EFFECT OF DIETARY BETA-1, 3 GLUCAN ON IMMUNOMODULATION ON DISEASED *OREOCHROMIS NILOTICUS* EXPERIMENTALLY INFECTED WITH AFLATOXIN B_1

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Abstract

A hundred and fifty samples of fish rations were collected and subjected to mycological examination. The total prevalence of *Aspergillus flavus* was 66.67%. Fifteen isolates among them were aflatoxinB1 producers. AFB_1 was extracted and tested for pathogencity. AFB_1 intoxicated fish showed off feed, sluggish swimming, dark skin, loss of reflexes, increased mucus secretion, loss of scales and ascities .Internally, liver displayed pale coloration with patches of congestion and pin point hemorrhages. The spleen and the Kidneys appeared enlarged, congested and dark in color.

The effects of aflatoxin B1 (AFB_1), beta-1, 3 glucans and their interactions on non-specific immunity and disease resistance of Nile tilapia (*Oreochromis niloticus*) were studied. The immunostimulant beta-1,3 glucan was fed at 0.1% and/or AFB_1 at 200µg crude /Kg feed for 21 days in Nile tilapia were investigated. Dietary supplementation of AFB1 group showed normocytic normochromic anemia, lymphopenia as well as alteration in some biochemical parameters related to organs damage induced by AFB_1. The AFB_1 treated group showed significant reduction in non-specific immunity as reduced superoxide anion production of blood phagocytes, serum lysozyme, serum bactericidal activity, and neutrophils glass adhesion and macrophage phagocytic indices. Also the specific immunity as measured through reduced protection against *Streptococcus iniae* challenge in comparison to control fish. Feeding of beta-1, 3 glucans to healthy fish raised the non-specific immunity and protection against bacterial infection compared with the control. Moreover feeding of beta-1, 3 glucans to AFB_1 induced immunocompromised fish significantly raised the degree of resistance against *S. iniae* challenge and the non-specific immunity level in comparison to non-treated AFB_1 exposed fish. This study showed that the β-glucans was able to enhance the non-specific immunity of the Nile tilapia (*Oreochromis niloticus*) immunocompromised with AFB_1.

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi which are chemically diverse. There are hundreds of mycotoxins known, but few have been extensively researched and even fewer have good methods of analysis available.

Worldwide, approximately 25% of crops are affected by mycotoxins annually (CAST, 1989 and Bandyopadhyay *et al.*, 2007). A practical definition of a mycotoxin is a fungal metabolite that causes an undesirable effect when animals or humans are exposed. Mycotoxicoses are diseases caused by exposure to foods or feeds contaminated with mycotoxins. Mycotoxins exhibit a variety of biological effects in animals such as: liver and kidney toxicity, central nervous system abnormalities, estrogenic responses and others (Nelson *et al.,* 1993 and Kovács, 2004).

Aflatoxins are a family of extremely toxic, mutagenic, and carcinogenic compounds produced by *Aspergillus flavus* and *A. parasiticus* (Deiner *et a1.,* 1987 and Kurtzman *et al.,* 1987). AFB_1 is classified as group I carcinogen by International Agency for Research on Cancer (Anon., 1993). Toxigenic *A. flavus* isolates produce aflatoxins B_1, and B_2 and toxigenic *A. parasiticus* isolates produce aflatoxins B_1, B_2, G_1, and G_2 (Cotty *et al.,* 1994, Pitt 2000, Kosalec and Pepeljnjak 2005, Cotty and Jaime-Garcia 2007).

The immune system is an important defensive mechanism against invading organisms; impaired immune functions will decrease resistance to infectious diseases. Suppression of immune responses and cause immunomodulation by aflatoxin B_1 has been demonstrated in domestic animals and fishes (Sharma, 1993, Marin *et al.,* 1996, Li *et al.,* 1999 and Shephard, 2008).

Β-glucans are wide spread in nature, plant, algea, bacteria, yeast and mushrooms (Dalmo and Seljelid, 1995). Β-glucans are non-antigenic in animals, but have been shown to be powerful activators of non specific defence mechanisms in a wide range of fishes (Kumari and Sahoo 2006 and Guselle *et al.*, 2007).

Limited information exists with respect to the effects of aflatoxin B_1 on the immune system of Nile tilapia *Oreochromis niloticus*. Also the literature concerning the protective effect of some available commercial products, that used as antifungal and detoxifying feed additives in fish rations are relatively scarce. So our studies aimed to investigate the adverse effect of aflatoxin B_1 and also evaluate the protective effects of Beta–glucan against mycotoxin contaminated rations in Nile tilapia *Oreochromis niloticus*.

MATERIALS AND METHODS

Ration Samples

A total number of 150 fish rations samples were collected from different Fish farms in Sharkia, El-Beharah and Kafr El-Sheikh Governorates. All samples were inoculated into Czapek–Dox Agar media, incubated at 25 C for 7 days for isolation and identification of fungi (Raper and Fennel, 1965). *Aspergillus flavus* (60 strains) was

tested for aflatoxin production according to Specitsiva (1964). Aflatoxin was extracted and purified from isolated strains after their growth on the natural media (crushed corn media) according to Roberts and Patterson (1975). Qualitative and qualitative estimation of AFB_1 as mentioned by Schuller and Van Egmond (1981).

Fishes

A total number of 270 apparently healthy Nile tilapia (*Oreochromis niloticus*) weighing 100 ± 10gm were obtained from a local commercial fish farm. They were maintained in glass aquaria filled with dechlorinated tap water which continuously aerated. The fish were supplied with a commercial fish ration previously analyzed without detectable AFB_1. They were acclimatized to the laboratory conditions for 2 weeks before the start of the experiment. The water temperature was kept at 24±2 ^{o}C throughout the experiment. About half of the water was changed daily in all the experimental aquaria. The fecal matters were siphoned out once daily. The biomass of the fish in each aquarium was measured at the beginning of the experiment and after each sampling to adjust the daily ration.

Experimental pathogencity of aflatoxin B_1

A total number of 30 *O. niloticus* were equally divided into three groups. The first group was kept as control. The second and third groups were injected with crude AFB_1 intraperitonealy at a rate of 0.8 and 1 ppm, respectively. All of the injected fish were observed for mortality and any clinical abnormalities.

Experimented Rations

A standard commercial ration was supplemented with AFB_1 and/or β-1.3glucan at the concentrations of 200μg and 1 gm/kg ration respectively. The dietary ingredients were thoroughly mixed in a mixer and extruded through a 2.5-mm diameter in a meat grinder. The pellets were air-dried at room temperature (28°C), broken into small pieces, sieved to obtain appropriate size, and stored at −5 °C until used.

Experimental Design

The triplicate fishes were randomly chosen into four equal groups (60 fish). Group (Gp. 1) was the control. Group (2) was fed on ration containing AFB_1 at the doses of 200μg /kg ration daily. Group (3) was fed on ration containing B-1.3 glucans 0.1% of the ration and AFB_1 at the doses of 200μg /kg ration. Group (4) was fed on ration containing β -1.3 glucans 0.1%. All the fish were fed twice daily for 3 successive weeks at the rate of 2% of their body weight. At the end of experimental trial, blood samples were taken for hematological and immunological assay and challenge infection tests performed. Prior to handling, sampled fish were euthanized in 200 mg/l MS-222.

Five fish were randomly sampled from each group in 3 triplicates at the end of experiment. Blood samples were collected by heart puncture in air-dried, heparinized sterile test tubes (500 U sodium heparinate /ml) to study the non-specific defense mechanism, total and differential leukocytic count and neutrophils adhesion test. The remaining whole blood samples were centrifuged at 3000 rpm for 5 minutes and plasma was stored at -80 ^{o}C to be used for plasma lysozyme assay. The peritoneal macrophages were isolated to assay the macrophage oxidative burst. The erythrogram (erythrocytes count, hemoglobin concentration, PCV value, blood indices, MCV, MCH and MCHC), total and differential leukocytic counts were performed in triplicate for each sample according to Stoskoph, (1993). Serum biochemical parameters, ALT, AST, total protein, albumin, glucose, urea, creatinine and uric acid were estimated following standard methods using commercial kits (Spinreact, Spain).

Immunological Studies

Superoxide anion production

The superoxide anion production of blood phagocytes was measured according to Chung and Secombes (1988) with some modifications of Sahu, *et al.*, (2007). Flat bottom 96-well microtitre plates were coated with 100 µL buffer containing poly-L-lysine solution (0.2% Sigma). Whole blood (100 µL) was added in five wells and incubated at 37°C for 2 h, then washed with Hanks balanced salt solution (HBSS). Then 100 µL of NBT (1 µg/ml HBSS) was added containing 104 *S. iniae* cells. After incubation for 30 min at 37 oC, the medium was removed after the reaction stopped by adding 100 µL of methanol. The formazone in each well was dissolved with 120 µL of 2 M KOH and 140 µL of DMSO and measured using a plate reader (Bio TEC, ELX800G, USA) at 630 nm, with 405 nm as reference.

Bactericidal activity

Serum bactericidal activity was done following the procedure of Kajita *et al.*, (1990). An equal volume (100 µL) of serum and bacterial suspension 2×10^{8} (CFU) was mixed and incubated for 1 h at 25 ^{o}C. Blank control was also prepared by replacing serum with sterile PBS. The mixture was then diluted with sterile PBS at a ratio 1:10. The serum–bacterial mixture (100 µL) was plated in blood agar and plates were incubated for 24 h at 37 ^{o}C. The number of viable bacteria was determined by counting the colonies grown in nutrient agar plates.

Plasma lysozyme

Plasma lysozyme was determined by the turbidometric assay according to Parry *et al.,* (1965). Briefly, the lysozyme substrate was 0.75 mg/ml of gram positive bacterium *Micrococcus lysodeikticus* lyophilized cells (Sigma, St. Louis, MO). The substrate was suspended in 0.1 M sodium phosphate/citric acid buffer, pH 5.8. Plasma or mucus (25

µl) was placed, in triplicate, into a microtiter plate and 175 µl of substrate solution was added to each well at 25 ^{o}C. The reduction in absorbance at 450 nm was read after 0 and 20 minutes using microplate ELISA reader (Bio TEC, ELX800G, USA). The units of lysozyme present in plasma or mucus (µg/ml) were obtained from standard curve made with lyophilized hen-egg-white-lysozyme (Sigma).

Neutrophils glass- adhesion assay

Neutrophils glass- adherent, using nitroblue tetrazolium assay, was determined according to Anderson *et al.,* (1992). Briefly, within 15 minutes after blood samples were collected, one drop of blood using heparinized capillary hematocrit tubes was placed onto a 22-mm square coverslip. The coverslips were placed individually in Petri-dishes humid chambers and incubated for 30 minutes in room temperature (25°C) to allow the neutrophils to stick to the glass. After incubation, the coverslips were gently washed with PBS (pH 7.4) and the cells were transferred upside down to a microscope slide containing a 50 µl drop of 0.2% filtrated NBT solution (Fluka Buchs, Co. Switzerland). After other 30 minutes of incubation, the positive, dark-blue stained cells were counted under the microscope. Two cover slips were examined for each fish. Three random fields were counted on each slide. The six fields were averaged. The mean and standard error of the mean of the fish lots were calculated

Lymphocytes transformation index

Lymphocyte transformation index was determined according to Ota, (1984). Briefly, equal whole blood RPMI mixture for lymphocytes isolated on cell separating medium Hiostopaque (Sigma). RPMI (Sigma) mixed with 40% bovine fetal serum. The harvesting cell washed 3 times with balanced Hank's salt solution (Sigma) without calcium and magnesium but with Hepes. Absence of calcium prevents clotting of lymphocytes isolated from heparinized blood. Standardizing the lymphocytes concentration were around $2X10^{6}$ /ml. Flat-bottom microtiter plates were set up with 200µ/l (100µ/l RPMI serum mixture and 100 µ/l lymphocyte cell suspension). Five µ/l of mitogen Phytohemagglutinin (PHA) 1mg/ml (in sterile PBS) was added to each well. Non stimulated cultures were prepared in the same manner except without added mitogen. All assays were performed in triplicate for each sample. The microtiter plates were incubated at 37°C in a humid atmosphere containing 5% CO_2 for 48 hours. Each culture medium was transferred in a micro-tube and centrifuged at 400g for 10 minutes. The supernatant was collected and glucose concentration was determined with semi-automatic spectrophotometer (BM-Germany 5010) using a standard (100 mg/dl) glucose solution (Werner *et al.,* 1970). The blast transformation index (TI) was calculated as follows: TI %=[(MG–SG)/MG] X100, where MG=glucose concentration

in the non stimulated culture medium and SG=glucose concentration in the sample after incubation (Khokhlova *et al.,* 2004).

Macrophage phagocyte indices.

Peritoneal macrophages were isolated according to Jill and Phillip, (1998). Mononuclear phagocytic percent has been determined according to Ota, (1984).

Challenge test

The challenge test was done in 3 replicates where 10 fish from each group, were randomly collected and injected with highly virulent pathogenic strain of *Streptococcus iniae*. *S. iniae* was originally isolated from natural outbreaks of streptococcosis in *Tilapia* farm. The isolate was identified as *S. iniae* by the standard bacteriological method as described by Krieg and Holt (1984) and studied for their pathogencity by re-isolated from experimentally infected Nile tilapia. The *S. iniae* isolate was grown in tryptic soy broth for 24 h at 28 °C. The concentration of the culture was adjusted by macffarine tubes at a concentration of 2×10^8 colony-forming units (CFU). The fish of each group were challenged by intraperitoneal injection with 100 µl of *S. iniae* culture containing 2×10^8 (CFU). The mortality rate was calculated after 15 days post challenged. Dead fish were removed once a day and subjected to bacterial re-isolation.

Statistical analysis

Data were statistically analyzed by ANOVA test with posthock LSD multiple comparison test using statistical software program State View 4.01 (1993). Differences were considered significant at $P < 0.05$.

RESULTS

The total percentage of *Aspergillus flavus* isolated from the examined fish rations was 66.67%. Fifteen isolates among them were aflatoxinB_1 producers. The results of experimental pathogencity of aflatoxin B_1 in *O. niloticus* (Table, 1) revealed that, the mortality rates were 80 and 100% in 0.8 and 1 ppm injected groups, respectively.

Clinically the experimentally infected fish with AFT1 was sluggish swimming, off food, loss of reflexes, increased opercular movements, darkness of the skin and the presence of excessive amounts of mucus on gills. Peticheal haemorrhages were seen at fin bases, tail and head region, loss of scales, sloughing of tail and sever abdominal distention. Finally, the moribund fish was dropped to the bottom of aquarium and dead. Internally, liver displayed pale coloration with patches of congestion and pin point hemorrhages. The gall bladder was distended with brownish bile. The spleen and the Kidneys appeared enlarged, congested and dark in color.

The effects of dietary of AFB_1 and β-glucgan on hematological, some serum biochemical and immunological parameters are tabulated in tables (2, 3, 4&5). The erythrogram results showed normocytic normochromic anemia in both AFB_1 (Gp. B) and AFB_1 & β-glucan. (Gp. C) in comparison with the control group. Leucopenia and lymphopenia observed in Gp. (B) compare to control. Lymphocytosis is reported in β-glucan treatment group (Gp. D) in comparison to other treatment groups.

Regarding to serum biochemical parameters, elevation liver transaminase (ALT & AST), glucose, and urea as well as uric acid blood level in Gp. (B) and Gp. (C) in comparison with control group.

The immunological studies showed decrease in the activities of superoxide anion production of phagocytes, bactericidal activity and serum lysozyme in Gp. (B) in comparison with control group. Also neutrophils glass adhesion, lymphocytes transformation index and macrophages phagocytic index is significantly decreased in AFB1 treatment group in comparison to control. All the immunological parameters are significantly increased in Gp. (C) when compare to Gp. (B), also in Gp. (D) when compare to other investigated groups. Mortality rate was significantly increased in Gp. B in compare with other groups.

DISCUSSION

Aflatoxin is one of the most important biological pollutants since the discovery of fungal toxins at 1960. In a developmental country, it is common to observe feeds contaminated with mycotoxins as well as AFB_1, this has been reported in human/animal feeds (Shank, 1989). Aflatoxin lowers the resistance to diseases and interferes with vaccine-induced immunity in livestock (Diekman and Green, 1992).

Our investigation showed that, the total prevalence of *A. flavus* was 66.67% among the examined samples. This is nearly in agreement with Moreno and Fernandez, (1986) who isolated the same genera from fish ration with incidence of occurrence for *Aspergillus* (70%). Fifteen isolates among them were aflatoxinB1 producers. Diener *et al.,* (1987) found that the significant variation in toxigenesis depends upon the substrates from which *Aspergillus flavus* strains have been isolated, consequently, isolates from fish meal, fish ration and yellow corn may vary in their growth rate and the ability to produce toxin.

AFB_1 intoxicated fish showed off feed, sluggish swimming, dark skin, loss of reflexes, increased mucus secretion, loss of scales and ascities .Internally, liver displayed pale coloration with patches of congestion and pin point hemorrhages. The spleen and the Kidneys appeared enlarged, congested and dark in color. Similar pictures were noticed by Halver, (1968).

Our study showed normocytic normochromic anemia in FB1 treated group at the end of the experiment. The main hemopiotic tissue in *O. niloticus* is anterior kidney (Stoskopf, 1993). Renal damage of AFB1 treated channel catfish was reported by Jantrarotai and Lovell (1990) and Jantrarotai *et al.,* (1990), as well as in common carp by Pepeljnjak *et al.,* (2003). Similarly, Hussein *et al.,* (2000) recorded anemia in *O. niloticus* exposed to 0.5 µg or 1.0 µg AFB1/Kg B.W. for 10 days. Also, anemia noticed in *O. niloticus* and *O. aureus* fed on ration supplemented with aflatoxins (Manal , 1993 and Rizkalla *et al.,* 1997, respectively).

The treated groups with AFB_1 showed leukocytosis, neutrophilia and lymphopenia. The main leukocytic picture of acute stress in teleost fish is neutrophilia, and lymphopenia (Robert 2001). Mycotoxins exposure can induce acute stress response by increasing blood cortisol levels (Chedid *et al.,* 1980 and Peng *et al.,* 2007). The lymphocytolysis was observed in haemopoietic organs (kidney and spleen) during AFB1 toxicity, in Labeo rohita under electronmicroscopic observations (Sahoo *et al.,* 1998). Leukocytosis had been reported by Jantrarotai and Lovell (1990) in channel catfish *Ictularus punctatus* fed AFB_1 (10 mg/Kg diet). They concluded that leukocytosis was probably as a result of a response to the necrosis of gastric glands. In contrast to the finding of Manal (1993), Rizkalla *et al.,* (1997) and Hussein *et al.,* (2000) who reported leukopenia in *Oreochromis* species exposed to 50µg crude AFB_1/Kg feed for 22 weeks, 3 mg AFB_1/ Kg diet for 90 days and 1.0 µg AFB_1/Kg BW for 10 days, respectively.

ALT and AST activities were significantly increased in the treated fish groups with AFB_1 in compared with control one. This elevation could be attributed to hepatic injury. Several authors concluded the increase of such enzymes to the hepatotoxic effect of AFB_1 (Jones *et al.,* 1997 and Soliman *et al.,* 1998). Our result in accordance with Pepeljnjak *et al.,* (2003) who observed elevation in liver transaminase enzymes in *Cyprinus carpio* fed 5.0 mg FB1/kg body weight for 42 days. In the same line, Hussein *et al.,* (2000) reported elevation of liver transaminase of *O. niloticus* that had been intoxicated with AFB_1.

Total protein and albumin concentrations were decreased in AFB_1 treated group. This decline in serum protein concentration may due to impaired protein synthesis and/or liver disorder caused by mycotoxins (Stoskoph 1993 and Buhler *et al.,* 2000). Sahoo *et al.*, (1998) concluded that the reduction in total protein level may be due to the hepatotoxic effect of AFB_1 in *Labeo rohita*. Several authors also reported declines in plasma protein concentration in fresh water fishes (*O. niloticus* and rainbow trout) exposed to mycotoxins (Hussein *et al.,* 2000 and Pepeljnjak *et al.,* 2003). Also,

Sahoo and Mukherjee (2001) observed decrease total plasma protein and albumin blood level in *Labeo rohita* exposed to AFB_1.

The plasma glucose concentrations significantly increased in treated fish group with AFB_1 as compared with control group. Jantrarotai *et al.,* (1990) concluded that aflatoxicosis could result in reducing activity and level of pancreatic enzymes in channel catfish *Ictalurus punctatus*. Meanwhile, hypoglycemia has also reported in *O. niloticus* that fed 0.5 µg or 1.0 µg AFB1/Kg B.W. for 10 days (Hussein *et al.,* 2000).

The elevation of urea in AFB_1 group was reported in this study. Urea in fish is produced by liver and excreted primarily by the gills rather more the kidney (Stoskoph, 1993). The elevation of urea in our investigation might be attributed to gill dysfunction. In the same aspect, Hussein *et al.,* (2000) reported hyperplasia at the basal part of secondary lamellae in *O. niloticus* after exposure to AFB_1.

The creatinine value in AFB_1 treated group was insignificant different in compared with control. The insignificant increased of creatinine level in AFB_1 treated group might be attributed to the renal damage was not severe to induce renal impairment. The hepatotoxixity of aflatoxin in rainbow trout, *Onchorynchus mykiss* and *Salmo gairdneri* was documented by Carlson *et al.,* (2001), Ottinger and Kaattari (2000) and Croy *et al.,* (1980), respectively.

Uric acid is formed by fish from exogenous and endogenous purines. It is converted in the liver to urea for excretion by the gills (Stoskoph, 1993). Elevation of uric acid levels in AFB_1 treated groups could be attributed to liver damage induced by aflatoxin (Carlson *et al.,* 2001 and Ottinger and Kaattari 2000).

The erythrogram and serum biochemical parameters in AFB_1 and β-glucan treated group (Gp. C) were insignificant differed from AFB_1 treated group (Gp.B). Meanwhile, dietary β-glucan supplement counteracts lymphopenia and leucopenia in AFB_1 treated group. Selvaraj *et al.*, (2006) reported elevation total leukocytes count in common carp (*Cyprinus carpio*) injected with β-glucan.

The AFB_1 had an immune-suppression effect indicated in this study by reducing the lymphocytes transformation. This reduction could be a result of the toxic effect of aflatoxins on the hematopoietic tissues (Ghosh *et al.,* 1990 and Sahoo *et al.*, 1998). Similarly, in an in vitro study, decreased lymphocyte proliferation and immunoglobulin production were marked, when rainbow trout peripheral blood leucocytes were exposed to AFB_1 (Ottinger and Kaattari, 1998). This suggestion was supported in our study by the lymphopenia in peripheral blood. Hannun and Linardic (1993) demonstrated the action mechanism of mycotoxins to inhibit humoral and cell-mediated immune responses. They concluded that the mycotoxins disruption of sphingolipid biosynthesis and increased sphingolipid down products resulted in

accumulation of free sphinganine and sphingosine in the T and B lymphocytes. Felding-Habermann *et al.,* (1990) and Martinova, (1996) concluded that the sphingolipid products had lymphocytes anti-prolipherative as well as inhibition of humoral and cell-mediated immune responses. In our study, fish treated with β-glucan showed increased lymphocytes transformation in compared with AFB1 and control groups. De Baulny *et al.,* (1996) concluded that the dietary β-glucan administration in turbot fish could indirectly stimulate lymphocyte activation process.

AFB_1 caused significant reduction in the population of glass-adherent NBT-positive cells which indicated the suppression of non-specific immunity level in *O. niloticus*. In the same manner, Sahoo and Mukherjee (2001) observed decreased glass adhesion NBT-positive neutrophils of Labeo rohita exposed to AFB_1. Neutrophils glass-adherent was significantly increase in β -glucan group in compared with AFB1 and control group. Palic *et al.,* (2005) found that β -glucan acted as stimulant for neutrophils function in *Pimephales promelas* fish.

Further, the degree of immunosuppression of AFB_1 was confirmed from the reduced bactericidal activity in the serum of AFB_1 treated fish. Reduced bactericidal activity may be due to the poor destroying capacity of serum antimicrobial factors to bacteria in aflatoxin-treated fish (Sahoo and Mukherjee 2001). As well as could be the possible suppression role of aflatoxin to release of antimicrobial factor to serum. This confirmed in our study by reducing serum lysozyme and superoxide anion production of blood phagocytes in AFB_1 treated group. Significant reduction was observed in the of AFB1-treated fish (*Labeo rohita*), indicated by lowered serum bactericidal and lysozyme activities (Sahoo and Mukherjee 2003). In our study, we observed raised serum bactericidal, lysozyme and superoxide anion activity in the β-glucan treated group in compared with AFB_1 and control groups. Selvaraj *et al.,* (2006) reported elevation of superoxide anion production by macrophages in common carp (*Cyprinus carpio*) injected with β-glucan. Enhanced macrophage respiratory burst activity in fishes fed on ration supplemented with β-glucan was documented by several authors, Toranzo *et al.,* (1995) in turbot, Dalmo and Seljelid, (1995) in Atlantic salmon , Yoshida *et al.,* (1995) in African catfish and in *Pseudosciaena crocea* by Ai *et al.,* (2007). Also Bagni *et al.,* (2005), Jorgensen *et al.,* (1993) and Ai *et al.*, (2007) reported that β-glucan had already significantly increased serum lysozyme levels in sea bass *Dicentrarchus labrax*, *Salmo salar* and *Pseudosciaena crocea*, respectively.

The macrophage phagocytic index was significantly decreased in AFB_1 treated group. This could attributed to that AFB_1 suppressed the macrophage phagocytic activity and macromolecular synthesis of macrophages as reported in *O. niloticus* (El-Enbaawy *et al.*, 1994), *Labeo rohita* (Sahoo and Mukherjee, 2002). Similarly, β-glucan

enhance phagocytic index had been reported by Wang *et al.*, (2007) and Ai *et al.*, (2007) in Nile tilapia (*O. niloticus*) and *Pseudosciaena crocea* after treatment with glucans.

Increased mortality rate in AFB_1 exposed group challenged with *S. iniae* could be due to damage in the immune system and other organs. Reduced mortality rate in AFB_1 and β-glucan treated group could be attributed to that β-glucan enhanced non specific immunity and diseases resistance. Cook *et al.,* (2001) , Kumari and Sahoo (2006), Selvaraj *et al.*, (2006), Guselle *et al.*, (2007) and Ai *et al.*, (2007) recorded that β-glucan increase diseases resistance in *Pagrus auratus, Clarias batrachus, Oncorhynchus mykiss, Cyprinus carpio* and *Pseudosciaena crocea* respectively.

We concluded that β-glucan has the ability to counteract immunosuppression induced by AFB_1 in Nile tilapia (*O. niloticus*).

Table 1. The mortality rate among the injected *O. niloticus* I / P with crude aflatoxin.

Group No.	Dose of crude aflatoxin ppm/kg	No. of fish in each group	Total No. of dead fish	Mortality %
1st	0	10	0	0
2nd	0.8	10	8	80
3rd	1.0	10	10	100

Table 2. Effect of dietary supplementation AFB1 and β-1.3glucan on erythrogram Picture (Mean ± S.E) in Nile tilapia (*Oreochromis niloticus*).

Groups	RBCs Mill/μL	Hb gm/dl	PCV %	MCV FL	MCH Pg	MCHC %
Gp. A (Cont.)	1.95 ± 0.14a	7.98 ± 0.41a	27.10 ± 1.15a	138.5 ± 3.82	40.1 ± 1.85	29.4 ± 1.12
Gp. B (AFB_1)	1.62 ± 0.12b	6.01 ± 0.24b	21.05 ± 1.05b	129.8 ± 4.95	37.2 ± 1.98	29.1 ± 1.21
Gp.C (AFB_1&Gluc.)	1.65 ± 0.10b	6.32 ± 0.28b	22.01 ± 1.21b	133.3 ± 4.28	38.2 ± 2.02	28.7 ± 2.31
Gp.D (Glucan)	2.12 ± 0.18a	8.14 ± 0.34a	29.04 ± 1.86a	136.8 ± 5.06	38.3 ± 2.74	28.3 ± 1.64

Table 3. Effect of dietary supplementation AFB_1 and β-1.3glucan on leukogram Picture (Mean ± S.E) in Nile tilapia (*Oreochromis niloticus*).

Groups	TLC $10^3/\mu L$	Neutro $10^3/\mu L$	Esinoph $10^3/\mu L$	Basoph $10^3/\mu L$	Lymph $10^3/\mu L$	Monocy $10^3/\mu L$	Mortality %
Gp. A (Cont.)	35.76 ± 2.15a	7.81 ± 0.58	0.92 ± 0.09	0.00 ± 0.00	25.4 ± 1.10a	1.63 ± 0.14	81 ± 3.4a
Gp. B (AFB_1)	29.52 ± 2.12b	7.16 ± 0.41	0.84 ± 0.11	0.00	19.98 ± 1.05b	1.56 ± 0.21	98 ± 1.2b
Gp.C (AFB_1&Gluc.)	34.08 ± 2.96ab	7.28 ± 0.38	1.01 ± 0.10	0.14 ± 0.10	23.94 ± 1.34a	1.72 ± 0.22	52 ± 6.8c
Gp.D (Glucan)	41.24 ± 3.75a	7.74 ± 0.50	0.98 ± 0.12	0.00	29.91 ± 1.21c	1.61 ± 0.18	28 ± 6.4d

Significant at $P > 0.05$

Table 4. Effect of dietary supplementation AFB_1 and β-1.3glucan on some biochemical parameters (Mean ± S.E) in Nile tilapia (*Oreochromis niloticus*).

Group	ALT U/ml	AST U/ml	T. Prot gm/dl	Album gm/dl	Gluco. mg/dl	Urea mg/dl	Creat. mg/dl	Uric Acid mg/dl
Gp. A (Cont.)	37.1 ±3.41a	121 ±9.4a	3.18 ± 0.21a	1.42 ± 0.11a	118 ± 7.2a	8.1 ±0. 52a	0.61 ± 0.10	2.01 ± 0.21a
Gp. B (AFB_1)	65.1 ±6.15b	174 ± 8.65b	2.65 ± 0.18b	1.01 ± 0.10b	146 ± 8.5b	11.8 ±0. 41b	0.71 ±0.09	3.48 ±0.37b
Gp.C (AFB_1&Glu)	59.4 ± 5.6b	162 ± 9.12b	2.81 ± 0.16ab	1.09 ± 0.14b	131 ± 9.1ab	11.4 ±0. 68a	0.68 ±0. 07	2.98 ±0. 28b
Gp.D (Glucan)	35.1 ± 2.8a	124 ± 7.24a	3.25 ± 0.24a	1.36 ± 0.16a	121 ± 8.6a	7.65 ±0. 71a	0.58 ±0. 12	2.14 ±0. 19a

Table 5. Effect of dietary supplementation AFB_1 and β-1.3glucan on some immunological parameters (Mean ± S.E) in Nile tilapia (*Oreochromis niloticus*).

Group	Superoxide anion (O. D.)	bactericidal activity %(CFU)/	Lysozyme µg/ml	Neutrophil glass adhesion	Lymphocyte transformation index	Macrophage phagocytic index
Gp. A (Cont.)	0.18 ±0. 04a	21.4 ±1. 48a	9.86 ±0.20b	14.1 ±1.21a	19.1 ± 0.91a	1.95 ± 0.21a
Gp. B (AFB_1)	0.08 ±0. 03b	12.6 ±1. 36b	6.41 ± 0.38a	8.1 ±0.91b	10.2 ±0. 81b	1.12 ±0. 11b
Gp.C (AFB_1&Gluc.)	0.16 ±0. 04ab	19.1 ±1. 28a	9.71 ± 0. 31b	12.4 ±1. 52a	16.8 ±1.12a	1.74 ±0. 14a
Gp. D (Glucan)	0.39 ±0. 08c	45.1 ±3. 52c	12.45 ± 0. 28c	16.1 2.61a	28.2 ±2.14c	2.86 ±0. 28c

Significant at P > 0.05

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