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Ameliorative effect of mycofix on infectious bursal disease virus antibody titer in broiler chicks fed aflatoxin

M. T. Gargees, A. M. Shareef

Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract

The effect of including Aflatoxin (AF) and mycofix in broiler diets on antibody titers against infectious bursal disease virus (IBDV) was evaluated. A total of 480 broilers were distributed into a completely randomized three experiments (160 birds/each) with four levels of AF (0, 2.5, 3.5 and 5 parts/10^6, and four levels of mycofix (0, 0.05, 0.15 and 0.25%), totaling eight treatments with two replicates for each experiment. Chicks were vaccinated at 7 and 14 days of age with infectious bursal disease live vaccine (CEVA, winter field 2521 G-16)). For antibody analyses, Enzyme-linked immunosorbent assay (ELISA), was used, and blood samples were collected at 28 days of age by jugular vein puncture. The inclusion of mycofix, was effective in stimulating the humoral immune responses against IBDV with each increase of its level of inclusion. Only the highest high level (0.25%) of mycofix inclusion, resulted in significantly ($P<0.05$) returning ELISA antibody titers against IBDV in chicks fed all levels of AF to those of the control values.

Keywords: Aflatoxin, Chicken, Mycofix, ELISA
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تاثيرالمايكوفكس في تخفيف تاثير سموم الافلاعلى تكوين الاضداد في أفراخ فروج اللحم الملقحة ضد مرض التهاب غدة فابريشيا

معن طاهر جرجيس، عقيل محمد شريف

فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

قيم تاثير اضافة سموم الافلا والمايكوفكس الى علائق فروج اللحم على معاييراضداد مرض التهاب غدة فابريشيا قسمت ٤٨٠ من افراخ فروج اللحم على ثلاث تجارب وبشكل عشوائي (١٦٠ طير /تجربة) باضافة اربعة مستويات من سموم الافلا (صفر2.5 3.5 5) و اربعة مستويات من المايكوفكس (0 و 0.15% 0.25%) 0.05% لثمان معاملات /تجربة وبمكررين /معاملة لقحت الافراخ بعمر٧ و١٤ يوم باللقاح الحي المضعف (CEVA,Winterfield 2521G-16) استخدمت تقنية الممتزالمناعي المرتبط بالانزيم ,(ELISA) حيث جمعت عينات الدم من الوريد الوداجي للافراخ بعمر ٢٨ يوم حفزالمايكوفكس على انتاج الاضداد ضد مرض التهاب غدة فابريشيا في الافراخ المستهلكه للعلائق الملوثه بسموم الافلا وفي جميع مستويات للمتز (0.05 %0.15,% 0.25,%) المضافة لمستويات سموم الافلا (2.5 3.5 و٥ جزء بالمليون). الا ان الممتز مايكوفكس و عند اعلى مستوى0.25 % فقط كان كفيلا باعادة مستوى الاضداد بصورة معنويه في مجاميع الافراخ المستهلكه لمستويات سموم الافلا الثلاثة لمستويات مجموعة السيطرة.

Introduction

Due to a lack of biosecurity and management practice, free-range chickens are more or less constantly exposed to the risk of immunosuppressants like aflatoxins and infectious bursal disease virus (IBDV), which might lead to increased susceptibility to diseases and vaccination failures (1). Infectious Bursal Disease (IBD) is an acute, highly

contagious viral infection of growing chickens. It is caused by a double stranded, bisegmented RNA virus belonging to the genus *Avibirna* virus of the family *Birnaviridae* (2). IBD virus (IBDV) primarily attacks the lymphoid tissue with a special predilection for the bursa of Fabricius, resulting in depletion of B cells by inducing apoptosis in severely immunodepressed chickens (3). The primary target of the virus is B-lymphocytes and T-cells (4). IBD is a serious menace in the development of poultry enterprise and has resulted in major worldwide economic losses (5). Such outbreaks have caused colossal losses to poultry farmers in Iraq (6-8) and may be due to the occurrence of antigenic variant strains, interference by maternal antibodies or to other immunosupressive afflictions such as aflatoxicosis/ mycotoxicosis (9). Aflatoxins are a group of related toxic metabolic byproducts in feeds produced by *Aspergillus flavus* and other species of *aspergilli*. *A.flavus* produces aflatoxin B1 only, the most important of the toxins (10). Aflatoxins are a concern to village poultry production because of the frequency of contamination of animal feeds and the threat they pose to animal health. Their economic impact on animal production and passage into the human food chain is of great concern globally (11). These toxins are particularly a problem in developing countries where hot and humid climates, poor post-harvest management (12-14)) combined with insect damage of feed resources and lack of regulatory systems for aflatoxin monitoring and control are conducive to growth of fungi in grains/cereals, which form the bulk of crop residues fed to free-range poultry. Aflatoxins reduce complement activity, which is the most sensitive aspect of the immune system they alter, reduce growth rate, cause depression of cell-mediated immunity (CMI) and general immunosuppression in chickens leading to poor vaccination response (15,16). 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 (17). Silica-containing compounds are practical and economical feed additives and can reduce the effects of aflatoxin (18). Bentonite clay also ameliorates aflatoxicosis, and aflatoxin induced reduction in antibody production (15,19). Various sorbents have different affinities for aflatoxins and therefore differ in preventing the biological exposure of aflatoxin to the animals consuming contaminated feeds. Mycofix was reported to be effective in ameliorating Aflatoxic negative effect on Newcatle antibody productionin broiler chickens (20). Therefore, our trial was conducted to evaluate mycofix, for alleviating aflatoxin negative effect on infectious bursal disease 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, Mosul,Iraq.

Broilers

Four hundreds and eighty, male one-day old broilers (cobb),were divided to three experiments,One hundred and sixty chicks for each (20 birds /groups, 10 birds / replicate). Broiler chicks 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.

Aflatoxin

Aflatoxin was prepared through inoculation of rice by *Aspergillus parasiticus* NRRL 2999 (21,22). Fermented rice was then autoclaved and ground. The flatoxin content was measured by spectophotometric analysis (23,24). 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.

Design of the experiments

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 plus 3; 0.05%;
3-Aflatoxin 2.5 ppm;
4-Aflatoxin 3.5 ppm;
5-Aflatoxin 5 ppm;
6- Mycofix plus 3; 0.05 % +Aflatoxin 2.5 ppm;
7- Mycofix plus 3; 0.05 % +Aflatoxin3.5 ppm;
8- Mycofix plus 3; 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 plus 3;0.15%;
3-Aflatoxin 2.5 ppm;
4-Aflatoxin 3.5 ppm;
5-Aflatoxin 5 ppm;
6- Mycofix plus 3; 0.15 % +Aflatoxin 2.5 ppm;
7- Mycofix plus 3; 0.15 % +Aflatoxin3.5 ppm;
8- Mycofix plus 3; 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 plus 3; 0.25%;
3-Aflatoxin 2.5 ppm;
4-Aflatoxin 3.5 ppm;
5-Aflatoxin 5 ppm;
6- Mycofix plus 3; 0.25 % +Aflatoxin 2.5 ppm;
7- Mycofix plus 3; 0.25 % +Aflatoxin3.5 ppm;
8- Mycofix plus 3; 0.25 % +Aflatoxin 5 ppm

Vaccine and vaccination

Live attenuated infectious bursal disease vaccine (CEVA IBDL, Winterfield 2512 G-61, France), has been used for vaccination at 7 and 14 days against infectious bursal disease. 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 (25).

Blood sampling and serum preparation

On day 3 and 28, labeled blood samples (number of birds and date) were taken from chickens and kept overnight under refrigerator temperature. For serum separation, test tubes containing blood samples were centrifuged at 3000 rpm. for 10 minutes.

Evaluation of immune response

Serum samples were used to evaluate IBD humoral immune response. Enzyme-linked immunosorbent assay (ELISA), using symbiotics corporation kits, to evaluate antibody titers in each group broiler chicks.

Statistical analysis

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

Results

levels of maternal antibodies

Maternal antibody titers of broiler chicks at 3 days of age are presented in table 1. ELISA Titers were ranged from 1500-6000, with mean of coefficient of variation (CV%) of 128.8%.

Experiment 1

The effects of 0.0 5% mycofix and AF on ELISA IBDV antibody titers are illustrated in table (2). From figure, it is evident that all groups of chickens fed three AF levels had significantly (P<0.05) low IBDV antibody titers compared with the control group. The addition of 0.05% mycofix to all three levels of Aflatoxin was not effective to counteract its negative effect on IBDV antibody titers, which were ranged between 2000-4000.

Aflatoxin part/10^6	Mycofix %	Mean ELISA Antibody titer	SEM
0	0	5200 a	115
0	0.05	5248 a	117
2.5	0	1199 b	155
3.5	0	1247 b	98
5	0	799 bc	79
2.5	0.05	2426 c	112
3.5	0.05	3548 c	148
5	0.05	2449 d	100

Experiment 2

In this experiment, as shown in table 3, all aflatoxin levels used were significantly (P<0.05) reduce ELIZA antibody titer to IBDV 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 no effectiveness to do so, although some improvement in antibody titer was noticed, being raised to 3000-4000.

Aflatoxin part/10^6	Mycofix %	Mean ELISA Antibody titer	SEM
0	0	5260 a	126
0	0.05	5069 a	115
2.5	0	1258 b	107
3.5	0	1238 b	117
5	0	879 bc	112
2.5	0.05	3999 c	112
3.5	0.05	3944 c	123
5	0.05	3296 c	129

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 IBDV 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 4). Titers were returned to those of the control one of 4000-5000.

Aflatoxin part/10^6	Mycofix %	Mean ELISA Antibody titer	SEM
0	0	5284 a	115
0	0.05	5199 a	107
2.5	0	1267 b	107
3.5	0	1218 b	179
5	0	814 b	122
2.5	0.05	4841 a	114
3.5	0.05	4645 a	100
5	0.05	4497 a	100

Discussion

In our study we vaccinated the experimental chicks at 7 and 14 days of age with IBDV as recommended by (26), when CV% of the chicks more than 80%, giving a heterogeneous individuals titers. It should be noted that if breeder animals are vaccinated and the chickens have maternal antibodies the day of the first vaccination should be postpone, if not, they should be vaccinated the first day of life. Munoz (2003) (27) recommends continuous monitoring of the vaccination responses and the levels of maternal antibodies to determine the best day for the first vaccination. Usually the variations in antibody responses after vaccination are higher with live vaccines than with inactivated ones. The half-life of maternal antibodies is approximately 3 days (28) and maternal protection often lasts 2-4 weeks after hatching (29).

Immunosuppression caused by AFB1 has been demonstrated in chickens (30). 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 infectious bursal disease vaccine by reducing them significantly ($P<0.05$) when compared with the control one. These results are in agreement with the results of ameliorating the negative aflatoxin effect on ND and IBDV antibody formation in broiler chicks during aflatoxicosis by the addition of mannanoligosaccharides (31).The results were in the same conclusion with the results reported by Azzam, and Gabal (1998) (32), 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(0.6-10ppm) can suppress immunoglobulin(Ig) IgG or IgA and antibody response to Salmonella and sheep RBCs (26). Edds *et al*.(1973) (34) reported that chickens whether vaccinated or not against Mareks disease (MD) receiving a diet containing 0.2ppm 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 (35).

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 (30). Feeding of aflatoxin containing feed to poultry has a detrimental effect on humoral and cell mediated immune response, leading to fulminating disease outbreaks even after vaccination. Mohiudin (1993) (36) documented a marked decline in antibody titres and phagocytic activity representing an impairment of humoral and cell mediated immune systems in experimentally-fed aflatoxin as low as 0.2 ppm. Similarly, (37) recorded a significant decrease in serum globulin component by feeding aflatoxin level in different feed samples from these poultry farms ranging between 31 and100 ppb. Such a high aflatoxin content in feed could have impaired the immune system, thereby resulting in vaccination failure. Earlier, (38) reported a severe IBD outbreak with amortality rate of up to 35.8% in grower pullets that were vaccinated withIBD vaccine, ascribable to immunosuppression owing to aflatoxicosis.

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 (39); impairment of lymphokines production and antigen processing by macrophages (40); as well as a decrease in or lack of the heat –stable serum factors involved in phagocytosis (41). Here, ELISA results, urged us to look in the value of vaccination against IBDV 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). The beneficial effect of Mycofix in ameliorating the negative effect of AF on IBDV antibody titers is related to its three strategies in protection birds from the effect of AF through its fist strategy in chemosorption of AF. Mycofix deactivates aflatoxin with its polar functional group, due to AF fixation to adsorping 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 (42). In addition, Mycofix in its second strategy contains phytogenic substances, a hepatoprotective flavolignins (silymarin), which prevents toxins from entering the liver cell membranes, and by the third strategy of containing the terpenoid complexes, which reduce inflammations and protect the mucous membranes. Strengthing body's natural immune response, by phycophytic constituents of Mycofix, could compensate the

immune-suppressive effect of AF by modulating immune responses by enhancing metabolic functions. These phycophytic 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 immunosuppressant most certainly occurs more frequently than is currently recognized. Therefore, the poultry industry must exercise to 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 confuse. Regretfully, 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 feedstuffs which is practically unavoidable, nevertheless the use of mold inhibitors and toxin binders provide practical solution.

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