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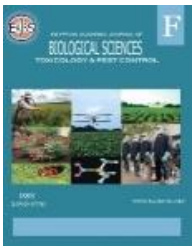

Effects of Chemical and Biological Anti-mycotoxins on Performance, Haematological, Biochemical and Immunological Parameters of Broiler Chickens during Aflatoxicosis

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ABSTRACT

Background:Contamination of feedstuffs with mycotoxins is a worldwide problem of great importance. Mycotoxins are secondary toxic fungal metabolites. There are great interests to create effective prevention and decontamination methods to diminish the toxic effects of AFs in animal and poultry production.

Objectives: The present study was designed to evaluate the toxic effects of aflatoxin on performance, some hematological, serum biochemical and immunological parameters and to determine the preventive effect of added anti-mycotoxins.

Methods:In this study, a total of 180 broilers were used and divided into 6 equal groups. They received zeocem (chemical synthetic anti-mycotoxin) at a dose of 1 kg/ton feed and nutritox (biological synthetic anti-mycotoxin) at a dose of 0.25 kg/ton feed, to treat broiler fed aflatoxin. All treatments were administered from 1- 42 days of age.

Results: By the end of the experiment, nutritox (biological synthetic anti-mycotoxin) alleviated the hazardous effects of aflatoxin on performance, hematological, serum biochemical and immunological parameters rather than zeocem (chemical anti-mycotoxin) fed broilers.

Conclusion:Treatment with biological synthetic anti-mycotoxin (nutritox) is better than using chemical synthetic anti-mycotoxin (zeocem) in control of aflatoxicosis in broilers.

INTRODUCTION

Contamination of feedstuffs with mycotoxins is a worldwide problem of great importance. Mycotoxins are secondary toxic fungal metabolites. Aflatoxins (AFs) are mainly formed during the growth of *Aspergillus flavus* and *A. parasiticus* and can be

harmful to human and animal health; causing economic losses in animal production especially in the poultry industry by suppressing immunity in flocks and increasing susceptibility to several infections, decreasing egg and meat production, decreasing feed consumption and growth inhibitory effect (Pimpukdee *et al.*, 2004), (Verma, 2004), (Bhat *et al.*, 2010) and (Gutleb *et al.*, 2015). AFs are recognized hepatotoxic, mutagenic, carcinogenic and immunosuppressive agents in animals and poultry (Richard, 2007), (Rawal *et al.*, 2010) and (Magnoli *et al.*, 2011). Other undesirable Biochemical, hematological, immunological effects of AFs have also been reported (Manafi *et al.*, 2014), (Nemati *et al.*, 2015) and (Magnoli *et al.*, 2017). There are four aflatoxins produced naturally, B1, B2, G1, and G2. Aflatoxin B1 (AFB1) is the most common in feed and believed to be the most biologically active form causing cytotoxicity, genotoxicity and oxidative stress (Sweeney and Dobson, 1998); (Vaamonde et al., 2003) and (El-Nekeety *et al.*, 2017). AFB1could be activated by cytochrome P450 (CYP450) into a highly reactive metabolite that reacts with DNA and proteins inducing genotoxicity and cytotoxicity (Cervino *et al.*, 2007) and (Muhammad *et al.*, 2017).

There are great interests to create effective prevention and decontamination methods to diminish the toxic effects of AFs in animal and poultry production. Many natural adsorbents and organic compound; such as zeolites, bentonites, lactic acid bacteria, yeast and yeast cell wall; have been used to control and reduce the negative effects of AFs (Daković *et al.*, 2008), (Onwurah *et al.*, 2013) and (Roto *et al.*, 2015). mycotoxicosis can be controlled by using mycotoxin binders to decrease their absorption and bioavailability. The most famous binders used are zeolite, hydrated sodium calcium aluminosilicate (HSCAS), bentonite, montmorillonite, and active carbons. Another control approach is converting mycotoxins into non-toxic metabolites using bacteria and yeast cell walls, enzymes, vitamins, amino acids, and synthetic polymers as cholestyramine and polivinil-polipirrolidon polymers

Zeocem is a mixture of zeolites and (HSCAS) while, Nutritox is a biological synthetic food additive composed of bacteria (*lactobacillus acidophilus*), organic acids, anti-inflammatory (papin), lipase and protease enzymes, vitamin B complex, vitamin E and propylene glycol.

The aim of the present study was to investigate the undesirable effects of aflatoxicosis on performance, blood hematology, serum biochemical and immunological parameters in addition to pathological changes. Also to evaluate and compare the preventive efficacy of the biological antimycotoxin (nutritox) and the chemical antimycotoxin (zeocem) against aflatoxicosis in broilers.

MATERIALS AND METHODS

Ethical Statement:

Animal Care and Ethics Review Committee at the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (The approval NO. 201710), approved animal care and housing, as well as the experimental protocol. In addition, the experimental protocol is in accordance with guidelines for the care and use of laboratory animals of the National Institute of Health. Appropriate procedures were used to reduce potential pain, distress, and discomfort. Animals were housed in groups in order to promote social behavior.

Experimental Chickens:

One hundred and eighty Isa Hubbard broiler chicks (one day old) were obtained from Ismailia Poultry Company Ismailia City, Egypt. Chicks were reared in litter under standard environmental and hygienic conditions and were fed on a balanced basal ration

formulated according to NRC (NRC, 1994) (Table1). Feed and water were given *ad-libitum*. The temperature was adjusted to 32°C during the first week of age and decreased by 2°C per week (Harrison and Harrison, 1986). All birds were vaccinated against Newcastle disease and against Gumboro disease at proper times (Giambrone and Clay, 1986).

Table 1: Composition of the experimental diets

Ingredients	Starter (0-3weeks)	Grower-Finisher (4-6 weeks)
Ground yellow corn	56.7	66.6
Soya bean meal (44% CP)	29.5	23.53
Fish meal (60.5% CP)	7.0	5.0
Soya bean oil	4.06	2.02
Dicalcium phosphate	0.88	0.6
Limestone	1.26	1.69
DL – Methionine (purity 96%)	0.1	0.06
Iodized sodium chloride	0.25	0.25
Vitamins &mineral premix*	0.25	0.25
Calculated composition		
Crude protein	22.0	19.0
ME kcal per kg	3060.0	3040.0
Calorie/protein ratio(C/P)	139.0	160.0

** Each 2.5 kg contain the following vitamins and minerals:*
Vit. A 12 mIU, vit. D3 2 mIU, vit. E 1000mg, vit. k3 2000mg, vit. B1 1000mg, vit. B2 5000mg, vit. B6 1600mg, vit. B12 10mg, biotin 50mg, pantothinic acid 10000mg, nicotinic acid 30000mg, folic acid 1000mg, manganese 6000mg, zinc 5000mg, iron 3000mg, copper 10000mg, iodine 1000mg, selenium 100mg, cobalt 100mg, carrier(CaCO3) to 2.5kg. (AGRI-VET. Under technical assistance of HELM Germany).

Protective Agents Against Aflatoxin:

a) Zeocem: is a chemical synthetic product of Agro Company, (Slovak Republic), composed of **a** mixture of mycotoxin binders, zeolites, and Hydrated Sodium Calcium Aluminium Silicates (HSCAS).

b) Nutritox: is a biological synthetic feed additive manufactured by Eldwelea company, composed of lactobacillus acidophilus bacteria (L-form), organic acids, papin (has proteolytic & anti-inflammatory activities), lipase and protease enzymes, vitamin B complex and vitamin E and propylene glycol (a source of energy).

Standard Aflatoxin:

Aflatoxin B1 (AFB1) was purchased from Sigma Chemical Co. (St. Louis, MO 63118, USA).

Experimental Design:

A total number of chicks (180) was divided into 6 equal groups (n = 30 each group with three replicates). Group (I): fed the normal diet and kept as control. Group (II): fed diet containing zeocem at a dose of 1 kg/ton feed. Group (III): fed diet containing nutritox at a dose of 0.25 kg/ton feed. Group (IV): fed 2.5mg aflatoxin/kg diet according to (Kececi *et al.,* 1998) with zeocem at a dose of 3 kg/ton feed. Group (V): fed diet containing 2.5 mg aflatoxin/kg diet with nutritox at a dose of 0.5 kg/ton of feed and group (VI): fed 2.5 mg aflatoxin/kg diet. All treatments were administered from 1- 42 days of age.

Growth Performance:

Body weight was individually determined on weekly basis. Body weight gain, feed consumption and feed conversion ratio (FCR) were calculated. Body weight gain, feed consumption, and FCR were calculated for the whole experimental period (Brady, 1968).

Sampling:

Two blood samples were obtained from each bird from wing vein at the end of the 2nd, 4th and 6th week. The first sample (1 ml of whole blood) was used for evaluation of the hemogram. Serum was prepared from the second sample and used for assays of serum biochemical and immunological parameters. After blood sampling, chicks were sacrificed to obtain livers and kidneys for histopathological examination.

Hemogram:

Parameters of the hemogram (RBC, Hb, PCV, MCV, MCH, MCHC, TLC and differential leucocytic count) were determined according to the standard techniques described by (Jain, 1986). Blood films were stained by Giemsa stain. The percentage and absolute value for each type of white cells were calculated according to (Feldman *et al.,* 2000).

Serum Biochemical Parameters:

Alanine aminotransferase (ALT), Gamma-glutamyl transfrase (GGT) activities, total and direct bilirubin were determined colorimetrically according to the methods of (Reitman and Frankel, 1957) and (1970)(Laemmli, 1970) respectively, using commercial kits of Randox co. UK. Serum total protein and albumin were determined using Stanbio kits according to (Gornall *et al.,* 1949) and (Bablok *et al.,* 1988) respectively. Globulin was estimated by subtract the total serum albumin from total serum protein according to (Knight *et al.,* 1972). Cholesterol was evaluated according to (Allain *et al.,* 1974) using kits obtained from Spinreact company. Serum glucose was assessed according to (Beach and Turner, 1958) from Spinreact company. Serum uric acid was determined according to (1990) by using Spinreact kits. Creatinine was determined according to (Larsen, 1972) using Human company, Germany.

Immunological Analysis:

(IgG, IgM) were evaluated using Elisa kits obtained from Bethyl laboratories, Inc. and were determined according to (Larsson *et al.,* 1993). Interleukins 1 and 10 (IL1& IL10) ELISA kits were purchased from Kamiya biomedical company and were determined according to manufacturer's protocol While, Tumor necrotic factor–α (TNF-α) and interleukin 6 (IL6) ELISA Kits were obtained from Genorise scientific, Inc**.**, and determined according to (Wajant *et al.,* 2003).

Histopathological Examination:

Specimens of livers and kidneys were taken from all groups then fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5-micron thickness and stained with Hematoxylin and Eosin (H&E) for histopathological examination (Mepham, 1991).

Statistical Analysis:

Data that were collected from performance parameters, hematological and serum biochemical analysis of treated and control groups were statistically analyzed. Results expressed as mean ± standard error (S.E.). Significance of the results was evaluated using the one-way analysis of variance (ANOVA). Results were considered statistically significant at a level of $p \leq 0.05$.

RESULTS

Growth Performance:

Final weight and body weight gain were significantly higher in groups I, II, and III compared to aflatoxin treated groups IV, V, and VI. Group V significantly had higher final body weight and gain compared to group IV. Feed consumption was significantly higher in groups III, IV. Significantly followed by groups VI, and I. Groups II and V had the lowest feed consumption. A significant improvement in FCR was seen in groups I, II, and III followed by group VI. The significantly worst FCR was seen in group IV (Table 2).

Table 2: The effect of Aflatoxicosis, nutritox and zeocem on growth performance parameters (mean ±SE) of different groups.

Groups / Parameters	I	II	III	IV	V	VI
Initial weight (g/bird)	46.92±0.93^{a}	45.25±0.50^{a}	45.91±0.73^{a}	45.07±1.41^{a}	45.80±1.21^{a}	46.03±1.90^{a}
Final weight (g/bird)	2328.79±30.91^{a}	2270.10±5.83^{a}	2278.63±6.06^{a}	1170.60±57.73^{d}	1760.18±53.56^{c}	2010.51±55.19^{b}
Body weight gain(g/bird)	2281.87±30.09^{a}	2224.85±6.33^{a}	2232.75±5.66^{a}	1125.53±58.91^{d}	1714.38±52.40^{c}	1964.48±53.40^{b}
Feed consumption (g/bird)	3418.00±13.05^{bc}	3353.67±3.71^{c}	3640.67±54.86^{a}	3635.00±57.83^{a}	3391.67±17.85^{c}	3524.33±20.63^{b}
FCR	1.50±0.02^{d}	1.51±0.00^{d}	1.63±0.02^{cd}	3.25±0.15^{a}	2.01±0.03^{b}	1.80±0.06^{c}

Within the same row, means with different superscripts significantly differ among the studied groups ($P \leq 0.05$).

Hematological Results:

After two weeks, total erythrocytic count, hemoglobin, PCV, heterophils, lymphocytes as well as total leucocytic count were significantly decreased in groups IV and VI while, insignificantly changed in the other groups compared with the control (Tables 3&4). The decreases in total leucocytic count, heterophils and lymphocyte in group VI were more than in group IV. No significant changes were recorded in MCV, MCH, MCHC, total eosinophilic and basophilic count.

After 4 weeks total erythrocytic count, hemoglobin, PCV, MCV, MCH and MCHC were significantly decreased in groups IV and VI, while significantly increased in groups III and V when compared to control one (Table 5). Also, total erythrocytic count and PCV were significantly increased in group V while, all these parameters were insignificantly changed in group II. As shown in table 6, total leucocytic count was significantly decreased in groups IV and VI and significantly increased in group III. The decrease in total leucocytic count in group VI was more obvious than in group IV. In groups II, V there was no significant changes. Heterophils were significantly decreased in groups IV and VI, while groups III and V were significantly increased in that parameter. Lymphocytes were significantly decreased in groups IV and VI. Monocytes were significantly decreased in group VI.

Table (3): The effect of Aflatoxicosis, nutritox and zeocem on some erythrogram parameters (mean ±SE) on different groups after two weeks .

Group / Parameter		II	III	IV	V	VI
RBC (10^6/µl)	2.74 ± 0.07^{ab}	2.72 ± 0.07^{ab}	2.81 ± 0.05^{a}	2.60 ± 0.06^{b}	2.78 ± 0.05^{a}	2.50 ± 0.03^{c}
Hb (gm/dl)	9.80 ± 0.23^{a}	9.70 ± 0.17^{a}	9.90 ± 0.09^{a}	9.31 ± 0.21^{b}	9.83 ± 0.11^{a}	8.81 ± 0.09^{c}
PCV (%)	34.4 ± 1.51^{b}	34.0 ± 2.07^{b}	35.1 ± 1.30^{a}	32.5 ± 1.67^{c}	34.8 ± 1.30^{a}	31.4 ± 0.89^{d}
MCV (Fl)	125 ± 1.44^{a}	125 ± 1.11^{a}	125 ± 0.90^{a}	125 ± 0.44^{a}	125 ± 0.89^{a}	126 ± 1.02^{a}
MCH (Pg)	35.8 ± 2.34^{a}	35.7 ± 2.19^{a}	35.2 ± 1.69^{a}	35.8 ± 1.06^{a}	35.4 ± 0.54^{a}	35.2 ± 1.43^{a}
MCHC (%)	28.5 ± 2.06^{a}	28.5 ± 2.00^{a}	28.2 ± 1.26^{a}	28.7 ± 0.83^{a}	28.3 ± 0.61^{a}	28.1 ± 1.22^{a}

Within the same row, means with different superscripts are significantly differ among the studied groups at ($P \leq 0.05$).

Table 4: The effect of Aflatoxicosis, nutritox and zeocem on leucogram parameters (mean ±SE) on different groups after two weeks

Group / Parameter	I	II	III	IV	V	VI
WBC (10^3 /µl)	35.6 ± 0.75^{a}	35.2 ± 1.01^{a}	36.4 ± 0.75^{a}	32.4 ± 0.75^{b}	35.6 ± 0.75^{a}	28.8 ± 1.01^{c}
Heterophils (10^3 /µl)	12.0 ± 0.25^{a}	11.4 ± 0.45^{ab}	11.9 ± 0.35^{ab}	10.7 ± 0.23^{b}	11.5 ± 0.42^{ab}	9.21 ± 0.36^{c}
Lymphocytes (10^3 /µl)	22.0 ± 0.42^{a}	22.1 ± 0.55^{a}	22.7 ± 0.56^{b}	20.0 ± 0.63^{b}	22.3 ± 0.46^{b}	17.9 ± 0.74^{c}
Monocytes (10^3 /µl)	0.79 ± 0.14^{a}	0.92 ± 0.10^{a}	0.94 ± 0.09^{a}	0.97 ± 0.02^{a}	0.99 ± 0.07^{a}	0.86 ± 0.03^{a}
Eosinophils (10^3 /µl)	0.57 ± 0.08^{a}	0.56 ± 0.08^{a}	0.51 ± 0.09^{a}	0.52 ± 0.70^{a}	0.49 ± 0.08^{a}	0.52 ± 0.09^{a}
Basophils (10^3 /µl)	0.28 ± 0.07^{a}	0.21 ± 0.08^{a}	0.36 ± 0.01^{a}	0.19 ± 0.08^{a}	0.28 ± 0.07^{a}	0.24 ± 0.06^{a}

Within the same row, means with different superscripts are significantly differ among the studied groups at ($P \leq 0.05$).

Table 5: The effect of Aflatoxicosis, nutritox and zeocem on some erythrogram parameters (mean ±SE) on different groups after four weeks.

Group / Parameter	I	II	III	IV	V	VI
RBC (10^6/µl)	2.80 ± 0.04^{b}	2.83 ± 0.07^{b}	3.10 ± 0.60^{a}	2.42 ± 0.60^{c}	2.90 ± 0.05^{a}	2.41 ± 0.06^{c}
Hb (gm/dl)	10.9 ± 0.21^{b}	11.0 ± 0.12^{b}	11.9 ± 0.21^{a}	8.62 ± 0.13^{c}	11.1 ± 0.12^{b}	8.60 ± 0.14^{d}
PCV (%)	35.1 ± 0.51^{c}	35.5 ± 0.58^{c}	38.8 ± 0.74^{a}	29.5 ± 0.71^{d}	36.3 ± 0.37^{b}	29.5 ± 0.66^{d}
MCV (Fl)	125 ± 0.71^{a}	125 ± 1.16^{a}	125 ± 0.75^{a}	122 ± 0.70^{b}	125 ± 1.19^{a}	122 ± 1.08^{b}
MCH (Pg)	38.9 ± 0.63^{a}	38.9 ± 0.99^{a}	38.4 ± 1.09^{a}	35.6 ± 0.71^{c}	38.2 ± 0.97^{a}	35.7 ± 0.92^{c}
MCHC (%)	31.1 ± 0.36^{a}	31.0 ± 0.58^{a}	30.7 ± 0.87^{a}	29.2 ± 0.62^{b}	30.5 ± 0.60^{a}	29.1 ± 0.79^{b}

Within the same row, means with different superscripts are significantly differ among the studied groups at ($P \leq 0.05$)

Table 6: The effect of Aflatoxicosis, nutritox and zeocem on leucogram parameters (mean ±SE) on different groups after four weeks .

Group / Parameter	I	II	III	IV	V	VI
WBC (10^3 /µl)	36.8 ± 1.01^{b}	36.8 ± 1.02^{b}	39.5 ± 0.75^{a}	29.6 ± 0.75^{c}	37.9 ± 0.75^{ab}	25.85 ± 0.89^{d}
Heterophils (10^3 /µl)	11.3 ± 0.32^{c}	11.5 ± 0.30^{bc}	12.8 ± 0.28^{a}	9.47 ± 0.34^{d}	12.4 ± 0.27^{ab}	8.27 ± 0.36^{e}
Lymphocytes (10^3 /µl)	23.6 ± 0.69^{a}	23.4 ± 0.74^{a}	24.7 ± 0.46^{a}	18.4 ± 0.64^{b}	23.3 ± 0.48^{a}	16.10 ± 0.45^{c}
Monocytes (10^3 /µl)	1.02 ± 0.07^{a}	0.96 ± 0.09^{a}	0.87 ± 0.08^{ab}	0.82 ± 0.05^{ab}	0.83 ± 0.08^{ab}	0.68 ± 0.08^{b}
Eosinophils (10^3 /µl)	0.58 ± 0.08^{a}	0.66 ± 0.07^{a}	0.78 ± 0.02^{a}	0.58 ± 0.13^{a}	0.67 ± 0.07^{a}	0.58 ± 0.07^{a}
Basophils (10^3 /µl)	0.23 ± 0.09^{a}	0.22 ± 0.09^{a}	0.32 ± 0.08^{a}	0.29 ± 0.01^{a}	0.37 ± 0.01^{a}	0.22 ± 0.05^{a}

Within the same row, means with different superscripts are significantly differ among the studied groups at ($P \leq 0.05$).

Tables 7&8 represented the hematological results after six weeks; total erythrocytic count, hemoglobin, PCV MCV, MCH and MCHC were significantly decreased in groups IV and VI when compared to control one, while groups III, V were significantly increased in total erythrocytic count, hemoglobin and PCV. The other groups of chicks were insignificantly changed in the erythrogram parameters (Table 7).

Total leucocytic count, lymphocytes and heterophils in table (8) were significantly decreased in groups IV and VI, while group III was significantly increased in those parameters. Significant monocytopenia was recorded in group VI whereas there was monocytosis in group III. Significant eosinopenia occurred only at group VI. Significant basopenia was recorded in groups IV and VI. The other groups were insignificantly changed in comparison to control.

Table 7: The effect of Aflatoxicosis, nutritox and zeocem on some erythrogram parameters (mean ±SE) on different groups after six weeks .

Group / Parameter	I	II	III	IV	V	VI
RBC (10^6/µl)	2.95 ± 0.09^{b}	2.89 ± 0.06^{b}	3.14 ± 0.06^{a}	2.66 ± 0.07^{c}	3.02 ± 0.07^{a}	2.43 ± 0.06^{d}
Hb (gm/dl)	10.9 ± 0.19^{c}	10.7 ± 0.07^{c}	11.4 ± 0.16^{a}	8.52 ± 0.19^{d}	10.9 ± 0.05^{b}	7.96 ± 0.50^{e}
PCV (%)	36.4 ± 0.51^{c}	35.7 ± 0.73^{c}	38.8 ± 0.66^{a}	31.3 ± 0.81^{d}	37.2 ± 0.80^{b}	28.8 ± 0.37^{e}
MCV (Fl)	123 ± 0.39^{a}	124 ± 0.72^{a}	124 ± 1.20^{a}	118 ± 1.17^{c}	123 ± 0.76^{a}	119 ± 1.37^{b}
MCH (Pg)	36.9 ± 0.98^{a}	37.1 ± 0.76^{a}	36.3 ± 0.84^{ab}	32.0 ± 0.37^{c}	36.2 ± 0.95^{b}	32.8 ± 0.82^{c}
MCHC (%)	29.9 ± 0.73^{a}	30.0 ± 0.67^{a}	29.4 ± 0.73^{a}	27.2 ± 0.55^{b}	29.4 ± 0.67^{a}	27.6 ± 0.57^{b}

Within the same row, means with different superscripts are significantly differ among the studied groups at ($P \leq 0.05$).

Table 8: The effect of Aflatoxicosis, nutritox and zeocem on leucogram parameters (mean ±SE) on different groups after six weeks **.**

Group / Parameter	I	II	III	IV	V	VI
WBC (10^3 /µl)	37.6 ± 0.75^{b}	38.4 ± 1.16^{b}	44.3 ± 0.75^{a}	26.2 ± 0.89^{c}	38.6 ± 0.89^{b}	22.6 ± 1.01^{d}
Heterophils (10^3 /µl)	12.3 ± 0.37^{b}	12.5 ± 0.48^{b}	13. 7 ± 0.29^{a}	8.56 ± 0.34^{c}	12.6 ± 0.26^{b}	7.43 ± 0.35^{d}
Lymphocytes (10^3 /µl)	23.5 ± 0.42^{b}	24.1 ± 0.72^{b}	28.2 ± 0.52^{a}	16.2 ± 0.36^{c}	24.1 ± 0.69^{b}	14.1 ± 0.69^{d}
Monocytes (10^3 /µl)	0.90 ± 0.09^{b}	0.84 ± 0.06^{b}	1.16 ± 0.12^{a}	0.73 ± 0.07^{bc}	0.93 ± 0.09^{ab}	0.55 ± 0.07^{c}
Eosinophils (10^3 /µl)	0.67 ± 0.08^{ab}	0.68 ± 0.07^{ab}	0.88 ± 0.01^{a}	0.53 ± 0.09^{bc}	0.70 ± 0.08^{ab}	0.38 ± 0.07^{c}
Basophils (10^3 /µl)	0.29 ± 0.08^{ab}	0.31 ± 0.08^{ab}	0.44 ± 0.01^{a}	0.20 ± 0.05^{c}	0.30 ± 0.8^{ab}	0.13 ± 0.05^{c}

Within the same row, means with different superscripts are significantly differ among the studied groups at ($P \leq 0.05$).

Biochemical Parameters:

Biochemical changes were showed in tables 9, 10 and 11. After 2 weeks (Table 9): GGT and ALT were significantly increased in groups IV and VI when compared to control one. Meanwhile, the other groups are insignificantly changed. Total and direct bilirubin was significantly increased in groups VI and also, direct bilirubin showed significant increase in group IV when compared to control one. Meanwhile, the other groups are insignificantly changed. Concerning total protein and albumin they significantly decreased in groups IV and VI and increased in group II while, they were not changed in groups III and V in compare with control group. Globulin showed insignificant changes. Cholesterol was decreased significantly in group VI only and insignificantly changed in other groups compared with control group. Glucose was not changed in group III while, decreased significantly in the other groups when compared with control. Uric acid was significantly increased in groups IV and VI and Creatinine was significantly increased in group VI while the other groups are insignificantly changed compared with control.

Table 9: The effect of Aflatoxicosis, nutritox and zeocem on some serum biochemical parameters (mean ±SE) on different groups after two weeks **.**

Group / Parameter	I	II	III	IV	V	VI
GGT (U/I)	27.6 ± 0.70^{c}	28.5 ± 0.71^{bc}	27.1 ± 0.45^{c}	30.2 ± 0.54^{ab}	26.6 ± 0.28^{c}	31.4 ± 1.02^{a}
ALT (U/I)	9.56 ± 0.28^{bc}	10.2 ± 0.27^{b}	10.1 ± 0.19^{b}	11.5 ± 0.19^{a}	9.22 ± 0.15^{c}	11.8 ± 0.25^{a}
Total Bilirubin (mg/dl)	0.36 ± 0.01^{b}	0.37 ± 0.01^{b}	0.36 ± 0.33^{b}	0.38 ± 0.35^{b}	0.36 ± 0.01^{b}	0.43 ± 0.01^{a}
Direct Bilirubin (mg/dl)	0.05 ± 0.01^{bc}	0.05 ± 0.01^{bc}	0.05 ± 0.01^{c}	0.08 ± 0.01^{a}	0.03 ± 0.01^{c}	0.07 ± 0.01^{ab}
Total protein (gm/dl)	2.85 ± 0.04^{b}	3.14 ± 0.06^{a}	2.81 ± 0.08^{b}	2.44 ± 0.11^{c}	2.82 ± 0.02^{b}	2.44 ± 0.13^{c}
Albumin (gm/dl)	1.66 ± 0.03^{b}	1.86 ± 0.03^{a}	1.62 ± 0.06^{b}	1.44 ± 0.07^{c}	1.69 ± 0.01^{b}	1.37 ± 0.08^{c}
Globulin (gm/dl)	1.18 ± 0.06^{ab}	1.28 ± 0.03^{a}	1.19 ± 0.03^{ab}	1.05 ± 0.05^{bc}	1.13 ± 0.01^{bc}	1.06 ± 0.06^{bc}
Cholesterol (mg/dl)	126 ± 1.20^{a}	128 ± 1.06^{a}	126 ± 0.66^{a}	127 ± 1.02^{a}	129 ± 0.86^{a}	122 ± 1.37^{b}
Glucose (mg/dl)	284 ± 1.32^{a}	270 ± 2.08^{c}	280 ± 0.86^{ab}	269 ± 1.90^{c}	277 ± 1.15^{b}	255 ± 1.68^{d}
Uric acid (mg/dl)	10.5 ± 0.35^{c}	10.6 ± 0.28^{bc}	10.0 ± 0.18^{c}	11.7 ± 0.26^{a}	10.1 ± 0.12^{c}	11.5 ± 0.52^{ab}
Creatinine (mg/dl)	0.36 ± 0.07^{b}	0.42 ± 0.01^{b}	0.41 ± 0.01^{b}	0.42 ± 0.01^{b}	0.44 ± 0.01^{b}	0.48 ± 0.01^{a}

Within the same row, means with different superscripts are significantly differ among the studied groups at ($P \leq 0.05$).

After Four Weeks: biochemical changes were showed in table (10); GGT and ALT were significantly increased in groups IV and VI when compared to control one. Meanwhile the other groups are insignificantly changed. Total bilirubin was significantly increased in groups IV and VI while, direct bilirubin was significantly increased in groups IV and VI and significantly decreased in groups II, III, and V. Total protein, albumin and globulin were decreased in groups IV and VI when compared with control group. Globulin was significantly increased in groups III and V. Cholesterol was significantly decreased in groups IV and VI especially in group VI. Glucose was significantly decreased in groups II, IV and VI, while significantly increased in group V. Uric acid and creatinine were significantly increased in groups IV and VI, while other groups are insignificantly changed when compared to control.

Table 10: The effect of Aflatoxicosis, nutritox and zeocem on some serum biochemical parameters (mean ±SE) on different groups after four weeks.

Parameter \ Group	I	II	III	IV	V	VI
GGT (U/I)	28.1 ± 0.73^{b}	27.5 ± 0.36^{b}	27.3 ± 0.49^{b}	33.0 ± 0.85^{a}	27.8 ± 0.41^{b}	34.4± 0.89^{a}
ALT (U/I)	9.96 ± 0.36^{b}	10.4 ± 0.23^{b}	9.96 ± 0.12^{b}	14.9 ± 0.47^{a}	10.2 ± 0.10^{b}	14.9 ± 0.41^{a}
Total Bilirubin (mg/dl)	0.44 ± 0.01^{b}	0.46 ± 0.01^{b}	0.40 ± 0.01^{b}	0.56 ± 0.01^{a}	0.44 ± 0.01^{b}	0.55 ± 0.02^{a}
Direct Bilirubin (mg/dl)	0.08 ± 0.01^{b}	0.05 ± 0.01^{c}	0.05 ± 0.01^{cd}	0.11 ± 0.01^{a}	0.03 ± 0.01^{d}	0.13 ± 0.01^{a}
Total protein (gm/dl)	3.28 ± 0.05^{a}	3.37 ± 0.07^{a}	3.38 ± 0.08^{a}	2.67 ± 0.11^{b}	3.40 ± 0.07^{a}	2.70 ± 0.07^{b}
Albumin (gm/dl)	1.90 ± 0.06^{a}	1.99 ± 0.05^{a}	1.87 ± 0.04^{a}	1.60 ± 0.07^{b}	1.85 ± 0.05^{a}	1.61 ± 0.04^{b}
Globulin (gm/dl)	1.38 ± 0.04^{b}	1.37 ± 0.02^{b}	1.51 ± 0.03^{a}	1.07 ± 0.04^{c}	1.55 ± 0.03^{a}	1.08 ± 0.03^{c}
Cholesterol (mg/dl)	137 ± 1.00^{a}	139 ± 0.75^{a}	139 ± 0.95^{a}	121 ± 1.28^{b}	138 ± 0.86^{a}	117 ± 0.80^{c}
Glucose (mg/dl)	276 ± 1.70^{b}	261 ± 1.20^{c}	278 ±1.07^{ab}	217 ± 1.01^{d}	279 ± 0.93^{a}	217 ± 0.86^{d}
Uric acid (mg/dl)	9.84 ± 0.24^{b}	10.1 ± 0.26^{b}	9.52 ± 0.20^{b}	13.9 ± 0.35^{a}	9.96 ± 0.27^{b}	14.3 ± 0.27^{a}
Creatinine (mg/dl)	0.45 ± 0.02^{b}	0.42 ± 0.02^{b}	0.45 ± 0.02^{b}	0.67 ± 0.03^{a}	0.46 ± 0.01^{b}	0.73 ± 0.03^{a}

Within the same row, means with different superscripts are significantly differ among the studied groups at ($P \leq 0.05$).

After Six Weeks: biochemical parameters were represented in table (11), GGT; total and direct bilirubins were significantly increased in groups IV and VI. ALT also increased significantly in groups IV and VI and significantly decreased in groups III and V. Total protein and albumin were significantly decreased in group IV and VI meanwhile, significantly increased in the other groups when compared to control. Globulin was significantly increased in groups II and III while, decreased significantly in groups IV and VI and unchanged in group V. Cholesterol was significantly decreased in groups IV and VI and significantly increased in group III meanwhile; the other groups are insignificantly changed. Glucose was significantly decreased in groups IV and VI especially in group VI, significant increases were found in groups II, III and V in comparison with control. Uric acid was significantly decreased in groups III and V and increased significantly in groups IV and VI. Creatinine was significantly increased in groups IV and VI, while the other groups were insignificantly changed.

Table 11: The effect of Aflatoxicosis, nutritox and zeocem on some serum biochemical parameters (mean ±SE) on different groups after six weeks **.**

Parameter \ Group	I	II	III	IV	V	VI
GGT (U/I)	28.6 ± 0.52^{b}	28.6 ± 0.54^{b}	27.1 ± 0.38^{b}	40.3 ± 0.55^{a}	27.8 ± 0.31^{b}	39.2 ± 1.01^{a}
ALT (U/I)	12.9 ± 0.27^{c}	12.1 ± 0.18^{cd}	11.1 ± 0.26^{de}	21.7 ± 0.52^{b}	10.9 ± 0.13^{e}	25.3 ± 0.67^{a}
Total Bilirubin (mg/dl)	0.43 ± 0.02^{c}	0.46 ± 0.01^{c}	0.41 ± 0.01^{c}	0.88 ± 0.04^{b}	0.44 ± 0.02^{c}	1.4 ± 0.10^{a}
Direct Bilirubin (mg/dl)	0.06 ± 0.01^{c}	0.05 ± 0.01^{c}	0.03 ± 0.01^{c}	0.11 ± 0.01^{b}	0.04 ± 0.01^{c}	0.14 ± 0.02^{a}
Total protein (gm/dl)	3.22 ± 0.03^{c}	3.92 ± 0.05^{a}	3.93 ± 0.05^{a}	2.22 ± 0.09^{d}	3.66 ± 0.08^{b}	2.38 ± 0.09^{d}
Albumin (gm/dl)	1.86 ± 0.03^{c}	2.41 ± 0.09^{a}	2.30 ± 0.05^{ab}	1.31 ± 0.04^{d}	2.18 ± 0.05^{b}	1.39 ± 0.04^{d}
Globulin (gm/dl)	1.35 ± 0.04^{c}	1.50 ± 0.08^{ab}	1.64 ± 0.03^{a}	0.90 ± 0.05^{d}	1.47 ± 0.04^{bc}	0.98 ± 0.05^{d}
Cholesterol (mg/dl)	129 ±1.02^{b}	127 ± 0.41^{b}	142 ± 0.71^{a}	109 ± 1.09^{c}	130 ± 0.86^{b}	107 ±1.15^{c}
Glucose (mg/dl)	247 ±1.28^{c}	255 ± 1.63^{b}	255 ± 0.86^{b}	213 ± 1.35^{d}	269 ± 0.70^{a}	200 ± 1.80^{e}
Uric acid (mg/dl)	8.34 ± 0.18^{c}	8.26 ± 0.19^{c}	6.75 ± 0.19^{d}	13.1 ± 0.38^{b}	6.70 ± 0.13^{d}	15.0 ± 0.25^{a}
Creatinine (mg/dl)	0.46 ± 0.01^{c}	0.44 ± 0.02^{c}	0.45 ± 0.01^{c}	1.16 ± 0.03^{b}	0.45 ± 0.01^{c}	1.20 ± 0.01^{a}

Within the same row, means with different superscripts are significantly differ among the studied groups at ($P \leq 0.05$)

Immunological Result:

The effects of aflatoxin, nutritox and zeocem on immunological parameters were evaluated and represented in table (12). Concerning levels of IgG and IgM were significantly decreased in groups IV and VI, while there were significant increases in groups III and V. TNF- α was significantly decreased in groups IV and VI, while there was significant increase in group III. IL-1, IL-6 and IL-10 were significantly decreased in groups IV and VI, while there was significant increase in groups III and V. Group II was insignificantly changed in all tested immunological parameters.

Table 12: The effect Aflatoxicosis, nutritox and zeocem on immunological parameters (mean ±SE) on different groups after six weeks.

Group / Parameter	I	II	III	IV	V	VI
IgG (mg/ml)	1.02 ± 0.01^{b}	1.04 ± 0.05^{b}	1.20 ± 0.01^{a}	0.63 ± 0.01^{c}	1.10 ± 0.02^{a}	0.65 ± 0.04^{c}
IgM (mg/ml)	0.30 ± 0.02^{c}	0.32 ± 0.02^{c}	0.41± 0.24^{a}	0.24 ± 0.01^{d}	0.37 ± 0.01^{b}	0.23 ± 0.01^{d}
TNF (pg/ml)	20.5 ± 0.63^{b}	21.2 ± 1.34^{b}	27.4 ± 1.20^{a}	17.9 ± 0.51^{c}	26.3 ± 0.36^{ab}	17.2 ± 0.35^{c}
IL-1 (pg/ml)	16.7 ± 0.19^{b}	16.3 ± 0.63^{b}	19.6 ± 0.98^{a}	13.0 ± 0.24^{c}	18.5 ± 0.63^{a}	10.4 ± 0.24^{d}
IL-6 (pg/ml)	107 ± 1.69^{c}	105 ± 2.13^{c}	118 ± 1.97^{a}	65.8 ±1.22^{d}	112 ± 1.08^{b}	62.6 ± 0.50^{e}
IL-10 (pg/ml)	25.4 ± 0.65^{c}	24.9 ± 1.29^{c}	35.7 ± 0.9^{a}	22.0 ± 0.56^{d}	30.5 ± 1.30^{b}	20.3 ± 0.44^{e}

Within the same row, means with different superscripts are high significantly differ among studied groups at ($P \leq 0.01$).

Histopathological Results:

Liver:

Group I: The hepatic parenchyma among all the sacrificed birds appeared normal (photo. A).

Group II: The majority of hepatic parenchyma appeared normal with numerous bile ductules and leukocytic infiltration mainly lymphocytes in some portal areas (photo B). A few birds exhibited mild degenerative changes in the hepatic cells mainly microsteatosis.

Group III: The majority of the examined showed normal hepatic tissue. Portal and interstitial lymphocytic aggregation, hyperplastic kupffer cells slight dilatation in hepatic sinusoids also could be seen in a few birds (photo C).

Group IV: The majority of sacrificed birds restore the normal morphological features of the hepatic tissue which represented by slightly dilated sinusoids and blood vessels and apparently normal hepatic cells (photo D). A few birds had pericellular fibroblastic proliferation accompanied by numerous bile ductules and some leukocytes in the portal area.

Group V: The administration of the antimycotoxin ameliorates the majority of the hepatic lesions of sacrificed chickens of this period. A few birds still exhibited minimal lesions represented by numerous bile ductules with portal fibroblast proliferation, mild congestion and normal hepatic parenchyma (photo E).

Group VI: Sever destruction and necrosis of the hepatic parenchyma accompanied by intense congestions of blood vessels and hepatic sinusoids were common (photo F). Portal and interstitial area lymphocytic aggregations together with fibrosis and newly formed bile ductules were prevalent.

Kidneys:

Group I: The renal tissue appeared normal (photo G).

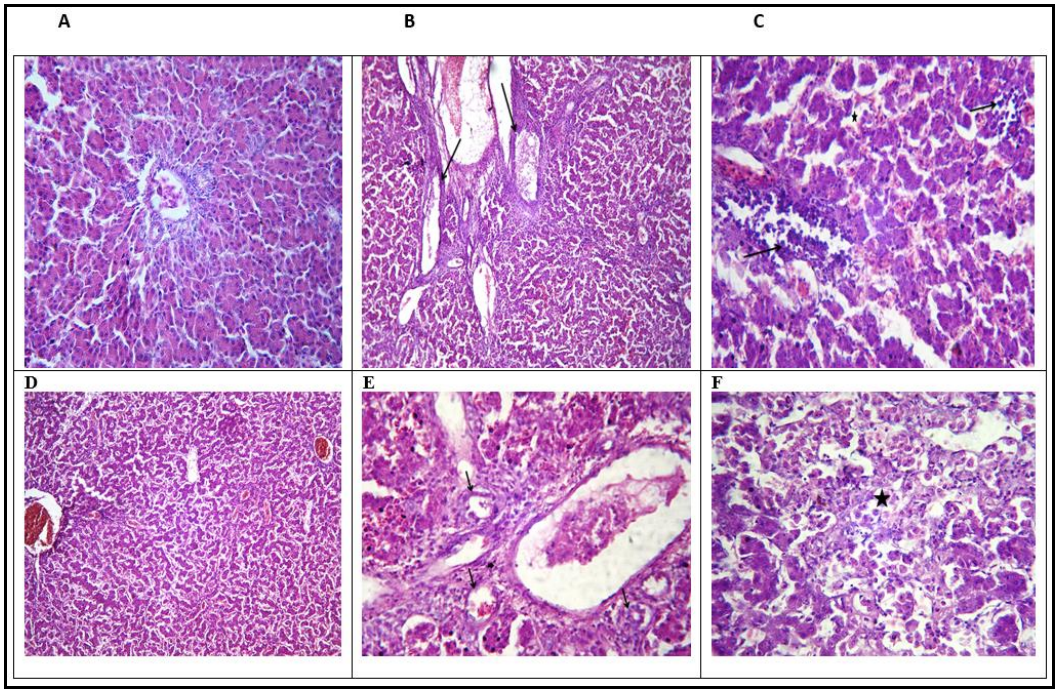
Group II: All the sacrificed chickens had normal renal parenchyma and few interstitial extravasated erythrocytes (photo H). A few birds had mild reversible degenerative changes in some renal tubules. Other birds revealed interstitial lymphocytic aggregations, mild congestions of blood vessels and capillaries and few hemorrhages.

Group III: The renal tissues of a few chickens revealed hypercellularity of some glomeruli, acute cell swelling of renal tubules and congestion (photo I).

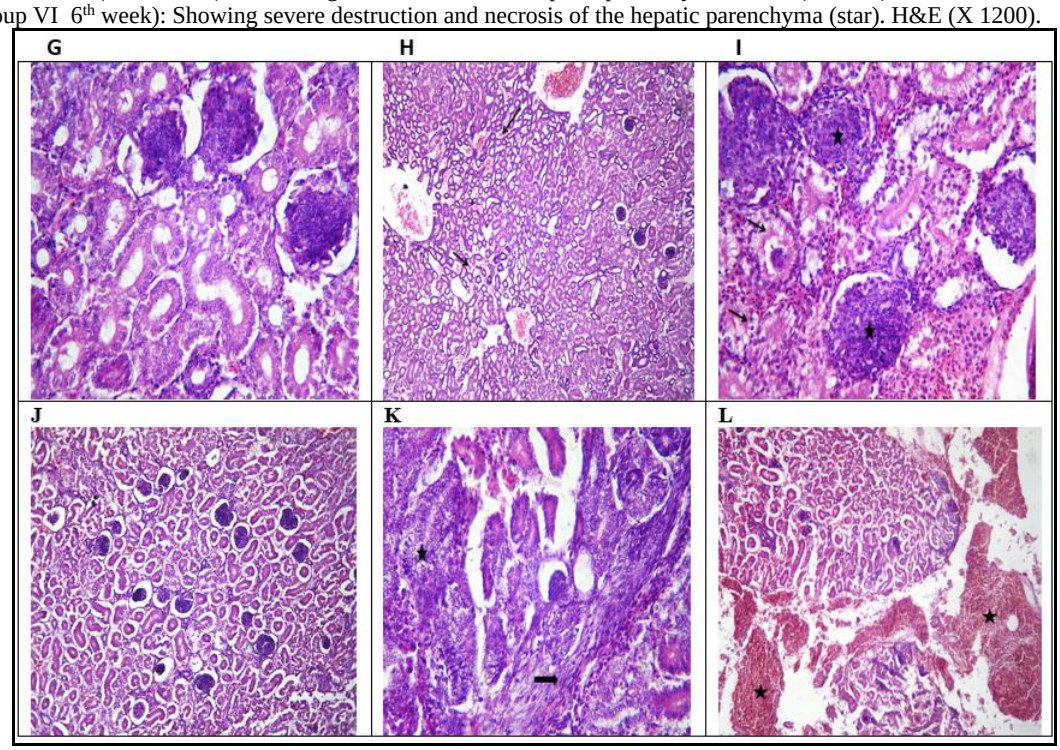
Group IV: All the examined kidneys appeared with normal microscopic segments of nephrons and interstitium with slightly dilated glomerular spaces in a few birds (photo J).

Group V: The examined renal tissue of all sacrificed chickens had apparently normal parenchyma. A few birds revealed fibrous tissue band, tubular epithelial and regenerative attempts in some renal tubules (photo K).

Group VI: Multiple scattered areas of hemorrhages associated with necrosis of renal tubules and fibroblast proliferation were common in the sacrificed birds (photo L). Glomeruli had contracted tufts and an increase of its spaces; interstitial aggregations of lymphocytes were observed.



A: - Liver of chicken (Group I): Showing normal hepatic parenchyma H&E (X 1200). **B:** Liver of chicken (group II): Showing apparently normal hepatic parenchyma and numerous bile ductules and leukocytic infiltration in some portal areas (arrows). H&E (X300). **C:-** Liver of chicken (Group III): Showing portal and interstitial lymphocytic aggregation (arrows), hyperplastic kupffer cells slight dilatation in hepatic sinusoids (star). H&E (X1200). **D:** Liver of chicken (Group IV 6th week): Showing slightly dilated sinusoids and blood vessels and apparently normal hepatic cells. H&E (X300). **E:** Liver of chicken (GroupV 6th week): Showing numerous bile ductules (arrows) with portal fibroblast proliferations (arrow head), mild congestion and normal hepatic parenchyma. H&E (X 1200). **F:** Liver of chickens (Group VI 6th week): Showing severe destruction and necrosis of the hepatic parenchyma (star). H&E (X 1200).



G:- Kidney of chicken (Group I): Showing normal renal parenchyma H&E (X 1200). **H:-** Kidney of chicken (Group II): Showing apparently normal renal parenchyma and few interstitial extravasated erythrocytes (arrows). H&E (X 300). **I:** Kidney of chicken (Group III): Showing hypercellularity of some glomeruli, acute cell swelling of renal tubules and congestion. H&E (X1200). **J:-** Kidney of chicken (Group IV 6th week): Showing normal renal tubules slightly dilated glomerular spaces H&E (X300). **K:-** Kidney of chicken (Group V 6th week): Showing fibrous tissue band (arrow), tubular epithelia and regenerative attempts (star) in some renal tubules H&E (1200). **L:** Kidney of chickens (Group VI 6th week): Showing intense hemorrhage (stars), necrosis of renal tubules and mild fibroblast proliferation H&E (X 300).

DISCUSSION

Poultry are considered one of the most sensitive birds to aflatoxin. Contamination of food of birds with these mycotoxins causes substantial losses among birds due to reduced rate of growth, reduced feed efficiency, marked drop in egg production and immune functions, liver damage, bile duct proliferation and most importantly decreased resistance to infectious diseases (Smith *et al.,* 1969), Pier and (Pier and Heddleston, 1970) and (Monson e*t al.,* 2015). In the present work, the protective effects of zeosem and nutritox against aflatoxicosis were evaluated in chickens.

Growth performance was significantly reduced in AFB1 treated group VI compared to the control. Results showed significantly decreased final weight, body weight gain, and bad FCR in AFB1 treated group VI compared to the control. This harmful influence on growth performance is in harmony with the results reported by other studies (Hedayati *et al.,* 2014), (Hussain *et al.,* 2008), and (Gowda *et al.,* 2009). The negative effect of AFB1 might be due to its damaging effects on carbohydrate, protein, lipid metabolism that results in poor energy utilization (Tessari *et al.,* 2010). Aflatoxins metabolites cause liver damage, and reduction in pancreatic digestive enzymes that impair nutrients absorption leading to reduction in feed consumption and poor performance (Nazarizadeh and Pourreza, 2019). The present data revealed that either Zeocem treated group II or Nutritox treated group III differed significantly from the control group I in final weight, body weight gain, and FCR. This agrees with previous study by Abdalla et al. (Abdalla, 2012) who observed a non-significant effect of Nutritox on poultry FCR in control diet. In addition, Uriyanghai (Uriyanghai, 2016) showed that addition of Zeolite to control diet did not affect chickens performance. Our results revealed significant improvements in final weight, body weight gain, and FCR in Nutritox/AFB_1 treated group V compared to Zeocem/AFB1 treated group IV. Supplementing Nutritox to AFB1 contaminated ration partially reduced the negative effects of AFB1 on growth performance of broilers in the present study. Abdalla et al. obtained the same result (Abdalla, 2012). The role of Nutritox to relive AFB1 toxic effect may be its adsorption mechanism(YAsUDA and Taga, 1980). Its L-bacterial fermentation extract (YAsUDA and Taga, 1980). It is bacterial ingredients can colonize chick's intestinal tract and secrete active substances that degrade AFs in ration, or decrease AFs absorption (Fan *et al.,* 2013). Our findings also is in agreement with previous research that indicated Zeolite did not ameliorate the toxic effect of AFB1 in chickens diet (Sova *et al.,* 1991), and, (Vekiru *et al.,* 2015). This negative result may be because Zeolite was ineffective in AFB1 binding (Vekiru *et al.,* 2015).

Evaluation of hemogram revealed significant changes only in chickens fed a ration supplemented with aflatoxin, while in groups that fed zeosem, nutritox, aflatoxin with zeosem or nutritox there were no significant changes along the experimental period. The picture of erythron mass in the present work after administration of aflatoxin was normocytic normochromic anemia at the 2nd week. Anemia in the 2nd week may be occurred due to the effect of aflatoxin on red cells or may be due to suppression of the bone marrow stem cell activity by the mycotoxin (myelotoxicity). Similar results were recorded by Tung (Tung *et al.,* 1975) who explained that aflatoxin associated anemia is due to decrease the life span of RBCs and by Murugesan (Murugesan et al., 2015). In the 4th and 6th week microcytic hypochromic anemia developed; this may be due to nutritional iron deficiency as a result of intestinal lesions and this also was noticed by Huff (Huff *et al.,* 1986) who mentioned that marked decline in PCV and Hb levels happened when broilers received 5 ppm of aflatoxin, They explained the decrease in occurred due to aflatoxin-induced cumulative toxicity. The rapid changes in PCV and Hb

levels induced by aflatoxin may be due to an inhibition of hematopoiesis in addition to defective hematopoiesis. Also, Jain (Jain, 1986) proved that in chronic toxicity; microcytic hypochromic anemia developed because the red cell life span is slightly shortened and there is no compensatory increase in red cell production. Abdel-Wahhab (Abdel-Wahhab *et al.,* 2002) reported that microcytic hypochromic anemia occurred due to many factors such as inhibition of protein synthesis as evidenced by lower serum albumin (JJ, 1989), decrease of the total iron binding capacity (Harvey *et al.,* 1991) and the hemopoietic cellular defects of aflatoxin (Abdel-Wahhab et al., 2002), (Van Vleet and Ferrans, 1992). Our results are in accordance with Ibtisam and Raghab (RR, 1997) and Oguz *et al.,* (Oguz *et al.,* 2000) as they reported that aflatoxicosis in broiler chickens caused microytic hypochromic anemia. Groups treated with nutritox and nutritox and aflatoxin showed erythrocytosis and also increases hemoglobin and PCV at 4 and 6 weeks, which may be attributed to the fact that, the probiotics used (lactobacillus acidophillus) increased the blood parameter values as a result of hemopiotic stimulation. These results matched the results of (SARMA *et al.,* 2003); (Manohar, 2005), and (Kumar *et al.,* 2006). Concerning total and differential leucocytic count there were leucopenia, lymphocytopenia and heteropenia in aflatoxin treated chicks. This result may be attributed to the toxic effect of aflatoxin on the circulating cells, sequestration of cells in the tissue and/or effect of aflatoxin on bone marrow and lymphoid tissue this result and explanation agree with earlier studies made by (Yaman *et al.,* 1988), (RR, 1997), (Kececi *et al.,* 1998). Who observed regression of the bursa of fabricius in young broilers exposed to aflatoxin. Also, (Espada *et al.,* 1992) recorded that chickens administered 0·2 and 3 μg aflatoxin B1 (afb1) / g bodyweight for 21 days showed cellular diminution in the medulla of bursa of Fabricius as well as significant decreases in the absolute weights of bursa of Fabricius and spleen. (Dönmez *et al.,* 2012) recorded significant reduction in erythrocyte count, leukocyte count, hemoglobin, and hematocrit levels with aflatoxin. It is important to note that histopathological examination performed on chicks of this group demonstrated lymphocytic infiltration in almost all of the examined organs. This result disagrees with (Sova *et al.,* 1991) who reported that leucocytosis was prominent in broiler treated with 2.5 ppm aflatoxin due to lymphocytosis and heterophilia and this difference may be attributed to differences in dose, duration of administration, type of aflatoxin and the breed of chickens. Chicks treated with zeocem (chemical synthetic antimycotoxin) and aflatoxin showed leucopenia, which in our opinion may be occurred due to the effect of aflatoxin that produced, since zeocem did not affect the toxin. Group treated with nutritox showed leukocytosis at 6 weeks, which may be attributed to lymphocytosis, which may be due to immunostimulatory activity of nutritox this result was in agreement with (Piard and Desmazeaud, 1991). Increase activities of serum GGT and ALT is accompanied with hepatocellular damage Even though, they are not liver specific in birds (Coles, 1986). The present study showed that aflatoxin induced significant increase in GGT and ALT, which may be due to the effect of aflatoxin on liver and heart. These results nearly similar to those reported by (Arshad *et al.,* 1993), (Nath *et al.,* 1996) and (Yang *et al.,* 2012). Also, increased serum total bilirubin was observed in birds fed aflatoxin diet which may be due to bile duct hyperplasia. This result is in agreement with (Rizvi and Shakoori, 2000) and (Soliman *et al.,* 2008). Our results confirmed histopathologically by degeneration and necrosis of hepatocytes with mononuclear leukocytes and heterophil infiltration. Meanwhile the liver transaminases were within normal values in groups treated with aflatoxin and nutritox, which means that nutritox has the ability to antagonize the side effects of aflatoxin. This conclusion approved histopathologically by the apparently normal hepatic tissues at 6th week. Serum total protein, albumin and globulin were significantly decreased after 4 weeks in aflatoxin

administrated group. This result may be due to decrease feed intake, utilization by intestine and metabolism by liver in addition to the effects of the toxin on the kidneys, which leads to descending albumin. (Lafarge and Frayssinet, 1970) explained hypoproteinemia and hypoalbuminemia as aflatoxin inhabit RNA polymerase and subsequently protein synthesis. Quezada et al., (Quezada *et al.,* 2000) stated that Serum total protein is considered as a marker of protein synthesis, and the hypoproteinemia generated by aflatoxin may contribute to the decline of immunoglobulins. The occurrence of this hypoproteinemia may be attributed to damage of endothelium of glomerular tuft or most likely associated with inhibition of protein synthesis in the liver (Tung *et al.,* 1975). This indicated histopathologically by diffuse hydropic degeneration and necrotic changes of tubular epithelium. Our results are in agreement with (Oguz et al., 2000), (Quezada *et al.,* 2000), (Rosa *et al.,* 2001) and (Magnoli *et al.,* 2017) as they reported hypoproteinemia and hypoalbuminemia with aflatoxicosis.

Histopathological findings revealed presence of sever destruction and necrosis in the hepatic parenchyma and intense congestion of glomerular blood vessels and inter tubular capillaries with lymphocytic aggregations together with fibrosis could be seen inside lumina of collecting tubules. Our histopathological results were in agreement with that reported by (Ortatatli *et al.,* 2005) and (Karimy *et al.,* 2017) as they reported hepatomegaly, hydropic degeneration of liver, hyperplasia of bile duct, fatty liver, and periportal fibrosis in addition to vaculation of renal epithelium in chickens given aflatoxin by different doses. This can support the observed hypoprotinemia. Groups treated with zeocem, fungus (aflatoxin) showed significant reductions in total protein, and albumin, which may be attributed to failure of zeocem to eliminate the fungus, and so aflatoxin, produced and performed its effect.

Total protein, albumin and globulin increased at 4, 6 week in group treated with nutritox and group treated with nutritox and aflatoxin due to the protective effect of nutritox may be attributed to its antioxidants effect, where nutritox prevent free radical formation and intervention to neutralize existing free radicals (Abe *et al.,* 1995).

Concerning serum cholesterol there were a significant decrease in cholesterol level in group treated with fungus (aflatoxin) and zeocem and group treated with fungus (aflatoxin) alone. This result may be due to inhibition of cholesterol biosynthesis, with liver involvement and perhaps a shift of concentration from blood to liver. This result agrees with (Oguz *et al.,* 2000); (Zhao *et al.,* 2010). More explanations presented by (Manning and Wyatt, 1984) who reported that cholesterol is synthesized primarily in the liver, and aflatoxin has been shown to competitively inhibit mitochondria transport carrier proteins that could result in decreased energy for cholesterol synthesis. Cholesterol biosynthesis also requires a specific sterol carrier protein that binds squalene and sterol precursors of cholesterol and activates the microsomal enzymatic steps of cholesterol synthesis (Nes, 2011). Decreased sterol carrier protein occurred due to decreased protein synthesis.

Group treated with nutritox only showed increase in cholesterol level at 6 weeks. This is due to the presence of proplyne glycol, which is consider a source of energy. Proplyne glycol increase cholesterol level by inhibition of adipose adenylate cyclase activity and lipolysis by elevated insulin concentration this result reported by (Juchem *et al.,* 2004) more explanation by (•Stephenson KA, 1997) which reported that increase cholesterol level due to increase non estrified fatty acid or B-hydroxybutric acid in blood as result of presence of proplyne glycol in diet.

Regarding to the effect of the mentioned treatments on serum glucose level, there was decrease in group treated with fungus (aflatoxin) and zeocem, and group treated with fungus (Aflatoxin) alone. This result matches with (Panda *et al.,* 1987) and (Zhao *et al.,*

2010). This due to the aflatoxin induced liver injury probably induces glycogen synthesis in the liver by inhibiting phosphorylase or by stimulating glycogen synthetase resulting in low levels of glucose in the aflatoxin fed quail. In-group treated with nutritox alone or group treated with nutritox and aflatoxin there is increase of glucose level at 4 and 6 weeks. This due to presence of propylene glycol in nutritox as a source of energy which lead to increase glucose level as propylene glycol is a glucogenic precursor, which is quickly absorbed from the intestinal wall or partly transformed to propionate before being absorbed and converted to glucose (Nielsen and Ingvartsen, 2004). Grummer *et.al.,* (Grummer, 1993) noted a significant increase in blood glucose concentration after glycol treatment.

In the present work Aflatoxin affect the renal tissue leading to renal damage. This effect was clearly investigated by both clinical and histopathlogical means. This renal damage was indicated by the increase in serum uric acid and creatinine. Uric acid is the primary catabloic product of protein, non-protein nitrogen and purines in birds (Rock *et al.,* 2013). Hyperurecemia in birds occur with starvation, gout, some medications, massive tissue destruction and renal diseases (Coles, 1986) and (Pham *et al.,* 2014).

Creatinine is not a major non-protein nitrogen component of avian blood (Bell and Freeman, 1971). Some investigators think that serum creatinine may become elevated in birds with renal diseases but less reliably than uric acid (George et al., 2006). In the present study aflatoxin produce increase in both uric acid and creatinine. These results were agree with those reported by (El-Shewy *et al.,* 1997) and differ from those reported by (Kececi *et al.,* 1998) and (Oguz *et al.,* 2000) as they reported decrease in both serum uric acid and creatinine during aflatoxicosis. The differences may be due to differences in dose and duration of treatment.

Such biochemical change in present work is an outcome of nephropathy. Nephropathy is manifested by diffuse sub-capsular and serrosal hemorrhage together with focal necrosis of tubules, focal proliferation of fibroblast, contracted glomerular tufts and dilations of glomeruli spaces were prevalent. Cystic dilations of some renal tubules that contained erythrocytes were observed.

The nephrotoxicity of aflatoxin was apparent in this study from increased serum levels of uric acid and creatinine, tubule-nephrosis and necrotic changes, which were observed histopathologically in most of the renal tissues. This increase in uric acid level and creatinine was sensitive indicator of aflatoxicosis. Combined treatment with zeocem and fungus (aflatoxin) caused increase in uric acid and creatinine values. The histopathological results of the renal tissues in chicks treated with fungus (aflatoxin) and zeocem showed focal areas of coagulative necrosis and replacement of tissues with mononuclear cells. While, there were no changes occurred at treatment with nutritox with fungus. Kaki et al., (Kaki *et al.,* 2012) recorded inhibitory activity of zeolites against different fungi including aspergillus flavus and aspergillus parasiticus. The histopathological results showed normal architecture of renal tissues in chicks treated with fungus (aflatoxin) and zeocem at the end of experiment.

In group treated with nutritox alone there was a decrease in uric acid level. This is due to presence of Lipase and protease enzymes, supplementation of these enzymes lead to reduce concentration of blood uric acid (Swennen *et al.,* 2005) who suggested that enzymes contained in nutritox (Lipase and protease) preparation increased nutrient metabolism, particularly protein anabolism of birds, therefore, promoting the growth of chickens.

Immunoglobulin (G, M) in the present work was decreased in aflatoxin treated group and in aflatoxin and zeocem treated group. This may be due to Immunosuppression caused by aflatoxin toxicity. (Agag, 2004)concluded that exposure of chicken to aflatoxin

suppress immunoglobulin bearing cells of bursa. Apart from this, aflatoxin also causes aplasia of the thymus, spleen, and bursa Fabricious in chicken, whereas larger quantities (0.6-10 ppm) cause the suppression of class G and M immunoglobulins during immunization (Karaman et al., 2005). Agha *et al.,* (Yunus *et al.,* 2011) reported that aflatoxicosis decreased the weight of bursa and thymus. Bondy and Pestka (Bondy and Pestka, 2000) concluded that mycotoxicosis suppress both innate and adaptive immunity.

Immunoglobulin levels in nutritox and aflatoxin and nutritox treated groups were insignificantly increased in comparing with control, where nutritox could prevent the immunosuppression effect of aflatoxin. In the same line, (Casas and Dobrogosz, 2000) recorded immunostimulant effects of lactobacillus spp. by enhanceing the phagocytosis of peritoneal macrophages and regulate immune function. Concerning serum interleukins 1, 6, 10 (IL1, 6, 10) and tumor necrosis factor-alpha (TNF-α) there were a significant decrease in their level in group treated with aflatoxin and zeocem, and group treated with aflatoxin alone.This result may be due to Tumor necrosis factor-α is a potent immunoregulatory cytokine produced by several types of cells, especially macrophages which augments the production of other cytokines as well as enhances polymorphnuclear leukocytes (PMNLs) functions, including O2 and H2O2 production and causes leukocyte adhesion and infiltration (Feuerstein *et al.,* 1994) and (Roilides *et al.,* 1998). Additionally , TNF-α stimulates PMNLs to damage aspergillus hyphae, enhances phagocytosis , augments PMNLs oxidative respiratory burst and degranulation and its role in the immune response to bacterial and certain fungal, viral, and parasitic invasions as well as its role in the necrosis of specific tumors (Tracey and Cerami, 1990); (Roilides et al., 1998) and (Filler *et al.,* 2005). At the end of this study, the examined serum samples taken from group treated with aflatoxin and zeocem, and group treated with aflatoxin alone expressed highly significant reduction of TNF- α release. In parallel to this respect (Dugyala *et al.,* 1994); (Adrian *et al.,* 1998) had demonstrated that inhibitory effects of aflatoxin on macrophage mediators could be a result of suppressed proliferation of the granulocyte-macrophage(GM) progenitor cells to granulocyte, macrophage and GM-colonies which primes macrophage to release proinflamatory mediators including IL (1, 6,10) and TNF-α. Hence, co-treatment of nutritox with aflatoxin appears to enhance the production of TNF-α because the ability of the included probiotic bacteria (Lactobacillus strains) to bind with aflatoxin (Peltonen *et al.,* 2000). Group treated with nutritox and group treated with nutritox and aflatoxin showed increases in IL (1, 6, and 10) and TNF-α levels. This due to that nutritox can act as immunomodulatory agent by activating specific and non-specific host immune responses in chicks, which in turn help in prevention and control of various infectious diseases this result is reported by (Fuller, 2012) and (Koenen *et al.,* 2004).

Conclusion

From the result of the research, it can be concluded that mycotoxicosis is one of the dangerous diseases that can result in great losses in poultry production. Antimycotoxin feed additives have a positive role and it must be added to the feed. Adding nutritox as antimycotoxin feed additive (biological synthetic) protects the chicks from the negative effect of mycotoxins. We found that the addition of zeocem as antimycotoxin feed additives (chemical synthetic) did not perform the desired effect in comparison with the nutritox (biological synthetic).

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