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Effect of probiotic supplement on aflatoxicosis and gene expression in the liver of broiler chicken

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ABSTRACT

The present study aimed to investigate the toxic effect of aflatoxin B1 (AFB1) and the effectiveness of a biological mycotoxin binder (Nutritox®) in detoxifying aflatoxicosis. Ninety one-day-old chicks were chosen and divided into 3 groups. The first group received standard basal diet only, the second group received a basal diet with AFB1 (0.25 mg/kg diet), and the third group received a basal diet with AFB1 (0.25 mg/kg diet) and Nutritox® (1 kg/ton diet). AFB1 feed contamination significantly reduced growth performance and deteriorated FCR. Moreover, it significantly increased serum AST, ALT, and malondialdehyde and significantly decreased serum total protein, albumin, globulin, SOD, CAT activities and glutathione peroxidase gene expression as well. Aflatoxin residues were detected in the liver tissues. Furthermore, the liver and kidney of AFB1 treated group showed pathological changes. The supplementation of Nutritox® significantly reduced aflatoxin levels in the liver and counteracted the negative effects of AFB1.

1. Introduction

The most serious problem in the commercial poultry breeding is mycotoxicosis (Abeera et al., 2009). Mycotoxins are secondary metabolites with a low molecular weight, produced by toxigenic fungi under specific environmental conditions (Charoenpornsook and Kavisarasai, 2006). Research has shown that the most deleterious mycotoxins are aflatoxins, which are mainly produced by *Aspergillus flavus, Aspergillus parasiticus* and sometimes by other *Aspergillus* species (Anjum et al., 2012; Wogan and Pong, 1970). Because of its carcinogenic and immunosuppressant effect, aflatoxins detrimentally influence animal and poultry health, leading to massive economic loss and depression (Fan et al., 2013; Yunus et al., 2011). Amongst aflatoxins types, aflatoxin B1 (AFB1) is the most active one (Chang et al., 2016; Wilson et al., 1994).

It was reported that the liver is considered the main target organ for aflatoxins. AFB1 generates reactive oxygen species (ROS), and hence causes oxidative damage, which could result in hepatic damage (Kheir Eldin et al., 2008). Oxidative stress is a major implication of aflatoxicosis (Umarani et al., 2008). Malondialdehyde (MDA) is an important index for lipid peroxidation and oxidative damage caused by ROS (Nielsen et al., 1997; Rahal et al., 2014). In humans and other susceptible animal species, the metabolism of aflatoxins, especially AFB1, occurs in the liver by cytochrome P450 (CYP450) microsomal enzymes. This results in the synthesis of aflatoxin 8,9-epoxide, which is a reactive molecule that binds to DNA and albumin in the blood serum, forming adducts which cause DNA damage. In addition, it was reported that aflatoxin is accumulated in the liver and the high content of microsomal cytochrome P-450 enzymes of hepatic cells favors the formation of DNA-aflatoxin adducts (Wild and Montesano, 2009; Wu and Khlangwiset, 2010). The most important biochemical effects of AFB1 are the inhibition of DNA replication and RNA synthesis (Kichou and Walser, 1994; Wu and Khlangwiset, 2010). Besides liver (Mutlu et al., 2010), aflatoxins were reported to induce significant pathological changes in other organs such as the kidneys and spleen (Bilgic and Yesildere, 1992). The regular low-level dietary intake of aflatoxin leads to chronic aflatoxicosis which causes a reduction in weight gain and a decrease in feed intake and feed efficiency (Yunus et al., 2011).

The negative effect of mycotoxins in broiler chicks was counteracted

Abbreviations: A group, aflatoxin group without Nutritox®; AFB1, aflatoxin B1; ALT, alanine aminotransferase; AN group, aflatoxin group with Nutritox®; AST, aspartate aminotransferase; CAT, catalase enzyme; C group, control group without aflatoxin or Nutritox®; DM, dry matter; DNA, deoxyribonucleic acid; FCR, feed conversion ratio; GPx, glutathione peroxidase; MDA, malondialdehyde; RNA, ribonucleic acid; RF, Retention Factor; SOD, superoxide dismutase enzyme; TLC, thin layer chromatography

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by many physical, chemical and biological methods. The biological approach, using microorganisms and their metabolites, was recommended as a promising alternative to detoxify mycotoxins. Some microbes, including fungal and bacterial isolates (Zhao et al., 2011; Zhang et al., 2014), were reported to effectively biodegrade aflatoxins *in vitro*. Nutritox®, one of many commercial mycotoxin binder products available in the Egyptian market and used in aflatoxin-contaminated diets, has been proven to improve growth performance in layers and broilers (Ma et al., 2012; Fan et al., 2013).

Since the role of the dietary Nutritox® in protecting hepatic structure, function and gene expression from AFB1 in broilers has not been assessed so far, the main objective of the present study was to investigate the toxic effect of AFB1 and the protective effect of Nutritox® on growth performance, antioxidant status, liver function, histopathology and liver gene expression of broiler chicks.

2. Materials and methods

2.1. Aflatoxin production and determination

AFB1 used in the present study was obtained by using a toxigenic strain of *Aspergillus flavus* (Gene bank accession number KP137700), isolated previously from broilers feed at the Mycology Department, Animal Health Research Institute, Giza, Egypt. The toxigenic *A. flavus* was subcultured and grown for 7 days on Czapek yeast extracts. The *A. flavus* was screened for aflatoxins (AFs) production using Thin Layer Chromatography (TLC) (Shotwell et al., 1981). The presence of various AFs was qualitatively confirmed by the appearance of blue fluorescence on the plate and the comparison of the spot's Retention Factor (RF) value versus the RF of a known standard. Crushed yellow corn, obtained commercially, was verified as completely free from fungal or mycotoxins contaminations by gross and TLC examination. The corn was placed in conical flasks and then autoclaved at 121 °C for 15 min for three successive days. The corn was then treated with 10 ml of the spore suspension of toxigenic *A. flavus* strain (10^6 spores/ml), and the whole mixture was fermented by incubation at 28–30 °C for 21 days. After the incubation period, the corn in flasks was dried in a 60 °C oven for 24 h to kill the fungus, and then the product was powdered using a grinder. A 25 g representative sample of the yield was assessed for AFB1 content according to A.O.A.C. (1980). The corn containing aflatoxins was then incorporated into the basal diet of broiler chicken at a level that would provide the desired dose of 0.25 mg of AFB1/kg diet.

2.2. Experimental design, feeding program, and management

A total of 90 one-day-old chicks were obtained from a private farm. The chicks were housed in a clean well-ventilated room and kept under good sanitation and hygienic management. Feed and water were available ad libitum. After an acclimatization period of three days, chicks (average body weight = 83.75 g/chick) were randomly allotted into 3 groups (2 replicates per treatment and 15 chicks per replicate) (see Table 1). The first group (C), the negative control group, was fed on a basal diet (NRC, 1994) (see Table 2). The second group (A), the positive control group, was fed on a basal diet containing moldy corn instead of normal corn at a level that would provide the desired dose of 0.25 mg AFB1/kg diet. The third group (AN) was fed on a basal diet containing moldy corn and supplemented with 1.0 Kg of Nutritox® /Ton feed (biological mycotoxin adsorbent).

All birds were weighted at the beginning of the experiment and once each week. The feed was weighted daily to evaluate the feed intake. The feed conversion ratio (FCR) was calculated according to Tacon (1987). All groups of the broiler chicks were kept under observation for symptoms, mortality, and postmortem (PM) during the 42-day experimental period.

Table 1
Experimental design for the broiler chicks groups.

Groups	Diet type	AFB1	Nutritox®[a] (Biological mycotoxin binder)
C	Basal diet	–	–
A	"	0.25 mg/kg diet	–
AN	"	0.25 mg/kg diet	1 kg/ton

[a] It is a biological commercial mycotoxin binder made in Agrarian Marketing Corporation Company, USA, imported by IFT (International Free Trade Company) for Animal Health Research Institute, Egypt.

Table 2
Ingredients and calculated chemical composition of the used basal diets.

Ingredients	Ingredients%		Chemical composition		
	Starter	Grower and finisher	Items	Starter	Grower and finisher
Yellow Corn	55	61.5	ME (Kcal/Kg)	3225.8	3209.3
Soybean (44%)	25.5	25.5	CP%	23.09	20.04
Corn gluten meal (62%)	11.22	5.59	Calcium %	1	0.9
Corn oil	4	3.8	AP%	0.45	0.35
DCP[a]	1.8	1.25	Lysine%	1.1	1
Limestone[b]	1.3	1.36	Meth. + Cyst%	0.9	0.72
Lysine[c]	0.16	0.1			
DL-Methionine[d]	0.1	0.03			
Common salt	0.4	0.4			
Choline chloride (60%)[e]	0.22	0.17			
Premix[f]	0.3	0.3			

[a] Di-calcium phosphate (DCP): contain 18% phosphorus and 25% calcium.
[b] Lime stone contains 37% calcium & locally produced.
[c] Lysine 87% produced by Archar Daniels method company De Caur LL. Made in U. S. A.
[d] DL-methionine produced by Evoink Co. Guranted analysis 99.5% DL- methionine.
[e] Choline: choline chloride 60% with vegetable carrier (corn powder) produced by Shandyuong Pharmaceutical Co. China.
[f] Premix: each 2.5 kg contains: Vit A (12000000Iu), vit D (2000000Iu), vit E (10 g), vit K3 (2 g), vit B1 (1 g), vit B2 (5 g), vitB6 (1.5 g), vit B12 (10 g), nicotinic acid (30 g), pantothinic acid (10 g), folic acid (1 g), biotin (50 mg), choline chloride50% (250 g), iron (30 g), copper (10 g), zinc (50 g), manganese (60 g), iodine (1 g), selenium (0.1 g), cobalt (0.1 g) and carrier Q. S up to 2.5 kg.

2.3. Chemical analysis of feed

Analytical dry matter (DM) contents of feed samples were determined by oven-drying at 105 °C for 3 h (A.O.A.C., 2000, method 930.15). Ash contents of the feed were determined by incineration at 550 °C overnight. The crude protein was determined by using the Kjeldahl method according to Randhir and Pradhan (1981). The ether extract was determined according to Bligh and Dyer (1959) technique, modified by Hanson and Olly (1963).

2.4. Sampling and biochemical parameters measurements

At Day 42, the blood samples were collected from 4 birds of each group. The blood samples were left to coagulate at room temperature. The separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 15 min. The clear serum was kept in a freezer (−20 °C) until use to detect the serum superoxide dismutase (SOD) (Nishikimi et al., 1972), catalase (CAT) (Ashru and Sinha, 1971), lipid peroxidation (MDA) (Beuge and Aust, 1978), total protein (Henry, 1964), albumin (Dumas and Biggs, 1972), globulin (calculated by mathematical subtraction of albumin value from that of the total protein), ALT and AST (Reitman and Frankel, 1957).