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MONITORING BIOACCUMULATION OF A PROBIOTICS *SACCHAROMYCES CEREVISIAE* IN *ARTEMIA NAUPLII* FOR TILAPIA FINGERLING

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Abstract

Artemia nauplii have been used successfully as biological carriers for transferring essential nutrients to predators. In the present study artemia was used as bioencapsulate for *Saccharomyces cerevisiae* and evaluate the immunological effect of this form in tilapia fingerling. Fish was divided into eight groups in three replicates; fed with 0‰, 5‰, 10‰ and 15 ‰ yeast with and without artemia coating. PCV indicate higher significantly in groups T5, T6, T7 and T8 (27.52, 27.62, 27.72 and 27.92) than T1, T2, T3 and T4 (25.60, 25.90, 26.93 and 27.10). Significant increase in the leukocyte count (10^3/µl) in T6 and T8 (34.66 and 34.40) than T3, T4, T5, T7 and the negative control T1 and T2 (32.12, 32.94, 32.80, 32.60, 30.22 and 30.50). NBT and Lysozyme was significantly higher in T6 and T8. Survival percentages among experimented fish groups challenged with virulent *A. hydrophila* were 17, 20, 50, 63.4, 66.7, 86.7, 76.7 and 86.7. Humoral immune response was measured by hemagglutination inhibition test and slide agglutination assays two times 15 days interval. The data was illustrated high immune response in T6 and T8.

It could be concluded that coated S. cerevisiae with artemia is more effective than uncoated one that overcomes the problems of its low digestibility of yeast cell walls and its protein content. The best level of S. cerevisiae was fish fed artemia enriched with 10‰ yeast

Key words: *Artemia*, bioencapsulate, *Saccharomyces cerevisiae*, immunological effect

INTRODUCTION

Fish is one of the richest sources of animal protein and is the fastest food producing sector in the world. Worldwide, people obtain about 25% of their animal protein from fish and shellfish and consumer's demand for fish continues to climb. Aquaculture sector has undergone a sea change in order to

meet the increasing demand. The production is maximized through intensification with addition of commercial diets, growth promoters, antibiotics, and several other additives. Application of these measures leads to high production beyond any doubt, but the most worrisome factor is that the routine use of these products causes severe complications and even a stage has come where its sustainability is in stake (Panigrahi and Azad 2007).

Over the years various strategies to modulate the composition of the gut microbiota for better growth, digestion, immunity, and disease resistance of the host have been investigated in various livestock as well as in human beings. The manipulation of the gut microbiota through dietary supplementation of beneficial microbes is a novel approach not only from nutritional point of view but also as an alternate viable therapeutic modality to over- come the adverse effects of antibiotics and drugs. Those beneficial microorganisms are usually referred as probiotics which after administration can able to colonize and multiply in the gut of host and execute numerous effects by modulating various biological systems in host (Burr *et al.,* 2007).

A wide range of microalgae (*Tetraselmis*), yeasts (*Debaryomyces* , *Phaffia* and *Saccharomyces*), gram-positive (*Bacillus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Micrococcus, Streptococcus* and *Weissella*) and gram negative bacteria (*Aeromonas, Alteromonas, Photorbodobacterium, Pseudomonas* and *Vibrio*) have been applied as probiotics to improve aquatic animal growth, survival, health and disease prevention. Yeasts are the most important and most extensively used microorganisms in aquaculture either in live form, or after processing as they are a cheap dietary supplement (Stones and Mills, 2014).

Saccharomyces cerevisiae as yeast microorganism can enhance growth performance, immune responses and/or disease resistance of various fish species. The cell walls of this yeast may provide very important non nutritive compounds that may benefit fish health, including mannose polymers

covalently linked to peptides (mannoprotein), glucose polymers (glucans), minor amounts of chitin, as well as nucleic acids (Lara *et al.,* 2003).

Growth and survival of fish and crustacean larvae is affected greatly by their nutrition and the occurrence of infectious diseases. The filter feeders *Artemia nauplii* can be used as food sources for the culture of fish larvae. These filter feeders have been used successfully as biological carriers for transferring essential nutrients to predator larvae, using the bioencapsulation technique (Leger *et al.,* 1986).

Natural substances are now generally preferred over chemical and synthetic compounds for the growth and immune enhancement of aquatic organisms so, the aim of this study was to evaluate the effect of coated *Saccharomyces cerevisiae* with *Artemia nauplii* on immunity of tilapia fingerling.

MATERIAL AND METHODS

Artemia Preparation:

Artemia cysts (E.G. grade, Great Salt Lake strain, Artemia International LLC center USA) were regularly hatched in filtered artificial sea water (35 ppt), at 28 ± 1°C under continuous aeration and illumination (2000 lux). After 24 hr, nauplii were separated from unhatched cysts and washed in clean seawater. 50 ml of an emulsion (Selco, INVE Aquaculture Artemia Systems, Belgium) in which 5, 10 and 15 ‰ (w/w) *S. cerevisiae* were added. *S. cerevisiae* concentrations (w/w) in the enrichment emulsion correspond to 0.25, 0.5 and 0.75 g *S. cerevisiae* /50ml of enrichment solution. Control Artemia samples were fed with the emulsion in which no yeast was added.

Artemia nauplii enriched with Selco containing different levels of yeast for 24 hr (Figure 1), were washed thoroughly with artificial seawater 35 ppt, then they were transferred in clean seawater under culture conditions at a density of 300 nauplii/ml and stored under continuous aeration at 20 °C (Dhont *et al.,* 1993).

Figure 1. *Artemia nauplii* enriched with different levels of yeast

Experimental Design:

A total of 360 (14±0.5g) apparently healthy *O. niloticus* randomly collected from fish farm of Central Laboratory for Aquaculture Research, Abbassa (CLAR). Fish were acclimated to the laboratory conditions for 2 weeks supplied with dechlorinzed tap water at 28±1^{o}C and aeration in the wet laboratory of fish diseases department. During acclimatize period fish were fed a commercial diet.

Fish was divided into eight groups in three replicates, T_1 a fish fed commercial diet free from any additives, T_2 fish fed artemia not enriched yeast (*S. cerevisiae*), T_3 fish fed diet with 5‰ yeast, T_4 fish fed artemia enriched with 5‰ yeast, T_5 fish fed diet with 10‰ yeast, T_6 fish fed artemia enriched with 10‰ yeast, T_7 fish fed diet with 15 ‰ yeast, T_8 fish fed artemia enriched with 15‰ yeast. Fish larvae kept at a density of 1 ind/4litre were given immediately a food concentration of 15 nauplii/ ml for 3 weeks.

Estimation of Blood Parameters:

At the end of feeding experiment three fish from each treated and control groups were anaesthetized with MS-222. Blood samples were collected from the caudal vein with heparin as anticoagulant for white blood cells count (WBCs), differential leukocytic count and packed cell volume (PCV) according to Wintrob (1967).

Another two blood samples were collected without anticoagulant first one was at 15^{th} day post challenged and the second after one month from challenged (15 days interval). This blood was centrifuged at 5000 rpm/10 min for serum separation which stored at -20^{o}C till use.

Nitroblue Tetrazolium Assay (NBT Assay):

The technique was carried out as previously described by He *et al.*, 2011. Collected Blood samples were placed in 2.5ml microcentrifuge tube then added an equal volume of 0.2% NBT. After incubation for 30 min, 1ml of dimethyl formamide was added and centrifuged. The supernatant was measured by spectrophotometer at wave length 540 nm.

Lysozyme Activity in Serum:

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 ١٥/١ M phosphate buffer (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) (Schaperclaus *et al.*, 1992).

Efficacy Trial:

Each of the experimented fish groups was subdivided into subgroups; each subgroup in three replicates (10 fish each). One of each subgroup was IP challenged with 0.5 ml of 24 hr culture suspension of containing $6x10^8$ cfu /ml live virulent *A. hydrophila*, and the other subgroup was injected with ٠.٥ ml sterile saline as control. The mortality was recorded up to ٧ days post challenge. The dead fish were removed once a day and subjected to bacterial re-isolation to verify death. The relative survival percentage was calculated for each group (Chu and Lu 2005).

Hemagglutination Inhibition (HI) Test:

For hemagglutination inhibition evaluation, fish blood was collected with heparin and washed successive times with phosphate buffered saline (PBS) till obtaining clear fish erythrocytes without fibrin. Then**,** suspension of *A. hydrophila* strain $6x10^8$ cfu /ml was serially diluted and mixed with 50µl 1% erythrocytes. The mixture was incubated at 37°C for 1hr. to determined agglutination point concentration of used bacteria. The serum was serial double fold diluted and titrated against suspension of *A. hydrophila* at agglutination point concentration, then mixed well and recorded the highest serum dilution inhibit agglutination of erythrocytes. The technique was done according to Mata *et al.* (2002) two times, first after one week from challenged and second measured with 15 days interval.

Slide Agglutination Assay:

The slide-agglutination assay was performed by mixing 20 µl of bacterial cell suspension in sterile saline solution (SS, 0.9 % NaCl, pH 7.4) ($6x10^8$ cfu/ ml) with 20 µl of serial dilutions of serum. A distinct and immediate agglutination was defined as positive. Agglutination titer was recorded as the reciprocal of the highest serum dilution giving a positive reaction. The technique was done according to Mata *et al.* (2002) two times, first after one week from challenged and second measured with 15 days interval.

Bactericidal Activity:

An equal volume (100µl) of serum and *A. hydrophila* suspension 6×10^8 cfu/ml was mixed and incubated for 1hr at 25ºC. Blank control was also prepared by replacing serum with normal saline. The mixture was diluted with saline at ratio 1:10. The serum-bacterial mixture (100µl) was plated in nutrient agar and plates were incubated for 24 hr at 25ºC. The viable bacterial number was determined by counting the colonies grown in nutrient agar (Amend, 1981).

Statistical Analysis:

The statistical analysis was performed by one way ANOVA analysis of variance according to Kachigan (1991). The multiple tests were carried out to determined difference between treatment means at significance level $P< 0.05$. The stander error was also determined.

RESULTS

Estimation of Blood Parameters:

Packed cell volume (PVC), total leucocytic count (TLC) and differential contained of the white blood cells were significantly higher in groups 6 and 8 than the other groups. While, groups 5 and 7 were significantly high in PVC and lymphocytes only. Groups 1 and 2 were significantly lower in all blood parameters (Table 1).

Nitroblue Tetrazolium Assay:

The value of the extinction from spectrophotometer was transposed according to the stander curve into mg of NBT/ 1ml of blood. Groups 6 and 8 were significantly higher than the other groups in the three measured. Group 7 was significantly high in 2^{nd} and 3^{rd} measure. While, groups 1 and 2 were significantly lower in the three measured (Table 2 and Figure 2).

Table 1. Blood Parameters in the Fish under Experimented.

Group	PCV%	TLC (10^3/µl)	Lymph.	Neut.	Eosin.	Baso.	Mono.
T_1	25.60 ^{C} ±0.16	30.22^{C} ±0.22	24.56 ^{C} ±0.22	3.52^{C} ±0.16	0.50 ^{C} ±0.02	0.20 ^{C} ±0.02	1.40 ^{C} ±0.02
T_2	25.90 ^{C} ±0.18	30.50 ^{C} ±0.20	24.82 ^{C} ±0.26	3.51 ^{C} ±0.14	0.49 ^{C} ±0.03	0.19^{C} ±0.03	1.47 ^{B} ±0.02
T_3	26.93 ^{B} ±0.18	32.12 ^{B} ±0.16	25.74 ^{B} ±0.25	4.12 ^{B} ±0.18	0.50 ^{C} ±0.03	0.23 ^{B} ±0.03	1.52 ^{B} ±0.02
T_4	27.10 ^{B} ±0.14	32.94^{B} ±0.25	25.92 ^{B} ±0.24	4. 16 ^{B} ±0.16	0.61 ^{B} ±0.02	0.22 ^{B} ±0.02	1.37 ^{B} ±0.03
T_5	27.52 ^{A} ±0.16	32.80^{B} ±0.15	26.12 ^{A} ±0.28	4.26 ^{B} ±0.21	0.65 ^{A} ±0.02	0.32 ^{A} ±0.03	1.39 ^{B} ±.03
T6	27.62 ^{A} ±0.20	34.66^{A} ±0.18	27.62 ^{A} ±0.28	4.42 ^{A} ±0.16	0.70 ^{A} ±0.03	0.36 ^{A} ±0.03	1.52 ^{A} ±0.04
T_7	27.72 ^{A} ±0.15	32.60^{B} ±0.2	26.02 ^{A} ±0.22	4.16 ^{B} ±0.21	0.65 ^{A} ±0.02	0.32 ^{A} ±0.03	1.4 ^{B} ±.03
T8	27.92 ^{A} ±0.20	34.40^{A} ±0.19	27.52 ^{A} ±0.28	4.41^{A} ±0.16	0.62^{A} ±0.03	0.33 ^{A} ±0.03	1.47^{A} ±0.04

Superscript letters explain degree of significantly at P <0.05. Means having the same superscript letters in the same column are not significantly different

Table 2. Nitroblue Tetrazolium Assay for Tilapia Fingerling One Week Interval.

Group	7th day	15th day	21st day
T_1	0.31416 ^{C} ±0.02	0.5236 ^{C} ±0.02	0.748 ^{C} ±0.04
T_2	0.33633 ^{C} ±0.02	0.56056 ^{C} ±0.02	0.8008 ^{C} ±0.04
T_3	0.78355 ^{C} ±0.02	1.30592 ^{B} ±0.02	1.8656 ^{B} ±0.04
T_4	1.00531 ^{B} ±0.02	1.67552 ^{B} ±0.02	2.3936 ^{B} ±0.04
T_5	1.19196 ^{B} ±0.02	1.9866 ^{B} ±0.02	2.838 ^{B} ±0.04
T6	1.88496 ^{A} ±0.02	3.1416 ^{A} ±0.02	4.488 ^{A} ±0.04
T_7	1.37676 ^{B} ±0.02	2.2946 ^{A} ±0.02	3.278 ^{A} ±0.04
T8	1.781472 ^{A} ±0.02	2.96912 ^{A} ±0.02	4.2416 ^{A} ±0.04

Superscript letters explain degree of significantly at P <0.05. Means having the same superscript letters in the same column are not significantly different

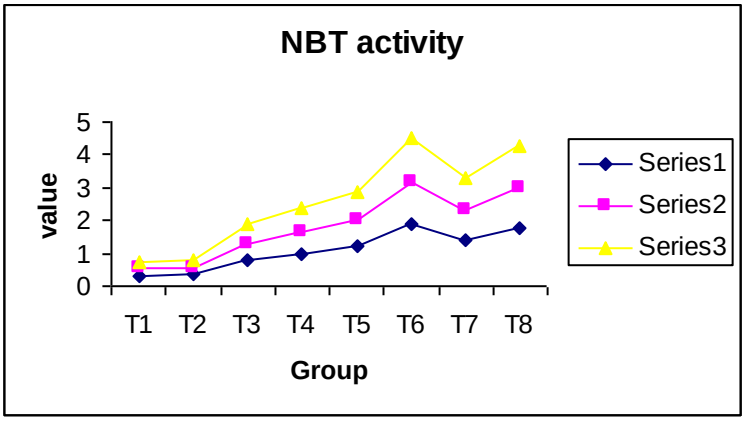

Figure 2. Three Measured Nitroblue Tetrazolium Assay One Week Interval

Lysozyme Activity in Serum:

The lysozyme content is determined on the basis of the calibration curve and the extinction measured. The values of the extinction from spectrophotometer were transposed according to the stander curve. Groups 6, 7 and 8 were significantly higher than the other groups in the three measured. While, groups 1 and 2 were significantly lower in the three measured (Table 3).

Table 3. lysozyme Activity for Tilapia Fingerling One Week Interval.

Group	7th day	15th day	21st day
T_1	$4.61^{C} \pm 0.02$	$4.71^{C} \pm 0.02$	$4.66^{C} \pm 0.02$
T_2	$4.86^{C} \pm 0.02$	$4.68^{C} \pm 0.02$	$4.76^{C} \pm 0.02$
T_3	$5.90^{B} \pm 0.02$	$6.06^{B} \pm 0.02$	$5.88^{C} \pm 0.02$
T_4	$5.96^{B} \pm 0.02$	$6.11^{B} \pm 0.02$	$6.00^{B} \pm 0.02$
T_5	$6.32^{B} \pm 0.02$	$6.42^{A} \pm 0.02$	$6.28^{B} \pm 0.02$
T6	$7.06^{A} \pm 0.02$	$7.10^{A} \pm 0.02$	$6.74^{A} \pm 0.02$
T_7	$6.46^{A} \pm 0.02$	$6.55^{A} \pm 0.02$	$6.66^{A} \pm 0.02$
T8	$7.11^{A} \pm 0.02$	$7.04^{A} \pm 0.02$	$6.86^{A} \pm 0.02$

Superscript letters explain degree of significantly at $P < 0.05$. Means having the same superscript letters in the same column are not significantly different

Efficacy Trial:

Survival percentage was 100% in the group injected with saline. Groups 6 and 8 were higher survival percentage (86.7%) than other groups. Groups 1 and 2 were lowest survival percentage (17% and 20%) Table (4).

Table 4. Mortality and survival percentage among experimented fish challenged with virulent *A. hydrophila*

Subgroup	No. of injected fish	Challenge dose	No. of dead fish	Mortality %	Survival %
1	30	0.5 ml bacteria	25	83	17
2	30	0.5 ml bacteria	24	80	20
3	30	0.5 ml bacteria	15	50	50
4	30	0.5 ml bacteria	11	36.6	63.4
5	30	0.5 ml bacteria	10	33.3	66.7
6	30	0.5 ml bacteria	4	13.3	86.7
7	30	0.5 ml bacteria	7	23.3	76.7
8	30	0.5 ml bacteria	4	13.3	86.7
9	30	٠.٥ml saline	0	0	100

Hemagglutination Inhibition Test:

The immune titer measured by HI was higher in groups 6 and 8 than other groups in the first and second measured with 15 days interval. While, groups 1 and 2 were lowest in the two measured (Figure 3).

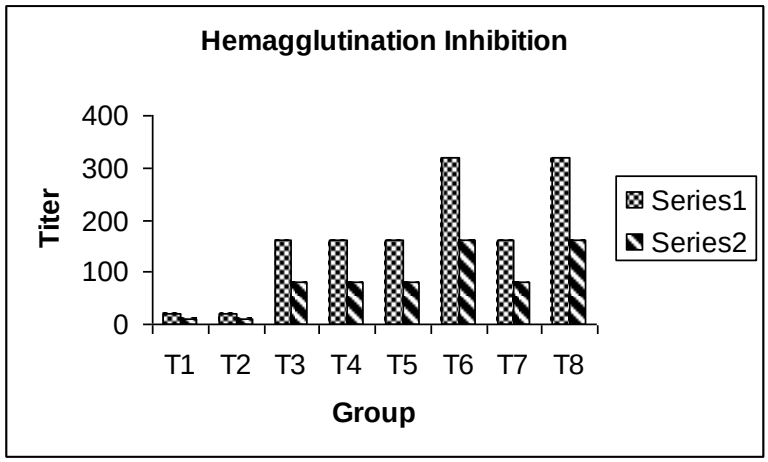

Figure 3. Immune titer measured by HI with 15 days interval

Slide Agglutination Assay:

The immune titer measured by slide agglutination assay was higher in groups 6 and 8 than other groups in the first and second measured with 15 days interval. While, groups 1 and 2 were lowest in the two measured (Figure 4).

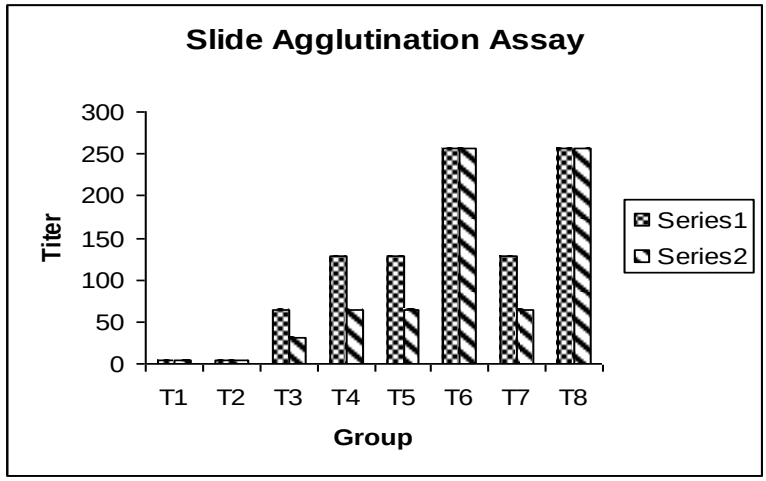

Figure 4. Immune titer measured by slide agglutination assay with 15 days interval

Bactericidal Activity:

There is bacterial growth in groups 1and 2 in all serum dilution. Groups 3, 4 and 5there is no bacterial growth up to 1/80 serum titer. Groups 6, 7 and 8 there is no bacterial growth up to 1/320 serum titer (Table 5).

Table 5. Bactericidal Activity against serum collected from Tilapia Fingerling.

Serum titer	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
T_1	G	G	G	G	G	G	G	G
T_2	G	G	G	G	G	G	G	G
T_3	NG	NG	NG	NG	RG	G	G	G
T_4	NG	NG	NG	NG	RG	G	G	G
T_5	NG	NG	NG	NG	RG	G	G	G
T6	NG	NG	NG	NG	NG	NG	RG	G
T_7	NG	NG	NG	NG	NG	NG	RG	G
T8	NG	NG	NG	NG	NG	NG	RG	G

G: growth NG: no growth RG: relative growth

DISCUSSION

Artemia spp. is highly palatable feed items that are nonselective filter feeders, providing an ideal transport mechanism of probiotics. In the present work live feed, as *artemia nauplii* are incubated in a *Saccharomyces cerevisiae* suspension after which they are fed to the tilapia fingerlings. Since these live organisms are non-selective filter feeders, they were accumulated the probiotic in their digestive tract and as such transform themselves into living microcapsules whish fed to tilapia fingerling and evaluate its effect on immune response and health conditions.

PCV used as an estimation of health status of the fish and help in detecting the improving changes through using of immunostimulants. Table 1 showed higher significantly in groups T_5, T_6, T_7 and T_8 (27.52, 27.62, 27.72 and

27.92) than T_1, T_2, T_3 and T_4 (25.60, 25.90, 26.93 and 27.10). Those indicate the better health state of this fish groups. This finding differed about the results recorded by Tukmechi and Bandboni (2014). The difference may be due to fish species (rainbow trout and *Oncorhynchus mykiss*), different concentration (0.5 and 1%) and yeast application.

The significant increase in the leukocyte count (10^3/µl) in T_6 and T_8 (34.66 and 34.40) than T_3, T_4, T_5, T_7 and the negative control T_1 and T_2 (32.12, 32.94, 32.80, 32.60, 30.22 and 30.50) mainly related to results obtained by Ortuno *et al.* (2002). The reason of the increasing of leukocyte in T_6 and T_8 related to cell walls of yeast organisms are mainly composed of β-linked glucan, a polysaccaride comprised of a backbone chain of β(1-3) glucose units that stimulate production of immunologic cells and able to activate reformation of hematopoietic stem cells. Significant increase in monocytes, macrophages and neutrophils consider a good indicator about activate case of non-specific defense mechanisms.

NBT estimates represent the activity of phagocytosis. It was significantly higher in T_6 and T_8 after one week from coated yeast addition and gradually increases with time in 2nd and 3rd weeks more than uncoated (T_3, T_5 and T_7) and control (T_1 and T_2) groups (Table 2 and Figure 2). The schedule results of uncoated groups supported with that obtained by Azza *et al.* (2014) which reported the medium gradually increase of NBT value with oral *Rhodotorula Minuta* yeast addition.

Lysozyme, one of the important bactericidal enzymes of innate immunity is an indispensable tool of fish to fight against infectious agents. *S. cerevisiae* is found to trigger the lysozyme level in teleosts. It was significantly higher in T_6 and T_8 after one week from coated yeast addition and gradually increases with time in 2nd and 3rd weeks more than uncoated (T_3, T_5 and T_7) and control (T_1 and T_2) groups (Table 3). The enhancement of lysozyme level has been proved by results of Taoka *et al.* (2006) who reported significantly high lysozyme level supplementing commercial yeasts through water in comparison to oral supplementation in *O. niloticus.*

S. cerevisiae therapy offers a suitable alternative for controlling pathogens thereby overcoming the adverse consequences of antibiotics and chemotherapeutic agents. In fish culture, probiotics either in diet or bioencapsulation help in achieving natural resistance and high survivability of larvae and post larvae of fishes. Survival percentages among experimented fish groups challenged with virulent *A. hydrophila* were 17, 20, 50, 63.4, 66.7, 86.7, 76.7 and 86.7(Table 4). The results of the challenge test was approved by Irianto and Austin (2003) who discussed the effectiveness of *S. cerevisiae* in terms of protection against infectious pathogens is often attributed to the elevate immunity. Also, it can protect against edwadsiellosis, enteric red mouth disease, furunculosis, lactococossi, streptococcosis, and several other diseases. The prevention of disease by stimulating the production of immunologic cell mediators appears to be due to sharing the effect of yeast cell wall glucans have an ability to stimulate certain aspects of the immune system. The mechanism for this effect has been characterized, and involves a specific glucan receptor which is present on peripheral blood leukocytes and extravascular macrophages. Activation of this receptor with glucans stimulates the amplification of host defenses which involves a cascade of interactions primarily mediated by macrophages and macrophage-derived products, thereby increasing a host resistance to infection.

Phianphak *et al.* (1999) mentioned that the use of *S. cerevisiae* provided disease protection by activating both cellular and humoral immune defenses in tiger shrimp *(P. monodon*). In this study the humoral immune response was measured by hemagglutination inhibition test and slide agglutination assays two times 15 days interval. The data was illustrated high immune response in T_6 and T_8 and the stability of immune titer after two weeks interval, medium immune response in T_4, T_5 and T_7 while low immune response in T_1, T_2 and T_3 (figure 3 and 4). The results is higher than that recorded **by** Rodrigues *et al.* (2000) who documents measuring stability of produced specific antibodies against *A. hydrophila* challenge, showed cut point at 1/160, 1/320 and 1/320 at first blood-

collection and 1/80, 1/80 and 1/160 at the second blood-collection after two weeks interval. That may be related to use artemia as coater for *S. cerevisiae*.

Bactericidal activity of innate immunity is an important tool of fish to fight against infectious agents. The collected serum of the experiment fish was tested against the challenged bacteria (*A. hydrophila*). The results was bacterial growth in groups 1and 2 in all serum dilution. Groups 3, 4 and 5 there is no bacterial growth up to 1/80 serum titer. Groups 6, 7 and 8 there is no bacterial growth up to 1/320 serum titer (Table 5). This finding supported by the results cited by Kesarcodi *et al.* (2008) who documented no bacterial growth up to 1/320 serum titer in *O. niloticus* oral feed with *S. cerevisiae*.

It could be concluded that *S. cerevisiae* able to enhance the *O. niloticus* cellular innate immune response, these results support the possible use of whole yeast as natural inmunostimulants in common fish diets. Coated *S. cerevisiae* with artemia is more effective than uncoated one that overcomes the problems of its low digestibility of yeast cell walls and its protein content. The best level of *S. cerevisiae* was fish fed artemia enriched with 10‰ yeast.

REFERENCES

Amend, D.F., 1981. Potency testing of fish vaccines. Developments in Biological standardization, 49: 447-454.

Azza, M.M.; M.M. Hazaa; F.M. Saleh; I.H. abass and A.M. Abeer Afifi, 2014. Study on The *Rhodotorula Minuta* In Cultured *Oreochromis Niloticus.* 4th Conference of Central Laboratory for Aquaculture Research, 203-218

Burr, G.D.; Gatlin and S. Ricke, 2007. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. J World Aquacul Soc, 36 (4): 425-436.

Chu, W.H. and Lu C.P., 2005. Multiplex PCR assay for the detection of pathogenic *Aeromonas hydrophila*. Journal of Fish Diseases, 28: 437-441.

Dhont, J.P. and P. Sorgeloos, 1993. Preparation and use of Artemia as food for shrimp and prawn larvae. In: CRC Handbook of Mariculture. 2nd Edition.

Vol 1: Crustacean Culture. J.V. Mc Vey (Ed). CRC Press, Inc., Boca Raton, Florida, USA, pp 61-93.

He, S.; Z. Zhou; K.Meng; H. Zhao; B. Yao; E. Ringo and I. Yoon, 2011. Effects of dietary antibiotic growth promoter and *Saccharomyces cerevisiae* fermentation product on production; intestinal bacterial community; and nonspecific immunity of hybrid tilapia (female × Oreochromis aureus male). J .Anim. Sci., 89: 84-92.

Irianto, A. and B. Austin, 2003. Use of dead probiotic cells to control furunculosis in rainbow trout; *Onchorhynchus mykiss* (Walbaum). J. Fish Dis, 26: 59e62.

Kachigan, S., 1991. Statisical Analysis: A conceptual introduction, Radius Press., 8 (16).

Kesarcodi, A.; H. Kaspar; M. J. Lategan and L. Gibson, 2008. Probiotics in aquaculture: the need; principles and mechanisms of action and screening processes. Aquaculture, 274:1e14.

Lara, M.; M.A. Olvera; B.E. Guzman and W. Lopez, 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*; and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 216:193-201.

Leger; P.; D.A. Bengston; K.L. Simpson and P.Sorgeloos, 1986. The use and nutritional value of Artemia as a food source. Oceanogr. Mar. Biol. Annu. Rev., 24: 521-023.

Mata, M.; A. Skarmeta and Y. Santos, 2002. A proposed serotyping sheme for *Flavobaterium psyhrophilum*. Letters in Applied Microbiology, (35) 166-170.

Ortuño, J.; A. Cuesta; A. Rodriguez; M.A. Esteban and J. Meseguer, 2002. Oral administration of yeast *Saccharomyces cereviceae* enhances the cellular

innate immune response of gilthead seabream (*Sparus aurata L*.). Vet Immunol Immunopathol, 85:41e50.

Panigrahi, A and I.S. Azad, 2007. Microbial intervention for better fish health in aquaculture: the Indian scenario. Fish Physiol Biochem, 33: 429-440.

Phianphak, W.; Rengpipat; S.; Piyatiratitivorakul; S.; P. Menasveta, 1999. Probiotic use of *Lactobacillus spp*. for black tiger shrimp; *Penaeus monodon.* J. Sci. Res. Chula Univ., 24: 42–51.

Rodrigues, ACP; D.C. Cara; S.H. Fretez; F.Q. Cunha; E.C. Vieira and J.R. Nicoli, 2000. *Saccharomyces boulardii* stimulates Ig production and the phagocytic system of gnotobiotic. J. Appl Microbiol, 89: 404e14.

Schaperclaus, W.; H. Kulow and K. Schreckenbach, 1992. Handbook of Mariculture. 2nd Edition. Fish disease. Rotterdam; the Netherlands: A.A. Balkema., 101 - 105.

Stones, M.M and S.R. Mills, 2014. A natural product from the bakery industry; baker's yeast. J. Appl. Ichthyol., 30: 55–61.

Taoka, Y; H. Maeda; S.M. Kim; S. Park and T. Yoshikawa, 2006. Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*. Fisher Sci, 72:755e66.

Tukmechi, A. and M. Bandboni, 2014. Effects of *Saccharomyces cerevisiae* supplementation on immune response; hematological parameters; body composition and disease resistance in rainbow trout; *Oncorhynchus mykiss* (Walbaum; 1792). Journal of Applied Ichthyology; February 10.1111/jai.12314

Wintrob, M.M., 1967. Clinical haematology.6th Edition; Lea and Febiger Inc.; Philadelphia, 415 – 427.

تقييم التراكم الحيوى لخميرة الخبز كبروبيوتك داخل الأرتيميا لأصبعيات البلطى

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الملخص العربى

تستعمل يرقات الأرتيميا كحامل بيولوجى للعناصر الأساسية للأسماك فى فترات النمو الأولى وذلك لما تتميز به من عدم الإختيار الغذائى. وفى الدراسة المقدمة تم إستعمال الأرتيميا لتكون حامل لخميرة الخبز تم تقديمها لأصبعيات البلطى وتقييم المستوى المناعى لتلك الأصبعيات المغذاه بهذه الصورة. ولهذا الغرض تم تقسييم أسماك التجربة إلى ثمانى مجموعات بثلاث تكرارات تم تغذيتها بثلاث تركيزات من الخميرة ٥، ١٠، ١٥% يتم تقديمها إلى الأصبعيات بطريقتين الأولى بصورة مباشرة فى العليقة والأخرى داخل الأرتيميا مع وجود المجموعة الضابطة لم يتم إضافة الخميرة لها.

أظهرت قياسات المحتوى الكلى لخلايا الدم وجود تفاوت معنوى بين المجموعات حيث كانت ٢٧.٥٢، ٢٧.٦٢، ٢٧.٧٢، ٢٧.٩٢ فى المجوعات الخامسة والسادسة والسابعة والثامنة على التوالى و٢٥.٦، ٢٥.٩، ٢٦.٩٣، ٢٧.١ فى المجموعات الأولى والثانية والثالثة والرابعة على التوالى. كما كان هناك تفاوت معنوى فى عدد كرات الدم البيضاء مقدرة بالألف فى الميكروليتر ٣٤.٦٦، ٣٤.٤ بالنسبة للمجموعتين السادسة والثامنة و٣٠.٢٢، ٣٠.٥، ٣٢.١٢ ، ٣٢.٩٤، ٣٢.٨، ٣٢.٦ بالنسبة للمجموعات الأولى والثانية والثالثة والرابعة والخامسة والسابعة. وأظهرت نتائج إختبار نشاط خلايا الدم البيضاء والنشاط الليزوزومى تميز المجموعتين السادسة والثامنة عن باقى المجموعات. كما كانت نسبة الإعاشة بعد إختبار التحدى هى ١٧، ٢٠، ٥٠، ٦٣.٤، ٦٦.٧، ٨٦.٧، ٧٦.٧، ٨٦.٧ بالنسبة للمجموعات من الأولى إلى الثامنة على التوالى.

كما أظهرت نتائج الإستجابة المناعية الذاتية من خلال إختبارى تثبيط التلازن الدموى وإختبار التجلط على الشريحة وجود مستوى مناعى مرتفع فى المجموعتين السادسة والثامنة.

ويمكن تلخيص نتائج الدراسة فى ان الإستفادة من خميرة الخبز تزداد بالنسبة لإصبعيات البلطى عندما تعطى فى صوررة مغلفة بالأرتيميا حيث يمكن التغلب فى هذه الحالة على ضعف هضم غلاف الخميرة الخلوى.كما كانت افضل نسبة إضافة للخميرة داخل الأرتيميا هى ١٠ %.