

Mycofix ameliorative effect on Newcastle disease antibody production in broiler chickens during aflatoxicosis

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Abstract

Three experiments were conducted to elucidate the alleviation effects of Mycofix plus 3.0 on Newcastle antibody formation during aflatoxicosis in broiler chickens. Three levels of Mycofix (0.05%, 0.15%, and 0.25%) and aflatoxin (2.5ppm, 3.5ppm, and 5ppm) were used. Chickens were vaccinated at 8 and 18 days of age. Enzyme-linked immunosorbent assay and Haemagglutination inhibition tests were employed for determination Newcastle antibody titers at 28 days. The results showed that, Mycofix , and only at its high level of addition (0.25%) was effective in ameliorating the negative effect of aflatoxin at the rates 2.5ppm and 3.5 ppm levels of inclusion on antibody production but not at the high level of 5ppm on antibody production, comparing with titers in control groups.

Keywords: Newcastle disease; Antibody; Broiler chicken; Aflatoxin

تأثير المايكوفكس في تخفيف تأثير سموم الأفلا على تكوين الأضداد لمرض نيوكاسل في أفراخ فروج اللحم

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الخلاصة

أجريت ثلاث تجارب لمعرفة الدور المخفف للممتز مايكوفكس بلص 3 على انتاج الأضداد لمرض نيوكاسل في الأفراخ المستهلكة لعلائق ملوثة بسموم الافلا. استخدمت ثلاث مستويات للممتز (0.05%، 0.15%، 0.25%) وثلاث مستويات لسموم الافلا (2.5، 3.5 و 5 جزء بالمليون). لقت الأفراخ بعمر 8 و 18 يوم. استخدم اختبار الممتز المناعي المرتبط بالإنزيم واختبار تثبيط التلازن الدموي لقياس معيار الأضداد عند 28 يوم. أوضحت النتائج أن الممتز مايكوفكس عند أعلى مستوى 0.25% فقط كان كفيلا برفع مستوى الأضداد بصورة معنوية في مجاميع الأفراخ المستهلكة ل2.5 و 3.5 جزء بالمليون لسموم الافلا وليس للأفراخ المستهلكة 5 جزء بالمليون مقارنة مع مجاميع أفراخ السيطرة.

Introduction

Poultry feeds and ingredients are vulnerable to fungal growth and aflatoxin formation by *Aspergillus flavus* and *A. parasiticus* which is relatively stable in normal feed products. Aflatoxins have two fused dihydrofuran rings with various moieties, and members are designated as B1,

B2, G1 and G2 (1). Aflatoxin – producing fungi and aflatoxin-contaminated animal feedstuffs are recognized worldwide (2), usually with adverse implications for poultry production (3). The immune system in poultry is the first target to be influenced by mycotoxins. Immunosuppression can be observed in poultry ingesting aflatoxins at levels below those that cause over

symptomatology, and explained, in part, by atrophy of the bursa of Fabricius, thymus, and spleen (4). In chickens, aflatoxin interferes with normal T and B cell immunity including suppression of antibody production, either by acting directly on the immune system or by weakening the birds, thus making them less receptive to vaccination (5). Aflatoxins increase susceptibility to, or severity of, cecal coccidiosis and Marek's disease (6), salmonellosis (7, 8), inclusion body hepatitis (9), and infectious bursal disease virus (10). Vaccination failures are emerging because of aflatoxicosis in chickens (11). The control of mycotoxicosis is based on preventing fungal development in the feedstuffs, and on detoxifying toxin-contaminated feed. Detoxification is an approach for utilizing aflatoxin-contaminated poultry feeds while preventing aflatoxicosis. Sorbent compounds can be part of an integrated approach (12). Silica-containing compounds are practical and economical feed additives and can reduce the effects of aflatoxin (13). Bentonite clay also ameliorates aflatoxicosis, and aflatoxin induced reduction in antibody production (14, 15). Various sorbents have different affinities for aflatoxins and therefore differ in preventing the biological exposure of aflatoxin to the animals consuming contaminated feeds. Therefore, our trial was conducted to evaluate mycofix plus 3.0, for alleviating aflatoxin negative effect on Newcastle antibody production in broiler chickens.

Materials and Methods

The experiments were carried out in the animal house research division and the department of veterinary public health in the college of veterinary medicine, university of Mosul.

Broilers

Four hundreds and eighty, male one-day old broilers (cobb), were divided to three experiments, One hundred and sixty chicks for each, They were weighted individually, wing banded, and housed in a heated battery brooders under continuous fluorescent lighting. Chicks were fed *ad libitum* for 4 weeks, a corn-soybean meal based diet obtained from a commercial mill. It contained 22% crude protein and 2950 kcal/kg metabolizable energy.

Aflatoxins

Aflatoxin was prepared through inoculation of rice by *Aspergillus parasiticus* NRRL 2999 (16, 17). Fermented rice was then autoclaved dried and ground. The Aflatoxin content was measured by spectrophotometric analysis (18, 19). Of the total aflatoxin content in the powder, 81% was AFB1, 14% was AFG1, 4% was AFB2, and 1% was AFG2. The rice powder was incorporated into the basal diet to produce the desired level of 2.5, 3.5, and 5 mg/kg feed in each experiment.

Experiment 1: One hundred and sixty, one-day old, male broiler chicks were randomly assigned into eight treatments (20 birds /group, 10 birds /replicate) as the following:

- 1- Control group; 0.0 mycofix or Aflatoxin.;
- 2- Mycofix 0.05%;
- 3- Aflatoxin 2.5 ppm;
- 4- Aflatoxin 3.5 ppm;
- 5- Aflatoxin 5 ppm;
- 6- Mycofix 0.05 % +Aflatoxin 2.5 ppm;
- 7- Mycofix 0.05 % +Aflatoxin3.5 ppm;
- 8- Mycofix 0.05 % +Aflatoxin 5 ppm

Experiment 2: One hundred and sixty, one-day, male broiler chicks were randomly assigned into eight treatments (20 birds/group, 10 birds/replicate) as the following:

- 1- Control group; 0.0 mycofix or aflatoxin.;
- 2- Mycofix 0.15%;
- 3- Aflatoxin 2.5 ppm;
- 4- Aflatoxin 3.5 ppm;
- 5- Aflatoxin 5 ppm;
- 6- Mycofix 0.15 % +Aflatoxin 2.5 ppm;
- 7- Mycofix 0.15 % +Aflatoxin3.5 ppm;
- 8- Mycofix 0.15 % +Aflatoxin 5 ppm

Experiment 3: One hundred and sixty, one-day, male broiler chicks were randomly assigned into eight treatments (20 birds/group, 10 birds/replicate) as the following:

- 1- Control group; 0.0 mycofix or aflatoxin.;
- 2- Mycofix 0.25%;
- 3- Aflatoxin 2.5 ppm;
- 4- Aflatoxin 3.5 ppm;
- 5- Aflatoxin 5 ppm;
- 6- Mycofix 0.25 % +Aflatoxin 2.5 ppm;
- 7- Mycofix 0.25 % +Aflatoxin3.5 ppm;
- 8- Mycofix 0.25 % +Aflatoxin 5 ppm

Vaccine and vaccination

Live attenuated La Sota strain vaccine (TAD), with 10⁶EID 50 ML -1 has been used for vaccination at 8 and 18 days against Newcastle disease (ND). A vial of vaccine has been diluted with distilled water and serial dilutions were made to get one dose of vaccine in 1 ml distilled water. The chickens have been given 1 ml containing one dose of the vaccine via mouth using 1 ml syringes (20).

Blood sampling and serum collection

On day 28, labeled blood samples (number of birds and date) were taken from main brachial vein of the chickens, using 1 ml syringes., kept in room temperature until clotted (almost 30 minutes), the clots were denatured and kept in a water bath at 56°C for 60 minutes in order to separate the sera for serological tests (21).

Evaluation of immune response

Serum samples were used to evaluate humoral immune response. ELISA (using symbiotic corporation kits) and β -procedure hemagglutination inhibition test were used to evaluate antibody titers of the serum samples in each broiler chicks group (22, 23).

Statistical analysis

The data were analyzed using computerized statistical program (SPSS, 2005).

Results

Experiment 1: The effects of 0.0 5% mycofix and AF on ELISA Newcastle antibody titers are illustrated in table (1). From table, it is evident that all groups of chickens fed three AF levels had significantly ($p < 0.05$) low ND antibody titers compared with the control group. The addition of 0.05% mycofix to all three levels of Aflatoxin was not effective to suppress its negative effect on ND antibody titers, expressed by Geometric mean (GMT) of HI (Figure1) and ELISA tests result (Table 1). Newcastle antibody titers obtained by ELISA test compared with those obtained by HI test are shown in figure (Figure 2). ELISA titers between 6000-7000, 3000-4000, and below 1000 were equivalent to HI titers of 1/160, 1/80 and 1/10, respectively.

Table 1: Effect of three levels of Aflatoxin and 0.05% Mycofix on ELISA Newcastle antibody titers *

Group	Aflatoxin ppm	Mycofix %	ELISA titers
1	0.0	0.0	6543.00 ± 128.804a
2	0.0	0.05	6470 ± 127.301a
3	2.5	0.0	452.80 ± 15.894b
4	3.5	0.0	426.00 ± 14.226b
5	5	0.0	382.40 ± 29.662b
6	2.5	0.05	430.60 ± 7.166b
7	3.5	0.05	458.80 ± 15.682b
8	5	0.05	342.00 ± 13.885b

a-b Means within a column with no common superscript differ significantly ($p < 0.05$).

* Values represent the mean of two groups of ten broilers per each treatment.

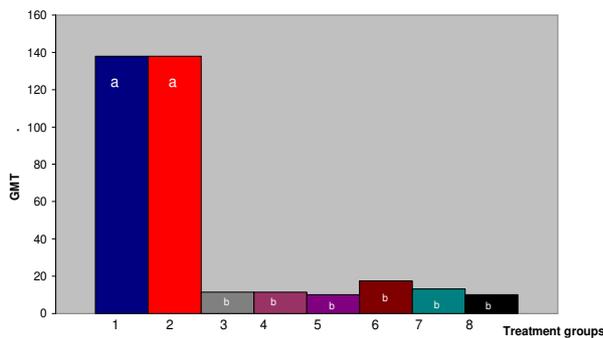


Figure 1: Effect of three levels of aflatoxin and 0.05% mycofix on GMT of ND antibody titers in broiler chickens.

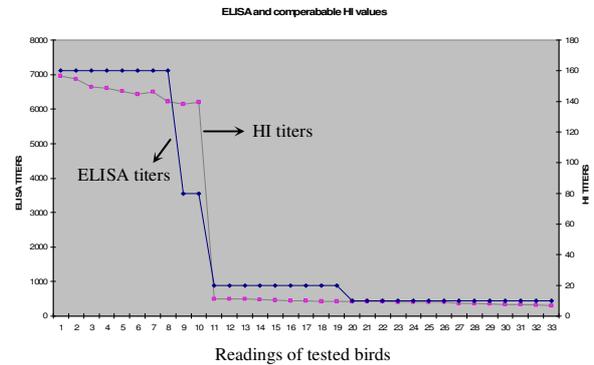


Figure 2: ELISA and comparable HI tests result of ND antibody titers in broiler chickens.

Experiment 2: In this experiment, as shown in table 2, all aflatoxin levels used were significantly ($p < 0.05$) reduce ELISA antibody titer to ND disease in orders less than that of the control one. The addition of 0.15% Mycofix in a trail to counteract the negative AF effect, revealed effectiveness only with the lower AF level (2.5 %). No positive effect noticed with the other higher AF levels of 3.5 and 5 ppm (Table 2). Improvement in GMT of ND Antibody titers was not recorded when 0.15% mycofix was added to all three AF levels (figure 3). Newcastle antibody titers obtained by ELISA test compared with those obtained by HI test are shown in figure 4. ELISA titers between 6000-7000, and below 1000 were equivalent to HI titers of 1/160, and 1/10 and 1/20, respectively.

Table 2: Effect of different Aflatoxin levels and 0.15% mycofix on ELISA antibody titers *

Group	Aflatoxin ppm	Mycofix %	ELISA titers
1	0.0	0.0	6365.60 ± 134.040a
2	0.0	0.15	6513.20 ± 151.946a
3	2.5	0.0	453.80 ± 121.187cd
4	3.5	0.0	420.40 ± 10.205cd
5	5	0.0	340.00 ± 8.549d
6	2.5	0.15	954.20 ± 17.405b
7	3.5	0.15	632.00 ± 9.279c
8	5	0.15	426.60 ± 15.114cd

a-b Means within a column with no common uperscript differ significantly ($p < 0.05$).

* Values represent the mean of two groups of ten broilers per each treatment.

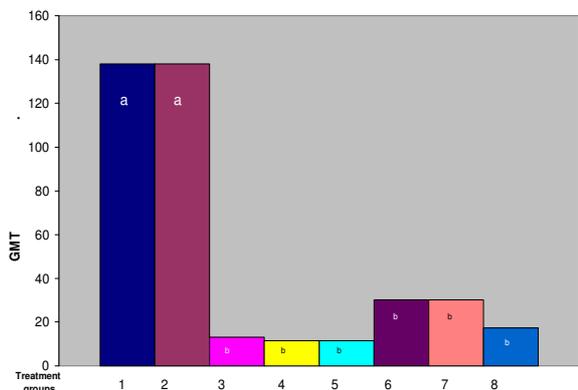


Figure 3: Effect of three aflatoxin levels and 0.15% mycofix on GMT of ND antibody titers in broiler chickens.

Experiment 3: In the third experiment, higher mycofix level, 0.25%, was added to the three AF levels in order to alleviate its negative effect on ND antibody production. Significant ($p < 0.05$) improvement was recorded here in all groups of chickens fed diets contaminated with AF and amended with 0.25% mycofix when compared with groups fed AF alone (Table 3). Haemagglutination inhibition results show the same results of those of ELISA, except that with the highest AF (5 ppm) effect on ND antibody production (figure 5). Newcastle antibody titers obtained by ELISA test compared with those obtained by HI test are shown in figure 6. ELISA titers between 6000-7000, 4000-5000, 2000-3000, and below 1000 were equivalent to HI titers of 1/160, 1/80, 1/40 and 1/10 and 1/20, respectively.

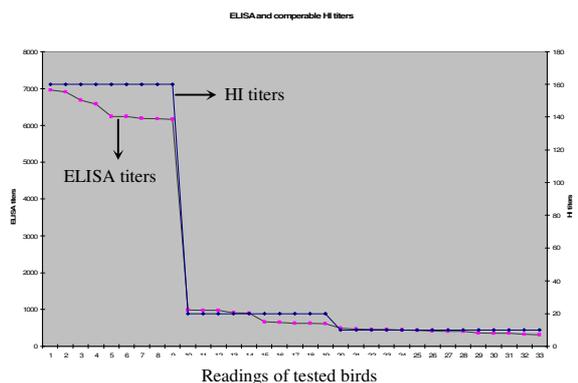


Figure 4: ELISA and comparable HI results on ND antibody titers in broiler chickens.

Table 3: Effect of different Aflatoxin levels and 0.25% mycofix on ELISA antibody titers *

Group	Aflatoxin ppm	Mycofix %	ELISA titers
1	0.0	0.0	6391.60 ± 159.379 b
2	0.0	0.25	6514.80 ± 117.978 a
3	2.5	0.0	450.00 ± 7.134e
4	3.5	0.0	429.80 ± 10.846e
5	5	0.0	427.41 ± 14.891e
6	2.5	0.25	1497.6 ± 100.152c
7	3.5	0.25	1427.00 ± 88.058cd
8	5	0.25	1190.00 ± 90.164d

a-b Means within a column with no common uperscript differ significantly ($p < 0.05$).

* Values represent the mean of two groups of ten broilers per each treatment.

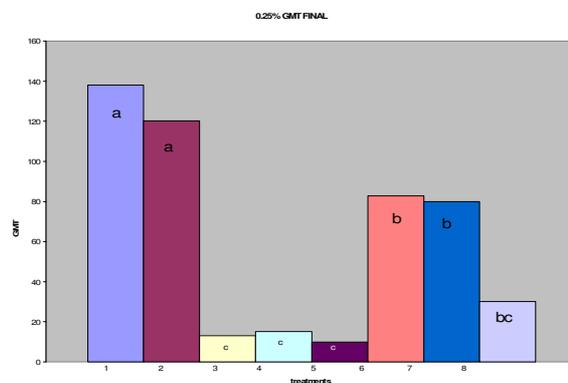


Figure 5: Effect of three aflatoxin levels and 0.25% mycofix on GMT of ND antibody titers in broiler chickens.

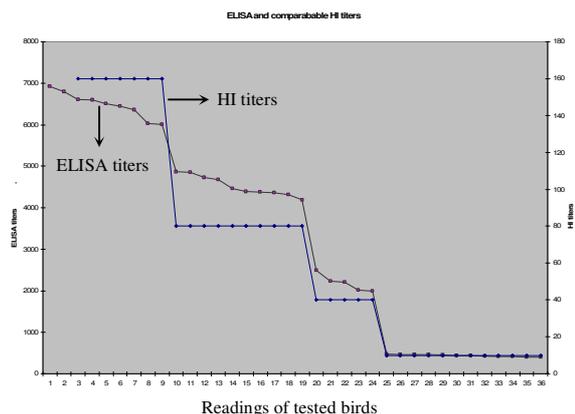


Figure 6: ELISA and comparable HI results on ND antibody titers in broiler chickens.

Discussion

Immunosuppression caused by AFB1 has been demonstrated in chickens (24). The adverse effects of aflatoxin on complement, interferon and serum proteins are presumably the result of liver injury and inhibition of protein synthesis. To counteract AF immunosuppression on antibody production, we tried in this experiment to evaluate the efficacy of mycofix plus 3.0, as a new applicable enterosorbent feed additive. All the performed three experiments confirmed the dose-response effect of aflatoxin on antibody titer profile against Newcastle disease vaccine by reducing them significantly ($p < 0.05$) when compared with the control one. These results are in agreement with our previous results of ameliorating the negative aflatoxin effect on ND antibody formation in broiler chicks during aflatoxicosis by the addition of sodium bentonite (15). The results were in the same conclusion with the results reported by Azzam, and Gabal (1998) (25), who reported reduction in antibody titers to vaccines for Newcastle disease, infectious bronchitis, and infectious bursal disease, in layers fed aflatoxin at a rate of 200 ppb for 40 weeks. The immunological suppression of aflatoxin has been documented by many authors, since antibody responses to *Pasturella multocida*, *salmonella pullorum* and Newcastle disease virus are normal in chickens exposed to low levels of dietary aflatoxin (0.2-0.5 ppm) but higher levels (0.6-10 ppm) can suppress immunoglobulin (Ig) IgG or IgA and antibody response to Salmonella and sheep RBCs (26). Edds *et al.* (1973) (6) reported that chickens whether vaccinated or not against Marek's disease (MD) receiving a diet containing 0.2 ppm AFB1 were more susceptible to challenge inoculation with MD virus than were controls. Similarly, chickens receiving 0.5 ppm dietary AFB1 and vaccinated against MD showed a significantly higher frequency of gross and microscopical lesions of MD than did chickens receiving aflatoxin-free diets (27). The presence of low levels of AFB1 in the feed appears to decrease vaccinal immunity and may therefore lead to the occurrence of disease even in properly vaccinated flocks. Immunosuppression caused by AFB1 has been demonstrated in chickens (24). The adverse effects of aflatoxin on complement, interferon and serum proteins are presumably the result of liver injury and inhibition of protein synthesis. The toxin could induce thymic aplasia (28); reduce T-lymphocyte function and number; suppress phagocytic activity; reduce complement activity (28,29); suppression of cell-mediated immune responses (30); thymic and bursal involution; suppression of lymphoblastogenesis; impairment of delayed cutaneous hypersensitivity (31); and graft-versus-host reaction (32); impairment of lymphokines production and antigen processing by macrophages (33); as well as a decrease in or lack of the heat-stable serum factors involved in

phagocytosis (34). Here, ELISA and Haemagglutination inhibition results, urged us to look in the value of vaccination against ND when chicks fed diets contaminated with aflatoxin. However, Mycofix, as one of the proposed solutions to the problem of poultry feed contamination with AF, and to counteract the negative aflatoxin effect on antibody production, should be added at its highest inclusion recommended level of 0.25%, to neutralize moderate levels of aflatoxin (2.5-3.5 ppm), but not high AF level of 5 ppm. The beneficial effect of Mycofix in ameliorating the negative effect of AF on ND antibody titers is related to its role in protection birds from the effect of AF through its chemisorption of AF. Mycofix deactivates aflatoxin with its polar functional group, due to AF fixation to adsorbing components in Mycofix, with stable binding capacity. Adsorption already starts in the oral cavity during salivation and continues in stomach and gut. The fixed mycotoxin being unable to enter the blood and subsequently excreted in feces after 98% adsorption of AF by Mycofix (35). In addition, Mycofix contains phytochemical substances, a hepatoprotective flavolignins (silymarin), which prevents toxins from entering the liver cell membranes, and contains also the terpenoid complexes, which reduce inflammations and protect the mucous membranes. Strengthening body's natural immune response, by phytochemical constituents of Mycofix, which compensate the immune-suppressive effect of AF by modulating immune responses and enhancing metabolic functions. These phytochemical substances support the synthesis of ribonucleic acids as well as the conversion and catabolism of amino acids, which are crucial factor in cell proliferation. The situation of immunosuppression most certainly occurs more frequently than is currently recognized. Therefore, the poultry industry must exercise extreme caution to manage mycotoxicosis with specific regard to maintenance of best health and immune status. In a field condition, a situation may arise which often confuses. Regrettably, the failure of bird to develop immunity is seldom linked to mycotoxins. From practical point of view, disease control means improved immunity, which obviously draws attention for mycotoxicosis. In spite of all attained efforts, mycotoxicosis invariably creep into the feed-stuffs which is practically unavoidable, nevertheless the use of mold inhibitors and toxin binders provide practical solution.

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