10) illustrated that relative level of protection (RLP) due to feeding fish on diet supplemented with *S. castelli* against *A. sobria* were 0, 83 and 83% for T_1, T_2 and T_3 respectively. While its protection against *Ps. fluorescens* was 0.0, 81.3, and 100% in T_1, T_2 and T_3 respectively.

Antibody titer:

Results of Fig (11) showed that the highest level of the antibody titer to *A. sobria* and *Ps. Fluorescens* infections was obtained with T_2 (8 $\log_{10}$). T3 increased antibody titer with *A. sobria* infection than *Ps. Fluorescens* (6 $\log_{10}$ and 5 $\log_{10}$) and the lowest value in T_1 (1 $\log_{10}$ and 3 $\log_{10}$) with *A. sobria* and *Ps. fluorescens* respectively.

DISCUSSION

Fish cultures are increasing to compensate for the shortage of animal protein all over the world. Fish under intensive culture conditions will be badly affected and often fall prey to different microbial pathogens that have been treated with chemotherapeutic substances of which antibiotics were intensively used. These curative substances produce the problem of bacterial drug fastness on one hand and the public health hazards on the other hands (Rebertson *et al.*, 2000). These awaited drawbacks enforced the fish pathologists to seek for other alternatives; the use of natural immunstimulants in the fish culture for the prevention of diseases is a promising new development (Anderson, 1992; Siwicki *et al.*, 1994 and Sakai, 1999). Natural immunstimulants are biocompatible, biodegradable and safe for the environment and human health. Moreover, they possess an added nutritional value. In this way, yeasts have been tested in fish diets as a possible alternative to commonly used animal protein (Rusmesy *et al.*, 1990 and 1992). Other advantages of using yeast cell their fast growth, low cost, high stability and the fact that they are not common constituent fish feed. This makes it easier to work with them at known concentrations, compared with using soluble substances such as vitamins, which exist as micronutrients in feed (Esteban *et al.*, 2001). In spite of all these advantages, there is little information on the use of whole yeast in fish diets and concerning the

hypothesis that the in vivo administration of whole yeast can enhance the fish immune system. Such a possibility would be of great interest for aquaculture research and the fish farming industry.

This study was planed to evaluate the using of *Saccaromyces castelli* as a probiotic in *O. niloticus* for the growth performance point of view as well as their effect on the immune response and fish resistance to pathogenic bacteria. (Edskes et al., 2009) *mentioned that S. castellii* has URE2 gene in which it was toxic to Escherichia coli.

S. castelli was isolated from intestine of black carp (*Mylopharyngodon piceus*). Gatesoupe (2007) reported that yeasts have been commonly isolated in the gastrointestinal tract. *S. castelli* had antibacterial activity against *Aeromonas hydrophila, A. veronii, A. sobria, A. jandaei, Pseudomonase anguilliseptica* and *Ps. fluorescens.* This result was in agreement with Gedek, (1999) and Castagliuolo et al., (1999) who reported that the yeast may be antagonistic to entero-pathogenic bacteria, due to adhesion of bacterial cells or by secreting proteases which inhibit bacterial toxins. Also yeasts produced extracellular proteases and siderophores, and they bound lactoferrin (Gatesoupe et al., 1997)

The improved fish growth and feed utilization may possibly be due to improved nutrient digestibility. In this regard, Tovar et al., (2002), Lara-Flores et al., (2003), and Waché et al., (2006) found that the addition of live yeast improved diet and protein digestibility, which may explain the better growth and feed efficiency recorded with yeast supplements. These results suggest that yeast supplementation plays a role in enhancing the average weight gains (AWG), specific growth rate (SGR) and feed efficiency ratio (FER) may have been due to increased fish appetite resulting in a higher feed intake and therefore improved growth. Moreover, due to the high feed intake, nutrient utilization, and the high nutrient digestibility, the deposited nutrients increased. Vazquez-Juarez et al., (1993) isolated yeast from the intestines of wild rainbow trout and introduced it with feed into the digestive tracts of domestic rainbow trout. They recorded that a significant increasing in the growth of the cultured trout.

Yeast is generally considered a good source of proteins, nucleic acids, vitamins and polysaccharides. Apart from cell wall glucans, the nucleotide content of the yeast might also contribute to immunostimulation (Carver and Walker, 1995).

The hepatosomatic and splenosomatic indices had not significantly differences between the treatments. The organosomatic indices are indicators of health (hepatosomatic index and splenosomatic index) (Goede and Barton., 1990).

Yeast supplemented diet had significantly increasing of hematocrit-level compared with control group. The elevated hematocrit-value could explain the efficiency of the used yeast on the health of the fish status (Abd El-Rhman, 2009).

The NBT was significantly increased with (T_2 and T_3), when compared with (T_1). This result is on contrary with Ortuno et al (2002) who reported that there was not significant of NBT value in sea bream due to feeding with whole yeast *S. cervisiae* and in agree with (He et al., 2009) who used S. *cervisiae* fermented product with hybrid tilapia.

The lysozyme activity is an important indicator of the immune defense of both invertebrates and vertebrates. The lysozyme is a fish defense element, which causes hydrolysis of the N-acetylmuramic acid and N-acetylglucosamine which are constituents of the peptidoglycan layer of bacterial cell wall and activation of the complement system and phagocytes by acting as an opsonin (Ellis., 1999 and Magnadóttir, 2006). In this study yeast significantly increased the serum lysozyme activity, so it stimulated the immune response in Nile tilapia. The increased lysozyme activity has been reported after supplementing the fish-feed, with probiotic (Panigrahi et al 2004 and Taoka et al. 2006) and β-glucan (El-Boshy et al. 2010).

The *S. castelli* supplementation diet significantly increased the serum bactericidal activity, against *A. sobria* and *Ps. fluorecence*. These results are triggered by an increased lysozyme-activity. Misra et al (2005) and El-Boshy et al. (2010) mentioned that, serum bactericidal activity in the fish injected with different dosages of β -glucan was always significantly higher than in control.

There were significant decreased in total bacterial count of fish intestine in T_2 and T_3 in compared with the control. This result was in agreement with Salminen et al., (1999) who defined a probiotic as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". The yeasts have to compete with other microorganisms and produced extracellular proteases and siderophores, and they bound lactoferrin. Iron availability is a key issue for fish microbiota (Gatesoupe et al., 1997). Andlid et al., (1995) observed reduced numbers of bacteria when yeast colonization peaked in rainbow trout intestine.

It is important to estimate the relative level of protection in the treated fish to determine the efficacy of an immunostimulant. The yeast supplemented diet groups reduced mortality which induced by *A. sabia* and *Ps. fluorecence* when compared with the control group. These results indicate that the yeast activated the immune system of the Nile tilapia and it became resistance to pathogenic bacteria. Kumari and Sahoo (2006);

Irianto and Austin (2002) and Villamil et al. (2002) reported that yeast or structural polysaccharides improved disease resistance in fish by reducing the mortalities which associated with infection by pathogens such as *Aeromonas* and *Vibrio anguillarum*.

The highest level of the antibody titer was obtained in T_2 and the lowest level in T_1. Siwicki et al. (1994) tested several immunostimulants on rainbow trout as lyophilised *Candida utilis*, lyophilized *S. cerevisiae*, β-glucans, deacylated chitin, a premix of selenium and vitamins C and E, and a premix of betaine and amino acids. These additives increased cellular immune response and immunoglobulin serum titer.

CONCLUSION

The present study indicated that live *Saccaromyces castelli* enhanced growth performance, feed utilization, immune responses as well as the resistance to *Aeromonas sabia* and *Pseudomonase fluorecence* infection of Nile tilapia. The optimum level of dietary live *Saccaromyces castelli* is about 5 g per kg diet.

Table (1): Inhibition zones diameter (mm) due to antibacterial effect of *Saccaromyces castelli* against some bacterial isolates.

Bacterial isolates	Inhibition zones (mm)
Pseudomonase anguilliseptica	38 ± 0.66a
Pseudomonase fluorecence	40 ± 0.33 a
Aeromonas veronii	13 ± 3.33 b
Aeromonas Sobria	42.5 ± 1.45 a
Aeromonas jandaei	0 ± 0.00 b
Aerobacter sp.	0 ± 0.00
Moraxalla kingil	0 ± 0.00
Actinobacter anitratus	0 ± 0.00
Haemophilus aphrophilus	0 ± 0.00
Enterobacter sp	0 ± 0.00

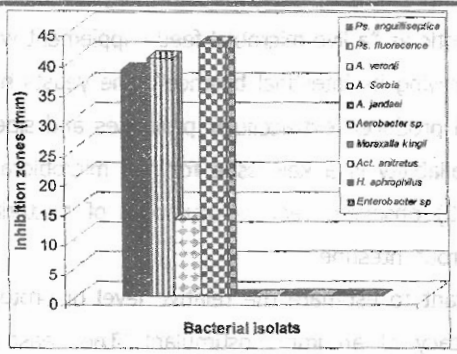

Fig (1): Antibacterial assay of *Saccaromyces castelli* against some bacterial isolates.

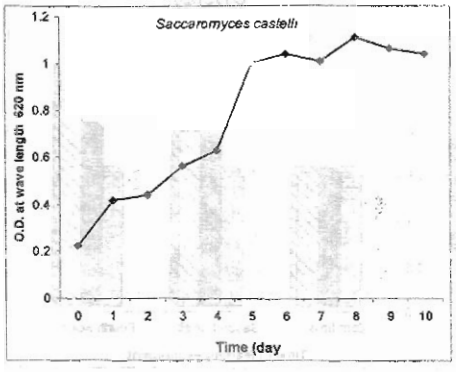

Fig (2): The growths curve of *Saccaromyces castelli* per day by determines the value of optical density using spectrophotometer at 620 nm wave length.

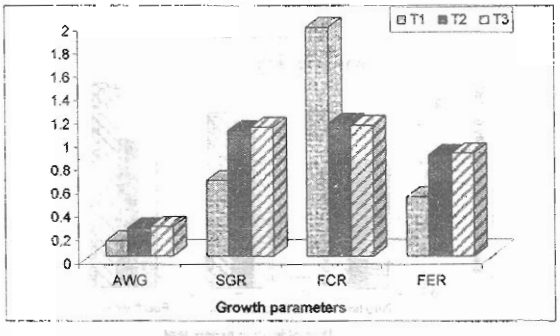

Fig (3): The effect of *Saccaromyces castelli* supplemented diet on growth parameters in *O. niloticus* fed for 28 day. AWG= daily weight gain per fish; SGR= specific growth rate (%); FCR= feed conversion ratio (gm); FER= feed efficiency ratio (gm).

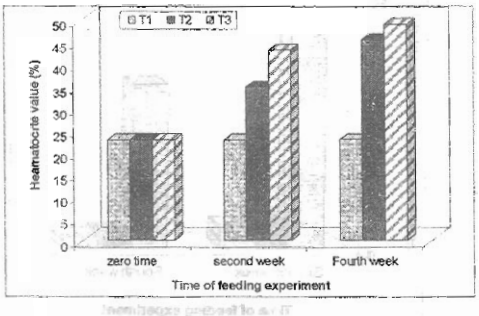

Fig (4): Effect of *Saccaromyces castelli* supplemented diet on heamatocrte value in *O. niloticus* at second and fourth week of feeding experiment.

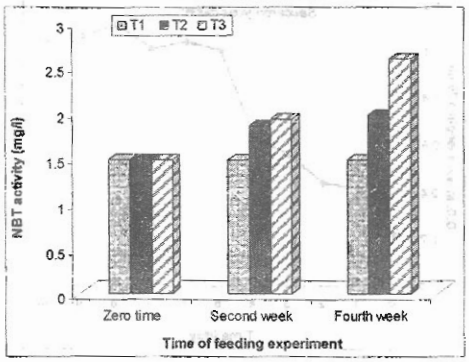

Fig (5): Effect of *Saccaromyces castelli* supplemented diet on respiratory burst by using Nitro Blue Tetrazolium activity (NBT) mg/ml in *O. niloticus* at second and fourth week feeding experiment.

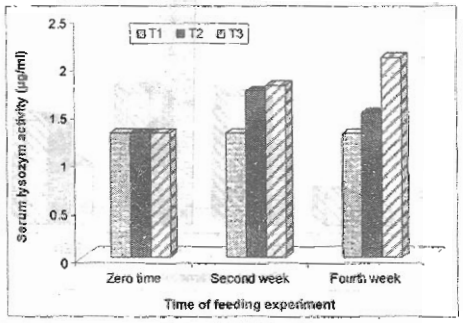

Fig (6): Serum lysozym activity µg/ml in *O. niloticus* due to feeding by *Saccaromyces castelli* supplemented diet at second and fourth week of experiment.

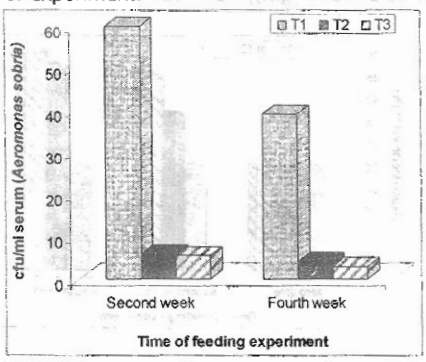

Fig (7): Effect of *Saccaromyces castelli* supplemented diet on Serum bactericidal activity of *O. niloticus* against *Aeromonas sobria* at second and fourth week of experiment.

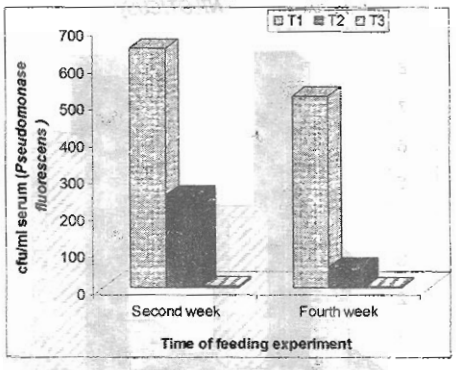

Fig (8): Effect of *Saccaromyces castelli* supplemented diet on Serum bactericidal activity of *O. niloticus* against *Pseudomonas fluorescens* at second and fourth week of experiment.

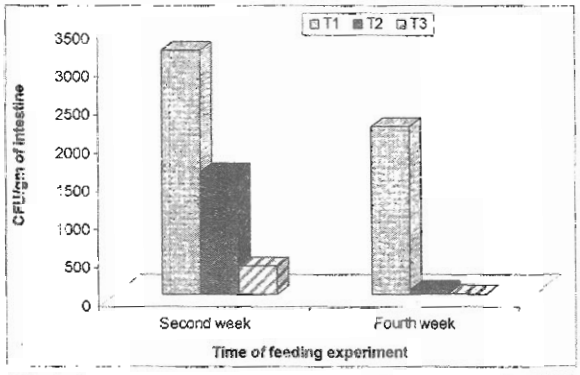

Fig (9): Effect of *Saccaromyces castelli* supplemented diet on total bacterial count of *O. niloticus* intestine at second and fourth week of experiment.

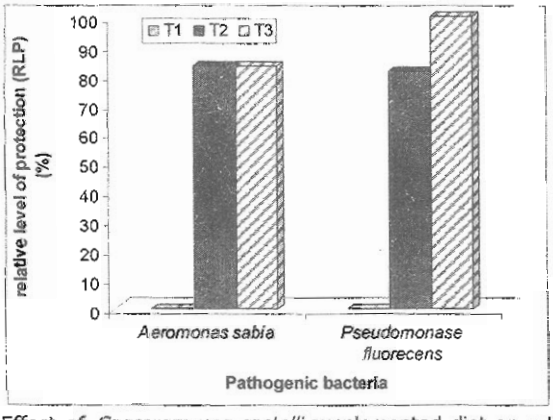

Fig (10): Effect of *Saccaromyces castelli* supplemented diet on relative level of protection in *O. niloticus* against *A. sobria* and *Ps. fluorescens*.

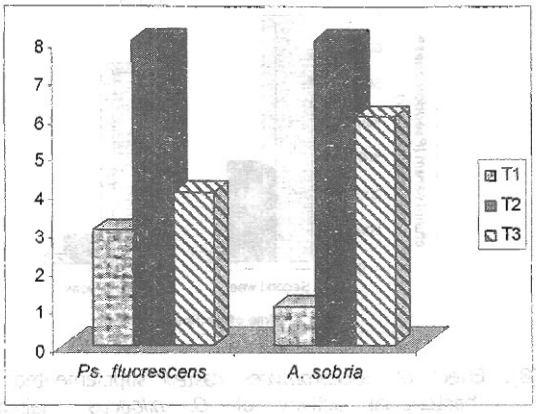

Fig (11): Effect of *Saccaromyces castelli* supplemented diet on the antibody titre level due to *A. sobria* and *Ps. Fluorescens* infection

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تقيم خميرة السكارومايسس كاستيلى كبروبيوتك فى أسماك البلطى النيلى

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١- قسم النبات شعبة الميكروبيولوجى كلية العلوم جامعة بنها.

٢- قسم أمراض الأسماك المعمل المركزى لبحوث الثروة السمكية بالعباسة.

أجريت هذه الدراسة بهدف تقيم تأثير خميرة سكارومايسس كاستيلى على الأستجابة المناعية الغير متخصصة و مقاومة الأصابة الأصتناعية لبعض البكتريا الممرضة و تأثيرها على النمو فى أسماك البلطى النيلى.

لقد تم أضافة السكارومايسس كاستيلى المعزولة من أمعاء أسماك المبروك الأسود على العلف التجارى حيث أنه يحتوى على ٣٠% بروتين لتغذية أسماك البلطى عليها. لقد تم تقسيم الأسماك الى ثلاث مجموعات كل مجموعة به ثلاث مكررات المجموعة الأولى تم تغذيتها على أعلاف خالية من الخميرة كمجموعة ضابطة و المجموعة الثانية تم تغذيتها على أعلاف تحتوى على ٥جم/ كجم من العليقة و المجموعة الثالثة تم تغذيتها على اعلاف تحتوى على ١٠جم/ كجم من العليقة. تم تغذية الأسماك مرتين فى اليوم الواحد لمدة ٢٨ يوما. فى نهاية التجربة تم عمل عدوى صناعية بميكروب الأريموناس سوبريا و السيديموناس فلورسنس بالحقن البروتونى ووضعت تحت الملاحظة لمدة ١٤ يوما.

لقد أسفرت النتائج عن:

١- وجود نشاط مثبط ضد أنواع البكتريا مثل السيديموناس انجوليسيبتكا ، السيديموناس فلورسنس، الأريموناس فيرونى و الأريموناس سوبريا و كانت قطر المنطقة المثبطة حوالى ٣٨،٤٠ ،١٣، ٤٢ مم على التوالى.

٢- كان له تأثير إيجابى على نمو الأسماك.

٣- كان له تأثير محفز للمناعة حيث عمل على زيادة الهيماتوكريت، الليزوزوم ، معدل أختزال صبغة النتربلوتترازوليم، نشاط السيرم المضاد للبكتريا وقلل من العدد الكلى لبكتريا الأمعاء.

٤- لوحظ انه زاد من مقاومة أسماك البلطى النيلى للأمراض البكتيرية حيث انه قلل من نسبة النفوق بالمقارنة مع المجموعة الضابطة.

٥- كان لها تأثير إجابى على الأجسام المضادة لكل من الأريموناس سوبريا و السيديموناس فلورسنس. لذلك تعتبر السكارومايسس كاستيلى واحدة من البروبيوتك الذى يعمل كمضاد للبكتريا و محفز للنمو و المناعة كما يجعل الأسماك أكثر مقاومة للأمراض البكتيرية و بالتالى فأنه يعمل على زيادة أنتاجية أسماك البلطى النيلى المستزرعة.