Kafrelsheikh Vet. Med. J. Vol. 9 No. 2 (2011) (165-177)

SOME STUDIES ON PROBIOTICS AS A FEED ADDITIVE IN TILAPIA (OREOCHROMIS NILOTICUS) FINGERLINGS

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ABSTRACT

The current study has been carried out to evaluate the potential benefits of adding either active or inactive yeast (Saccharomyces cerevisae) to the Nile tilapia (Oreochromis niloticus) feed on its performances. The experiment lasted for a 100 days period, using a total number of 150; about 16 g Nile tilapia finger ling which were randomly distributed into 15 aquaria representing 5 groups, each group of 3 replicates. Fish were fed a balanced ration at a rate of 3% of fish body weight along the experimental period. The five groups were fed basal diet, (T1) without any addition as control, supplied with 0.1 % and 0.2 % active yeast for (T2) and (T3) or supplied with 0.2 % and 0.4 % inactive yeast for T4 and T5 respectively. Results indicated that, the fish fed yeast supplemented diets revealed significant improvement in growth parameters (body weight gain, feed conversion ratio, protein efficiency ratio) and better serological parameters. Also a positive non specific immune induction was realized from the increased amount of total globulins. Meanwhile, feeding the lower level of active yeast 0.1 % for (T2) group gave the best results and an economical approach to improve aquaculture outputs. It could be concluded that feeding a ration supplemented with 0.1 % active yeast, Saccharomyces cervicae, could improve both health status and growth performance of Nile tilapia.

Key Words: Nile tilapia, active and inactive Yeast, Saccharomyces cerevisae, growth parameters, health status, protein profile, and activities of serum enzymes, creatinine.

INTRODUCTION

In Egypt, the production of fish coming from aquaculture represents about 65% of total fish production sources ***(GAFRD, 2009)***. This activity requires both high-quality feeds (which should contain not only necessary nutrients but also complementary feed additives to keep organism´s healthy and favor growth) and a friendly environment for aquaculture. Some of the most utilized growth-promoting feed additives include hormones, antibiotics, probiotics, prebiotics, ionospheres and some salts ***(Klaenhammer and kullen, 1999)***. Probiotics are Zootechnical additives having several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds, improvement of water quality, enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes ***(Carnevali* et al., *2006 and El-Dakar,* et al., *2007)***. Some common strains used as probiotics products; such as *Lactobacillus acidophilus, L. bulgaricus, L.plantariu* , *Streptococcus lactis* and *Saccharomyces cerevisiae* ***(FAO, 2004)***. More over, *Saccharomyces cerevisae* of the most widely commercialized yeast has long been fed to animals and fish as a rich source of protein and B-Complex vitamins and for reducing mortalities, ***(Abd El-halim et al., 1992 and Nilson et a.l, 2004)*.** 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-products ***(Lee and Kim 2001)***. Feeding active yeast significantly increased growth of tilapia and carp than inactive yeast, ***(Omar et al., 1989)***. Concerning the immune-stimulation ability; many researches showed improvement in the immune response of fishes treated with probiotics ***(Zhou. 2006 and Watson et al., 2008)***. This study was planned to evaluate the effect of both active and inactive *Saccharomyces cerevisae* on Nile tilapia (Oreochromis niloticus) health and growth performances.

MATERIALS AND METHODS

The present work was applied in the Aquaculture Research Unit, Sakha, Kafr El-Sheikh governorate in year 2011 in order to evaluate two commercial, active and inactive state, yeast products (*Saccharomyces cerevisae*) addition to the feed of Nile tilapia (*Oreochromis niloticus*) fish through investigating of growth performance, feed efficiency, body composition, protein profile and some serum enzymes activities indicating the efficiency of both kidney and liver functions.

1- Fish culture system:

About 150 fish (mono sex males) Nile tilapia (O. niloticus) with an average body weight of about 10 grams, were obtained from the earthen nursing pond of a private local farm. Fishes were randomly allotted into 5 groups which distributed in 15 aquaria (three replicate for each group). Fish were raised in the aquaria for two weeks before the beginning of the experiment (acclimation period). Water was partially changed once every day, using de-chlorinated fresh water. Aeration was provided using air blowers. This management was done according to ***Abdelhamid et al (2002)***.

2- Experimental diet:

Fish were fed during the acclimation and along the experimental periods on a basal diet ***(NRC, 1993)*** (table 1) at a rate of 3% of fresh biomass in each aquarium (six days per week) and offered at 2 meals (8 am and 3 pm) daily. The five groups were fed basal diet without any addition for control (T1), supplied with 0.1 % and 0.2% active yeast[(1)]

for (T2) and (T3) or with 0.2% and 0.4% inactive yeast[(2)] for T4 and T5 respectively. The ration was prepared every two weeks according to ***Shimeino, et al (1993)*.**

[(1)] *Active yeast Saccharomyces cerevisae (BIOSAF Sc 47 a commercial product containing 100% Saccharomyces cerevisae distributed by a French company; LES AFFRE-FRANCE).*

[(2)] *Inactive Saccharomyces cerevisae(DIAMOND V XP a commercial product containing 100% dried Saccharomyces cerevisae distributed by DIAMOND V mills, Cidar Rapids, IOWA, USA.).*

3- Proximate analysis:

Dry matter, crude protein, ether extract, crude fiber, ash contents of the diet ingredients and the whole body of fish at the beginning and the end of the experiment were performed according to ***A.O.A.C. (1984).***

4- Growth parameters:

Average total gain (ATG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV %) and survival rate (SR %) were calculated according to ***Annet, (1985) and Pouomonge and Mbonglang, (1993)*.**

5- Biochemical examinations:

At the end of the experimental period, blood samples were taken from the caudal vein for serological analysis. Serum was obtained by centrifugation of blood at 3000 rpm for 15 min then stored in deep freezer for the biochemical analyses. Serum total protein content was determined according to ***Henry (1964)***. Serum albumin was estimated according to ***Dumas and Biggs (1972)***. Globulin content was calculated by mathematical subtraction of albumin value from that of the serum total protein. Activities of Aspartate Amninotansferase (AST), Alanine Aminotransferase (ALT) and Creatinine were determined according to ***Reitman and Frankel (1957)***.

The obtained numerical data were statistically analyzed using ***SPSS (1997)*** for one-way analysis of variance. When F- test was significant, least significant difference was calculated according to ***Duncan (1955)*.**

Table (1): Ingredients and composition of the basal diet

Physical composition	Ingredient %
Fish meal	6
Yellow corn	33
Soya bean meal	37
Wheat bran	12
Sunflower oil	5
Vit. & Min.mix[1].	0.3
Na Cl	0.20
Di Ca Phosphate	1.5
Glutein	5
Chemical composition	Nutrient content
Dry matter.	90.71 %
Crude protein.	32.35 %
Ether extract.	8.07 %
Crude Fiber	11.49 %
Ash	5.52 %
Nitrogen Free Extract	42.57 %
DE[2]	3450.37 *Kcal/Kg*

1- *Vitamin and mineral mixture (product of HEPOMIX) each 2.5 kg contain: 12.000.000 IU Vit.A; 2.000.000 IU Vit. D3; 10 g Vit. E; 2g Vit. K3; 1g Vit. B1; 5g Vit. B2; 1.5 g Vit. B 6 ; 10g Vit.B12; 30 g Nicotinic acid ; 10 g Pantothenic acid ; 1g Folic acid; 50g Biotin; 250g Choline chloride 50% ; 30g Iron; 10g Copper; 50g Zinc; 60g Manganese; 1g Iodine; 0.1g Selenium and Cobalt 0.1g.*

2- *Digestible energy caculatedl (Kcal/Kg), based on 5.0 kcal/g protein, 9.0 kcal/g lipid, 2.0 kcal/g carbohydrate. (**Wee and Shu. 1989**).*

RESULTS AND DISCUSSION

1- Growth performance and survival rate:

At the end of experimental period results demonstrated in (Table 2) showed that both groups fed diets 2 and 3 revealed significant increase in the parameters of growth performance and survival rate. This improvement may be as a result of the ability of yeasts to keep the integrity of intestine making it more able to absorb nutrients; yeasts also are considered as an added nutritive value for being a rich source of protein and vitamin B-complex. This interpretation came close to that emphasized by ***Zhou (2006), Marzouk et al., (2008) and Watson et al., (2008)***. More over, there were significant ($P \leq 0.05$) differences among various groups of fish as groups 2 and 3 seemed better than 1, 4 and 5. This may be attributed to the ability of active yeasts to adhere to the gut and the secretion of amylase enzymes; which increase the digestibility of the ration more than that of inactive yeast containing rations. This is in agreement with the results of ***Omar et al., (1989)*** who compared the improvement of both active and inactive yeast on sea bass growth performance.

Table (2): Growth performance of the experimented tilapia fish as affected by the dietary treatments for 100 days

Groups	Initial weight (g)	Final weight (g)	TWG g / fish	ADGg /fish/day	FCR	SGR%/day	SR %
T1	16.00±0.01	54.26±0.00^{b}	38.26±0.006^{b}	0.38±0.00^{b}	2.97±0.00^{b}	1.22±0.05^{a}	100.00±0.00^{a}
T2	16.06±0.03	64.00±0.07^{a}	47.94±0.003^{a}	0.47±0.001^{a}	2.37±0.01^{b}	1.38±0.04^{a}	100.00±0.05^{a}
T3	16.20±0.004	56.00±0.09^{b}	39.80±0.009^{b}	0.39±0.005^{b}	2.86±0.05^{b}	1.24±0.05^{a}	100.00±0.03^{a}
T4	16.33±0.06	46.50±0.08^{c}	30.17±0.008^{c}	0.30±0.005^{c}	3.77±0.03^{a}	1.03±0.04^{b}	95.66±0.66^{b}
T5	16.46±0.005	55.50±0.04^{b}	39.04±0.006b	0.39±0.005^{b}	2.92±0.07^{b}	1.21±0.01^{b}	100.00±0.00^{a}

**Means within the same column with different superscripts are significantly different (P≤0.05).*

(T1) group fed Diet 1 =Control (basal diet without yeast), (T2) group fed Diet 2= (basal diet + 0.1 active yeast), (T3) group fed Diet 3 = (basal diet + 0.2 % active yeast), (T4) group fed Diet 4= (basal diet +0.2 % inactive yeast) and (T5) group fed Diet 5 = (basal diet + 0.4 % inactive yeast).

TWG : total weight gain, ADG : average daily gain, FCR: feed conversion ratio,

SGR: specific growth rate, SR : Survival ratio.

2- Body composition:

The results of carcass composition of Nile tilapia (Table 3) showed no significance in dry matter and crude protein among fish treatments. Meanwhile, the percentage of ether extract and ash differed significantly ($p>0.05$) among fish groups which may be attributed to the presence of *Saccharomyces cerevisae* in both active and non-active state. These results agree with the finding of ***Verschuere et al., (2000)*** *and* ***Yan-Bo, (2008).***

Table (3): Means ± standard error of proximate analysis (% on the dry matter basis) of experimental fish fed on graded levels of vaccine.

Treatment	DM	CP	EE	Ash
1- Diet 1	26.38±0.18 a	60.62±0.83 a	18.62±0.95b	20.16±0.88b
2- Diet 2	25.89±0.16 a	59.85±0.30 a	19.43±0.27a	23.39±0.07a
3- Diet 3	25.61±0.20 a	59.61±1.20 a	20.46±0.02a	22.22±1.68a
4- Diet 4	25.23±0.11 a	59.42±0.25 a	20.16±0.02a	22.18±0.23a
5-Diet 5	25.22±0.20 a	59.78±0.25 a	16.42±0.02c	20.16±0.95b

Means in the same column bearing the same letter do not differ significantly at (p>0.05) levels.

(T1) group fed Diet 1 =Control (basal diet without yeast), (T2) group fed Diet 2= (basal diet + 0.1 active yeast), (T3) group fed Diet 3 = (basal diet + 0.2 % active yeast), (T4) group fed Diet 4= (basal diet +0.2 % inactive yeast) and (T5) group fed Diet 5 = (basal diet + 0.4 % inactive yeast).

4- Biochemical examinations:

It is obvious from (Table 4) that values of the liver and kidney enzymes for both control and various treated groups in our study are higher than that recorded by many authors such as ***Nayak et al. (2004)*** and ***Marzouk et al. (2008).*** This difference may be due to the different sources of water as our cultured fishes were reared in agriculture drainage water till reached the weight of 10g (outdoor reared tilapia fry may take few months to reach that size especially in the autumn, winter and spring seasons; characterized by lower water temperature than summer season) which is polluted with many residues of agriculture and industrial activities having stressful impacts on both liver and kidneys. The previous interpretation may match those mentioned by ***Gabriel et al. (2001), Fernandes and Mazon (2003) and Stefan (2004) .***On the other hand, results of protein profile showed a significant increase in total protein and globulin and significant decrease in albumin especially in the T2 fed a lower dose of active yeast. This increased non specific immune response may be due to the ability of the probiotics to increase the nutritional value of ration, modify the fish associated gut microbial community. Moreover, the cell wall of yeasts stimulated the innate immunity and the ability of yeast cells to energy substrates in the gut of fish increasing the ability of overcoming enteric diseases. Such remarks were also recorded and emphasized by ***Zhou (2006) and Watson et al., (2008).***

Table (4): Results of biochemical tested parameters of different dietary treatments fish groups.

Treatment	**ALT (u/L)**	**AST (u/L)**	**Serum albumin (g/dl)**	**Serum total protein (g/dl)**	**Serum globulin (g/dl)**	**Creatinine (mg/dl)**
Diet 1	178	17	2.63	4.9	2.27	3.2
Diet 2	104	21	3.7	7.2	3.50	2.5
Diet 3	134	12	1.7	2.6	0.90	2.6
Diet 4	46	8	1.64	3.1	1.46	1.6
Diet 5	46	8	2.26	5.0	2.74	1.7

It could be concluded that adding *Saccharomyces cerevisae* to the diet of Nile tilapia improved growth performances, feed utilization and health status and the diet supplemented with 0.1% of active *Saccharomyces cerevisae* was the economically best treatment.

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الملخص العربي

صممت هذه الدراسة لتقييم تأثير إضافة الخميرة النشطة وغير النشطة إلى علائق البلطي النيلي على أداء النمو والمناعة ووظائف الأعضاء وصحته العامة.

ولقد أجريت هذه التجربة باستخدام 150 إصبعية متوسط وزنها حوالي 16 جم لمدة 100 يوم حيث تم تقسيمها إلى خمس مجموعات تم تغذيتها على خمس معاملات غذائية مختلفة وكل معاملة ممثلة في 3 تكرارات.

وكانت المعاملة الأولى هي عليقه ضابطة تم تركيبها لتغطى الاحتياجات الغذائية للبلطي بدون إضافات والمعاملة الثانية والثالثة تم إضافة 0.1 و 0.2 % من الخميرة النشطة على التوالي أما المعاملة الرابعة والخامسة فلقد تم إضافة 0.2 و 0.4 % من الخميرة غير الشطة على التوالى.

أظهرت النتائج تحسن في كفاءة معدلات النمو والتحويل الغذائي في جميع المعاملات التي تم إضافة الخمائر إليها وكذا في المؤشرات المناعية ممثلة في نسبة الجلوبيولين في بروتين الدم والصحة العامة ممثلة في تحسن وظائف الكبد والكلى وانخفاض معدلات النفوق.

ومن هذه الدراسة يمكن استخلاص أن إضافة الخمائر لعلائق اصبعيات البلطي النيلي وخصوصا بنسبة 0.1% من الخميرة النشطة تحسن أداءه الإنتاجي والمناعي والصحي وتكون هذه النسبة أكثرها توفيرا لتكلفة المنتج.