Abbassa International Journal for Aquaculture, ISSN 1687-7683,
Special Issue for Global Fisheries & Aquaculture Research Conference ,
Cairo International Convention Center, 24 – 26 October 2009, pp 453 - 465

EFFECT OF GARLIC AND YEAST IN THE CULTURE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract

Garlic and yeast were evaluated experimentally through using 1200 Nile tilapia (*Oreochromis niloticus*) fingerling that distributed into 3 equal groups (each of four replicates). Fish of the first group served as a control and received a balance diet. The second and third groups fed on basal diet supplemented with garlic (2%) and yeast (0.02%); respectively for 1 and 2 months. The survival and growth performances were evaluated. Blood samples were collected from the experimented tilapia, one and two months from the onset of the experiment, to measure the haematocrite values (HCV), Nitroblue tetrazolium (NBT), nuetrophil adherence and lysozyme activity. The protective effect of these compounds on fish was evaluated via challenge infection using pathogenic A. hydrophila.

The body weight gain, daily body gain, specific growth rates and survival rate were significantly higher in all treated groups when compared with the control group at one and two months of feeding experiment when compared with the controls. The two supplemented groups showed non-significant increase values of hematocrite and nitroblue tetrazolium and significantly increased Neutrophil adherence and lysozymes at one and two months of exposures when compared with the controls but no significant differences were noticed between the two treated periods. The challenge infection showed an improved relative level of protection (RLP) in the 2 supplemented groups when compared with the control .

It could be concluded that the supplementation of either garlic or yeast for one month is more potential, less expensive and promising for the production of *Oreochromis niloticus* in aquaculture.

Key words: Garlic, yeast, *Oreochromis niloticus*, innate immunity, growth and survival performances, challenge infection .

INTRODUCTION

It is widely demonstrated that farmed fish are more susceptible to disease agents than their wild counterparts due to the artificial conditions posed by intensive rearing (Salinas et al., 2006). The immune system of aquatic organisms, such as fish, is continuously affected by periodic or unexpected changes of their environment. Aquatic animal diseases control in

Egypt includes a limited number Government-approved antibiotics and chemotherapeutics, beside limited vaccines that can be used to assist the environmental management (Aly *et al.,* 2008). However, the approach which focus on the treatment upon disease appearance using antibiotics are sometimes of little value or less successful due to emergence of antibiotic resistant microorganisms which makes this method of control less successful (Anderson, 1992 and Stoskopf, 1993.

Immune therapy is an approach that has been actively investigated in recent years as a method for disease prevention. It does not involve recognition of a specific antigen or targeting the immune response toward a specific pathogen, but causes an overall immune response that hastens recognition of proteins (Camps *et al*., (1993), Secombes (1994) and Sordello *et al*., (1997). So, the use of immune stimulants for prevention of diseases in fish is considered an alternative and promising idea (Sakai, 1999) .Immune stimulants include a wide range of chemical agents, bacterial components, polysaccharides, animal or plant extracts, yeasts, nutritional factors and cytokines. Few data are available on the use of garlic extract as immune stimulant in fish in Egypt (Aly *et al.,* 2008), except, the application of some exported commercial product as Biogen which have garlic "Alliein" in its components (Khalil *et al.,* 2001 and Soliman *et al,* 2002). The usage of Saccharomyces cerevisae in fish was studied by many researchers, who noticed it as a good enhancer of fish immune system (Schoz *et al.,* 1991 and Tovar *et al.,* 2002), it also improve the survival and growth rate of supplemented fish (Abd El-Tawab *et al.* 2008 and Marwa Fathy 2009)

The present study aimed to investigate the effect of both garlic and yeast (*Saccharomyces cervecesa*) on survival and growth performance of cultured Nile tilapia (*Oreochromus niloticus*), besides studying its immune stimulatory and disease control effect via changes in hematocrite value, Nitrobule tetrazolium, neutrophil adherence and lysozome activity, and its impacts on resistance to challenge infection using pathogenic *A. hydrophila*.

This study aimed to examine the effect of inulin and vitamin C as probiotic on the growth, survival, and immunomodulation of the cultured Nile tilapia (Oreochromis niloticus), besides their possible protective effects against a challenge infection using *A. hydrophila* .

MATERIALS AND METHODS

-1Fish and facilities

1200mixed sex Tilapia nilotica (*Orechromis niloticus*) with average weight of 20.0 ± 0.02 grams were obtained from the Abbassa Hatchery. They were divided into 3 equal groups each of 4 replicates. The replicates were randomly distributed in 12 hapas within an earthen ponds in the WorldFish Center, Abbassa, Egypt .

.2Basal and supplemented diets:

The ingredients of the basal diets (Table I) were obtained from local markets and mixed mechanically with a horizontal mixer (Hobarts model D300T) at a low speed for 30 min after crushing of corn to the size of 0.5 mm using a Thomas-Willey Laboratory Mill Model 4. Then, oil was added gradually to ensure an even distribution of the ingredients with increase in the mixer speed for 5 min during which time 600 ml water was added. Pellets (0.5 cm) were prepared using pellet machine (CPM California Pellet mill Co .(.

The garlic obtained as crushed garlic (*Allium sativum*), these sulfer-containing amino acids (1-3%) named alliin and was procured from the local market. Vet YeastTM is a commercial product available in market and manufactured by Complimentary Industry Co., Egypt. It is used to improve the growth and resistance of poultry and large animals in Egyptian farms. One gram of this product contains 1 x 109 dried Saccharomyces cerevisiae cells according to the manufacturers. The two immunostimulants were added to the basal diet (2% garlic and 0.2% Vet YeastTM) for growing *O. niloticus*. They were mixed with the basal diet and pellets were made.

The obtained pellets were allowed to air dry at room temperature for 24 h. The required diet was prepared biweekly and stored in a refrigerator (4°C) for daily use.

.3Experimental design

Fish of first group served as a control and fed on a basal diet. The 2nd and 3rd groups fed on same diet supplemented with garlic and Vet YeastTM 2 % and 0.2 % for the 2nd and 3rd groups respectively. The fish fed on respected diet 2 times daily with 5% of the total body weight till the end of experiment (2 months). The water quality was within the normal range

along the period of experiment {NO3 (0.20 mg/L,), NH4 (0.2mg/L), Chla (42.27 mg/L), available P (0.02 mg/L.{(

By the end of the first month of experiment, the survival and growth parameters were measured and challenge infection was done. By the end of the experiment (2 months), the survival and growth parameters as well as hematocrit, nitroblue titrazoluim (NBT) test, Lysozyme activity test and Neutrophil adherence of experimented fish were calculated and another challenge infection was done. Mortality and relative level of protection after first and second challenge infections were also counted.

.4Laboratory tests

-Survival and growth performance

Fish were counted and survival (%) of fish was calculated. All individuals from each pond were sampled at 1 and 2 months of experiment. Growth in terms of length and weight,), Body gain (BG), daily body gain (DBG), Specific Growth Rate (SGR), Food conversion rate (FCR) and condition factor (CF) was estimated according to the following equations:

BG = FW-IW

DWG = FW – IW/culture period in days

SGR = 100 X ([Ln (FW) – Ln (IW)]/culture period in days.

FCR= FW – IW / F X 100.

CF = (weight / total length3) X 100.

Where: IW= initial weigh (g/fish), FW= final weight (g/fish), F= amount of food (g.(

-Hematological analysis and immunological tests :

Sixty fish from each group (15 fish from each replicate) were anaesthetized with MS-222, taken for blood collection. Blood was collected from the caudal vein. The blood and serum samples were used for the estimation of hematocrit value (Smith 1967), Nitroblue titrazolium (Siwicki *et al.* 1985), Lysozyme activity test (Parry *et al.,* 1965)and Neutrophil adherence (Anderson *et al,* 1992)

-Challenge experiment

Sixty fish from each group (15 fish from each replicate) were randomly collected and challenged, by inoculating 0.5 ml culture suspension of pathogenic Aermonas hydrophila

containing 4 x 108 bacteria ml-1 via intraperitoneal (I/p) route. After inoculation fish of each challenged group were transferred to 6 aquaria (50 X 60X 70 cm) and observed throughout 7 days for mortality.

4 Statistical analysis

One way analysis of variance (ANOVA) and Duncan's Multiple Range Test (Duncan 1955) were used to determine differences among treatments (mean at a significant level of $P<0.05$). Standard errors were also estimated. Analysis was carried out using the SAS package (SAS 2005).

Table 1.Composition of the basal diet used throughout the experiment.

Ingredients	Diet (%)	Protein (%)		Metabolic energy (Joules)	
		ingredients	feed	Ingredients	feed
Fish meal	7.85	0.72	5.76	4000	32000
Soybean meal	52.9	0.48	25.392	2870	151823
Ground corn	29.1	0.109	3.1719	1240	36084
Wheat flour	5.00	0.134	0.67	2700	13500
Vegetable oil	2.00	0.00	0.00	9100	18200
Cod liver oil	2.00	0.00	0.00	9100	18200
Di calcium phosphate	1.00	0.00	0.00	0.00	0000
Mineral mix.	0.07	0.00	0.00	0.00	0000
Vitamin mix.	0.05	0.00	0.00	0.00	0000
Total	100	0.00	34.9939	0.00	269807

RESULTS

After one month of the experiment, where the fish were given either basal diet (control) or basal diet supplemented with immunostimulants and probiotics (Garlic and Vet Yeast), the body weight gain, daily body gain, specific growth rate and survival showed significant increase values at 1 and 2 months of experiment. The condition factor showed varied results along the two months while the food conversion rate were significantly lower in all treated groups when compared with the control group at both 1 and 2 months of treatment. As a general observation, no significant differences were recognized in the growth tests between supplementation of these products for 1 and 2 months (Table 2)

The hematocrit, nitroblue tetrazolium (NBT), neutophil adherence and lysozymes values were increased in probiotics and immunostimulants supplemented groups when compared with the control group at both 1 and 2 months of experiment. The increase was non-significant in the haematocrit values and nitroblue tetrazolium (NBT) in Vet yeast and garlic supplemented groups at both 1 & 2 months of experiment. The neutophil adherence and lysozymes values were significantly increased in Vet yeast and garlic supplemented group at 1 and at 2 months of experiment, but no significant differences were found between 1 and 2 months of treatment (Table 3)

The relative level of protection (RLP) after challenge infection using A. hydrophila was 23.35 % and 35.29 % for garlic and Vet yeast supplemented groups, respectively after 1 month and 25 % and 43.75 % for same treatments after 2 months of experiment (Table 3)

Table2. Growth performance and survival after feeding of immunostimulants (Mean ± SE):

Parameters	One month			Two months		
	Control	Garlic	Yeast	Control	Garlic	Yeast
Body gain (g)	13.74^{B} ± 0.85	23.82^{A} ± 1.22	23.80^{A} ± 1.12	24.98^{B} ± 0.84	38.11^{A} ± 1.38	37.50^{A} ± 1.57
Daily body gain (g)	0.46^{B} ± 0.03	0.79^{A} ± 0.04	0.79^{A} ± 0.04	0.41^{B} ± 0.01	0.63^{A} ± 0.02	0.62^{A} ± 0.03
Condition factor (%	1.70^{C} ± 0.02	1.83^{B} ± 0.05	2.13^{A} ± 0.04	1.80^{B} ± 0.01	1.79^{A} ± 0.06	1.69^{B} ± 0.07
Specific growth rat	1.64^{B} ± 0.08	2.26^{A} ± 0.90	2.37^{A} ± 0.08	1.29^{B} ± 0.02	1.73^{A} ± 0.04	1.70^{A} ± 0.05
Food conversion ra	1.84^{A} ± 0.10	1.12^{B} ± 0.02	1.12^{B} ± 0.05	1.84^{A} ± 0.03	1.35^{B} ± 0.04	1.36^{B} ± 0.05
Survival (%)	79.50^{B} ± 2.50	96.36^{A} ± 1.83	95.85^{A} ± 1.54	75.25^{B} ± 2.87	96.3^{A} ± 7.70	92.00^{A} ± 2.26

Mean _ SE having the same letter in the same row are not significantly different.

Table 3. Hematological and immunological parameters after feeding of immunostimulants:

Parameters	One month			Two months		
	Control	Garlic	Yeast	Control	Garlic	Yeast
HCV	29.4^{A} ± 1.23	32.6^{A} ± 0.99	31.56^{A} ± 1.63	30.60^{A} ± 1.96	36.39^{A} ± 2.41	35.29^{A} ± 2.54
NBT	0.223^{A} ± 0.02	0.243^{A} ± 0	0.217^{A} ± 0.03	0.258^{A} ± 0.03	0.342^{A} ± 0.03	0.333^{A} ± 0.03
Neutophil adheren	8.59^{B} ± 0.74	11.01^{A} ± 1.04	11.11^{A} ± 1.11	9.1^{B} ± 0.74	12.11^{A} ± 1.04	11.56^{A} ± 1.11
Lysozyme Activity	8.73^{B} ± 0.39	10.34^{A} ± 0.13	10.22^{A} ± 0.18	9.84^{B} ± 0.41	11.82^{A} ± 0.66	10.97^{A} ± 0.60
Challenge Mortality	85	65	55	80	60	45
RLP %	0	23.53	35.29	0	25	43.75

Mean ± SE having the same letter in the same row are not significantly different..

DISCUSSION

Probiotic used as antimicrobial feed additives which were commonly used as gross promoters (Brander *et al.,* 1991), compete for nutrients and adhesion site of the pathogenic bacteria and stimulate immune system of the fish (Gatesoup, 1991), therefore, decreasing the economic losses due to diseases, through decreasing the mortalities (Nikoskelanien *et al.,* 2001 and Marwa Fathy, 2009).

In the present study the *O. niloticus* were feed with two types of probiotics (Crushed garlic and *Saccharomyces cervisiae*) supplemental diet for one and two months. The results revealed that, the garlic and *S. cerevisiae* potentiate the immune response stronger than the basal diet supplemented groups. The immuno-potentiating effect covers both the humeral and cell mediate immune response concerning the growth performance of the *O. niloticus* feed on the garlic and *S. cereveciae* where a significance increase in the body gain was seen (23.82± 1.22, 23.80 ± 1.12 and 13.74± 0.85 in control); respectively, the average daily body gain (0.79± 0.04,0.79±0.04 and 0.46±0.03 in control); respectively, the specific growth rate (2.26± 0.90, 2.37 ±0.08 and 1.64±0.08 in control); respectively and the survival percent (96.36±1.83, 95.85±1.54 and 79.50±2.50 in control); respectively, after one month and two months as showing in table(2) and that may be supported by Ramadan et al., (1991) in tilapia; Abd El Hamid and Mohamed, (2008) in monosx Nile tilapia fingerlings; Abd El-Tawab *et al.,* (2008) in galiliee tilapia and Marzouk *et al.,* (2008) in Nile tilapia. Nunes (1994) explained such improvement as a positive effect of probiotic on the intestinal flora that increase the digestive enzymes which improves the digestibility, the absorbability and the utilization of the dietary nutrients.

Concerning the hematological investigation and immunological parameters as well as bacterial diseases due to challenge with *Aeromonas hydrtophila* as shown in table(3), there was a significance increase in haematocrite, nitrobluetetrazolium, neutrophil adhearance and lysozyme values of the blood of *O. niloticus* feed on the garlic and *S. srevecaie* when compared with control group at both the one and two months of experiment. Similar findings were reported by Nevien (2005) in Nile tilapia after yeast supplementation, Panigrahi *et al*., (2005) by using the viable forms of *Lactobacillas rhamnosus*, Marzouk et al., (2008) when used probiotics (dead saccharomyces and life

Bacillus sabtillus and *Saccharomyces cervisae*) in *O. niloticus* and Tseng et al., (2009) after using *Bacillus sabtillus* in white shrimp. In contrast *Verlhak et al.,(*1998) found no increase in lyzosyme activities in rainbow trout (*Oncorhnchus myksis*) fed with *S. cerevisae* and Nevien (2005) after whole yeast supplementation in *O. niloticus*. Lysozyme is mainly produced by macrophages Gordon et al., (1974), also, the lysozyme gene during maturation of macrophages (Cross *et al*., 1988).

In the present investigation, the result cleared that garlic and *S. cerevisaei* potentiate the immune response after intraperitoneal (I.P) injection with virulent strain of *Aeromonas hydrophila* where the protection increase.

The lower mortalities 65% and 55% in garlic and yeast; respectively when compared with the control (85%) control indicating the protective effect of these product in fish against aeromonas infection. A lower mortalities were also, reported by Yano et al., (1989) in carp., Robertsen et al., (1999) in salmon. El-Kafoury (2006) in Nile tilapia and male monosex tilapia; Safinaz (2006) and Marzouk *et al*., (2008) in Nile tilapia. The lower mortalities may be attributed to the improvement of the health condition and enhanced immune responses both humeral and cell- mediated.

From the previous results we noticed that the addition of crushed garlic by 2% and yeast (*S. cervesiae*) by 0.02% improve the health status, growth and immune status of the cultured *O. niloticus*; building protection against diseases to minimize the economical losses due to out breaks. The supplementation of either garlic or yeast for one month is more potential, less expensive and promising for the production of *Oreochromis niloticus* in aquaculture.

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تأثير الثوم والخميرة على استزراع البلطي النيلي

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تم تقييم تأثير الثوم والخميرة تجريبيا على استزراع عدد 1200من اسماك البلطي النيلي التي تم توزيعها على ثلاثة مجموعات. اسماك المجموعة الأولى غذيت على عليقه متوازنة فقط كمجوعة ضابطة. أما اسماك المجوعتين الثانية والثالثة فقد غذيت على عليقه متوازنة مضافا إليها الثوم (2%) والخميرة (0.02%) على التوالي لمدة شهر وشهريين. ثم تم تقييم معدل الإعاشة والنمو كما تم اخذ عينات دم بعد شهر وشهرين لقياس الهيماتوكريت وبعض الاختبارات المناعية. كما تم تقييم مقدرة كل من الثوم والخميرة على وقاية الأسماك من بعض الأمراض وذلك من خلال العدوى التجريبية بميكروب الايروموناس هيدروفيللا.

وقد تبين ارتفاع معنوي في زيادة الوزن الكلى ومعدل النمو اليومي وكذلك نسبة الإعاشة في المجموعتين المعاملتين بالثوم والخميرة بالمقارنة بالمجموعة الضابطة. كما لوحظ زيادة غير معنوية في الهيماتوكريت و الاختبارات المناعية المختبرة لنفس المجموعتان المعاملتان بالثوم والخميرة بعد شهر وشهرين بالمقارنة بالمجموعة الضابطة ولم يلاحظ اى فرق معنوي بين تلك المجموعتان. وقد نتج عن العدوى التجريبية تحسن في معدل الوقاية في تلك الموجوعتين عن المجموعة الضابطة.

وقد خلص البحث إلى أن إضافة أي من الثوم أو الخميرة لمدة شهر أكثر واقعية وتأثيرا واقل تكلفة لإنتاج البلطي النيلي في المزارع السمكية.