Effect of some feed additives against toxicity induced by aflatoxin contaminated diet in broiler

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Abstract

The effects of the *Saccharomyces cerevisiae* (SC) and Fructooligosaccharide (FOS) on body weight, feed conversion, body weight gain and immune response was investigated in broiler fed on aflatoxin (AF). A total number of 180 one day old broiler chickens (Hubbard classic) were randomly distributed into 6 experimental groups; G1: control (basal diet), G2: (basal diet + SC 2%), G3: (basal diet + FOS 0.25%), G4: (basal diet + AF100µg), G5: (basal diet + AF100µg + SC 2%), G6: (basal diet + AF100µg + FOS 0.25). The weight range, feed conversion, body weight gain and humoral immunity of birds were evaluated. Results of (G2) and (G3) show a significant (P<0.05) increase weight range, body weight gain, fed conversion ratio and the antibody titers in compare to control, while the AF contaminated diet (G4) showed decrease in body weight gain, feed conversion ratio and the antibody titers against Newcastle disease virus. Aflatoxin groups treated with 2% *Saccharomyces cerevisiae* (G5) and 0.25% fructooligosaccharide (G6) show increased in the broilers performance value and humoral immune response of broilers vaccinated against Newcastle virus disease as compared to the aflatoxin group (G4). In conclusion the addition of *Saccharomyces cerevisiae* and fructooligosaccharide had a beneficial effects on broilers health and can minimize the effect of aflatoxin on broiler performance and immunity.
Key words: Aflatoxin, broiler, feed additives, Probiotic, prebiotic.

دراسة تأثير بعض الإضافات الغذائية ضد التسمم المستحدث بالأفلاتوكسين في علف فروج اللحم

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الخلاصة

جرى دراسة تأثير اضافة الخميرة و سكر الفركتوز على معدل الوزن والتحويل الغذائي والزيادة الوزنية والاستجابة المناعية مع دراسة تأثير اضافة البروبايوتك والبريبايوتك عليها مع او بدون تلوث العليقة بسموم الافلاتوكسين على فروج الحم حيث تم تربية 180 فرخة بعمر يوم واحد وتم تقسيمها عشوائيا الى 6 معاملات؛ المعاملة الاولى (G1) تمثل مجموعة السيطرة تناولت العليقة الاساسية ، المعاملة الثانية (G2) تناولت العليقة الاساسية + 2% من الخميرة ، المعاملة الثالثة (G3) تناولت العلقة الاساسية + 0,25 % من السكر المعقد ، المعاملة الرابعة (G4) تناولت العليقة الاساسية + 100 مايكروغم / كغم الافلا توكسين ، المعاملة الخامسة (G5) تناولت العليقة الاساسية + 2% من الخميرة + 100مايكروغم/كغم الافلا توكسين ، المعاملة السادسة (G6) تناولت العلقة الاساسية + 0,25 % من السكر الاحادي + 100مايكروغم/كغم الافلا توكسين ، تم قياس معدل الاوزان ومعدل التحويل الغذائي ، الزيادة الوزنية والمناعة المصلية. اظهرت نتائج المعاملة الرابعة انخفاض في معدل الاوزان ومعدل التحويل الغذائي ، الزيادة الوزنية ، ومعدل الاجسام المضادة لمرض النيوكاسل بالمقارنة مع مجموعة السيطرة (G1). اظهرت نتائج المعاملة الثانية والثالثة ارتفاع معنوي في معدل الاوزان والتحويل الغذائي ، الزيادة الوزنية ومعدل الاجسام المضادة لمرض النيوكاسل بالمقارنة مع مجموعة السيطرة ، معاملتي السم الفطري التي تم معالجتها ب 2% من الخميرة و0,25% من السكر المعقد (G5) و(G6) اظهرت ارتفاع معنوي في معدل التحويل الغذائي ، الزيادة الوزنية ومعدل الاجسام المضادة لمرض النيوكاسل بالمقارنة مع المعاملة الرابعة. نستنتج من الدراسة ان اضافة 2% من الخميرة و0,25% من السكر الفركتوزي قد يكون لها تأثيرات ايجابية ونفعية على أداء فروج اللحم يمكن ان تؤدي الى التقليل من التأثيرات السلبية لهذا السم الفطري على فروج اللحم.
الكلمات المفتاحية: السموم الفطري ، فروج اللحم ، الاضافات الغذائية ، المعزز الحيوي ، السابق الحيوي.

Introduction

Contamination of feed with mycotoxin is a global problem. Poultry industry suffers greater economic losses due to greater susceptibility of the species compared to other animals to the toxin and also due to continuing intermittent occurrences in diet (1). Mycotoxins are a group of extremely toxic and biologically active substances. Among them aflatoxins (AF) which are produced by the fungi *Aspergillus flavus* and *A. parasiticus* (2). .*A. flavus* the most common contaminant of many grains used in human and animal nutrition and also birds (3). AFB1 is considered to be the most potent hepatotoxins and well-known hepato-carcinogens (4). The report on contamination of grains with AF showed that 77% was due to B1, while the rest are contaminated with other AF types (2). In birds Aflatoxin causes poor growth and feed conversion, increased mortality, decreased egg production, leg problems, liver damage and carcass condemnations (5). There is high correlation between outbreaks of Newcastle disease (ND) and aflatoxin contamination of broiler rations. Humoral immune response of broilers could increase and decrease depending upon the level and length of exposure to the toxin (6,7). The probiotic (live yeast; SCE) is used to control the severity of AF and provided significant improvements (8). The probiotic term was first used for the substances produced by microorganisms that stimulate the growth of man and animals. The name is taken from the Latin words "pro" and "bios"(9). Prebiotic partly and/or completely reversed the adverse effect of AF on performance, biochemistry, hematology and immune responses of birds (10). A prebiotic compound is defined as a non-digestible food ingredient that can be utilized by intestinal micro flora, which beneficially affects the host (11), also used for detoxification of other mycotoxins such as zearalenone (12).

Materials and methods

Aflatoxin preparation

Pure crystalline AFB_1 (Sigma Chemical Co. USA) was incorporated into the diets by dissolving it in chloroform (13), dissolving AFB1 (1 mg/10 mL) (14). Ten (10) ml of AFB1solution completing to 250 ml of chloroform then the solution mixing with 250 grams of ground feed then mixed into the basal diet to provide the desired level of AFB1/kg of diet (100 ppb of AFB1/kg of feed), all above steps a cure in a deep freezer. The diets were analyzed for aflatoxin content. The toxin was calculated using Neogen ELISA kit (Neogen Corporation) with XL800 reader (15).

Probiotic

Live yeast (*Saccharomyces cerevisiae*) (Daehan new pharm company-korea) mixed with the ration at a rate of 2 g/kg (0.2 %).

Prebiotic

Fructooligosacred from (sigma aldrich company; U.S.A) mixed with the ration at a rate of 0.25 g/kg.

Chickens and diets

One-hundred and eighty (1-day-old), Hubbard classic-strain chicks were provided from a commercial hatchery. Individually weighed chicks were divided into 6 groups of 30 birds. The chicks were housed in floor pen. The chicks were assigned to the following treatment; Group (G1) control diet, basal diet without additive; (G2) basal diets + 2% *Saccharomyces cerevisiae*; (G3) basal diet + 0.25% fructooligosaccharide; (G4) basal diet + AFB1100µg; (G5) basal diet + 0.2% *Saccharomyces cervisiae* + AFB1100µg; (G6) basal diet + 0.25% fructooligosaccharide + AFB1100µg. The chicks were housed in a temperature-controlled room with continuous lighting and were fed with a commercial ration. To maintain accurate and safe control the diets containing the various treatments were placed in plastic feed containers in the growing house. Basal diet was formulated according to the National Research Council (16) requirements of all nutrients, without antibiotics, coccidiostats, or growth promoters: (Table 1). Feed and water were provided ad libitum throughout the experiment. Chickens were vaccinated at the 1^{st} day of age against Newcastle Disease (ND) using clone 30 vaccine via eye drop, and at the 12^{th} day of age against Newcastle

Table (1): Composition and nutrient content of basal diets (%)

Ingredients	Starter % 1-14days	Grower % 15-42days
Maize	43.5	44.3
Wheat	25.8	1.8
Soybean meal	27.8	27.2
Protein concentrate	10	10
Salt (Nacl)	0.3	0.3
Di-calcium phosphate	0.4	0.4
total	100	100
Calculated nutrients composition		
Crude protein (%)	22.11	21.20
ME (cal/kg)	3005.5	3105.6
Percent of ME/ crude protein	136	146.5
Lysine (%)	1.14	1.08
Methionine and cystine (%)	0.78	0.75
Calcium (%)	1.03	1.03
Phosphorous available (%)	0.47	0.47
Crude fiber (%)	3.603	3.466
Fat(%)	5.23	6.64

(NRC1994)

Disease (ND) using Lasota vaccine via drinking water and at 14th day against infectious bursal disease using Winterfield strain in drinking water. At 27th day old, vaccination was done against ND using clone 30 respectively by eye drop and at 28th day against infectious bursal disease using winterfield strain in drinking water. Body weight gain were determined weekly, and mortality was recorded as it occurred. Feed was weighed on the same days above to evaluate the feed conversion ratio (FCR).

Samples collection

Blood samples

Five birds from each group were selected randomly and blood was collected via wing vein for evaluation of humoral immunity after vaccination. For separation of the serum, blood was left to clot, centrifuged at 3000 rpm for 10min. and then the samples were preserved at -20°C till submission for further analysis. Serum samples were used to evaluate humoral immune response. ELISA (using symbiotic corporation kits) (17).

Statistical analysis

Statistical analysis was done by using two way ANOVA method as suggested by (18). The data were analyzed using computerized statistical program (SPSS, 2011). All statements of significance are based on the level of probability $P<0.05$.

Results

Growth performance

Data presented in Tables 2,3 and 4 show the effects of dietary treatments on performance**.** Feeding AF (G4) caused significant ($P<0.05$) decreases in BW and BWG from week 3 onwards compared with the control (G1), and caused poor feed conversion ratio compared with the control (G1) diet. Group feed *S. cervisiae* (G2) and Fructooligosaccharide (G3) show significant ($P<0.05$) increase body weight and body weight gain compare to control (G1). G2 and G3 show significant ($P<0.05$) low feed conversion ratio compare to control (G1). The addition of *S. cervisiae* (G5) and Fructooligosaccharide (G6) to an AF-containing diet significantly ameliorated the adverse effects of AF on body weight, body weight gain and feed conversion ratio in compared with G4**.**

Table (2): Effect of Fructooligosaccharide and *S. cerevisiae* on body weight of broiler chicks fed diet containing100µg aflatoxin B1/kg at 1 to 42 days of age.

Groups	Age				
	Wk. 1	Wk. 2	Wk. 3	Wk. 4	Wk. 5
G1	110±5.77AC	293±1.73AB	620±11.54A	1100±28.56A	1600±28.86A
G2	111±6.35AC	307±4.04AB	655±2.88B	1300±57.73B	1900±28.86B
G3	94±2.3BC	316±4.16A	650±2.88B	1250±28.86B	1910±5.77B
G4	92±1.15BC	310.33±28.47A	529±2.3C	850±28.86C	1500±28.86C
G5	101±0.57C	370±2.88B	575±2.88D	1150±28.86A	1780±11.54D
G6	113±1.73A	290±2.88AB	580±2.88D	1100±28.86A	1800±28.86D

Mean in the same column with different superscript letters (A,B,C and D) are significantly different (P<0.05) .
G1; (basal diet), G2; (basal diet+SC), G3; (basal diet+FOS), G4; (basal diet+AFB1), G5; (basal diet +SC +AFB1), and G6; (basal diet+ FOS+AFB1).

Table (3): Effect of Fructooligosaccharide and *S. cerevisiae* on body weight gain of broiler chicks fed diet containing100µg aflatoxin B1/kg at 1 to 35 days of age.

Groups	Age				
	Wk. 1	Wk. 2	Wk. 3	Wk. 4	Wk. 5
G1	70±2.88A	178.33±2.02A	327±1.15A	450±5.77A	450±14.43A
G2	66±2.51A	196±1.15B	348.33±1.66B	645±2.88B	600±14.43B
G3	49±3.46Bc	221±3.48c	334±2.3C	600±28.86BC	550±28.86C
G4	47±1.73B	175±28.47AD	262±1.15D	320±11.54C	400±28.86A
G5	56±1.73C	169±2.3D	305±2.88E	575±14.43C	530±17.32C
G6	68±1.15A	177±4.04AD	285±2.88F	520±11.54D	600±14.43B

Mean in the same column with different superscript letters (A,B,C and D) are significantly different (P<0.05) .
G1; (basal diet), G2; (basal diet+SC), G3; (basal diet+FOS), G4; (basal diet+AFB1), G5; (basal diet +SC +AFB1), and G6; (basal diet+ FOS+AFB1).

Table (4): Effect of Fructooligosaccharide and *S. cerevisiae* on feed conversion ratio of broiler chicks fed diet containing100µg aflatoxin B1/kg at 1 to 35 d of age.

Groups	Age				
	Wk. 1	Wk. 2	Wk. 3	Wk. 4	Wk. 5
G1	1.69±0.13A	1.67±0.15AB	1.55±0.014A	1.59±0.02A	1.77±0.05A
G2	1.69±0.02A	1.47±0.02A	1.45±0.016B	1.12±0.02B	1.4±0.04B
G3	1.64±0.11A	1.49±0.02A	1.29±0.01C	1.19±0.05B	1.51±0.09B
G4	1.75±0.09A	1.84±0.01B	1.67±0.014D	2.02±0.09C	2.02±0.02C
G5	1.74±0.07A	1.52±0.01A	1.44±0.018B	1.34±0.02B	1.51±0.07B
G6	1.6±0.04A	1.63±0.04A	1.57±0.014A	1.52±0.03A	1.38±0.03B

Mean in the same column with different superscript letters (A,B,C and D) are significantly different (P<0.05) .
G1; (basal diet), G2; (basal diet+SC), G3; (basal diet+FOS), G4; (basal diet+AFB1), G5; (basal diet +SC +AFB1), and G6; (basal diet+ FOS+AFB1).

Immune responses

The effects of experimental diets on antibody titers against NDV are presented in table (5). The lowest antibody titer against NDV was found in the birds of AF group (G4) at 3th and 5th week and the different was significantly (P<0.05) compare to control, groups feed diet with *S. cerevisiae* (G2) and Fructooligosaccharide (G3) show high antibody titer against NDV compared to control (G1) and other groups at 3th and 5th week. The addition of *S. cerevisiae* (G5) and Fructooligosaccharide (G6) to the AF-containing diets (100 ppb) show increase antibody titer against NDV compared to AF group (G4) and control, while both are significantly lower than G2 and G3 at 3th and 5th week.

Table (5): Effect of Fructooligosaccharide and *S. cerevisiae* on maternal and humoral immune response (Mean ELISA Antibody titer) of broiler chicks fed diet containing 100µg aflatoxin B1/kg.

Age	Groups					
	G1	G2	G3	G4	G5	G6
MDA 1/day	7850±28.86 B	6955±2.88B	7850±28.8 6B	7600±28.76B	6955±2.88B	6910±5.77B
Wk. 1	2566.33±300.8 2AB	3252.33±423. 95A	3237.66±407.9 4A	1259.33±810. 46B	1913.33±700. 7AB	2656±867.47 AB
Wk. 3	7158±106.14A	9879±43.49B	11024.66±303. 69C	5822.66±132. 12D	7097±80.77A	6962.66±162 .46 .A
Wk. 5	2238.33±229.0 05A	5195.33±473. 29B	7881±304.06C	1279.66±185. 6D	1978.33±184. 97A	2375.33±64. 54A

Mean in the same column with different superscript letters (A and B) are significantly different (P<0.05). G1; (basal diet), G2; (basal diet +SC), G3; (basal diet +FOS), G4; (basal diet +AFB1), G5; (basal diet +SC +AFB1), and G6; (basal diet+ FOS+AFB1).

Discussion

The toxicity of AF in poultry has been well documented, as specified by (19). Severe economic losses have been reported in the commercial poultry industry due to aflatoxicosis (20). Contamination of AF in feed causes aflatoxicosis in poultry that is characterized by reduced feed intake, decreased weight gain, poor feed conversion ratio (21). Adverse effects of AFB1 on studied production traits are consequent to protein synthesis and lipogenesis inhibition (22). The weak post vaccinal immune response in chickens supplemented with aflatoxin B via their feed could be attributed to the regressive development of the thymus and the bursa of Fabricius (23). Our results of growth performance below to chickens of group (4) that fed on ration contaminated with aflatoxin B1only showed the significant ($p<0.05$) poorest performance compared with other groups. These obtained results are consistent with previous reports on the performance depressing effects of aflatoxin B1 (24). The adverse effect of aflatoxin B1on feed conversion ratio in present study agree with (25), who report no effects of low aflatoxin diet on the feed consumption of broilers but reduced feed conversion ratio. In our study the lower body weight and body weight gain of birds agree with results obtained by (26), and increase feed conversion ratio that constant with the results of (27) in broiler and other poultry species. The adverse effects of AFB1 on growth performance have been associated with a decrease in protein and energy utilization, probably as a significance of a deterioration of the digestive and metabolic efficiency of the birds (14). In our study addition of *S. cerevisiae* in broilers' diet improved body weight gain and feed conversion ratio, that agreement with (28). The role of *S. c.* in aflatoxins detoxification may be recognized to its toxin binding ability, in addition to stimulation the immune system and by competing for binding sites on enterocytes which inhibit colonization of the intestine by pathogens, indicating its possible beneficial effect on mycotoxicosis in broiler chickens (29). Aflatoxin B1group plus *S. cerevisiae* showed a significant ($p<0.05$) increase the performance compare to group feed aflatoxin alone, this results agreement with previous study (30). Group treated with Fructooligosaccharides alone showed a significant ($p<0.05$) high performance value compared with control group, that agree with (31). The physiological effects of related to Fructooligosaccharides have been shown to enhance the growth of *Bifidobacterium*, *Lactobacillus* and inhibit *Escherichia coli* in large intestine and stimulation of the immune system (32). Fructooligosaccharides was decrease the adverse effects of Aflatoxin, that agree with (10,33) they have been observed that the addition of prebiotics to AF containing diet significantly recovered the adverse effects of AF on performance through binding toxic molecules and prevent their absorption from the gastrointestinal tract. In our study we report the adverse effect of Aflatoxin on the antibody titers against Newcastle Disease virus (NDV), decrease in the antibody titer values against ND vaccine was detected upon feeding Aflatoxin, that depression in titer values is a clear indicator of immune-depressing effects of Aflatoxin on humoral antibody, that agree with the previous reports of (27, 34). The reduction of antibody titers could be due to inhibition of DNA and protein synthesis by aflatoxin through deficiency of amino acid transport and m-RNA transcription, resulting in lowered level of antibody production (34). Group treated with *S. cerevisiae* alone (G2) show an increase in antibody titer against NDV compared with control group (G1),that agree with previous research (35) they reported that using a *Saccharomyces cerevisiae* fermentation product showed an increase in immune function of broilers when fed for 42days. Antibody titer against NDV significantly increased in group fed diet containing Fructooligosaccharide alone as compared with the control group (G1) and other groups, that agreement with (36). How showed that addition of prebiotics to the diet of broilers chicks stimulating the humoral immune response against IBDV and NDV vaccine viruses. Antibody titer against NDV increased in group fed diet containing aflatoxin plus *S. cerevisiae* (G5) as compared with the AF group (G4) that agree with previous study report that the combinations from *Saccharomyces cerevisiae* reduce the immunosuppressive effects of the mycotoxins and stimulate the immune response resultant in higher levels of the antibody in response to the vaccines (37). in group fed diet containing aflatoxin plus Fructooligosaccharide show increase antibody titer against NDV compare to AF group (G4) and that agree with (10).

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