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Reducing the Toxicity of Aflatoxin in Broiler Chickens' Diet by Using Probiotic and Yeast

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Abstract: The aim of this study was evaluating the aflatoxins (AFS) removal ability of probiotic bacteria and yeast. Two hundred and seventy one-day-old (Ross 308) broiler chicks were randomly divided into nine experimental groups. The dietary treatments were: 1 control, 4 and 5 artificially contaminated diets with 0.5 and 1 mg of AFS, respectively; 2 and 3 received control plus probiotic or yeast 1%, respectively. 6 and 7, received AFS contaminated diets (0.5 mg) plus yeast and yeast plus probiotic, respectively. The criteria of evaluation included body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR). The toxic effect of aflatoxins were apparently in groups received AFS contaminated diet which showed significantly lower BWG and FC and impaired FCR compared with control diet. Such effects could be overcame by adding probiotic bacteria and yeast to aflatoxin treated groups. On the other hand, AFS treated group showed significant increase in liver and kidney functions. The intakes of probiotic with yeast significantly alleviated the elevation of ALT, AST, urea and creatinine levels. The results indicated the effect of probiotic plus yeast as a potential protective agent against aflatoxin toxicity which decrease the risk of occurrence of liver and kidney dysfunction.

Key words: Probiotic, yeast, broilers, aflatoxins, body weight, feed intake, feed conversion

INTRODUCTION

Poultry is an important source of protein to the ever-expanding population in rural areas (Munyawu *et al*., 1998). Poultry farming is a subsector of livestock, which has been developed from backyard chick rising to commercial farming during the past five decades among the agriculture sector, it is considered as one of the most progressive and innovative business (Rahman *et al*., 2009).

Aflatoxin is the common name for a group of extremely toxic chemically related compounds produced by the mould *Aspergillus flavus* and *Aspergillus parasiticus* during growth, harvest or storage of different foods and feeds (Wood, 1989). Aflatoxins cause clinical illness and death when consumed in high quantity; at lower levels they reduce the growth rate, feed intake, feed efficiency and suppress immunity of young animals (Harvey *et al*.,1989; Abdelhamid *et al*., 1992a,b). Aflatoxins are the most important problems and the most toxic mycotoxins to animals and man (Abdelhamid *et al*., 1990, 1999; Abdelhamid and Saleh, 1996). The aflatoxins have been shown to be potent hepatotoxic, carcinogenic, teratogenic and mutagenic, which can lead to genetic damage (Wogan, 1973).

In poultry, aflatoxicosis is characterized by acute death, sever hepatic injury, extensive hemorrhages, lowered productivity and lowered immunity to diseases (Dalvi, 1986). In addition, affected birds retain residues of the aflatoxin in their tissues (Chen *et al*., 1984). These residues are highest in the liver, gizzard and kidney, but there is a large individual bird variation in the amount of residues retained and in the duration of time required for their total clearance (Wolzak *et al*., 1986). The determination of these residues is necessary for the diagnosis of aflatoxicosis in poultry and in quality control of poultry product.

Scientific efforts were directed towards using physical, chemical and biological techniques for detoxification or inactivation of aflatoxins (Muller, 1983; Abdelhamid *et al*., 1985-92a, b; Abdelhamid, 1993; Abdelhamid and Mahmoud, 1996).

Beneficial probiotic microorganisms species belonging to *Lactobacillus, Streptococcus*, *Bacillus*, *Enterococcus*, *Aspergillus*, *candida* and *Saccharomyces* have been reported to enhance the beneficial intestinal microflora and broiler performance in some studies (Fritts *et al*., 2000). Some probiotic microorganisms may be reduced or eliminated by the low pH in the gizzard and thus have little effect in the lower intestinal tract where pathogens pose problems.

Feed additives are added either singly or in combination to basic feed, usually in small quantities as premixes. These are called as non-nutrients feed additives. Probiotic are one of the feed additives which are the live culture of useful microorganisms (Rahman *et al*., 2009). Natural aflatoxin inhibitors, e.g. carotenoids and benzoxazolinone compound from corn (Norton, 1998) and glucomannan from yeast cell wall can reduce also

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aflatoxin level and its effects (Bintvihok, 2001). Also, peptide (D4E1) may help defeat aflatoxin in cotton (STAT, 2001). Additionally, yeast (Stanley *et al*., 1993), bacteria (El-Nezami *et al*., 1998) and fungi (El-Sayed, 1996) even toxigenic *Aspergillus flavus* (Cotty and Bhatnagar, 1994) prevent aflatoxin contamination.

Increasing dietary protein above the required concentration diminished the reduction in body weight of chicks fed 5 ppm dietary AFB, (Smith *et al*., 1971). Addition of dietary fat decreased broiler mortality during aflatoxicosis (Hamilton *et al*., 1972; Abdelhamid *et al*., 1994). Accordingly, the aim of the current study was to evaluate aflatoxins (AFS) removal ability of probiotic bacteria and yeast.

MATERIALS AND METHODS

Birds: Total number of two hundreds and seventy one-day-old Ross 308 broiler chicks were obtained from a commercial farm (El-Wadee). The trial was carried out at a poultry house in Regional Center for Foods and Feeds, Giza, Egypt. The chicks were randomly distributed among nine treated groups of five replicates each (6 chicks per replicate), in a washed fumigated batteries. The temperature was controlled; feed, water and light were provided for 24 h. Birds were vaccinated against New Castle, AI and Gamboro diseases.

Diets formulation and composition: The experimental diets were formulated to cover the chick requirements as recommended by the management guide data (Ross 308). Feed and water were provided ad-libitum. Diets were formulated in regional center for food and feed to be isonitrogenous, isocaloric and mycotoxins-free as well as free from any medication as growth promoter or antibiotics. The diets which used were formulated to meet the nutrient requirements of the broiler chicks during starter, grower and finisher periods according to the national research council (NRC, 1994). All birds were fed a starter diet from one to 14d of age containing 23% CP and 3060 Kcal ME/Kg diet. From 15 to 28 d of age, the birds were switched to grower diet containing 22% CP and 3175 Kcal ME/Kg diet. While, during 29 to 42 d of age, they were fed finisher diet containing 20% CP and 3200 Kcal Me/Kg diet (Table 1). Calcium and available phosphorus were adjusted using di-calcium phosphate and limestone. Vitamins and trace minerals were added to cover broiler's requirements.

Table 2 shows that control group was fed the basal diet (G_1) while, the other experimental groups (G_4, G_6, G_7) were provided with low dose of AFS contaminated diet (0.5 mg/kg), groups (G_5, G_8, G_9) were received high dose of AFS contaminated diet (1.0 mg/kg). Oral gavage with 5 x 10^{10} cfu/mL of self-developed undefined anaerobic culture/chick was administrated at day 3 post-hatch (G_2). Dry yeast 1% was provided to group (G_3). AFS at dose of 0.5 and 1 mg/kg were provided to groups G_6 and G_8 with dray yeast 1%. Groups G_7 and G_9 were fed AFS dose 0.5 and 1 mg/kg, respectively and treated with yeast 1% and once with anaerobic caecal culture on the day 3 of hatch only. Live body weight gain and feed conversion ratio were calculated.

Table 1: Ingredients and nutrient composition of the control experimental diet (%)

Ingredients	Starter	Grower	Finisher
Yellow corn	55.81	55.98	63.0
Soybean meal (45.7%)	30.0	29.0	22.0
Corn gluten meal (60.5%)	7.50	6.75	8.00
Vegetable oil	2.625	4.335	3.275
Dicalcium phosphate	1.74	1.93	1.78
Limestone	0.82	0.55	0.56
Vit. and Min. premix[1]	0.40	0.40	0.40
Salt	0.40	0.40	0.40
L-Lysine HCl	0.42	0.39	0.37
DL-Methionine	0.21	0.19	0.14
Choline chloride	0.075	0.075	0.075
Total	100	100	100
Calculated composition[2]			
Crude protein (%)	23.20	22.30	20.30
ME (Kcal/kg)	3065.3	3179.5	3208.6
Lysine (%)	1.36	1.30	1.13
Methionine (%)	0.61	0.57	0.50
Methionine+Cystine (%)	0.98	0.94	0.85
Calcium (%)	0.96	0.90	0.85
Available P (%)	0.46	0.48	0.46

[1]Vitamin-mineral mixture supplied per Kg of diet: Vit. (A). 1200 I.U., Vit. (D_3), 2000 I.U., Vit. (E), 10 mg, Vit. (K3), 2 mg Vit, (B_1) 1 mg, vit. (B2) 5mg; vit. (B_6), 1.5 mg; vit. (B_{12}) 10 µg; Biotin, 50 µg; Pantothenic acid, 10 mg; Niacin, 30 mg; Folic acid, 1 mg Manganese, 60 mg; Zinc, 50 mg; iron, 30 mg; copper, 10 mg; iodine, 1 mg; Selenium, 0.1 mg and Cobalt, 1 mg

[2]Calculated based on feed composition tables of NRC (1994)

Table 2: Experimental treatments

Groups	Treatment
G1	Control (-) fed AF-free diet
G2	Control (-) treated with once anaerobic caecal culture on the day 3 of hatch only
G3	Control (-) treated with yeast 1%
G4	Control (+) fed low dose of AF-contaminated diet (0.5 mg/kg)
G5	Control (+) fed high dose of AF-contaminated diet (1 mg/kg)
G6	Fed low dose of AF and treated with 1% yeast
G7	Fed low dose of AF and treated with yeast 1% and once with anerobic caecal culture on the day 3 of hatch
G8	Fed high dose and treated with 1% yeast
G9	Fed high dose and treated with yeast and once with anaerobic caecal culture on the day 3 of hatch

Aflatoxin production and assessment: Aflatoxin production was carried out according to Davis *et al*. (1966) using liquid yeast medium and *Aspergillus flavus* strain (NRRL 3145). The media which contain detectable amount of aflatoxin was mixed well with the basal diet to get the aflatoxin-contaminated diet.

The total aflatoxin content in liquid medium diet and tissues was determined according to Roos *et al*. (1997) and AOAC (2006) method using monoclonal antibody columns for total aflatoxins (VICAM Science Technology, Watertown, MA, USA). Aflatoxin identification was performed by a modified HPLC-AFLATEST procedure

Agillent 1200 Series USA. HPLC equipment with two pumps, column C18, Lichrospher 100 RP-18, (5 µm x 25 cm) was used. The mobile phase consisted of water, methanol acetonitrile (54:29:17, v/v/v), at flow rat of 1 mL/min. The excitation and emission wave lengths for all aflatoxins were 362 and 460 nm (fluorescence detector), respectively.

Preparation of anaerobic inoculums: The caecal contents from three adult broilers were obtained after slaughtering the birds, mixed and immediately transferred into an anaerobic chambers (cox laboratory products, Ann. Arbor, Mioch). This material was mixed with five parts of sterile glycerin and 0.2 mL amounts of the suspension then was placed in test tubes and stored at-70°C until use. Before inoculation, 200 mL of liver veal lactose (LVL) broth (10 g/L of tryptose, 5 g/L of NaCl, 2.4 g/L of beef extract, 5 g/L of yeast extract, 0.4 g/L of cystine hydrochloride, 25 g/L of lactose and 0.6 g/L of agar with a final pH of 6.67) was inoculated with a lapful from the anaerobic inoculums and incubated anaerobically for 24 h, after which plate count was done using LVL ager (the same as LVL broth with the addition of 1.5% agar). The inoculums contained about 10^{10} cfu/mL (Bailery *et al*., 1988).

Caecal sample which was used in the preparation of anaerobic culture was examined to ensure its freedomness from *Salmonella* using peptone water as pre-enrichment broth selective broth selective broth *Salmonella shigella* (S.S) ager as plating medium, biochemical test for identification (NMKL, 1999).

Haematological and biochemical examination: At the end of the experiment, blood samples were collected from three birds of experimental group via wing vein puncture. Serum was separated and stored at -20°C for analysis of the activities of the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), while, uric acid and creatinine using commercial kits from Diamnond diagnostic Company, Egypt.

Histopathological techniques: Samples of different internal organs of the experimental birds for histopathological examination were fixed in 10% formal saline for 2 days, washed in tap water, dehydrated in ascending grades of ethyl alcohol and finally cleared with xylene and embedded in paraffin wax. The paraffin blocks were five-micron cut and stained by haematoxylin and eosin as described by Pearse (1968).

Statistical analysis: The data obtained were subjected to one way analysis of variance using the linear model (GLM) of SAS (1990). Means were compared using Duncan's new multiple range test ($p<0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

The toxic effects of aflatoxins were apparently in grower period of age (Table 3, 4 and 5). G_4 and G_5 received low and high dose of AFS contaminated diet, respectively show lower BWG and FC compared with basal diet which may be due to loss of animals' appetite caused by aflatoxins. Similar results were obtained by Parlat *et al*. (1999) who found that BWG and FCR were decreased significantly by AFB1 treatment compared with control. Decreasing of growth rate by aflatoxin may be due to disturbance of one or more of basic metabolic processes (carbohydrate, lipid and protein metabolism) in the liver and loss of appetite (Cheeke and Shull, 1985). Also, it might be due to detoxification process in the body utilizing glutathione enzyme which partly composed of methionine and cysteine, hence this detoxification processes depletes the metabolic availability of methionine leading to poor growth and feed efficiency (Devegowda *et al*., 1998). In addition BWG and FCR in the groups G_2 and G_3 received probiotic bacteria and yeast were higher compared with negative control and FC and FCR were around negative control. Probiotics are living microorganisms that when ingested may help to maintain the bacterial balance in the digestive tract and may be included in the treatment of pathological conditions, such as diarrhea, candidiasis, urinary infections, immune disorders, lactose intolerance, hypercholesterolemia and food allergy (Shah, 2000; Mombelli and Gismondo, 2000). They also have antigenotoxic effects, as well as an ability to decrease the level of DNA damage in colon cells treated with N-methyl-N-nitro-N-nitrosoguanidine (Pool-Zobel *et al*., 1993, 1996). *Saccharomyces cerevisiae*, in particular has proven to benefit health in several ways including stimulation of growth of intestinal microflora in mammals, pH modulation in ruminants (which gives rise to an increase in the rate of cellulitic bacteria), improvement of reproductive parameters in milk cows and fowls (fertility and fetal development) as well as reduction in the number of pathogenic microorganism in monogastric animals (Dawson, 1993; Wallace, 1994, 1998). Biological detoxification of mycotoxins works mainly via two major processes, sorption and enzymatic degradation, both of which can be achieved by biological systems (Halasz *et al*., 2009). Live microorganisms can absorb either by attracting the mycotoxin to their cell wall components or by active internalization and accumulation. Dead microorganisms too can absorb mycotoxins and this phenomenon can be exploited in the creation of biofilters for fluid decontamination or probiotics to bind and remove the mycotoxin from the intestine. Enzymatic degradation can be performed by either extra or intracellular enzymes. The degradation can be complete, the final product being CO_2 and water. Cell wall polysaccharide and peptidoglycan are the two main elements responsible for the binding of AFB_1

Table 3: Effect of aflatoxins contaminated diets, yeast and probiotic on broiler chickens performance (at starter period, 0-14 days of age)

	Item			
Treat.	Final body weight (g)	Body weight grain (g)	Feed consumption (g)	Fad conversion ratio (g feed/g gain)
G1	261±21^{ab}	221±21^{ab}	272±4	1.23±0.11
G2	267±41^{ab}	222±41^{ab}	265±15	1.22±0.17
G3	258±23^{a}	245±23^{a}	287±17	1.17±0.11
G4	251±18^{b}	208±18^{b}	264±15	1.27±0.01
G5	245±12^{b}	205±12^{b}	257±10	1.25±0.08
G6	257±39^{ab}	217±39^{ab}	269±20	1.24±0.07
G7	263±17^{ab}	218±17^{ab}	266±19	1.22±0.17
G8	248±20^{b}	201±20^{b}	253±16	1.26±0.07
G9	259±13^{b}	211±13^{b}	261±23	1.24±0.06

^{a,b}Means within a column for each statistical with common supers scripts are significantly not different (p>0.05), Treat: Treatment

Table 4: Effect of aflatoxins contaminated diets, yeast and probiotic on broiler chickens performance (at grower period, 15-28 days of age)

	Item			
Treat.	Final body weight (g)	Body weight grain (g)	Feed consumption (g)	Fad conversion ratio (g feed/g gain)
G1	1115±51^{ab}	858±61^{ab}	1289±52^{ab}	1.50±0.07^{ab}
G2	1134±45^{ab}	873±57^{ab}	1362±51^{a}	1.56±0.05^{ab}
G3	1153±39^{a}	868±49^{a}	1285±66^{ab}	1.48±0.11^{ab}
G4	1080±18^{ab}	834±27^{ab}	1393±61^{a}	1.67±0.10^{a}
G5	1038±17^{b}	793±32^{b}	1229±50^{b}	1.55±0.06^{ab}
G6	1110±62^{ab}	847±31^{ab}	1364±39^{a}	1.61±0.09^{a}
G7	1108±29^{ab}	849±33^{ab}	1350±11^{a}	1.59±0.04^{a}
G8	1042±57^{b}	775±56^{b}	1301±97^{ab}	1.66±0.88^{a}
G9	1098±49^{ab}	847±51^{a}	1381±49^{a}	1.63±0.08^{a}

^{a,b}Means within a column for each statistical with common supers scripts are significantly not different (p>0.05), Treat: Treatment

Table 5: Effect of aflatoxins contaminated diet, yeast and probiotic on broiler chickens performance (at finisher period, 29-42 days of age)

	Item			
Treat.	Final body weight (g)	Body weight grain (g)	Feed consumption (g)	Fad conversion ratio (g feed/ g gain)
G1	2153±71^{ab}	1036±81^{ab}	1889±55^{a}	1.82±0.09^{a}
G2	2108±28^{ab}	998±52^{ab}	1806±91^{a}	1.81±0.08^{a}
G3	2190±18^{a}	1037±36^{a}	1856±62^{a}	1.79±0.05^{a}
G4	2023±19^{b}	925±67^{b}	1776±59^{a}	1.92±0.06^{a}
G5	1989±31^{b}	951±27^{b}	1779±70^{a}	1.87±0.03^{a}
G6	2079±61^{ab}	999±62^{ab}	1828±37^{a}	1.83±0.10^{a}
G7	2050±47^{b}	942±51^{b}	1762±51^{a}	1.87±0.09^{a}
G8	2030±88^{b}	896±67^{b}	1702±71^{a}	1.90±0.12^{a}
G9	2001±49^{b}	979±19^{b}	1829±63^{a}	1.87±0.07^{a}

^{a,b}Means within a column for each statistical with common supers scripts are significantly not different (p>0.05), Treat: Treatment

Table 6: Effect of aflatoxins contaminated diet, yeast and probiotic on broiler chick serum parameters

Group	ASTU/L	ALTU/L	Uric acid mg/dL	Creatinine mg/dL
G1	69.33±4.93^{b}	5.67±0.58^{bc}	3.54±0.21^{bc}	0.22±0.06
G2	71.67±9.29^{b}	5.67±0.59^{bc}	3.95±0.61^{bc}	0.23±0.04
G3	71.67±16.50^{b}	6.33±1.26^{bc}	3.39±1.01^{c}	0.21±0.03
G4	83.00±14.93^{ab}	8.33±1.30^{a}	4.88±0.23^{a}	0.29±0.04
G5	91.67±10.11^{a}	8.67±1.06^{a}	5.40±0.69^{a}	0.30±0.06
G6	75.33±11.65^{b}	7.00±1.16^{b}	4.31±0.59^{bc}	0.23±0.05
G7	86.33±12.13^{ab}	6.67±0.58^{bc}	4.04±0.90^{b}	0.24±0.07
G8	87.33±17.10^{ab}	8.33±0.56^{a}	4.65±0.49^{bc}	0.25±0.02
G9	91.33±16.40^{ab}	8.00±0.70^{a}	4.38±0.67^{bc}	0.28±0.05

^{a,b,c}Means within a column for each statistical with common supers scripts are significantly not different (p>0.05), Treat: Treatment

(Qatley *et al*., 2000). Bacteria obtained from feces reduced AFB1 by 82.5% after incubation in the liquid medium at 37°C for 72 h AFB1 degradation was pH sensitive. The highest degradation 84.8% was observed at pH 8.0 and it decreased gradually as the pH value were down, to the lowest at pH 4.0 (14.3%). The correlation of AFB1 degradation with pH values is typical for enzymatic reactions. The AFB1 degradation varied under different temperature. The degradation was lower at 20°C (60.8%) and 30°(63.5C%) than at 37°C (78.7%). Temperature at 37°C should be more suitable for the survival and growth of the bacterium, thus optimal for its enzyme system (Shu *et al*., 2008).

Because the microbes present in probiotic would secrete analyze, protease and lipase. Which would enhance the catalytic activities of the endogenous enzymes to liberate more energy from hydrolyzing the energy feed nutrients. Such higher quantity of liberated energy would help to improve BWG of the chicks fed probiotic added rations as compared to the chicks fed

the rations containing no probiotic. Also, the microbes of probiotic in the intestinal tract of the inoculated birds may be secreting the amylolytic cellulolytic, proteolytic and lipolytic enzymes (Jozefiak *et al*., 2004) which provide maximum help to enhance the digestibility of starch, protein and fat components in the sequential way and liberated maximum energy. Such energy would not only improve the overall vital activities in the birds, but also improve the BWG. The mechanism of reducing aflatoxins by yeast and lactic acid bacteria is due to their adhesion to cell-wall components (Blanco *et al*., 1993). Yeasts *Saccharomyces cerevisiae*are capable to bind AFB1. The addition of yeast cell walls into feed can lead to a decrease of aflatoxin toxic effect in broilers (Santin *et al*., 2003).

Results in Table 6 revealed that positive controls (G_4 and G_5) gave a significant increase in liver function enzymes, alanine amino transferase (ALT) and aspartate amino transferase (AST). Also, showed significantly ($p<0.05$) higher urea and creatinine levels, as compared to healthy group fed the basal diet indicated the toxicity of aflatoxin on liver and kidney functions. These results are in coincide with those reported by Matri (2001) in Japanese quail birds and those of El-Afifi *et al*. (2013) in broiler chickens received contaminated feed with aflatoxin and showing significantly higher ($p<0.05$) serum AST, ALT, creatinine and urea. On the other hand, the intakes of probiotic bacteria and yeast reflected significantly higher ($p<0.05$) activity of serum AST and ALT. On the other hand, the intakes of probiotic bacteria and yeast significantly ($p<0.05$) eliminated the elevation ALT, AST, urea and creatinine level in aflatoxins treated broiler chickens. This result showed the detoxification activity of probiotic bacteria alone or with yeast. The probiotic and yeast with AFB_1 bound to their surfaces likely to adhere to the intestinal wall and prolong exposure to dietary aflatoxin. Hence, specific probiotics and yeast may be potent and safe means to reduced adsorption (Gratz *et al*., 2006). In addition, the protective effects of probiotic and yeast against aflatoxin induced intestinal and systemic toxicity via binding and reducing its transport in different used system (Gratz *et al*., 2007). Results indicated also that the protective action of probiotic and yeast as a potential protective agents against aflatoxin toxicity as well as their beneficial health effect and may thereby offered an effective dietary approach to decrease the risk of occurrence of liver and kidney dysfunctions.

Figure 1 shows the results of aflatoxin residues analysis. Results showed that no. AFs residue was detected in tissues of groups G_1, G_2, G_3. However, the levels of AFS residue in groups G_4 of G_5 which fed 0.5 and 1.0 mg AFS/kg contaminated diet were 4 and 7 ppb, respectively.

Toxic residues of AFs in animal products were harmful to public health. Aflatoxins tend to infiltrate most of the

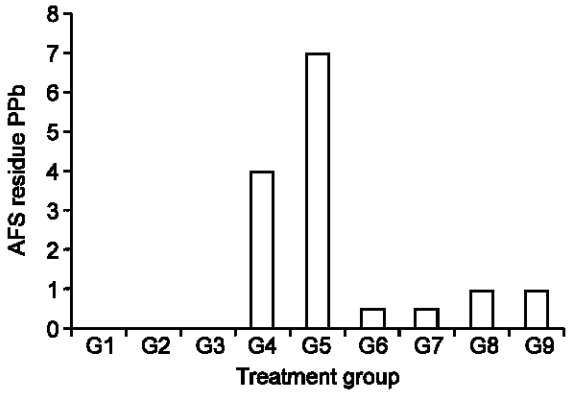

Fig. 1: Residue of aflatoxin in thigh muscle (ug/kg wet tissue) of broiler of different tested groups

soft tissues and fat depends of the chicken (Leeson *et al*., 1995). One day after administration of a single oral dose of C^{14}-labelled AFB1 to laying hens, the highest concentration of C^{14}-activity was detected in the liver, followed by muscle, pancreas, skin adipose tissue, lungs and spleen (Sawhney *et al*., 1973 a,b).

Histopathological examination: Microscopically, examination of chicken liver from G_1 revealed no histopathological changes (Fig. 2). Meanwhile, liver of chicken from G_4 showed accompanied with focal aggregations of inflammatory cells. Mild perivascular coagulative necrosis also occurred. Examined sections from G_5 showed that hepatocytes existed vascular degradation thickening in the wall of arteries and veins with edema with distended hyperplastic bile ductules (Fig. 9).

Examined sections from G_2 showed slight congestion of blood vessels, portal areas showed aggregation of macrophages and bile ductules hyperplasia (Fig. 13). The G_3 showed no definite lesions in all organs. Liver of chicken from G_6 showed congested blood vessels. Moreover, sections of liver from G_7 revealed congested blood vessels, thickening of hepatic capsule (Fig. 15) with macrophage aggregations. There was bile ductules hyperplasia. On the other hand, examined sections from G8 revealed mild aggregation of inflammatory cells. Examined sections from G_9 which treated with high concentration of AFS and probiotic plus yeast showed slight congested blood vessels and aggregation of inflammatory cells.

Although the liver is known to be the target organ of AFB1, respiratory exposure to AFB1 contaminated dust has been linked with increased incidence of tumor in the respiratory tract of animals and humans. Biodegradation of AFB1 by lung cells and by nasal mucosal epithelial cells, with subsequent formation of B_1-DNA adducts has been reported (Daniels and Massey, 1992; Tjalve *et al*., 1992).

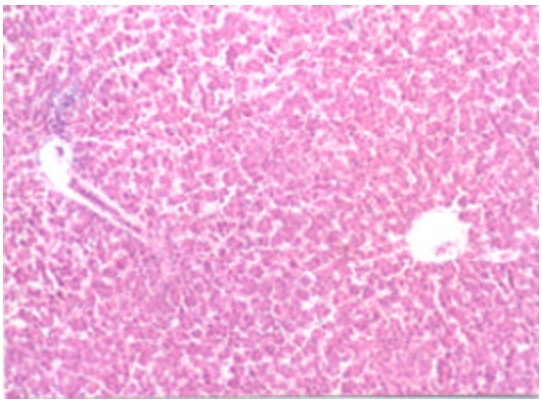

Fig. 2: Liver of chicken fed control diet showed no histopatholgical changes

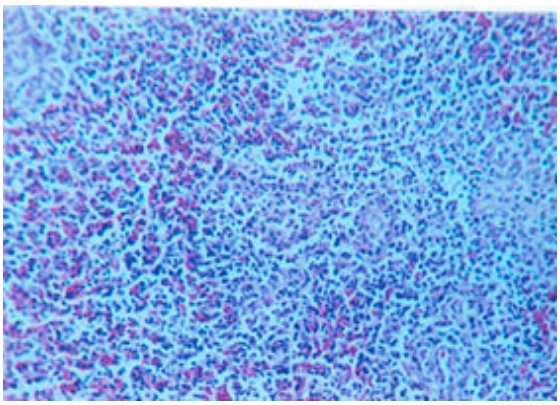

Fig. 5: Spleen of chicken treated with toxin (0.5 mg/kg) capillary sheath proliferation (H and E and 250)

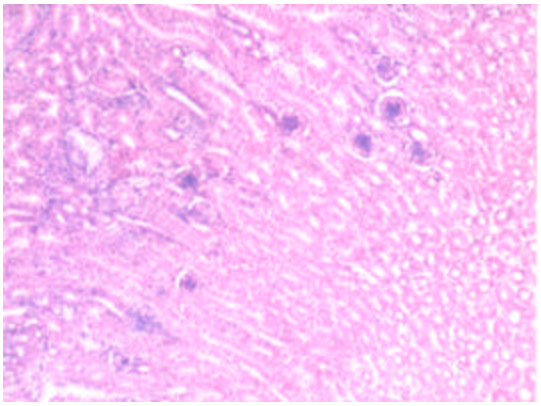

Fig. 3: Kidneys of chicken fed control diet showed no histopatholgical changes

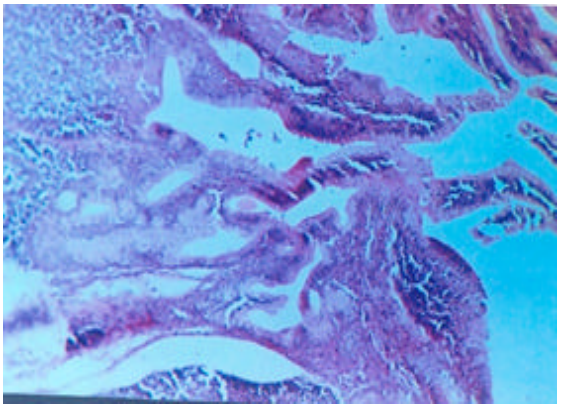

Fig. 6: Bursa of chicken treated with toxin (0.5 mg/kg) showed epithelial hyperplasia (H and E X 100)

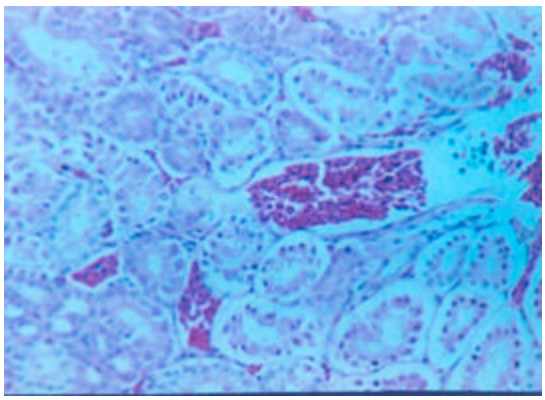

Fig. 4: Kidney of chicken treated with toxin (0.5 mg/kg) showed necrobiotic changes of renal tubular cells and congested blood vessels (H and E X 250)

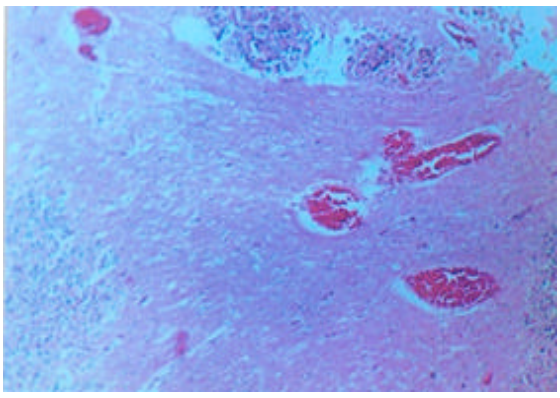

Fig. 7: Brain of chicken treated with toxin (0.5 mg/kg) showed haemorrhage and congestion of blood vessels (H and E X 100)

Kidneys of chicken from G_1 revealed no histopathological changes (Fig. 3). Whereas kidneys of chicken from G4 showed the majority of the renal tubular cells suffered from necrobiotic changes (Fig. 4), in

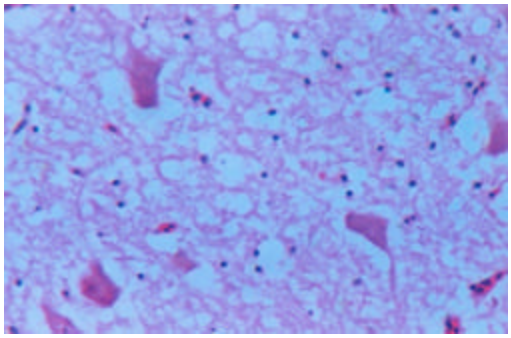

Fig. 8: Brain of chicken treated with toxin (0.5 mg/kg) showed spongiosis and neurons necrosis (H and E X400)

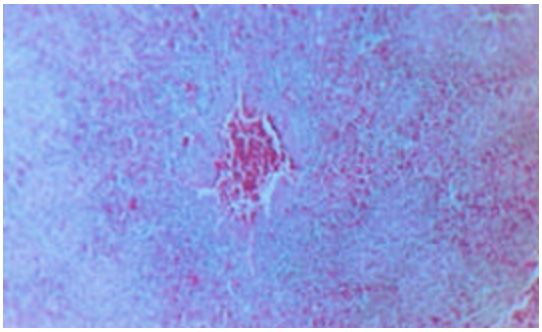

Fig. 11: Spleen of chicken treated with toxin (1 mg/kg) showed pronounced haemorrhage (H and EX100)

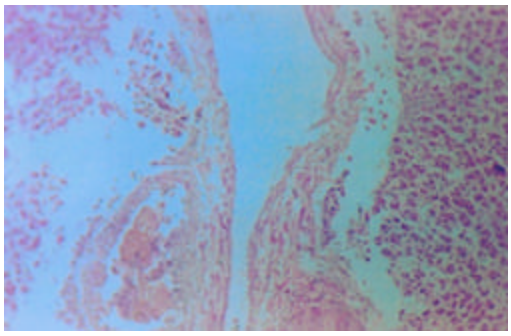

Fig. 9: Liver of chicken treated with toxin (1 mg/kg) showed bile ductal hyperplasia (H and EX100)

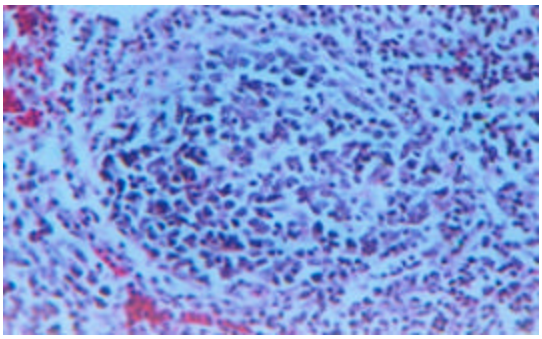

Fig. 12: Spleen of chicken treated with toxin (1 mg/kg) showed macrophages (H and E X 400)

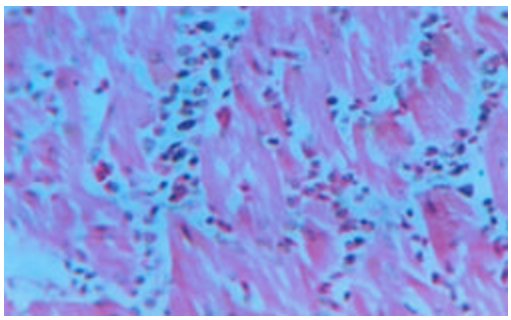

Fig. 10: Heart of chicken treated with toxin (1 mg/kg) showed heterophiles and macrophages infilteration between cardiac muscle (H and EX400)

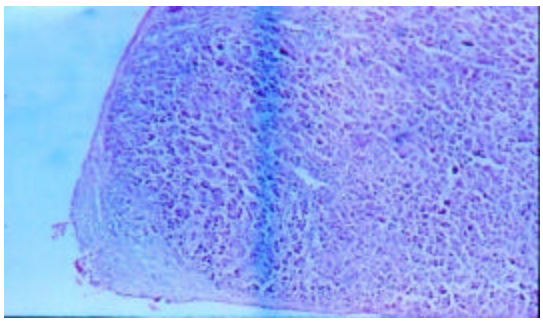

Fig. 13: Liver of chicken treated with probiotic showed bile ductules hyperplasia (H and E X 250)

addition to vacuolar degeneration. There was peripheral haemorrhage. Examined section from G_5 showed haemorrhage and congestion of renal blood vessels and granular degeneration of tubules epithelial cells. Examined sections from G_2 showed congested blood vessels and haemorrhage were existed (Fig. 14). The G_3 showed no definite lesions. All renal blood vessels of kidneys from, G_6 were congested. Section of kidney from G_7 showed severed haemorrhage (Fig. 16). On the other hand, examined sections from G_8 showed slight congested blood vessels. Kidneys of chicken from G_9 showed congested blood vessels and slight granular degeneration of renal tubules.

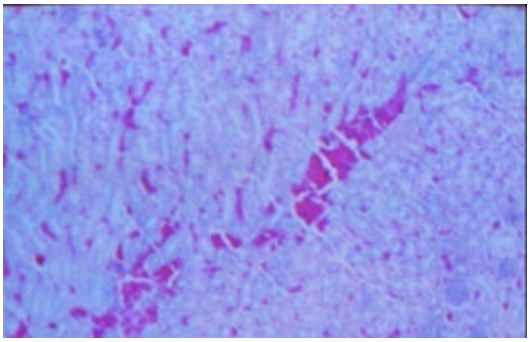

Fig. 14: Kidney of chicken treated with probiotic showed severe hemorrhage (H and E X 10)

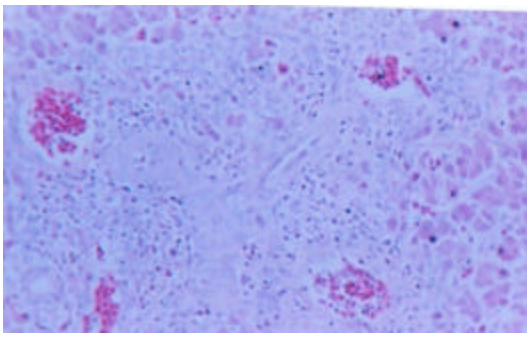

Fig. 15: Liver of chicken treated with toxin (0.5 mg) and yeast and 1 dose probiotic showed thickening of hepatic capsule (H and X 100)

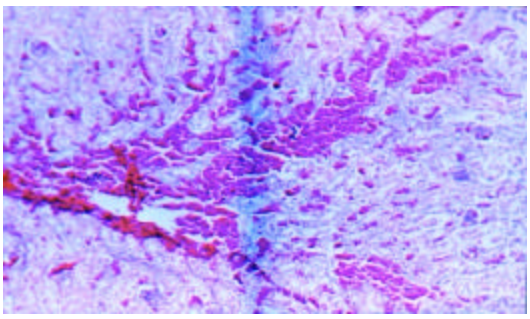

Fig. 16: Kidney of chicken treated with toxin (0.5 mg) and yeast and 1 dose of probotic showed severe haemorrhage (H and E X 100)

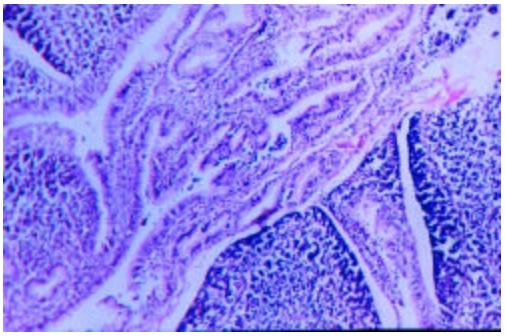

Fig. 17: Bursa of chicken treated with toxin (0.5 mg) and yeast and probiotic showed pronounced epithelial hyperplasia (H and E X 100)

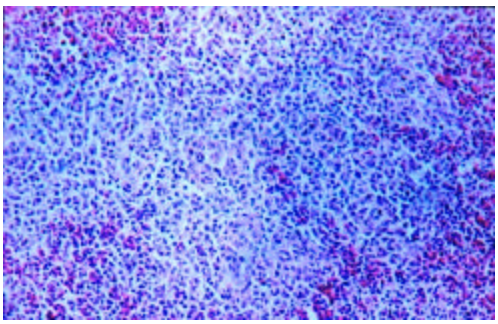

Fig. 18: Spleen of chicken treated with toxin (1 mg) and yeast showed aggregation of macrophages and haemorrhage (H and E X 250)

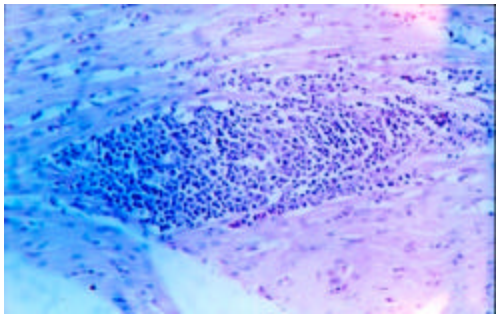

Fig. 19: Heart of chicken treated with toxin 1 mg), yeast and 1 dose of prophetic showed aggregation of macrophages (H and E X 250)

The results of histopathology obtained indicated the toxicity of aflatoxins in liver and kidney, these results walk in the same line with numerous animal studied which have shown that the liver in the main target organ and therefore the main symptoms of aflatoxin exposure in domestic laboratory animals are hepatic injuries (IARC, 1993). In addition, Matri (2001) reported that sever histopathological changes was observed in the liver, kidney, heart, ovary and oviduct during aflatoxicosis. Also, these results agree with (El-Afifi *et al*., 2013), who reported that the livers of the AFS treated group were slightly pale, enlarged and grayish mottled in appearance. However, addition of probiotic strain to aflatoxin treated chicken showed improvement in the

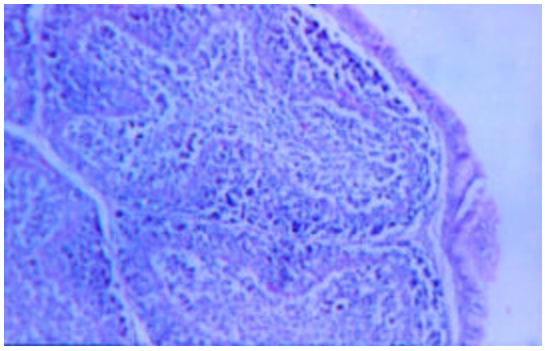

Fig. 20: Bursa of chicken treated with toxin (1 mg), yeast and probiotic (1 dose) showed marked lymphocytic depletion (H and E X 100)

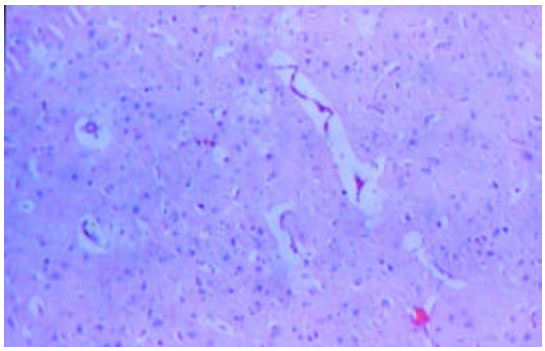

Fig. 21: Brain of chicken treated with toxin (1 mg, yeast and probiotic (1 dose) showed perivascular edema (H and E X 100)

liver sections and showed no histopathological changes in kidneys as negative control. These results agree with Bekhatro (2008) and Yener *et al.* (2009) who reported that liver of rate fed on probiotic showed no histopathological changes except minute vacuoles in the cytoplasm of some hepatocytes.

In G_4 heart, it showed intermyocardial haemorrhage and myomalacia. Spleen showed depletion of lymphocytes with profilteration of reticuloendothelial cells. Capillary sheathes were proliferation (Fig. 5) with haemorrhage. Bursa reflected epithelial lining exhibited hyperplasia (Fig. 6). Brain blood vessels were congested with haemorrhage mainly in the cerebellum (Fig. 7). Spongiosis and neurons necrosis were observed (Fig. 8). In G_5 heart, myomalacia was observed with perivascular edema. Few heterophils and reticular cells detected in between muscle fibers (Fig. 10). Spleen showed pronounced haemorrhage (Fig. 11) and aggregation of reticuloendothial cells (macrophage) (Fig. 12). Bursa epithelial hyperplasia was marked Brain was affected with spongiosis and necrosis degeneration. In G_2 and G_3 heart, no definite lesions. Bursa with no definite lesions. Spleen showed no definite lesions. Brains showed no definite lesions. In G6, spleen showed congested blood vessels and heart, Bursa and brain showed no definite lesions. In G7 heart and spleen showed no definite lesions, but Bursa slight lymphocytic depletion as well as epithelial hyperplasia was observed (Fig. 17). In G_8 heart no definite lesions. Spleen haemorrhage was exhibited (Fig. 18). In addition to macrophages aggregations with activation of capillary sheathes. Bursa showed mild depression of lymphocytes. Brain showed congested blood vessels and spongiosis. In G_9 heart revealed swelling muscles fibers with aggregation of macrophages (Fig. 19). Spleen showed no definite lesions Bursa showed pronounced depression of lymphocytes (Fig. 20). Brain showed congested blood vessels perivascular edema and neurons degeneration were detected (Fig. 21).

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