Clinicopathological Studies on the Effect of Some Antimycotoxin as Feed Additives in Broiler

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Abstract
The objective of the present study was to evaluate the toxic effects of aflatoxin on some hematological and immunological parameters and to determine the preventive effectiveness of added antimycotoxins.
In the study, a total of 150 broilers were separated equally into 6 groups. group (I): fed the normal diet, group (II): fed diet contains antimycotoxin (chemical synthetic), group (III): fed diet contains antimycotoxin (biological synthtic), group (IV) fed diet contains 500 µg aflatoxin/ kg diet + diet mix antimycotoxins (chemical synthetic), group (V): fed diet contains 500 µg aflatoxin / kg diet + diet mix antimycotoxin (biological synthtic), group (VI): fed diet contains 500 µg aflatoxin / kg diet. Concerning the hematological findings at the end of experiment of the groups (VI) and (VI) revealed microcytic hypochromic anemia with leucopenia, neutropenia and lymphocytopenia compared to control healthy group. While groups (III) and)V) revealed an increase in RBC_S count, Hb concentration and PCV, beside increased leucocyte, neutrophils and lymphocytes counts. The immunological parameter results showed a significant decrease in all measured parameters (IgG, IgM, IL-1, IL-6, IL-10 and TNF-alpha) in groups IV and VI when compared with control healthy group .But increased in groups (III and V) when compared with control healthy one.

Introduction

Aflatoxins (AF) is a dangerous mycotoxins released from *Aspergillus flavus* and *Aspergillus parasiticus* which is important in the poultry production. The AF toxicity in broiler chicken is dangerous due to their carcinogenic, mutagenic, teratogenic and growth inhibitory effects ***(Oguz and Kurtoglu, 2000).*** (AF) toxic effect on hematology ***(Oguz et al., 2000a)*** and immunity ***(Qureshi et al., 1995*)**. Presence of aflatoxin in feed causes contamination of poultry feed and aflatoxicosis in poultry production which cause suppression of the humoral and cellular immune responses , hence chicks become susceptible to some environmental and infectious agents ***(Oguz et al.,2003)*** .The adsorbent-based researches have been done at the beginning of 1990s to adsorb

aflatoxin from contaminated diet and decrease the toxin of aflatoxin in poultry feed ***(Ibrahim et al., 2000).*** as Zeolites ***(Miazzo et al., 2000),*** bentonites ***(Rosa et al., 2001)*** and clinoptilolite (CLI), which is a synthtic zeolite and a member of heulandite. Also, it has been reported that, aflatoxin production is inhibited by lactic acid bacteria ***(Gourama and Bullerman, 1995).*** Lactobacillus spp is a lactic acid producing bacteria that decrease aflatoxin production ***(Karunaratne et al., 1990).*** The aim of the present work is to investigate: The effects of aflatoxin, nutritox (synthetic biological antimycotoxin) and zeocem (synthetic chemical antimycotoxin) on blood hematology and immunity.

Material and Methods

This study was carried on 150 one-day old broiler chicks, Isa Hubbard breed which were obtained from El-dakhlea Poultry Company Meet Ghmer City, Egypt. Chickens were reared in litter under standard environmental and hygienic conditions. The temperature was adjusted according to the age (the first week 32°C, then decreased 2°C per week till reach 26°C), ***(Harrison and Harrison, 1986).*** Chicken were fed on a balanced commercial ration (basal diet) and water. All chicken were vaccinated against Newcastle disease at 7 and 18 days old and against Gumboro disease at 15 days old ***(Giambrone and Ronald, 1986).***

Blood Sampling

Two blood samples were obtained from each bird from wing vein. The first sample (one ml of blood) was collected in a clean tube containing potassium salt of EDTA as anticoagulant. This sample was used for evaluation of the hemogram. The second sample (3 ml of blood) was collected in a plain centrifuge tube and was used for preparation of serum and assay of immunological parameters.

Hematological parameters analysis:-

Parameters of the hemogram were determined according to standard techniques described by ***Jain (1986),*** which includes RBC, Hb, PCV, MCV, MCH, MCHC, TLC and differential leucocytic count. 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).***

Immunological parameters analysis:-

(IgG and IgM) were determined according to ***Larsson (1993).*** Interleukin 1 and interleukin 10 (IL1, IL10) were determined from undiluted serum samples according to ***Chan and Perlstein (1987)***. Tumor necrotic factor–α (TNF-α) and interlukin 6(IL6) were determined according *to* ***Wajant et al., (2003)***.

Statistical analysis

Data collected from the hematological and immunological analyses of treated groups of chicks

were statistically analyzed in compare to control group .Significance of the results was evaluated by calculating the ANOVA (F-test) according to ***Tamhane and Dunlop (2000)***.

Table (1) *Experimental design: Birds were subjected for different examinations at 2, 4, and 6 weeks from the beginning of the experiment. Birds take antimycotoxins from one-day old.*

group	Drug	Asprigillus flavus + Aflatoxin
Group I	No drug.	Basal diet
Group II	zeocem 1 kg/ ton	Basal diet
Group III	Nutritox 0.25kg/ton	Basal diet
Group IV	zeocem 3kg/ton	Mouldyfood + (500µgAF/kgdiet)
Group V	Nutritox 0.5kg/ton	Mouldy food + (500µgAF/kgdiet)
Group VI	No drug	Mouldy food + (500µgAF/kgdiet)

Results

After Two Weeks

Hematological results

Total erythrocytic count, hemoglobin and PCV are significantly decreased in groups IV and VI when compared to control one. Meanwhile the other groups of chicks are insignificantly changed. Calculation of red cells indices in groups IV and VI revealed development of normocytic normochromic anemia. Total leucocytic count is significantly decreased in groups IV and VI, while the other groups are insignificantly changed. Heterophils and lymphocyte are significantly decreased in groups IV and VI There is a significant decrease in total heterophile and lymphocyte count in group VI more than group IV. Meanwhile other groups are insignificantly changed. as shown in tables (2 and 3).

After Four Weeks

Hematological results

Total erythrocytic count, haemoglobin and PCV are significantly decreased in groups IV and VI, when compared to control one, while groups III, V showed a significant increased in the previous mentioned parameters. The other groups of chicks are insignificantly changed. Calculation of red cells indices in groups IV and VI revealed development of microcytic hypochromic anemia. Total leucocytic count is significantly decreased in groups IV and VI, while group III is significantly increased in that parameter. Group II and V were insignificantly changed. Heterophils are significantly decreased in groups IV and VI, while significantly increased in groups III and V in that parameter. Groups IV and VI are significantly decreased in lymphocytes, while the other groups

showed unsignificant changes. as shown in tables (4and 5).

After Six Weeks

Haematological results

Total erythrocytic count, hemoglobin and PCV were significantly decreased in groups IV and VI, and significantly increased in groups III and V but insignificantly changed in other groups of chicks. Microcytic hypochromic anemia was observed in groups IV and VI. Total leucocytic count significantly decreased in groups IV and VI, while significantly increased in group III .Heterophils are significantly decreased in groups IV and VI, while group III showed significant increase, The other groups are insignificantly changed. Lymphocytes are significantly decreased in groups IV and VI; meanwhile, groups III showed significantly increased. The other groups are insignificantly changed. Significant monocytopenia , eosinopenia and basopenia were recorded in groups IV and VI. Meanwhile; the other groups were insignificantly changed. as shown in tables (6 and 7).

Immunological result:

The seurm levels of IgG, IgM, TNF- α, IL-1, IL-6, IL-10 there were significantly decreased in groups IV and VI, there were significant increases in the previous parameters in groups III and V, meanwhile the other groups showed insignificantly changed. as shown in table (8).

Table (2): *The effect of aflatoxicosis, nutritox and zeocem on some erythrogram parameters (mean ±SE) in different groups after two weeks.*

Groups /Parameters	I	II	III	IV	V	VI
RBC ($\times 10^6/\mu l$)	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 (%)	34.40±1.51^{b}	34.00±2.07^{b}	35.1±1.30^{a}	32.5±1.67^{c}	34.80±1.30^{a}	31.40±0.89^{d}
MCV (Fl)	125.55±1.44^{a}	125.00±1.11^{a}	124.91±0.90^{a}	125.00±0.44^{a}	125.17±0.89^{a}	125.6±1.02^{a}
MCH (Pg)	35.76±2.34^{a}	35.66 ±2.19^{a}	35.23 ±1.69^{a}	35.81±1.06^{a}	35.36 ±0.54^{a}	35.24 ±1.43^{a}
MCHC (%)	28.49 ±2.06^{a}	28.53±2.0^{a}	28.21 ±1.26^{a}	28.65 ±0.83^{a}	28.25 ±0.61^{a}	28.05 ±1.22^{a}

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

Table (3): *The effect of aflatoxicosis, nutritox and zeocem on leucogram parameters (mean ±SE) in different groups after two weeks*

Groups /Parameters	I	II	III	IV	V	VI
RBC($\times10^6$/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)	10.88 ±0.19^c	10.72±0.07^c	11.41 ±0.16^a	8.52 ±0.19^d	10.94±0.05^b	7.96 ±0.50^e
PCV (%)	36.40 ±0.51^c	35.70±0.73^c	38.84±0.66^a	31.31±0.81^d	37.21±0.80^b	28.80 ±0.37^e
MCV (Fl)	123.38±0.39^a	123.52±0.72^a	123.69±1.20	117.71 ±1.17^c	123.21±0.76^a	118.51±1.37^b
MCH (Pg)	36.88±0.98^a	37.09±0.76^a	36.34±0.84^{ab}	32.03±0.37^c	36.22 ±0.95^b	32.75 ±0.82^c
MCHC (%)	29.89 ±0.73^a	30.02±0.67^a	29.37±0.73^a	27.21 ±0.55^b	29.40±0.67^a	27.63±0.57^b

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 some erythrogram parameters (mean ±SE) in different groups after four weeks***.**

Groups /Parameters	I	II	III	IV	V	I
RBC($\times10^6$/µl)	2.80 ±0.04^b	2.83 ±0.07^b	3.10±0.6^a	2.42±0.6^c	2.90 ±0.05^a	2.41 ±0.06^c
Hb(gm/dl)	10.9 ±0.21^b	11.00 ±0.12^b	11.90 ±0.21^a	8.62 ±0.13^c	11.08 ±0.12^b	8.60 ±0.14^d
PCV(%)	35.1 ±0.51^c	35.50±0.58^c	38.8 ±0.74^a	29.50 ±0.71^d	36.30 ±0.37^b	29.53 ±0.66^d
MCV(Fl)	125.36 ±0.71^a	125.44±1.16^a	125.16±0.75^a	121.91 ±0.7^b	125.17 ±1.19^a	122.53±1.08^b
MCH(Pg)	38.92 ±0.63^a	38.87±0.99^a	38.38±1.09^a	35.62 ±0.71^c	38.21±0.97^a	35.69±0.92^c
MCHC(%)	31.06 ±0.36^a	30.98 ±0.58^a	30.68 ±0.87^a	29.22±0.62^b	30.53±0.60^a	29.12 ±0.79^b

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 leucogram parameters (mean ±SE) in different groups after four weeks.*

Groups /Parameters	I	II	III	IV	V	VI
WBC ($\times10^3$ /µl)	35.59±0.75^a	35.20±1.01^a	36.39 ±0.75^a	32.39 ±0.75^b	35.58 ±0.75^a	28.75 ±1.01^c
Heterophils ($\times10^3$ /µl)	11.96 ±0.25^a	11.41 ±0.45^{ab}	11.86 ±0.35^{ab}	10.74±0.23^b	11.54 ±0.42^{ab}	9.21 ±0.36^c
Lymphocytes ($\times10^3$ /µl)	21.99 ±0.42^a	22.09 ±0.55^a	22.71 ±0.56^b	19.97 ±0.63^b	22.28 ±0.46^b	17.92 ±0.74^c
Monocytes ($\times10^3$ /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 ($\times10^3$ /µl)	0.57 ±0.08^a	0.56 ±0.08^a	0.51^a±0.09^a	0.52 ±0.7^a	0.49 ±0.08^a	0.52 ±0.09^a
Basophils ($\times10^3$ /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 (6): *The effect of aflatoxicosis, nutritox and zeocem on some erythrogram parameters (mean ±SE) in different groups after six weeks.*

Groups /Parameters	I	II	III	IV	V	VI
IgG (mg/ml)	1.02 ± 0.0١b	1.0٤ ± 0.0٥b	1.20 ±0.01٤a	0.63 ± 0.013c	1.10 ± .02a	0.65 ± .04c
IgM (mg/ml)	0.30 ± 0.0٧c	0.32 ± 0.0٧c	0.41 ± 0.24a	0.24 ± 0.013d	0.37 ± 0.01b	0.23 ± 0.0١d
TNF (pg/ml)	20.46 ± 0.63b	21.22 ± 1.34b	27.44 ± 1.20a	17.88 ± 0.51c	26.30 ± 0.36ab	17.22 ± 0.35c
IL-1 (pg/ml)	16.68 ± 0.19b	16.34 ± 0.63b	19.58 ± 0.98a	13.00 ± 0.2٤c	18.48 ± 0.63a	10.42 ±0.2٤d
IL-6 (pg/ml)	106.68 ±1.69c	105.08 ± 2.13c	118 ± 1.97a	65.84 ± 1.22d	112.04 ± 1.08b	62.58 ± 0.50e
IL-10 (pg/ml)	25.44 ± 0.65c	24.88 ± 1.29c	35.74 ±0.9١a	22.03 ± 0.56d	30.48 ± 1.30b	20.26 ± 0.4٤e

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

Table (7): *The effect of aflatoxicosis, nutritox and zeocem on leucogram parameters (mean ±SE) in different groups after six weeks.*

Groups /Parameters	I	II	III	IV	V	VI
WBC ($\times 10^3$ /µl)	36.78±1.01^{b}	36.79 ±1.02^{b}	39.52 ±0.75^{a}	29.60±0.75^{c}	37.94±0.75^{ab}	25.85±0.89^{d}
Heterophils ($\times 10^3$ /µl)	11.33 ±0.32^{c}	11.54±0.30^{bc}	12.83 ±0.28^{a}	9.47 ±0.34^{d}	12.40±0.27^{ab}	8.27 ±0.36^{e}
Lymphocytes ($\times 10^3$ /µl)	23.62 ±0.69^{a}	23.41±0.74^{a}	24.72±0.46^{a}	18.36±0.64^{b}	23.31±0.48^{a}	16.10±0.45^{c}
Monocytes ($\times 10^3$ /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 ($\times 10^3$ /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 ($\times 10^3$ /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 ($P \leq 0.05$).

Table (8): *The effect aflatoxicosis, nutritox and zeocem on immunological parameters (mean ±SE) in different groups after six weeks.*

Groups /Parameters	I	II	III	IV	V	VI
WBC ($\times 10^3$ /µl)	37.57±0.75^{b}	38.39±1.16^{b}	44.30 ±0.75^{a}	26.17 ±0.89^{c}	38.63 ±0.89^{b}	22.59±1.01^{d}
Heterophils ($\times 10^3$ /µl)	12.26 ±0.37^{b}	12.45 ±0.48^{b}	13.67 ±0.29^{a}	8.56 ±0.34^{c}	12.63 ±0.26^{b}	7.43 ±0.35^{d}
Lymphocytes ($\times 10^3$ /µl)	23.45 ±0.42^{b}	24.11 ±0.72^{b}	28.15 ±0.52^{a}	16.15 ±0.36^{c}	24.07 ±0.69^{b}	14.10±0.69^{d}
Monocytes ($\times 10^3$ /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 ($\times 10^3$ /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 ($\times 10^3$ /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 high significantly differ among studied groups at ($P \leq 0.01$).

Discussion

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 circulating red cells or may be due to suppression of the bone marrow stem cell activity by the mycotoxin (myelotoxicity). In the 4 and 6 week microcytic hypochromic anemia was developed; this may be due to nutritional iron deficiency as a result of intestinal lesions. Also ***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 concerning total and differential leucocytic count such as leucopenia, lymphocytopenia and heteropenia which resulted in fungus (aflatoxin) treated chicks. This result may be attributed to presence of the aflatoxins on the circulating cells, and these toxins effect reach to bone marrow and lymphoid tissue. Our results and explanation are agreed with earlier studies ***(kececi, 1998).*** Chicks treated with nutritox and nutritox and fungus (aflatoxin) showed erythtocytosis and also increase 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 supported by the results of ***Rajesh Kumar et al. (2006)***. Chicks treated with zeocem and fungus (aflatoxin) showed leucopenia which in our opinion may be occurred due to the effect of aflatoxin that produced by the fungus, since zeocem did not affect on the toxin. Group treated with nutritox showed leucocytosis that may be due to lymphocytosis and heterophilia at 2nd week. Also groups treated with nutritox, and fungus showed leucocytosis with lymphocytosis at 4 and 6 weeks, which may be attributed to immunostimulatory activity of nutritox, These results were in aggrement with ***Bal et al. (2004).***

Immunoglobulins (G, M) in the present work was decreased in both fungus (aflatoxin) and zeocem treated groups and fungus (Aflatoxin) treated group. This may be due to immunosuppression caused by aflatoxin toxicity ***(Agag, 2004).*** And also it reported that liver injuries result in reduced immunoglobulin production ***(Celik, et al., 2000).*** Meanwhile immunoglobulin levels in nutritox , aflatoxin and nutritox-treated groups were insignificantly increased in compare with control, where nutritox could prevent the immunosuppression effect of aflatoxin. In the same line ***Casas and Dobrogosz (2000),*** recorded the immunostimulant effects of lactobacillus sp. by enhancing the phagocytosis of peritoneal

macrophages and regulating immune function. Concerning serum interleukins 1, 6 and10 and also tumor necrosis factor-alpha (TNF-α) there were significant decreases in their levels in group treated with aflatoxin and zeocem, and those treated with aflatoxin alone. 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 O_2 and H_2O_2 production ***(Roilidies et al., 1998).*** In group treated with nutritox and group treated with nutritox and aflatoxin ,there are increase in IL (1, 6, 10) and TNF-α. This may be due to that the nutritox can act as immunomodulatory agent ***(Koenen et al., 2004).***

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دراسات باثولوجية إكلينيكية علي تاثير بعض المواد المضادة لسموم الفطريات كإضافات أعلاف في بداري التسمين

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قسم الباثولوجيا الإكلينيكية –كليه الطب البيطري – جامعة قناة السويس

يعتبر التسمم الفطري من أخطر الأمراض التي تصيب الحيوان والدواجن ويؤدى إلى خسائر فادحة في الثروة الداجنة. ولذا أجريت هذه الدراسة على اثنين من إضافات الأعلاف (مضادات سموم) بيولوجي و الأخركيميائي لدراسة مدى تأثيرهما على الفطر.

تم استخدام ١٥٠ كتكوت لإجراء هذه الدراسة تم تقسيمهم إلى ٦ مجموعات متساوية (المجموعة الأولى) ضابطة أخذت العليقة الأساسية.(المجموعة الثانية) تناولت اضافات اعلاف كيميائيه. (المجموعة الثالثة) أخذت اضافات اعلاف بيولوجيه. (المجموعة الرابعة) أخذت اضافات أعلاف كيميائيه + سموم الفطر. (المجموعة الخامسة) إضافات أعلاف بيولوجيه+سموم الفطر.(المجموعة السادسة) أخذت سموم الفطر وحده استمرت تلك المعاملات من سن يوم حتى ٤٢ يوم.وأسفرت النتائج عن التالي أظهرت دراسة خلايا الدم حدوث أنيميا مع نقص معنوي في عدد كريات الدم البيضاء في المجموعة التي أخذت الفطر وحده (الأفلاتوكسين) وكذلك قل معدل النمو معنويا كما حدث ذلك في المجموعة التي أخذت مضادات السموم الكيميائية مع الفطر. و كان هناك تأثيرا ايجابيا على المجموعات التي اخذت مضادات السموم البيولوجية. كما اسفرت اختبارات تحليل البروتين الكهربائي والجلوبيولين المناعي الي انخفاض في كلا من الاجسام المناعية G,M (IgG-IgM) وأيضا انخفاض في عامل نخر الورم الفا α (TNF α) وكذلك انخفاض كلا من الانترلوكين ١ و٦ و١٠ (IL1,6,10) في المجموعة التي أخذت الفطر وحده (الأفلاتوكسين) و كذلك في المجموعة التي أخذت مضادات السموم الكيميائية مع سموم الفطر.وكان هناك زياده في معدلات أنزيمات المناعة في المجموعه التي أخذت مضاد السموم البيولوجي مقارنة بباقي المجموعات.